GENETIC AND HISTOLOGICAL STUDIES ON EFFECT OF MESENCHYMAL STEM CELL THERAPY ON EXPERIMENTAL RENAL INJURY INDUCED BY CISPLATIN IN MALE ALBINO RATS

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ABSTRACT

Acute kidney injury (AKI) represents a major clinical problem with high mortality and limited treatment protocols. This study was planned to evaluate the therapeutic effectiveness of bone marrow–derived mesenchymal stem cells (BM-MSCs) in a rat model of cisplatin-induced AKI. The aim of the present investigation is to determine whether mesenchymal stem cells can restore renal tubular structure and ameliorate cisplatin-induced clastogenesis in the bone marrow cells of rat. The scoring of chromosomal aberrations was undertaken in the current study as markers of clastogenicity. The Study was carried on 36 male white albino rats, of average weight 120-150 gm. The animals were divided into six groups, Group one: - Served as control and received normal saline throughout the experiments. Group two (model control) received a single dose of cisplatin (5 mg/kg interapritoneally) after 5 days. Group three, four, five and six: - Male albino rats with induced ARF received single interapritoneal injection of PKH26 labeled BM-MSCs immediately after induction of renal failure at different time intervals. Serological measurements included serum urea and creatinine, bone marrow preparation for chromosome aberrations were carried out and kidney specimens were processed for H&E. MSCs-treated group exhibited protection against renal injury serologically and histologically. The main result obtained in the present study revealed that cisplatin when given at a single dose of 5 mg/kg cause high incidences of the percentage of total chromosomal aberrations 62.33% and abnormal metaphases in the bone marrow cells of rat. This percentage was reduced nearly to 17.33%
after injection with mesenchymal stem cells for 30 days, the reduction was highly significant (P<0.001) when compared with the control and model control groups. Results of the present study suggest a potential reno-protective capacity of MSCs which could be of considerable therapeutic promise for cell-based management of clinical AKI.

**Keywords:** Acute renal failure; chromosome; cisplatin; mesenchymal stem cells; Renal regeneration.

**INTRODUCTION**

Cisplatin is currently used as a first-line chemotherapeutic agent for the treatment of testicular, ovarian, bladder and other carcinomas. [1] The current accepted paradigm about cisplatin mechanism of action is that the drug induces its cytotoxic properties through reacting with nucleophilic bases in DNA and form intra- and interstrand cross-links (cisplatin–DNA adducts) and subsequent interference with normal transcription and/or DNA replication mechanisms. [2] This has led to propose the involvement of multi-step and multi-level effects of cisplatin in the tumor cell/host during cisplatin-mediated cancer chemotherapy. [3] However, full therapeutic efficacy of this drug is limited due to the development of acquired drug resistance by the cancer cells and various side effects in the host, including nephrotoxicity, damages the bone marrow cells and cumulative myelosuppression, the latter frequently presenting as severe anemia.

These adverse effects cause intolerable discomfort in cancer patients and worsen their quality of life, becoming the major difficulty in continuing cancer chemotherapy. The clastogenic potential of cisplatin has become of great interest because of its serious effects on the chromosomes of non-tumor cells. In patients treated with long-term cisplatin, genetic damage can be observed during chemotherapy or many years later. [4–6] Oxidative stress is believed to be important mechanisms in the development of cisplatin toxicity. [7]

Acute kidney injury (AKI) is a critical clinical condition associated with a high degree of morbidity and mortality despite the best supportive care. However, at present, no effective treatment-improving disease outcome is available. [8] A great number of patients with AKI require hemodialysis. The mortality rate of patients requiring dialysis as a result of AKI is twice as high compared to patients without AKI .[9]
Clinical management of AKI has improved over the last years, but a specific therapy to improve renal function after AKI has not been developed yet. Complications arise from the inability of the kidney to regenerate lesions with functional tubular epithelial cells. [10] Failure to replace damaged cells gives rise to tubulo-interstitial fibrosis and scarring, increasing the susceptibility for chronic renal injury. [11]

Stem cells are biological cells found in all multicellular organisms. Bone marrow contains at least two kinds of stem cells, hematopoietic stem cells and stem cells of non hematopoietic tissue . [12] variously referred to as mesenchymal stem cells (MSCs). MSCs are interesting because they are easily isolated from a small aspirate of bone marrow and readily generate single-cell-derived colonies. [13] they can differentiate into different types of cell and for these reasons they can currently being tested for their potential use in cell and gene therapy for a number of diseases. [14, 15]

Bone marrow–derived mesenchymal stem cells (MSCs) are known to naturally support hematopoiesis by secreting a number of trophic molecules, including soluble extracellular matrix glycoproteins, cytokines, and growth factors. [16, 17] Recent studies in models of myocardial infarction, acute kidney failure, and stroke have shown that MSC therapy has the potential to inhibit cell death and stimulate endogenous regeneration programs. [18-21] The aim of this study was to evaluate the therapeutic potential of BM-MSCs on induced acute kidney injury in a rat model of cisplatin, monitored by, histological and cytogenetic examination.

MATERIALS AND METHODS

Chemical agents

Cisplatin (cis-diamminedichloroplatinum (II), CDDP) was obtained from sigma-Aldrich (Germany). Provided as a powder dissolved in saline (1mg/ml).

Dosage and treatment

The single dose of cisplatin (5 mg/kg b.w.) was selected on the basis of its effectiveness in inducing chromosomal aberrations. The therapeutic doses of cisplatin for cancer patients were (40-175mg/m2). [22] Control groups were similarly treated with saline. For each group six animals were used.

Animals: Male Wister albino rats 6-7 week old, weighing (120 – 150 gm) were used throughout the study. Animal house with specific pathogen-free Conditions, rats were housed
in standard laboratory conditions and 12-hours light-dark cycle and had free access to standard food and tap water. Rats were housed separately in groups (each of six animals) in plastic cages, they were allowed to adapt to the laboratories for two weeks before beginning the experiment. All the ethical protocols for animal treatment were followed and supervised by the animal facilities, Faculty of Women's, Ain Shams University. They were divided in to six groups as follow:

**Group 1:** received normal saline (5 mg/kg), injected I.P. and served as control group (n=6).

**Group 2:** received a single dose of cisplatin (5 mg/kg) and served as a model group (n=6) (the animals will be injected with cisplatin to induce acute renal failure. At the fifth day, all animals were anaesthetized with ether; blood samples were taken out from retro-orbital sinus of rat.

**Group 3:** It is consisted of 6 albino male rats with induced acute renal failure received bone marrow mesenchymal stem cells (BMMSCs) in a dose of \((10^7\) cells) by intraperitoneal infusion 24 hours after the induction of ARF. At the planned time 7 days after BMMSCs injection rats were scarified.

**Group 4:** It is consisted of 6 albino male rats with induced acute renal failure received BMMSCs in a dose of \((10^7\) cells) by i.p infusion 24 hours after the induction of ARF. At the planned time 15 days after BMMSCs injection rats were scarified

**Group 5:** It is consisted of 6 albino male rats with induced acute renal failure received BMMSCs in a dose of \((10^7\) cells) by i.p infusion 24 hours after the induction of ARF. At the planned time 21 days after BMMSCs injection rats were scarified.

**Group 6:** It is consisted of 6 albino male rats with induced acute renal failure received BMMSCs in a dose of \((10^7\) cells) by i.p infusion 24 hours after the induction of ARF. At the planned time 30 days after BMMSCs injection rats were scarified.

The kidney tissues were immediately removed and were examined for

Histopathological examination of kidney tissue. -

-Detection of the MSCs homing in kidney tissue after it's

Labeling with PKH26 dye by fluorescent microscope to detect its red fluorescence.

**Bone marrow chromosome preparation**

The bone marrow preparations for the analysis of chromosome aberrations in metaphase cells were carried out according to Preston, et al. \(^{[23]}\) Animals were injected intraperitoneally with 0.04 colchicine (Sigma) equivalent to 0.1 ml120gm b.w.,120 min before sacrifice by
decapitation, which occurred 24 h after cisplatin administration. The bone marrow cells were collected in buffer solution and then transferred to the hypotonic solution (0.075 M KCl) for 20 minutes at 37°C and then fixed with cold fixative (one part glacial acetic acid to 3 parts methanol). The slides were stained with Giemsa (Sigma Chemical Co.). Fifty metaphase cells were scored per animal to determine the percentage of total chromosomal aberrations.

**Light microscopic studies:** At the end of each experimental period, right sided kidney specimens were fixed in 10% buffered formalin solution for 48 hours, dehydrated in ascending grades of ethanol and embedded in paraffin. Serial sections of 5~7 µm thickness were cut, mounted on glass slides and subjected to the following technique:

- H&E stain for histological assessment.\(^{[24]}\)
- Examination of PKH26 labelled MSCs using Fluorescent Microscope (Japan, 7M03285) in unstained sections.

**Preparation of BM-derived mesenchymal stem cells from rats**

Bone marrow was harvested by flushing the tibiae and femurs of 6-week-old male white albino rats with Dulbecco’s modified Eagle’s medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine serum (GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia)] and resuspended in complete culture medium supplemented with 1% penicillin–streptomycin (GIBCO/BRL). Cells were incubated at 37°C in 5% humidified CO2 for 12–14 days as primary culture or upon formation of large colonies. When large colonies developed (80–90% confluence), cultures were washed twice with phosphate buffer saline (PBS) and the cells were trypsinized with 0.25% trypsin in 1mM EDTA (GIBCO/BRL) for 5 min at 37°C. After centrifugation, cells were resuspended in serum supplemented medium and incubated in 50 cm2 culture flasks (Falcon). The resulting cultures were referred to as first-passage cultures.\(^{[25]}\) Cells was identified as being MSCs by their morphology, adherence, their power to differentiate into osteocytes and chondrocytes and by their surface marker as CD29, CD45& CD90 by flow cytometry analysis.

**Labeling of MSCs with PKH26**

Mesenchymal stem cells were harvested during the 4th passage and were labeled with PKH26 (26), which is a red fluorochrome. It has excitation (551nm) and emission (567 nm) characteristics compatible with rhodamine or phycoerythrin detection systems. The linkers are physiologically stable and show little to no toxic side-effects on cell systems. Labeled
cells retain both biological and proliferating activity, and are ideal for in vitro cell labeling, in vitro proliferation studies and long, in vivo cell tracking. In the current work, MSCs were labeled with PKH26 from Sigma Company (Saint Louis, Missouri USA). Cells were centrifuged and washed twice in serum free medium. Cells were pelleted and suspended in dye solution. Cells were injected intravenously into rat tail vain. After one month, kidney tissue was examined with a fluorescence microscope to detect and trace the cells.

**Injection of MSCs:** Immediately after induction of acute renal failure, rats of group 3-6 received labeled MSCs diluted with 1 ml of saline, loaded in a 1-ml sterile syringe and injected via intraperitoneal for each rat. [22]

**Laboratory investigations:** Using capillary tubes, blood samples were drawn from retro orbital veins. Serum urea and creatinine were measured for all rats, before and after intervention throughout the period of the experiment. Measurements were estimated by conventional colorimetric method using Quanti Chrom TM assay kits based on the improved Jung and Jaffe methods, respectively (DIUR-500 and DICT-500). [27] Measurements were done at Biochemistry Department, Kasr Al-Ainy Medical School.

**Statistical analysis**

Data were coded and entered using the statistical package SPSS version 21. Comparisons between groups were done using analysis of variance (ANOVA). Data was summarized using frequencies (number of cases) and relative frequencies (percentages) for categorical variables. For comparing categorical data, Chi square ($\chi^2$) test was performed. Exact test was used instead when the expected frequency is less than 5. P value <0.05 was taken as statistically significant.

**RESULTS AND DISCUSSION**

1. **Confirmation of homing of MSCs into the renal tissue**

MSCs showed strong red auto fluorescence after transplantation into rats, confirming that these cells were seeded into kidney tissue. Sections in the renal cortex of group three-six (MSC injected, 1week-4week) showed many MSCs labeled with the PKH26 detected within the corpuscles and tubules (Fig. 1).
"Fig. 1": Detection of MSCs labeled with PKH26 fluorescent dye in kidney tissue. MSCs labeled with the PKH26 showed strong red auto fluorescence after transplantation into rats, confirming that these cells were seeded into the kidney tissue.

2. Results of laboratory investigations

Measurements of serum urea and creatinine levels in the studied Groups

The results of the present study show a significant improvement in kidney function. Serum and creatinine were decreased in the ARF/MSC groups compared to the ARF group (P<0.05). As shown from the Table (1), there was a significant increase (P <0.05) in the mean urea, creatinine levels in the acute renal failure group (84.43±10.02), (1.18±0.41) respectively compared to the control group (34.85±8.19), (0.10±0.05) Fig. (2).

Moreover, there was a significant increase (P <0.05) in the mean urea, creatinine, level in the group receiving MSCs for one week (59.30±3.57), (0.56±0.19) respectively compared to the control group (34.85±8.19), (0.10±0.05). Moreover There was a significant decrease (p<0.05) in the mean urea and creatinine level in the group receiving MSCs for 1w (59.30±3.57), (0.56±0.19) compared to the acute renal failure group (84.43±10.02), (1.18±0.41).

In addition, the mean urea level in the acute renal failure group receiving MSCs for two week (55.87±4.17), showed a significant increase (P <0.05) when compared to the control group (34.85±8.19). The mean urea and creatinine level in the acute renal failure group receiving MSCs for 2w (55.87±4.17), (0.43±0.18) showed a significant decrease (P<0.05) when compared to the acute renal failure group (84.43±10.02), (1.18±0.41).

On the other hand, there was a significant increase (P <0.05) in the mean urea level in the group receiving MSCs for three weeks (55.17±6.71) compared to the control group
(34.85±8.19) moreover, There was a significant decrease (P <0.05) in the mean urea, creatinine level in the group receiving MSCs for 3w (55.17±6.71), (0.44±0.18) compared to the acute renal failure group (84.43±10.02), (1.18±0.41).

Finally, the mean urea level in the acute renal failure group receiving MSCs for four weeks (47.58±2.38) showed a significant increase (P <0.05) when compared to the control group (34.85±8.19). The mean urea, creatinine level in the acute renal failure group receiving MSCs for 4w (47.58±2.38), (0.24±0.09) showed a significant decrease (P <0.05) when compared to the acute renal failure group (84.43±10.02), (1.18±0.41).

Table (1): Mean of Serum Urea and Creatinine levels in Different Studied Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD Urea (mg/dl)</th>
<th>Mean ± SD Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Control</td>
<td>34.85±8.19</td>
<td>0.10±0.05</td>
</tr>
<tr>
<td>2- Acute renal failure (ARF)</td>
<td>84.43±10.02*</td>
<td>1.18±0.41*</td>
</tr>
<tr>
<td>3- ARF+MSCs (for one week)</td>
<td>59.30±3.57*#</td>
<td>0.56±0.19*#</td>
</tr>
<tr>
<td>4- ARF+MSCs (for two week)</td>
<td>55.87±4.17*#</td>
<td>0.43±0.18 #</td>
</tr>
<tr>
<td>5- ARF+MSCs (for three weeks)</td>
<td>55.17±6.71*#</td>
<td>0.44±0.18 #</td>
</tr>
<tr>
<td>6-ARF+MSCs (for four weeks)</td>
<td>47.58±2.38*#</td>
<td>0.24±0.09 #</td>
</tr>
</tbody>
</table>

ARF (Acute Renal Failure)  
MSCs (Mesenchymal Stem Cells)  
*: statistically significant compared to corresponding value in control group (I) (P<0.05)  
#: statistically significant compared to corresponding value in positive control group (ARF) (II) (P<0.05)
"Fig. 2": Histogram Show the Comparison between the Mean values of Urea and Creatinine Serum in the Studied Groups

3. Chromosomal aberrations

Microscopical examination of the prepared samples showed that cisplatin administration has a highly effect on the induction of structural chromosomal aberrations (SCAs) in bone marrow cells. The frequency of total SCAs after injection of cisplatin was 168 (56%) Table (2). The most frequent types of SCAs were break 40 (13.33%), gap (chromosome gap, isochromatid gap) 38 (12.66%), deletion 26 (8.66%) and centric separation 20 (6.66%) Fig. (4). Ring 13 (4.33%), dicentric 10 (3.33%), a centric fragment 9 (3%) and centric fusion 8 (2.66%) were became next whereas diradial 2 (0.66%) and chromatid exchange were hardly speculated (fig. 4). On the other hand, the numerical aberrations showed up in the form of polyploidy, endoreduplication and aneuploidy. It recorded 1.66%, 1.66% and 3%, respectively (Fig. 5).

The present study showed that administration of cisplatin has a very highly effect on the induction of SCAs and NCAs. From Table (4), the percentage of total aberrations was highly significant (P<0.001) 187 (62.33%) compared to control group 10 (3.33%).

It is clearly noticed from the results, post treatment with mesenchymal stem cells (group three) induced decrease of structure chromosomal damage to 131 (43.33%) comparing cisplatin group 168 (56%). Metaphase analysis in rat post treated with \(10^7\) of mesenchymal stem cells for one week revealed decrease in NCAs 15 (5%) as compared to cisplatin group 19 (6.33%) Table (3). The present study demonstrated that the percentage of total chromosomal aberrations resulted from animals received \(10^7\) of mesenchymal stem cells for one week was highly significant (P<0.001) 146 (46.66%) decreased comparing with cisplatin group 187 (62.33%) and significant (P<0.05) comparing to control group 10 (3.33%) Table (4).
The present study demonstrated that the percentage of total chromosomal aberrations resulted from animals received \(10^7\) of mesenchymal stem cells for two week was highly significant \((P<0.001)\) 109(36.33\%) decreased comparing with cisplatin group and control group 187(62.33\%), 10(3.33\%) respectively.

It was noticed that intraperitoneal administration of \(10^7\) mesenchymal stem cells for three weeks induced very highly decrease \((P<0.001)\) of the frequently of total chromosomal aberration 78(26\%) as compared with the cisplatin and control group 187(62.33\%), 10(3.33\%) respectively.

Finally the percentage of total chromosomal aberrations (CAs) in MSCs for four weeks was highly significant \((P<0.001)\) decreased 52(17.33\%) comparing with cisplatin and control group 187(62.33\%), 10(3.33\%) respectively. From the previous results treatment with mesenchymal stem cells for four weeks decreased total chromosomal aberrations more than treatment with mesenchymal stem cells for three, two, one week Table (2), figure (3).

"Fig. 3": Histogram Show the Percentage of Structural and Numerical Chromosomal Aberrations Induced by 5mg/kg Cisplatin in Rat Bone Marrow Cells and Treated With Mesenchymal Stem Cells.

- A* significant compared to control group \((P<0.05)\)
- A** highly significant compared to control group \((P<0.001)\)
- B* significant compared to positive control group \((P<0.05)\)
- B** highly significant compared to positive control group \((P<0.001)\)

**Histological results**

Hematoxylin and Eosin-stained sections: Sections in the renal cortex of the control group exhibited normal histological architecture demonstrating Malpighian renal corpuscles (MRCs) formed of tuft of glomeruli and Bowman's capsule enclosing Bowman's space,
proximal (PCT) and distal convoluted tubules (DCT). Proximal convoluted tubules showed narrow Lumina and were lined with high cuboidal (pyramidal) cells with rounded basal pale nuclei. The lining cells exhibited apical brush border and basal striations (Fig. 6 A). Distal convoluted tubules (DCTs) showed wider Lumina and were lined with cubical cells with rounded central nuclei and less prominent basal striations (Fig. 6 A, B). Examination of kidney sections in group II showed many forms of affection and damage of renal tissue, both cortical and medullary. Marked destruction of renal corpuscles was noted in some fields with loss of almost all glomerular tufts. Most of the lining cells of the cortical tubules exhibited vacuolated cytoplasm, pyknotic nuclei of most of the lining tubular cell and cellular debris in Lumina. Some tubules exhibited widened Lumina (Fig. 6C). Acidophilic hyaline casts were detected in the Lumina of some cortical tubules. Moreover peritubular congestion was noted (Fig. 6D).

On examination of renal cortical sections of group three, distortion of renal corpuscles was noted, in the form of atrophy of glomerular tuft. Some areas of tubular cytoplasm exhibited vacuolation. Cellular debris in tubular Lumina was noted (Fig. 7A). On the other hand renal cortical sections of group four showing thickening of parietal layer of Bowman’s capsule and cystic dilatation of renal tubules (Fig. 7B). Moreover examination of renal cortical sections of group five exhibit focal interstitial nephritis. Cellular debris in Lumina of tubule (Fig. 8).

Sections in the renal cortex of group treated with mesenchymal stem cell for four week (group six) showed apparently normal histological architecture of renal corpuscles with well formed capillary tufts and normal appearance of Bowman’s space. Most of the tubules; PCT and DCT, exhibited apparently normal histological architecture; except for few areas of cytoplasmic vacuolation. No peritubular congestion was noted. Regenerated tubule also observed (Fig. 9 A, B).
Table (2): The Percentage of Structural and Numerical Chromosomal Aberrations Induced by 5mg/kg Cisplatin in Rat Bone Marrow Cells and Treated With Mesenchymal Stem Cells.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Examined cells</th>
<th>Structural chromosomal aberration /300 metaphase</th>
<th>B</th>
<th>Gap</th>
<th>Iso.G</th>
<th>Del</th>
<th>C.S</th>
<th>R</th>
<th>A:F</th>
<th>Dic</th>
<th>C.F</th>
<th>Dir</th>
<th>Chr.ex</th>
<th>Total SCA</th>
<th>Mean± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group %</td>
<td>300</td>
<td></td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>1.75±.96</td>
</tr>
<tr>
<td>Cisplatin group %</td>
<td>300</td>
<td></td>
<td>18</td>
<td>26</td>
<td>20</td>
<td>6.66</td>
<td>6.66</td>
<td>13</td>
<td>9</td>
<td>10</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>168</td>
<td>15.27±</td>
</tr>
<tr>
<td>Cis+MSCs (1 Week) %</td>
<td>300</td>
<td></td>
<td>14</td>
<td>18</td>
<td>16</td>
<td>6.66</td>
<td>6.66</td>
<td>3</td>
<td>4.33</td>
<td>3</td>
<td>3.33</td>
<td>2.66</td>
<td>0.66</td>
<td>56</td>
<td>11.21</td>
</tr>
<tr>
<td>Cis+MSCs (2Week) %</td>
<td>300</td>
<td></td>
<td>14</td>
<td>18</td>
<td>16</td>
<td>6.66</td>
<td>6.66</td>
<td>3</td>
<td>2.66</td>
<td>2</td>
<td>2</td>
<td>0.33</td>
<td>.66</td>
<td>43.66</td>
<td>11.91±</td>
</tr>
<tr>
<td>Cis+MSCs (3Weeks) %</td>
<td>300</td>
<td></td>
<td>14</td>
<td>18</td>
<td>16</td>
<td>6.66</td>
<td>6.66</td>
<td>3</td>
<td>1.33</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td>7.51</td>
</tr>
<tr>
<td>Cis+MSCs (4Weeks) %</td>
<td>300</td>
<td></td>
<td>14</td>
<td>18</td>
<td>16</td>
<td>6.66</td>
<td>6.66</td>
<td>3</td>
<td>1.33</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>46</td>
<td>4.18±</td>
</tr>
</tbody>
</table>

B = break, G = gap. Iso.G = isochromatid gap, del = deletion, c.s = centromeric separation, R = ring, A.F = Acentric fragment, del = deletion, c.s = centromeric separation, Dir = diradial configuration, ch.ex = chromatid exchange.

SCA: structural chromosomal abnormalities

Table (3): The Percentage of Numerical Chromosomal Aberrations Induced by 5mg/kg Cisplatin in Rat Bone Marrow Cells and Treated With Mesenchymal Stem Cells.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Examined cells</th>
<th>numerical chromosomal aberration /300 metaphase</th>
<th>Aneuploidy</th>
<th>Hyperploidy</th>
<th>Polyploidy</th>
<th>Endoreduplication</th>
<th>Total NCA</th>
<th>Mean± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group %</td>
<td>300</td>
<td></td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>3</td>
<td>1.50±.50</td>
</tr>
<tr>
<td>Cisplatin group %</td>
<td>300</td>
<td></td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>19</td>
<td>4.75±.63</td>
</tr>
<tr>
<td>Cis+MSCs (1 Week) %</td>
<td>300</td>
<td></td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>15</td>
<td>3.75±.75</td>
</tr>
<tr>
<td>Cis+MSCs %</td>
<td>300</td>
<td></td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>10</td>
<td>2.50±.29</td>
</tr>
</tbody>
</table>
(2Week) % | 1 | 0.66 | 1 | 0.66 | 3.33 |
--- | --- | --- | --- | --- | --- |
Cis+MSCs (3Weeks) % | 300 | 3 | 1 | 1 | 8 | 2.00±0.58 |
Cis+MSCs (4Weeks) % | 300 | 2 | 1 | 0.66 | 0.33 | 2 | 1.50±0.5 |

NCA: numerical chromosomal abnormalities

Table (4): Total Chromosomal Aberrations in the Studied Groups Detected by Chi-square.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Aberration %</th>
<th>Mean± SE</th>
<th>Chi square (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>3.33</td>
<td>1.67±0.33</td>
<td>-----</td>
</tr>
<tr>
<td>Cisplatin group</td>
<td>62.33</td>
<td>12.47±2.75</td>
<td>P1=&lt;0.001 A**</td>
</tr>
<tr>
<td>Cis+MSCs (1 Week)</td>
<td>48.66</td>
<td>9.73±2.28</td>
<td>P1=&lt;0.001 ( P2=0.001 \ A** \ B* )</td>
</tr>
<tr>
<td>Cis+MSCs (2Week)</td>
<td>36.33</td>
<td>7.27±1.81</td>
<td>P1, P2=&lt;0.001 \ A** \ B**</td>
</tr>
<tr>
<td>Cis+MSCs (3Weeks)</td>
<td>26</td>
<td>5.13±1.12</td>
<td>P1, P2=&lt;0.001 \ A** \ B**</td>
</tr>
<tr>
<td>Cis+MSCs (4Weeks)</td>
<td>17.33</td>
<td>3.47±0.79</td>
<td>P1, P2=&lt;0.001 \ A** \ B**</td>
</tr>
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A* significant compared to control group \( P<0.05 \)

A** highly significant compared to control group \( P<0.001 \)

B* significant compared to positive control group \( P<0.05 \)

B** highly significant compared to positive control group \( P<0.001 \),

\( P1=\) compared to control group, \( P2=\) compared to positive control group.
"Fig 4": Photograph Showing Metaphases Plate (Structural Abnormalities) from Bone Marrow Cells of Rats Treated With Cisplatin

"Fig 5": Metaphases Plate from Bone Marrow Cells of Rats Treated With Cisplatin Showing Numerical Abnormalities
"Fig. 6": Histological changes from the control, cisplatin-treated rats. (A, B) Representative hematoxylin and eosin (H&E, ×400) staining of kidney sections from the control, showing Malpighian renal corpuscle containing glomerulus (G) surrounded by Bowman's space (arrowhead). PCT (P) is lined with high cuboidal cells having rounded basal nuclei. DCT (D) is lined with cubical cells having rounded central nuclei. (C, D) Photomicrograph of a section in the renal cortex of a cisplatin-treated rat showing distorted glomerular capillary tufts (G) and widening of Bowman's space (arrowhead) of Malpighian renal corpuscle. The lining tubular epithelial cells show marked cytoplasmic vacuolation (v), pyknotic nuclei (black arrows) and cellular debris in Lumina (black stars). Widened tubular lumen is noted (green star). In picture D, many tubules show dense hyaline casts (black arrows). Peritubular congestion(c) is noted (H&E, ×400).

"Fig. 7": Photomicrograph of a section in the renal cortex of cisplatin + MSCs for one week (A) showing atrophy of glomerular tuft (arrow head) and focal regenerated
vacuolated renal tubules (black arrow) and cellular debris in Lumina (green stars) (H&E, ×400). Photomicrograph of a section in the renal cortex of cisplatin + MSC for 2w group (B) showing thickening of parietal layer of Bowman’s capsule (arrowhead) and cystic dilatation of renal tubules (black arrows) (H&E, ×400).

"Fig. 8": Photomicrograph of a section in the renal cortex of cisplatin + MSC for three weeks showing focal interstitial nephritis (black arrows), Cellular debris in Lumina of tubule (black stars) (H&E, ×400).

"Fig. 9": Photomicrograph of a section in the renal cortex of cisplatin + MSC for four weeks group (A, B) showing apparently normal histological architecture of renal corpuscles with well formed capillary tufts. Dividing cells are noted within the lining tubular epithelium (black arrows) in picture A (H&E, ×400).
The recovery from AKI is incomplete, and long-term consequences of AKI can be severe.\[28, 29\] This calls for the development of new and more effective treatment for AKI. The following summarizes the role of bone marrow stem cells in renal repair and discusses the recent results of the therapeutic administration of exogenous stem cells in experimental models of acute tubular and glomerular injury in rat. One of the best methods to induce experimental AKI was by using the nephrotoxin cisplatin. It is widely used as anti cancer drug\[30\] and the kidney is its target organ.\[31\]

In the present study, serological analysis went parallel with morphometric examination for all groups. A statistically significant elevation of serum levels of urea and creatinine was reported in groups II (cisplatin group) after induction of acute renal failure. Such measurements confirmed the occurrence of renal dysfunction. These results are consistent with the findings of researchers who reported that animals that underwent renal injury exhibited significant increase in the serum concentrations of urea and creatinine, compared with control animals.\[32\]

As regards the renal tubules, severe tubular damage was detected where the lining tubular epithelial cells vacuolated cytoplasm and pyknotic nuclei. Dislodgment and shedding of epithelial cells was evident with accumulation of cellular debris in tubular Lumina as well as acidophilic hyaline casts. Similar changes were previously reported by other investigators who demonstrated severe acute tubular damage in kidney sections of the I/R group.\[33\] The influx of neutrophils, macrophages and lymphocytes during reperfusion initiates a cascade of chemokines liberation with the subsequent production of reactive oxygen species and nitric oxide resulting in further tubular damage.\[34\]

Moreover,\[35\] explained the presence of pyknotic nuclei as a result of apoptosis which causes condensation of nuclear chromatin. Apoptosis occurs as a result of activation in caspase-3 accompanied with DNA fragmentation in kidney tissues of cisplatin treated rats. They explained this by the fact that, the reactive oxygen species generated by cisplatin may trigger the opening of the mitochondrial permeability transition pores and permits the release of cytochrome c from mitochondria to cytosol and hence activates the mitochondria dependent pathway leading to apoptosis.\[36\]

Acidophilic hyaline casts were demonstrated in the tubular lumen of the cisplatin treated group. This finding is consistent with\[37\], who postulated that they might be detached cells
from the tubular basement membrane which combine with proteins present in the tubular lumen such as Tamm-Horsfall protein resulting in cast formation. In addition to impaired sodium reabsorption by the injured tubular epithelial cells leads to increase sodium concentration in the tubular lumen causing polymerization of Tamm-Horsfall protein forming a gel and contributing in cast formation.

Concerning the mechanism of action of cisplatin [38] explained the particular sensitivity of the renal proximal tubule to cisplatin toxicity, as this segment exhibits one of the highest densities of mitochondria in the kidney and cisplatin is hydrolyzed to generate a positively charged metabolite which preferentially accumulates within the negatively charged mitochondria. Thus, the sensitivity of cells to cisplatin appears to correlate with the density of mitochondria.

Developments of chromosomal aberrations have been commonly used as sensitive biological indicator in the clastogenic bioassays of a drug. [39] In present study, the development of these clastogenic parameters was seen after cisplatin treatment and it supports earlier findings of its clastogenic properties. [40-42] The clastogenicity of cisplatin in bone marrow cells was well investigated by Edelweiss et al. [43] and Choudhury et al. [44], who observed that the most impressive effect of a single dose of cisplatin was an increase in the frequency of chromosome aberrations and in the number of abnormal metaphases obtained after cisplatin treatment. Cisplatin applied intraperitoneally induced clastogenesis in bone marrow cells and persisted for 48 h after treatment. This damage, particularly in the population of undifferentiated cells that constitutes bone marrow, is dangerous because it can lead to mutations and DNA rearrangements. If such cells survive and proliferate, the risk of secondary acute myeloid leukemia and other drug-related cancers can increase. [45–47]

Data in the present study revealed that, the percentage of total chromosomal aberrations was 62.33 % in rats treated with cisplatin alone (5 mg/kg. b.w) for 5 days, which was highly significant (P< 0.001) when compared with the control group. This suggests that cisplatin induced multiple chromosomal damage possibly in the form of break fragments; gap and deletion. These results were corroborated by previous studies in mice and rats bone marrow cells. The authors observed that the total of chromosomal aberrations' and abnormal metaphases induced by cisplatin were increased after treatment. [48- 50]
Cisplatin is a heavy metal complex with two labile chlorides groups which upon hydrolysis in aqueous solution from various reactive species, which are recognized as one of the pathogenic intermediates following chemotherapy. Accumulation of these reactive species results in cellular oxidative stress and if not corrected can lead to the damage of important biomolecules such as membrane lipids, proteins and DNA. Cisplatin is able to generate reactive oxygen species, which cause damage to cellular genome and also the cell membrane leading to lipid peroxidation. Malondialdehyde (MDA), the product of lipid peroxidation, also interacts with DNA causing strand breaks that in turn develop into chromosomal breaks.

On the other hand, when the rats received mesenchymal stem cells after cisplatin the percentage of total chromosomal aberrations after two weeks (36.33%) was less than treatment for one week (48.66%). This might be due to various possible reasons such as death of damaged cells to hurry the clearance of the drug from the body or post replication repair process which was reported by previous investigators. In fact, an involvement of post replication repair process in cisplatin induced DNA damage has been established.

However, in the group of rats treated with mesenchymal stem cells for four week, the percentage of total chromosomal aberrations was reduced (17.33%) more than in other groups. A critical point of this study is the possibility that there may be a therapeutic window for the use of cisplatin. The deleterious effects of cisplatin might be, at least in part, mediated by an oxidative stress mechanism. As mentioned in the Introduction, cisplatin has a direct effect on nucleophilic bases in DNA, an important component of its anti-tumor activity, and this will be unchanged by any manipulations that alter the redox reaction. Bone marrow suppression and apoptogenic effects of cisplatin could contribute to the anemia that follows therapy with cisplatin. MSC was effective in reducing clastogenesis and apoptosis induced by cisplatin in bone marrow cells and may possibly decrease the risk of secondary tumors in cells that were not originally neoplastic. Based on the data presented here, strategies can be developed to decrease the deleterious effects of cisplatin in normal cells by using MSC.

Hematoxylin and Eosin stained sections of stem cell therapy group which obtained 4 weeks after MSC administration showed disappearance of glomerular atrophy and restoration of the normal architecture of the glomeruli and this was confirmed in the present study by fluorescence microscopy which showed homing of the injected cells in the kidneys of stem cell therapy group and the regenerative changes which were detected could be attributed to
the remarkable plasticity of adult bone marrow-derived stem cells. They could differentiate into multiple lineages other than the tissue of origin as vascular endothelium leading to recovery of the glomerular damage. \[54, 55\]

MSCs migrated to injured tissue might act by paracrine effects and/or differentiation. The role of exogenous BM-MSCs might be also attributed to production of substances that stimulate renal stem cells. This was consistent with other researchers who suggested that exogenous MSCs paracrine activity may stimulate the endogenous renal stem cell population, leading to cellular recovery and renal injury repair. \[56\]

Moreover, \[57\] found significant levels of VEGF, HGF and IGF-1 in MSC-conditioned media, which were capable of enhancing endothelial cell proliferation and differentiation. When MSCs were infused just prior to Ischemia reperfusion injury (IRI), these cells quickly homed to the renal microvascular circulation, and endogenous cell apoptosis was decreased in regions that contained MSCs. Because of that authors proposed that this is an important aspect of MSC-induced renoprotection.

In the present work, H&E stained sections of stem cell therapy group also showed regenerated proximal and distal convoluted tubules although there was very minimal residual vacuolation in the cytoplasm of some cells lining medullary tubules which might be due to diminished vascularity of the medulla than the cortex. \[58, 59\]

The tubular cell repair in acute kidney injury treated with bone marrow mesenchymal stem might be attributed to paracrine mechanisms through the production of different growth factors such as VEGF, basic fibroblast growth factor, monocyte-chemoattractant protein-1, IGF-1, and HGF. \[57\]

Moreover, \[60, 61\] evidenced that some growth factors, such as HGF, can have an anti-inflammatory action. HGF has been reported to inhibit renal inflammation by attenuating tubular cell apoptosis, inhibiting endothelial injury and neutrophil extravasation. Homing of MSCs to sites of injury might be attributed to certain substances released at sites of tissue damage. Increased chemokine concentration at the inflammation site likely directs MSCs migration to these sites. Chemokines are released after tissue damage and MSCs express the receptors for several chemokines. Stromal-derived factor-1, platelet-derived growth factor and CD44 are likely candidates in the regulation of MSCs homing since their receptors are up-regulated after renal injury. \[62\]
MSCs migrated to injured tissue might act by differentiation. MSCs have been proved to integrate into damaged tubules and differentiate into renal epithelial cells in cases of cisplatin and glycerol induced acute kidney injury. However, other studies showed protection from injury by exogenous MSCs with little or no tubular incorporation.

CONCLUSION
In this rat model of cisplatin induced acute renal injury, BM-MSCs administration proved to have a potential reno-protective capacity which could be of considerable therapeutic promise in the management of AKI. Renal-protective potential of MSCs in the current study was proved at both morphological and functional levels. MSCs were capable of homing to injured kidney when injected intraperitoneal soon after injury. Endogenous stem cells need to be augmented by exogenous administration of stem cells for more rapid improvement to attain normal function as early as possible.

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