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Treatment of Cisplatin Induced Kidney Injury in Rats by Bone Marrow-derived Mesenchymal Stem Cells

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Abstract

Background: Cisplatin is one of the most important antineoplastic drugs used in the treatment of many cancers; however the main dose-limiting side effect of cisplatin is nephrotoxicity. It has multiple cellular targets and modes of action that lead to nephrotoxicity. The present study aims to test whether bone marrow-derived mesenchymal stem cells (BM-MSCs) may play a therapeutic role in the treatment of nephrotoxicity.

Material and methods: Adult male Sprague Dawley rats (SD), were divided into four groups. The first group was injected (i.p) with **1ml** normal saline alone and kept as control, the second group was injected (i.p) with **1ml** normal saline containing **5mg/kg** cisplatin, the third group was injected (i.p) with **1ml** normal saline containing **5mg/kg** cisplatin and **0.5 ml** of culture media containing **5 x10^6** BM-MSCs into the tail vein in the first day post cisplatin injection and the fourth group was injected with **1ml** normal saline containing **5mg/kg** cisplatin and **0.5 ml** of culture media into the tail vein in the first day post cisplatin injection.

Results: Serum creatinine, blood urea nitrogen, creatinine clearance and malonadialdehyde were decreased in the kidney that received BM-MSCs while; superoxide dismutase and glutathione were increased to reach the normal values. Infusion of BM-MSCs ameliorated the renal dysfunction and enhanced tissue injury caused by cisplatin.

Conclusion: The present study shows BM-MSCs can exert a protective effect on cisplatin nephrotoxicity and suggests that BM-MSCs might be a new therapeutic approach for patients with kidney injury.

Key words: Cisplatin, Kidney, Nephrotoxicity, Stem cells.

Introduction

Acute kidney injury (AKI) is a common condition that affects up to 7% of hospitalized patients **[1]** Actually, the mortality rate in hospital acquired AKI still ranges from 30 to 80% **[2]**. It is caused by toxic or ischemic insult from chemotherapy, antibiotics, or shock occurring from infection. AKI can lead to dysfunction and apoptosis of renal tubular epithelial cells, in addition to a loss of renal endothelial cells **[3, 4]**.

Cisplatin is an antineoplastic drug used in the treatment of many solid-organ cancers. While toxicities include ototoxicity, gastro toxicity, myelosuppression, and allergic reactions **[5, 6],** the main dose-limiting side effect of cisplatin is nephrotoxicity **[7, 8]**. The nephrotoxicity of cisplatin has been recognized since its introduction over years ago. Yet, in spite of intense efforts over the ensuing decades to find less toxic but equally effective alternatives, cisplatin continues to be widely prescribed. It remains as a standard component of treatment regimens for head and neck cancers **[9],** testicular and ovarian cancers **[10, 11],** cervical cancer **[12]** and bladder cancer **[13]**.

Many strategies have been used to improve clinical outcomes in patients treated with cisplatin. The most common protocol for administering cisplatin consists of pre- and post-hydration with concurrent saline diuresis. The maintenance of adequate hydration is important for decreasing nephrotoxicity, but the mechanism of protection is unknown **[14]**.

Other common methods used for decreasing the nephrotoxicity of cisplatin include mannitol or furosemide administration **[15]**. Diuretic administration significantly improved renal function however; some degree of tubular necrosis was still present. Protection from nephrotoxicity was also seen when cisplatin was dissolved in a hypertonic NaCl solution (4.5%) relative to distilled water with no effect on the antitumor action of cisplatin **[16]**. Additional drugs have been administered in conjunction with cisplatin to reduce nephrotoxicity. Amifostine is a SH-containing compound that when injected before cisplatin

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in rats decreased nephrotoxicity but inhibited its antitumor effect **[17]**. Diethyldithiocarbamate (DDTC) is a chelating agent that potentially removes platinum bound to renal tubules; however, several side effects have limited the clinical application of this drug **[18]**. Sodium thiosulfate was used most commonly in conjunction with cisplatin to reduce toxicity **[19]**. In a rat model, sodium thiosulfate was found to provide protection from cisplatin ototoxicity, but was not consistently protective against nephrotoxicity **[20]**.

Cell-based therapeutic approaches have several potential advantages over specific drugs in the treatment of complex disorders. This is due to the broad functional repertoire of cells, including the secretion of various bioactive mediators, integration into host tissues, and differentiation within the injured organ. An increasing body of evidence suggests that mesenchymal stem cells (MSCs) possess potential effects in the treatment of AKI **[21, 22]**.

The functional benefit of MSC may be due to their ability to produce growth and tropic factors **[23]**. The local production of factors by stem cells may occur and play a role in tissue repair has been suggested by data in a mouse model of pancreatic regeneration **[24]**. Among those factors, *in vitro* MSC express hepatocyte growth factor and bone morphogenetic proteins acting to promote mitogenic, antiapoptotic, and morphogenic activities of renal tubular epithelial cells **[25]**.

MSCs-based therapies are currently being investigated for the treatment of AKI, although the mechanisms involved remain controversial. Numerous studies have demonstrated that *in vitro* expanded MSCs have therapeutic effects in experimental models of AKI **[26, 27]**. The isolation and characterization of stem cells offer new opportunities for expanding the repertoire of therapeutic options for this chronic illness.

Bone marrow derived mesenchymal stem cells (BM-MSCs) are an attractive therapy for renal tissue regeneration due to their pleuropotency, easily isolated, modified in vitro by vector-mediated gene delivery, and reintroduced as an autologous cell transplant reducing the risk of an immunogenic reaction. The use of adult stem cells also avoids the ethical ambiguities of using embryonic stem cells **[28, 29, 30, 31]**. Therapeutic administration of BM-MSCs in animal models of AKI suggests that a stem cell-based therapy may improve the recovery of both glomerular and tubular compartments **[32, 33]**.

The possibility that stem cells contribute to the repair and regeneration of kidney following AKI is of great interest for basic and translational research, therefore the present work was designed to assess the therapeutic ability of BM-MSCs to improve renal function and attenuate tubular injury in SD rats with cisplatin- induced AKI, as there are only limited therapeutic options available for kidney diseases.

Material and Methods

Cisplatin(*cis-D*iamminedichloroplatinumII) is a metallic coordination compound with a white or deep yellow to yellow-orange crystalline powder at room temperature (David Bull Laboratories 'DBL' Limited, Hospira, UK). It was dissolved in saline and injected intra peritoneal to SD rats at a dose of 5 mg cisplatin /kg/ body weight.

Adult Sprague Dawley rats (weighing 180 to 210 g, 8 weeks old) were maintained with food and water *ad libitum* in The Medical Experimental Research Center, Mansoura University, Egypt. They were housed in polyethylene cages (5 rats / cage) with stainless steel wire tops under standard environmental conditions (25 ± 3°C, 45 - 75% relative humidity, 12 h dark/light cycle).

BM-MSCs were isolated from bone marrow of long bones of adult SD rats. The bones were sterilized by immersion in 70% ethanol. The ends of the bones were cut and bone marrow was extruded by inserting a needle in one end of the bone shaft and injection of tissue culture media (Dulbecco's modified Eagle's medium, DMEM, Sigma) containing 10% fetal bovine serum (FBS, Sigma), penicillin (100U/ml) and streptomycin (100µg/ml). The effluent was collected in sterile tubes. Gentle pipetting resulted in obtaining of a single cell solution **[34].**

Bone marrow cells were plated in a concentration of 10^6 cells/ ml in (T-75) flasks. The cells were cultured in DMEM at 37°C in a humidified atmosphere that contained 5% CO2. Medium was changed after 4 days and every 3 days thereafter. Non adherent hematopoietic cells were removed when medium was changed. After a mean of 7days, cells reached sub confluence and was detached with trypsin/EDTA, reseeded at 4 x

10^3cells/cm^2, and used for experiments after the third passage.

Flow cytometric Phenotype analysis for MSC was carried out using Flowcytometer (Beckman, 500) at Oncology institute, Mansoura University, Egypt. Cells were harvested and washed in flow cytometry buffer and incubated for 30 min in flow cytometry buffer containing fluorescent-conjugated monoclonal antibodies directed against differentiation of MSC antigens (CD90, CD29, CD44) and against hematopoietic antigens (CD45, CD34). After the third passage, cells were used for in vivo experiments.

SD rats were classified into four experimental groups (each group containing 25 rats) according to the following scheme:-

Control group: in which each rat was injected (i.p) with 1ml normal saline alone and kept as normal control.

Cisplatin treated group: in which each rat was injected (i.p) with 1ml normal saline containing a single dose of 5 mg cisplatin /kg/bw.

Cisplatin + MSCs treated group: in which each rat was injected (i.p) with 1ml normal saline containing a single dose of 5 mg cisplatin /kg/bw and 0.5 ml of culture media containing 5 x10^6 MSCs into the tail vein in the first day post cisplatin injection.

Cisplatin + DMEM treated group: in which each rat was injected (i.p) with 1ml normal saline containing a single dose of 5 mg cisplatin /kg/bw and 0.5 ml of culture media in the tail vein in the first day post cisplatin injection.

Rats were fasted over night and were placed in metabolic cages for 24-h for urine collections then, anesthetized by diethyl ether inhalation (diethyl ether for anesthesia, Codex, Carlo Erba, Milan, Italy). Five rats were sacrificed at different time intervals in all groups at days 0, 4, 7, 11, and 30 post cisplatin injection for obtaining urine, blood samples and kidney tissue.

Biochemical parameters were studied in the serum and urine of adult SD rats from all experimental groups using the Perkin Elmer Lambda1 UV/VIS spectrophotometer, (Beckman, USA). Biochemical analysis was processed as creatinine clearance, creatinine, and blood urea nitrogen. Superoxide dismutase activity, glutathione and malonadialdehyde were processed on homogenate renal tissues.

Kidneys were perfused by neutral buffered formalin 10%, then after being excised they were kept in formalin till processing. For histological analysis, 3-µm sections were cut and stained with hematoxylin-eosin. Ten randomly selected sections were chosen, and screened for damage scoring. Changes were counted per 10 high power field (10 HPFs), and scored as follows:

Necrotic tubules: They were counted as number of necrotic tubules/10 HPFs: 1-3 necrotic tubules/10 HPFs were considered as (1), 4-5 necrotic tubules/10 HPFs were considered as (2), 5-10 necrotic tubules/10 HPFs were considered as (3) and more than 10 necrotic tubules/10 HPFs were considered as (4).

Solid sheets of cells in the interstitium: They were counted as number solid sheets/10 HPFs: 1-2 solid sheets /10 HPFs were considered as (1), 3-5 solid sheets/10 HPFs were considered as (2) and more than 5 solid sheets/10 HPFs were counted (3).

Regenerated tubules: They were counted as number of regenerated tubules/10 HPFs: 1-2 regenerated tubules/10 HPFs were considered as (1), 3-5 regenerated tubules/10 HPFs were considered as (2) and more than 5 regenerated tubules/10 HPFs were considered as (3).

Mitotic figures: They were counted as number of mitotic figures/10 HPFs: 1-2 mitotic figures/10 HPFs were considered as (1), 3-5 mitotic figures/10 HPFs were considered as (2) and more than 5 mitotic figures/10 HPFs were considered as (3).

Inflammatory cells: In each case if inflammatory cells are 1-3 rows of inflammatory cells between the tubules they were considered as (1), 4-5 rows of inflammatory cells between the tubules considered as (2) and 6 or more rows of inflammatory cells between the tubules considered as (3).

Interstitial fibrosis: If interstitial fibrosis occupies<25% of the field, they were considered as (1), if it occupies 25-50% of the field, they were considered as (2), if it occupies 50-75 % of the field, they were considered as (3) and if it occupies 75% or more of the field, they were considered as (4).

Atrophic tubules: Atrophic tubules (flat lining with casts & thick basement membranes) were counted as number of atrophic tubules/10 HPFs: 1-5 atrophic tubules/10 HPFs were considered as (1), 5-10

atrophic tubules/10 HPFs were considered as (2) and more than 10 atrophic tubules/10 HPFs were considered as (3). The other findings were commented upon as present or absent.

Data of each parameter of all experimental groups were tabulated as mean ± S.D. The results were analyzed using SPSS program software (version 17.0). Student-t-test was used for the calculation of probability (p) taking in consideration the control results as basal value. Statements of significance are based on probability (p) levels of ≤ 0.05 was considered statistically significant.

Phenotypic analysis.

Sprague Dawley rat bone marrow derived mesenchymal stem cells (BM-MSCs) at passage 3 were used for all in vivo experiments. BM-MSCs were grown in culture as previously described **[34].** The cultured BM-MSCs consisted of a heterogeneous cell population with a predominant spindle-shaped morphology and were able to form fibroblast like colonies (**Figure 1A**), and flow cytometric analysis was performed to confirm the identity of the cells as BM-MSCs. Cultured BM-MSCs expressed high levels of the MSC-specific markers CD29, CD90 and CD44 (**Figure 1B, C and D**) but did not express the hematopoietic markers CD45 and CD34 (**Figure 1E and F**).

Results

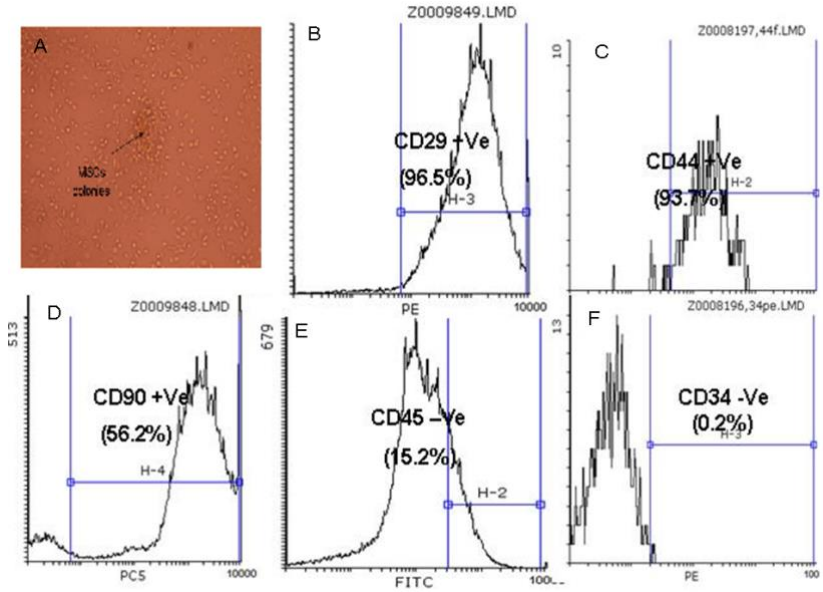

Figure (1): Flow cytometric analysis of surface markers on cultured SD rats bone marrow - derived mesenchymal stem cells. A: Photomicrograph of cultured SD rat BM-MSCs after one week (original magnification × 100). Specific surface markers CD29, CD44 and CD90 were expressed on cultured BM-MSCs (B, C & D), whereas hematopoietic stem cell markers CD45 and CD34 were not expressed (E&F).

Renoprotective effect of BM-MSCs.

The renoprotective potential of rat BM-MSCs against renal functional impairment was assessed in rats with cisplatin- induced AKI. Renal function, assessed as Creatinine, Creatinine clearance and Blood Urea Nitrogen, were evaluated at different time intervals (0, 4, 7, 11 and 30 day) after cisplatin injection. The intraperitoneal injection of cisplatin resulted in a significant increase in serum creatinine, creatinine clearance and BUN in the 4^{th}, 7^{th}, 11^{th} and 30^{th} day post cisplatin injection. Intravenous infusion of $5x10^6$ BM-MSCs markedly protected SD rats with cisplatin-induced AKI from renal function impairment, as reflected by significantly lower serum creatinine (**Figure 2**), creatinine clearance (**Figure 3**) and BUN (**Figure 4**). Whereas intravenous injection of DMEM into SD rats with cisplatin-induced AKI failed to improve their renal function.

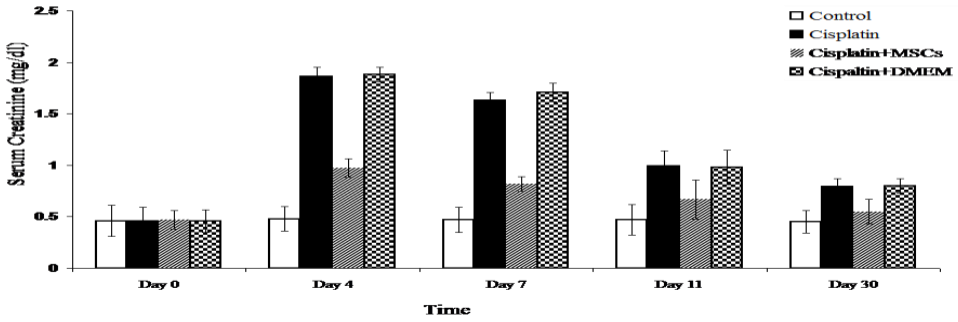

Figure (2): Changes in serum **creatinine** of rats with cisplatin-induced acute kidney injury and treated with BM-MSCs.

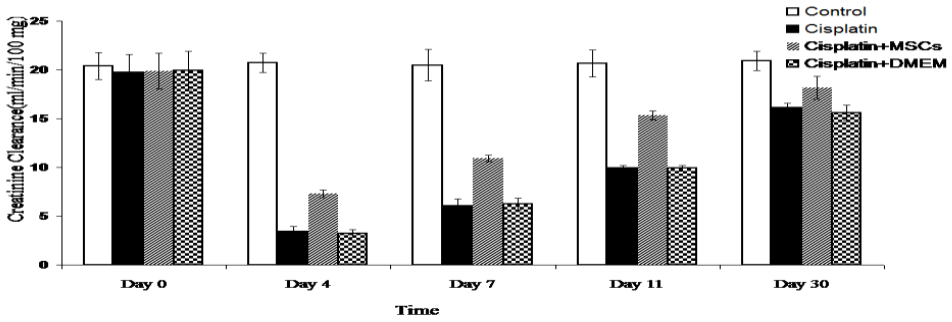

Figure (3): Changes in **Creatinine Clearance** of rats with cisplatin-induced acute kidney injury and treated with BM-MSCs.

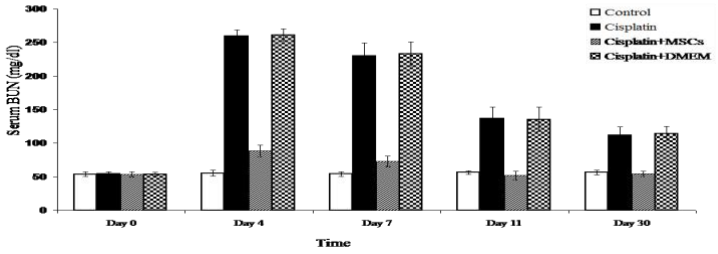

Figure (4): Changes in **Blood Urea Nitrogen** of rats with cisplatin-induced acute kidney injury and treated with BM-MSCs.

The experimental model of acute renal failure induced by cisplatin was also assessed in the renal antioxidant enzymes such as superoxide dismutase enzyme, glutathione and malonadialdehyde. The intraperitoneal injection of cisplatin resulted in a significant decrease in superoxide dismutase and glutathione while there was an increase in malondialdehyde in the 4^{th}, 7^{th}, 11^{th} and 30^{th} day post cisplatin injection. By contrast, intravenous infusion of $5x10^6$ BM-MSCs markedly protected SD rats with cisplatin-induced AKI from renal function impairment, as reflected by significant increase in super oxide dismutase (**Figure 5**) and glutathione (**Figure 7**) in addition to a significant decrease in malondialdehyde (**Figure 6**). Whereas intravenous injection of DMEM into SD rats with cisplatin-induced AKI failed to improve the previous parameters.

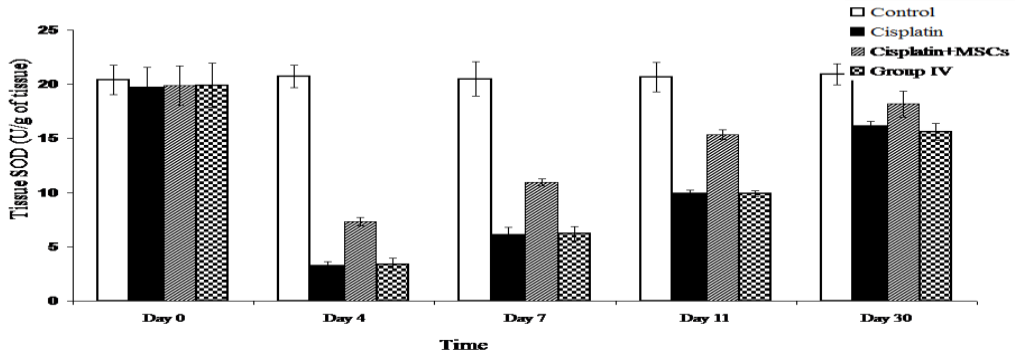

Figure (5): Changes in **superoxide dismutase enzyme** in renal tissue of rats with cisplatin-induced acute kidney injury and treated with BM-MSCs.

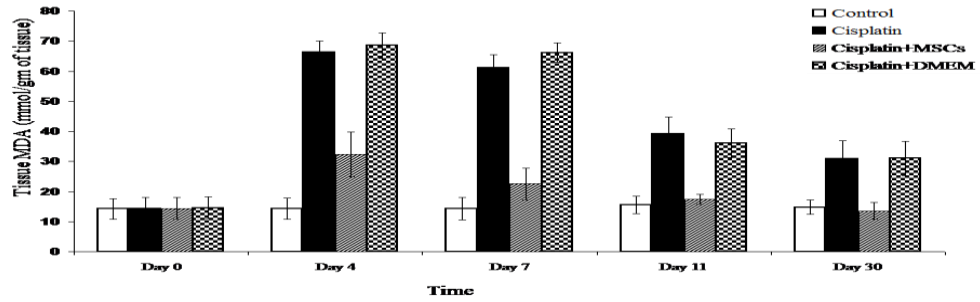

Figure (6): Changes in **Malondialdehyde** in renal tissue of rats with cisplatin-induced acute kidney injury and treated with BM-MSCs.

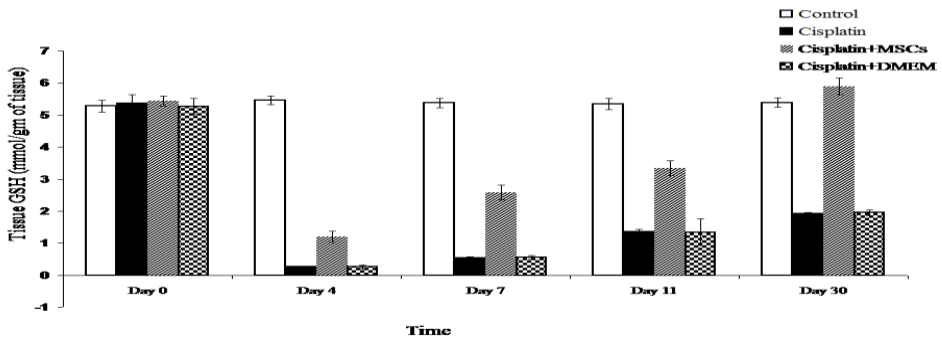

Figure (7): Changes in **Glutathione** in renal tissue of rats with cisplatin-induced acute kidney injury and treated with BM-MSCs.

Histological Examination

- **Renal histopathology at 0 day post cisplatin injection.**

Histopathological examination of the kidney of SD rats of different studied groups in the 0 day post cisplatin injection did not show any detectable changes and revealed normal renal tissue.

- **Renal histopathology at 4 day post cisplatin injection.**

Histopathological examination of the kidney of SD rats of cisplatin treated and cisplatin+DMEM treated groups in the 4th day post cisplatin injection, revealed changes in outer strip outer medulla (OSOM) with proximal tubules showing necrotic tubules with loss of brush border, cytoplasmic vacuolization, flatting and loss of the epithelial lining cells, luminal casts and cell debris (**Plate 1 A4**). As regard cisplatin with BM-MSCs treated group, there was lesser necrotic tubules and lesser dilated tubules. Also, there were regenerative changes in the form of mitosis and hyper chromatic large nuclei of the lining cells. The interstitium was the seat of mild inflammatory cells (**Plate 1 B4**).

- **Renal histopathology at 7 day post cisplatin injection.**

Kidney sections from cisplatin treated and cisplatin+DMEM treated groups in the 7th day post cisplatin injection, revealed lesser degenerative and more regenerative changes in the OSOM than day 4. Regenerative changes varied from tubular cell enlargement, mitosis and interstitial solid sheet formation dilated regenerating tubular cells have nuclei bulging in the lumen giving hobnail appearance (**Plate 1 A7**). In cisplatin with BM-MSCs treated group, there were lesser degenerative changes &lesser tubular necrosis with tubular dilatation than cisplatin treated group only. There was more regenerative changes in the form of solid sheets (intraluminal and interstitial), hyper chromatic cells and mitosis. The interstitium was the seat of mild round cells and mild fibrosis (**Plate 1 B7**).

- **Renal histopathology at 11 day post cisplatin injection.**

Sections obtained from cisplatin treated and cisplatin+DMEM treated groups in the 11th day post cisplatin injection. The degenerative changes were more marked and varied from tubular cell vascular degeneration, up to complete tubular necrosis with apoptosis and shedding of tubular cells in more than 90% of the tubules. Also there is mild interstitial round cell infiltrate. Regenerative changes were also detected and varied from tubular cell enlargement with, mitosis up to (5/10HPF**)** and interstitial solid sheet formation (**Plate 1 A11**). While cisplatin and BM-MSCs treated group revealed degenerative changes mainly in the form of tubular dilatation with focal tubular necrosis (2-4 tubules/10HPFs). Regenerative changes were detected in the form of regenerating tubules with prominent lining cells having a hobnail appearance & papillae, solid tubules and solid sheets in the interstitium (**Plate 1 B11**).

- **Renal histopathology at 30 day post cisplatin injection.**

Examination of the representative slides prepared from kidney samples obtained from cisplatin treated and cisplatin+DMEM treated groups in the 30th day post cisplatin injection cisplatin treated group or cisplatin and culture media treated group. No evidence of necrosis or degenerative changes was observed at this time 30 days. There is some regenerating large tubules in the OSOM lined by large atypical cells with prominent nucleoli and with occasional mitosis are seen (about 5-10% of all fields). There is mild peritubular and perivascular fibrosis (1/10 HPFs). Some dark tubules in the cortex were also seen (**Plate 1 A30).** Cisplatin and BM-MSCs treated group revealed intact tubular architecture with focal degenerative changes (5% of all studied fields in the group), mainly in the form of tubular atrophy and dilatation. Regenerative changes were detected (about 10% of all studied fields in the group) mainly in the form of tubular regenerative. The interstitium was the seat of mild round cells and mild fibrosis (**Plate 1 B30).**

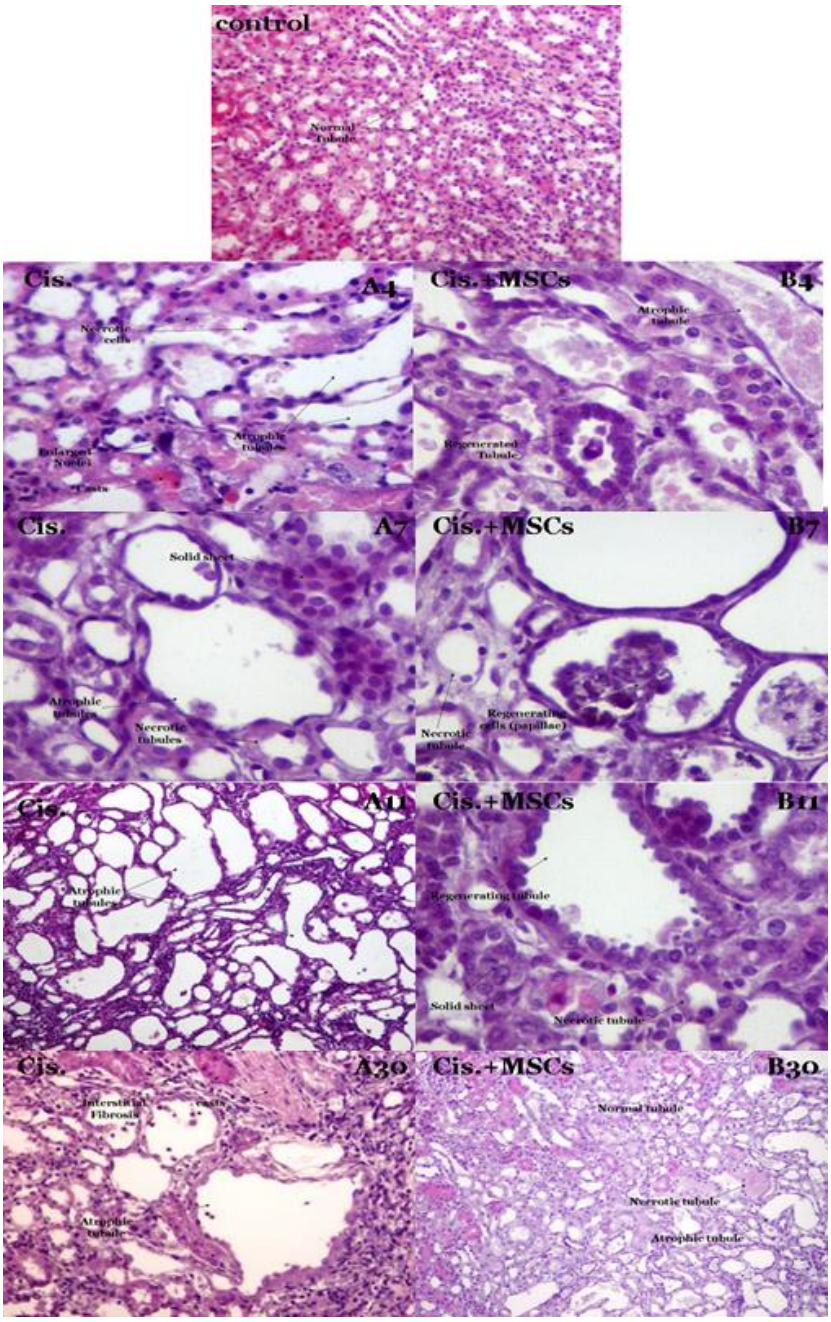

Plate (1): photomicrographs of SD rat kidney at 4th, 7th, 11th and 30th day post cisplatin injection (A4, A7, A11, A30 respectively) and treated with BM-MSCs (B4, B7, B11, B30 respectively). (H&E, x 200 in A11, A30, B30 and x 400 in A4, B4, A7, B7, B11).

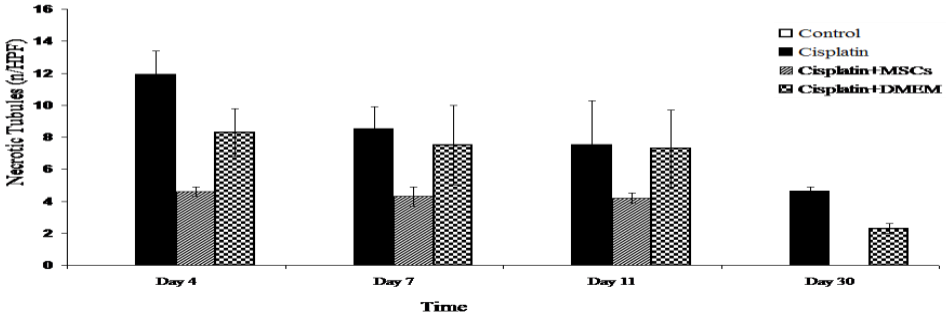

Figure (8): Changes in the quantitative assessment of **necrotic tubules** in renal tissue of rats with cisplatin-induced acute kidney injury and treated with BM-MSCs.

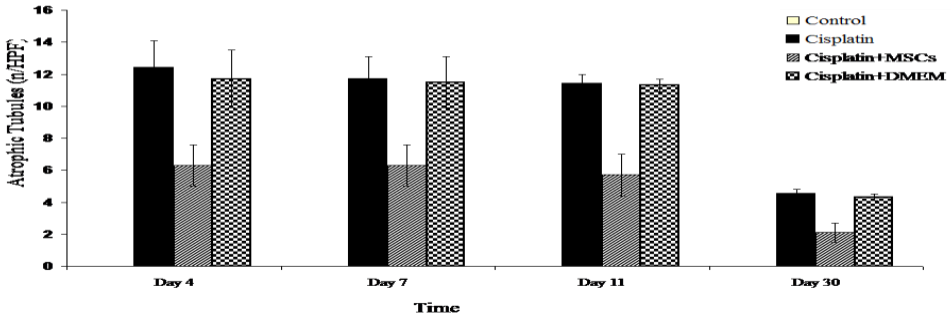

Figure (9): Changes in the quantitative assessment of **atrophic tubules** in renal tissue of rats with cisplatin-induced acute kidney injury and treated with BM-MSCs.

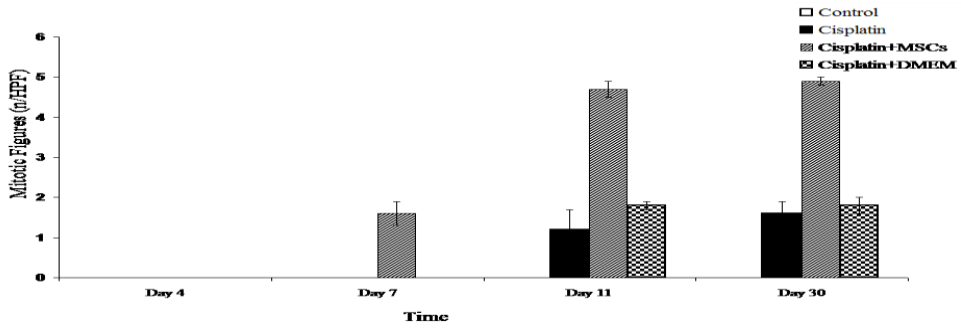

Figure (10): Changes in the quantitative assessment of **mitotic figures** in renal tissue of rats with cisplatin-induced acute kidney injury and treated with BM-MSCs.

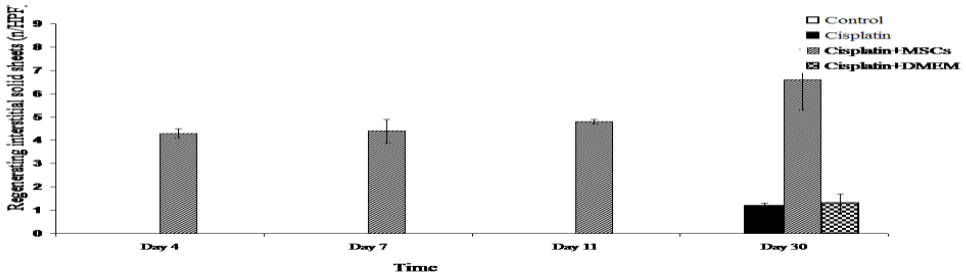

Figure (11): Changes in the quantitative assessment of **regenerating interstitial solid sheets** in renal tissue of rats with cisplatin-induced acute kidney injury and treated with BM-MSCs.

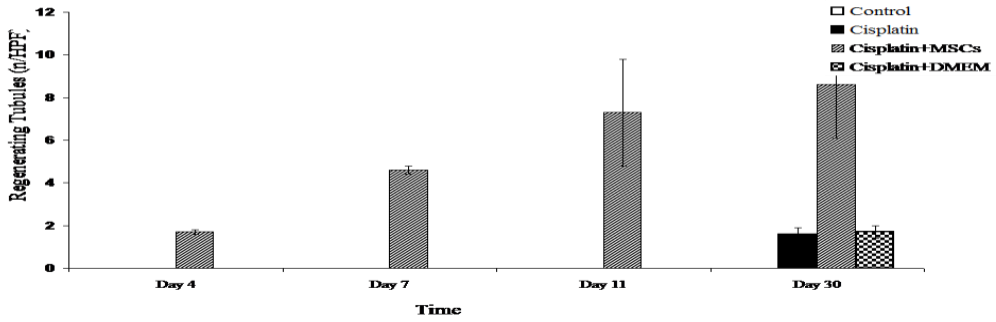

Figure (12): Changes in the quantitative assessment of **regenerating tubules** in renal tissue of rats with cisplatin-induced acute kidney injury and treated with BM-MSCs.

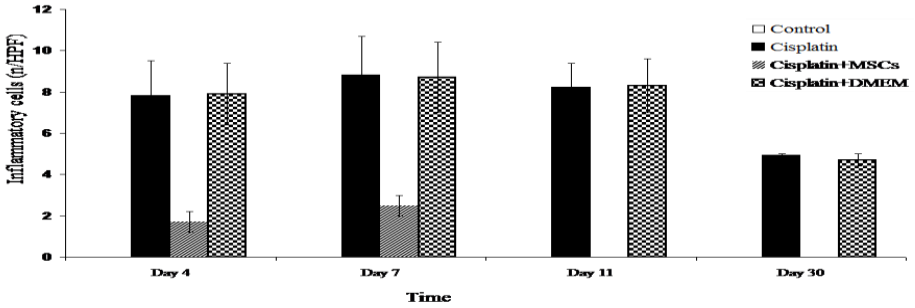

Figure (13): Changes in the quantitative assessment of **inflammatory cells** in renal tissue of rats with cisplatin-induced acute kidney injury and treated with BM-MSCs.

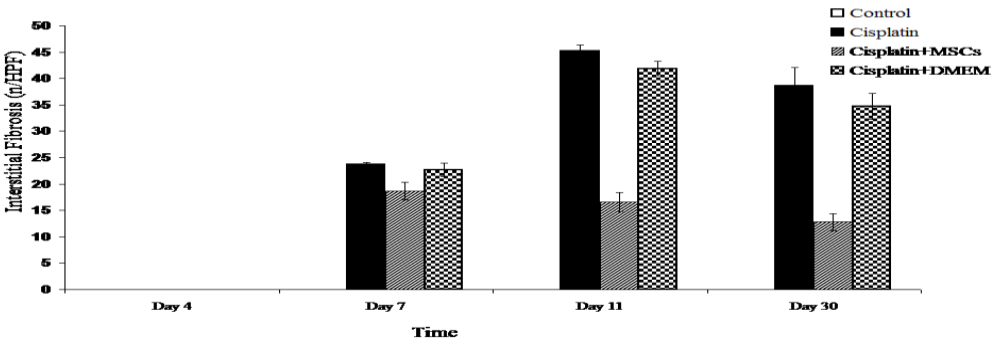

Figure (14): Changes in the quantitative assessment of **interstitial fibrosis** in renal tissue of rats with cisplatin-induced acute kidney injury and treated with BM-MSCs.

Discussion

Cisplatin is a major antineoplastic drug used for the treatment of many tumors. However, its clinical use as a chemotherapeutic agent is severely limited because of its serious adverse effects, particularly nephrotoxicity. Adult human kidney is an organ with limited normal cell turnover and no capacity for true regeneration **[35]**. The nephrotoxicity of cisplatin has been recognized since its introduction over years ago. Yet, in spite of intense efforts over the ensuing decades to find less toxic but equally effective alternatives, cisplatin continues to be widely prescribed. Therefore, blocking of cisplatin effects provide partial protection against nephrotoxicity **[36]**.

Despite advances in modern medical technology such as hydration and glycine, diethyldithiocarbamate, calcium channel blockers, sodium thiosulphate, glutathione, other sulfidryl compounds, etc. No effective therapies for AKI beyond supportive treatment are currently available **[14**, **37, 38]**. Cell-based therapies offer new potential therapeutics for the treatment of AKI **[39]**.

The present study showed that intravenous infusion of bone marrow-derived mesenchymal stem cells (BM-MSCs) have a protective effect against functional and structural renal injury of SD rats with cisplatin-induced AKI. The levels of serum creatinine, blood urea nitrogen, creatinine clearance and malonadialdehyde were decreased in the kidney that received BM-MSCs while; superoxide dismutase and glutathione were increased to reach the normal values. It also decreased renal tissue injury caused by cisplatin as reflected by changing in quantitative assessment of histological parameters (decreased number of atrophic tubules, necrotic epithelia, inflammatory cells and interstitial fibrosis in addition to increased regeneration in tubular epithelia.

Cisplatin has multiple cellular targets and modes of action that lead to nephrotoxicity. The main recognized direct target of cisplatin nephrotoxicity is the proximal tubular epithelial cells, which is endowed with highly cationic transporters for basolateral uptake of cisplatin from the adjacent peritubular capillary **[40]**. Cisplatin activates the intrarenal expression of vasoactive mediators **[41, 42]** and pro inflammatory factors, particularly tumor necrosis factor, which perturbs the peritubular endothelium **[43],** favoring the migration of inflammatory cells and leukocyte-mediated changes in vascular tone and perfusion **[44]**. Peritubular endothelial ultrastructural changes and polymorph nuclear leukocyte infiltration developed later than the tubular epithelial damage, indicating that endothelial cell dysfunction amplifies deleterious effects of tubular cell damage, as suggested in ischemic AKI **[45]**.

Because cisplatin has multiple cellular targets, blocking its effect on a single target may only provide partial protection against nephrotoxicity **[36]**. This suggests that multifaceted strategies may be required to improve clinical outcomes in patients treated with cisplatin. Cell-based therapeutic approaches have several potential advent ages over specific drugs or growth factors in the treatment of complex disorders such as acute kidney injury. This is due to the broad functional repertoire of cells, including the secretion of various bioactive mediators, integration into host tissues, and differentiation within the injured organ. An increasing body of evidence suggests that mesenchymal stem cells (MSCs) possess potential in the treatment of AKI **[46, 47, 48]**.

Therapeutic administration of exogenous BM-MSCs in animal models of AKI suggests that a stem cell-based therapy may improve the recovery of both glomerular and tubular compartments. Several studies showed a beneficial effect of mesenchymal stem cell administration in models of acute tubular injury and of endothelial progenitors in acute glomerular injury **[49, 50]**. In agreement with the present study, some workers using rat BM-MSCs found a decrease in necrotic tubules in the kidney of SD rats with cisplatin induced AKI. This supports the concept that BM-MSC was protective by contributing locally to tubular regeneration, acting mainly via paracrine mechanisms to promote strong proliferative and antiapoptotic responses [**51]**. Also, **Nicoletta Eliopoulos *et al*.** proposed that human MSCs secrete factors, such as vascular epithelial growth factor (VEGF) which has renoprotective abilities **[52]**.

The regeneration of surviving tubular epithelial cells has recently been shown to represent the predominant mechanism of kidney repair after acute tubular injury **[53]**. In this respect, the release of insulin growth factor-1(IGF-1) and probably other pro survival growth factors by BM-MSC stimulates tubular cell proliferation and exerts a powerful inhibitory

action on caspase-dependent tubular epithelial cell apoptosis induced by cisplatin **[54].**

Experimental studies have shown homing of BM-MSC beneficial effects of stem cell-based therapy on tissue structural repair in myocardial infarction **[55, 56],** neurologic diseases and acute kidney injury **[57]**. Many studies using MSCs have shown that damages to renal tubular as well as to renal endothelial cells can be prevented or repaired by MSCs. In rodent models of AKI caused by ischemia-reperfusion, or chemically induced such as through the use of cisplatin **[58, 59]**. Rodent MSCs have been found to be beneficial through their ability to differentiate into renal tubular cells, but most significantly through their ability to secrete beneficial factors in response to tissue injury **[60, 61, 62]**. These factors secreted by MSCs can engender antiapoptotic, anti-inflammatory, mitogenic, and angiogenic actions on injured tissue **[63, 64]**.

In conclusion, we have shown that injection of BM-MSCs can reduce cisplatin-induced kidney injury, thus intravenously BM-MSCs injection in SD rat with cisplatin-induced AKI can lead to less kidney function impairment and that factors secreted by BM-MSCs, may be involved in the paracrine protective effect of BM-MSCs on kidneys. We therefore deduce that promising cellular therapy applications employing BM-MSCs for AKI.

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