The role of bone marrow-derived mesenchymal stem cells and vitamin C in the treatment of HgCl2-induced renal tubular damage in albino rats: a histological and biochemical study
Ayaa R. Hassan, Aisha E. Mansy and Dina A. Sabry

Background
Acute renal failure is associated with 50–80% mortality. Currently, treatment options for this life-threatening disease are limited. However, stem cells offer an exciting potential for kidney regeneration.

Aim
In the present study we evaluated whether bone marrow stem cells and vitamin C, either in combination or alone, are effective in the regeneration of kidney tubules following acute tubular necrosis in rats.

Materials and methods
Eighty adult male albino rats divided into five groups (15 rats each) were used in this study. Group I was the control group; group II was treated with mercuric chloride (HgCl2); group III was treated with bone marrow-derived mesenchymal stem cells; group IV was treated with vitamin C; and group V was treated with bone marrow-derived mesenchymal stem cells and vitamin C. One set of animals was sacrificed on day 5 after injection of HgCl2, another set at 8 weeks after treatment, and one set at 12 weeks after treatment. Kidney tissue samples were processed for histological, histochemical, and immunohistochemical study and for biochemical assay. The obtained results were analyzed morphometrically and statistically.

Results
Group II showed several tubular changes in the form multiple vacuoles and darkly stained nuclei. Some tubules revealed karyolytic nuclei, denudation of the basement membrane, and acidophilic casts engorged in their lumens. The glomerular capillaries were congested and enlarged with decrease in filtration space and well-observed atrophic changes. Bone marrow stem cells and vitamin C were found to play a role in the regeneration of tubules of the renal cortex. H&E, Periodic acid–Schiff, and survivin expression revealed a significant improvement in the functional and structural recovery of the kidney from acute kidney injury (AKI) in groups III and V compared with group II.

Conclusion
The present results demonstrate that bone marrow stem cells contribute to the regeneration of the renal tubular epithelium in HgCl2-induced AKI. This study also suggests the antioxidant and antiapoptotic functions of bone marrow stem cells and vitamin C together in treating AKI.

Keywords:
acute kidney injury; bone marrow-derived mesenchymal stem cells, mercuric chloride, vitamin C

Introduction
Despite the recent developments and expansions in therapeutic choices, acute renal failure (ARF), which presents clinically with a rapid reduction in the glomerular filtration rate, continues to be associated with high mortality and morbidity [1,2]. Intrinsic ARF and acute tubular necrosis are responsible for at least 40% of hospitalizations in patients with a diagnosis of acute kidney injury (AKI). Moreover, 76% of patients hospitalized with ARF are in ICUs [1].

Clinicians are faced with a challenge in producing a positive treatment response in renal patients and in slowing down or halting the progression towards kidney failure [3].

The pathological manifestations can regenerate but without forming new nephrons [4].

Mesenchymal stem cells (MSCs) have potential regenerative capacity, and hence could have a considerable therapeutic role in cell-based management
of clinical AKI. Several preclinical animal studies have investigated the effects of different adult stem cell types, including hematopoietic, mesenchymal, endothelial, and kidney stem/progenitor cells, in the treatment of ARF [5-9].

A few studies on fetal kidney cell transplantation in rodents also support the regenerative potential of these cells after renal injury [10,11]. However, a suitable Renogics cell type to obtain a clinically relevant therapeutic effect in ARF has not yet been achieved and no cell-based clinical therapy has been established.

Recently it was shown that the rat fetal heart contains mesenchymal-like stem cells that exhibit rapid proliferation, multipotent differentiation potential, and constitutive expression of markers of cardiovascular lineage, indicating their precommitment toward the tissue of origin and thereby a greater efficacy in cardiac regeneration compared with other stem cell types [12].

MSCs ameliorate the injury due to ARF predominantly because of their antiapoptotic, anti-inflammatory, and antioxidative effects [13].

Vitamin C is an essential vitamin present in plant and animal cells [14]. Vitamin C acts as a potent water-soluble antioxidant in biological fluids [15] by scavenging physiologically relevant reactive oxygen species and reactive nitrogen species [16]. It may thereby prevent oxidative damage to important biological macromolecules such as DNA, lipids, and proteins [17]. In addition, it can regenerate the reduced form of α-tocopherol, perhaps accounting for the observed sparing effects of these vitamins [18]. However, few studies have researched the coadministration of vitamin C and its effect in improving AKI by reducing the damage to the proximal and distal convoluted tubules [19].

Survivin, a member of the inhibitor of the apoptosis protein family, suppresses apoptosis and regulates cell division. Survivin, which is the smallest member of the inhibitor of apoptosis protein family, is a 16.5-kDa intracellular protein that has been implicated in the regulation of apoptosis, cell division, and cell cycle in cancer cells as well as normal tissues through caspase-dependent and caspase-independent mechanisms [20].

Survivin is usually expressed at very low or undetectable levels in normal tissues but is highly expressed in most cancer tissues [21]. Upregulation of survivin gene expression is closely associated with the activation of several oncogenic pathways, including growth factor receptor signaling, STAT activation, and the phosphatidylinositol 3 kinase/Akt signaling pathway [22-24].

Of interest, survivin is a component of the centrosomes and microtubules of the metaphase and anaphase spindles, and it plays an important role in cell division. Survivin also functions as an apoptosis inhibitor by modulating caspase-9-dependent pathways [25]. Survivin interacts with multiple cellular signaling networks related to cancer development and progression, such as regulation of kinetochore attachment and spindle formation, modulation of the p53 checkpoint, regulation of angiogenesis, modulation of cellular stress responses, and inhibition of apoptosis. Because of its pleiotropic effects, survivin has been identified as a potential cancer drug target [26]. In this study we investigated survivin expression by immunohistochemistry as an index of apoptosis.

The present study aimed to investigate the relation between the possible therapeutic effect of adult bone marrow-derived mesenchymal stem cells (BMSCs) and vitamin C on renal tubular cells. This was accomplished by using mercuric chloride (HgCl2)-induced nephrotoxicity as a model in albino rats.

Materials and methods

Eighty male albino rats weighing 150-200 g were divided into five groups and placed in separate cages in the Animal House of Kasr El Aini. The rats were treated in accordance with the guidelines approved by the Animal Use Committee of Cairo University.

1. Control group: this group consisted of 15 rats, each animal received a single intraperitoneal injection of distilled water.
2. Group II (the HgCl2 group): this group included 15 rats, and constituted the AKI model. AKI was induced by a single subcutaneous injection of HgCl2 (7 mg/kg body weight) in the male rat (27). Animals were sacrificed on day 5 after injection.
3. Group III (the BMSC-treated group): in this group 20 rats received HgCl2 for 5 days (7 mg/kg body weight) by the same route, at the same frequency of administration, and at the same dose as in the previous group. They were injected with 0.5 ml of cultured and labeled BMSCs suspended in PBS in the tail vein (28). The injection was performed on 2 successive days following confirmation of tubular damage (29).

These rats were sacrificed as follows:
(a) Subgroup IIIa: 10 rats were sacrificed 8 weeks after BMSC injection.
(b) Subgroup IIIb: 10 rats were sacrificed 12 weeks after BMSC injection.

Before sacrifice, blood was collected from the eyes of the animals using capillary tubes for the assessment of serum creatinine and blood urea nitrogen.
4. Group IV (the vitamin C-treated group): in this group 15 rats received HgCl2 (7 mg/kg body weight) by the same route, at the same frequency of administration, and at the same dose as in the previous group. They were treated with vitamin C (250 mg/kg body weight) orally for 12 weeks (30).
5. Group V (BMSC+vitamin C-treated group): in this group 15 rats received HgCl2 (7 mg/kg body weight) by the same route, at the same frequency of administration, and at the same dose as in the previous group. They were injected with 0.5 ml of cultured and labeled BMSCs suspended in PBS in the tail vein.
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Vein and vitamin C (200 mg/kg body weight) orally for 12 weeks [30].

t-Ascorbic acid were purchased from Sigma (St Louis, Missouri, USA). Serum samples were collected for the estimation of creatinine and blood urea nitrogen levels using respective kits (Transasia Bio-Medicals, New Delhi, India) to prove acute tubular damage.

Bone marrow collection and stem cell isolation were performed at the Biochemistry Department, Faculty of Medicine, Cairo University, Cairo. Egyp. Culture and labeling were also performed at the Biochemistry Unit.

No mortality was detected during the entire duration of the experiment.

Isolation and preparation of mesenchymal stem cells

The bone marrow was flushed by flushing the tibiae and femurs of 6-week-old male albino rats with Dulbecco’s modified Eagle’s medium (Gibco/BRL, Life Technologies, California, USA). Nucleated cells were isolated with a density gradient (Ficoll/Paque; Pharmacia Fine Chemicals) and resuspended in complete culture medium supplemented with 1% penillin-streptomycin (Gibco/BRL). Cells were incubated at 37°C in 5% humidified CO2 for 12–14 days until the formation of large colonies (80–90% confluence). The culture was washed with PBS and released with 0.25% trypsin in 1 mM EDTA (Gibco/BRL) (5 min at 37°C). After centrifugation, the cells were resuspended in serum-supplemented medium and incubated in a 50 cm2 culture flask (Falcon) [31] for differentiating into osteocytes [19] and neurones [20]. Differentiation into osteocytes was achieved by adding 1–1000 nM/ml dexamethasone, 0.25 ml ascorbic acid, and 1–10 mM/ml d-glycerocephosphate to the medium. Kinetic quantitative determination of alkaline phosphatase was carried out in the medium of differentiated cells using a commercial kit provided by Stanbio Laboratory (Boerne, Texas, USA). Differentiation into neurones was achieved by adding b-mercaptoethanol, dimethyl sulfoxide, and conditioned medium for neurone induction. Differentiation was confirmed by detection of nerve growth factor gene expression in the cell homogeneous MSCs from passage 4 were used in this study upon reaching 80–90% confluence [26].

Labeling of stem cells with PKH26 dye

BMSGs were harvested during the second passage and were labeled with the PKH26 fluorescent linker dye [28]. PKH26 was purchased from Sigma. Cells were centrifuged and washed twice in a serum-free medium. Cells were pelleted and suspended in dye solution. Fluorescent-labeled BMSGs were injected intravenously.

Light microscopic studies

At the end of each experimental period, right-sided kidney specimens were fixed in 10% buffered formalin solution for 48 h, 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 techniques:

1. H&E staining for histological assessment [32].
2. Periodic acid–Schiff (PAS) staining to assess the brush border and basement membrane of proximal convoluted tubules (PCTs) [30].
3. Immunohistochemical staining for survivin using the avidin–biotin–peroxidase complex [30]. Kidney sections were incubated with mouse monoclonal antibody and counterstained with Meyer’s hematoxylin [33]. Survivin-positive cells showed brown cytoplasmic or nuclear deposits.

Negative controls were obtained by processing additional kidney specimens in the same way but skipping the application of the primary antibody.

4. Examination of PKH26-labeled BMSGs using an inverted fluorescent microscope. Renal tissue was examined using a fluorescent microscope (Olympus BX50F4, No.7M0326; Olympus, Tokyo, Japan) on unstained sections.

Morphometric study

Using a Leica Qwin 500 image analysis computer system (Leica Microsystems Ltd. Cambridge, UK) the following parameters were measured:

1. Mean area% of PAS-positive reaction in PAS-stained sections, measured in 10 random fields for each specimen at a magnification of x400. The area% represented the area of positive reaction, which was marked by a binary color to the area of the standard measuring frame.

2. Mean area% of positive immunoreactivity for survivin, measured in 10 high-power fields for each specimen at a magnification of x400.

Statistical analysis

The data obtained were expressed as means±SD and analyzed using an independent t-test. The significance of the data was determined by P values; P values greater than 0.05 were considered nonsignificant (NS), P values less than 0.05 were considered significant (S), and P values less than 0.001 were considered highly significant (HS).

Mean values of the data obtained from the image analyzer were calculated and compared statistically using the statistical package for the social sciences (version 9 for Windows; SPSS Inc., Chicago, Illinois, USA). Statistically significant differences between groups for the various parameters were examined using one-way ANOVA. This test is used to find a significant difference between more than two groups. A P value less than 0.05 was considered significant. Data were tabulated and represented graphically [31].

Results

Measurements of serum urea levels in the studied groups

There was no significant difference in the mean serum urea values between group V and group I. In group II, the mean serum urea showed a significant increase (P<0.05)
when compared with the corresponding group I, group III and group V. Mean serum urea values for group III showed a significant decrease when compared with group IV (vitamin C-treated only) (Histogram 1 and Table 1).

**Measurements of serum creatinine levels in the studied groups**

There was no significant difference in the mean serum creatinine level between group V and the corresponding group I. In group II, the mean serum creatinine level showed a significant increase (P<0.05) when compared with the corresponding group I, group III, and group V. Mean serum creatinine levels in group III showed a significant decrease when compared with group IV (vitamin C-treated only) (Histogram 2 and Table 2).

**Assessment of cell viability of bone marrow-derived mesenchymal stem cells**

Viability of cells was confirmed by trypan blue staining using a light microscope to differentiate between living and dead cells. The living cells were rounded, bright, glinting, and unstained, with a clear cytoplasm.

**Morphological changes of expanded undifferentiated bone marrow-derived mesenchymal stem cells**

Under an inverted microscope (Leica, Germany) undifferentiated BMSCs were typical of adherent spindle and fibroblast-like cells at 1 week of culture and reached 80-90% confluence at 2 weeks of culture (Fig. 1a and b, respectively).

**Confirmation of homing of bone marrow-derived mesenchymal stem cells into the renal tissue**

BMSCs showed strong red autofluorescence after transplantation into rats, confirming that these cells were seeded into kidney tissue. Sections in the renal cortex of group III and group V (BMSC-injected groups) showed homing of BMSCs labeled with the PKH26 within the tubules (Fig. 2a and b).

**Histological results**

**Periodic acid–Schiff stained sections**

Group I showed positive PAS reaction in the cortical tubules with preserved brush border and basement membrane (Fig. 10). Group II showed negative PAS reaction in parts of the basal lamina with loss of brush border in most of the tubules. However, the mesangial matrix of tubules showed PAS-positive reaction (Fig. 11). Subgroups IIIa and IIIb revealed positive PAS reaction at the basal lamina of the glomeruli, cortical tubules, and brush border of the tubules (Figs. 12 and 13). Group IV showed decrease in PAS reactivity as compared with group I and group V with partial loss or complete loss of the brush border in most of the tubules. The basement membrane was also interrupted at some sites (Fig. 14). Group V showed a PAS-positive reaction that was more or less similar to that of group I. Many cortical tubules had preserved brush border. A continuous basement membrane was detected in nearly all tubules (Fig. 15).

**Immunohistochemical results**

**Survivin-immunostained sections**

Group I showed some positive survivin immunoreactive nuclei among the lining tubular epithelial cells (Fig. 16). There was an increase in positive survivin immunoreactivity in the lining tubular epithelial cells of group II (Fig. 17) and group IV (Fig. 20) when compared with other treated groups (Figs. 18, 19, and 21).
Mean area% of periodic acid–Schiff-positive reaction in different groups

Group II and group IV showed a significant increase (P<0.05) in the mean area% of survivin immunoreaction when compared with group I, group IIIb, and group V. No statistically significant difference was recorded between group I and group V. However, group IIIb showed a decrease in the mean area% of positive survivin reaction when compared with group IV (Table 4, Fig. 1, and Histogram 4).

### Table 1. Serum urea levels in the studied groups

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group IIIa</th>
<th>Group IIIb</th>
<th>Group IV</th>
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<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>23.5</td>
<td>61.2</td>
<td>43.9</td>
<td>39.0</td>
<td>52.2</td>
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<td></td>
<td>14.5</td>
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<tr>
<td></td>
<td>13.5</td>
<td>62.8</td>
<td>32.5</td>
<td>20.3</td>
<td>41.1</td>
<td>13.9</td>
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<tr>
<td>Mean</td>
<td>14.8</td>
<td>60.2</td>
<td>40.0</td>
<td>37.8</td>
<td>46.1</td>
<td>19.1</td>
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### Table 2. Serum creatinine levels in the studied groups

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<th>Group IIIa</th>
<th>Group IIIb</th>
<th>Group IV</th>
<th>Group V</th>
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<tr>
<td>Creatina (mg/dl)</td>
<td>0.4</td>
<td>3.9</td>
<td>0.9</td>
<td>0.7</td>
<td>1.4</td>
<td>0.6</td>
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<tr>
<td></td>
<td>0.3</td>
<td>3.2</td>
<td>1.3</td>
<td>0.9</td>
<td>2.6</td>
<td>0.7</td>
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<tr>
<td></td>
<td>0.4</td>
<td>3.7</td>
<td>0.9</td>
<td>5.8</td>
<td>1.10</td>
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<td></td>
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<td>2.9</td>
<td>1.6</td>
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<tr>
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<td>1.4</td>
<td>1.1</td>
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<td>0.8</td>
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<tr>
<td></td>
<td>0.7</td>
<td>3.1</td>
<td>1.5</td>
<td>1.0</td>
<td>1.16</td>
<td>0.6</td>
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<td>0.7</td>
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<td>1.5</td>
<td>1.2</td>
<td>2.3</td>
<td>0.97</td>
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<td></td>
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<td>0.9</td>
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<td>0.7</td>
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<td></td>
<td>0.32</td>
<td>3.1</td>
<td>1.5</td>
<td>1.2</td>
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<td>0.8</td>
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<td></td>
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<td>1.5</td>
<td>1.0</td>
<td>2.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Mean</td>
<td>0.56</td>
<td>3.1</td>
<td>1.2</td>
<td>1.0</td>
<td>1.9</td>
<td>0.8</td>
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Table 3. Mean area±SD of Periodic acid–Schiff-positive reaction in the studied groups

<table>
<thead>
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<th>Groups</th>
<th>PAS-positive reaction (mean area±SD)</th>
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<tbody>
<tr>
<td>Group I</td>
<td>65.00±4.98</td>
</tr>
<tr>
<td>Group II</td>
<td>12.00±4.88</td>
</tr>
<tr>
<td>Group IIIa</td>
<td>45.33±2.03a</td>
</tr>
<tr>
<td>Group IIIb</td>
<td>48.3±2.62a</td>
</tr>
<tr>
<td>Group IV</td>
<td>19.09±3.62a</td>
</tr>
<tr>
<td>Group V</td>
<td>80.09±3.62a</td>
</tr>
</tbody>
</table>

*a: significant difference from other groups; PAS, Periodic acid–Schiff.

Table 4. Mean area±SD of survived positive reaction 0 in the studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean area±SD in the affected tubules</th>
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<tbody>
<tr>
<td>Group I</td>
<td>2.12±0.42</td>
</tr>
<tr>
<td>Group II</td>
<td>32.8±1.32a</td>
</tr>
<tr>
<td>Group IIIa</td>
<td>13.56±2.09a</td>
</tr>
<tr>
<td>Group IIIb</td>
<td>8.7±1.4a</td>
</tr>
<tr>
<td>Group IV</td>
<td>27.09±2.13a</td>
</tr>
<tr>
<td>Group V</td>
<td>4.09±3.62a</td>
</tr>
</tbody>
</table>

Figure 1. (a) Bone marrow–derived mesenchymal stem cells (BMSCs) at 1 week of culture (30–40% confluent); (b) BMSCs at 3 weeks of culture (90–95% confluent).

Figure 2. (a) Homing of labeled bone marrow–derived mesenchymal stem cells (BMSCs) detected using PKH26 red fluorescent dye (group III) (in vivo after 8 weeks); (b) homing of injected labeled BMSCs by detecting PKH26 red fluorescent dye in the rat kidney (group V) (in vivo after 12 weeks).
Figure 3. A photomicrograph of a section of the renal cortex from the control group showing renal corpuscles with glomerular capillaries (G), parietal layer of Bowman’s space (arrow), and filtration sparses. Proximal convoluted tubules (P) with narrow lumen, acidophilic cytoplasm, and basal rounded nuclei. Distal convoluted tubules (D) with wide lumen, less acidophilic cytoplasm, and rounded nuclei. H&E, x400.

Figure 4. A photomicrograph of a section of the renal cortex from group II 10 days after injection. The tubules were widely separated with loss of normal morphology. Some tubules showed multiple vesicles (V) and darkly stained nuclei, whereas others showed karyolytic nuclei. The glomeruli became expanded, congested (arrow heads), and enlarged (G). H&E, x200.

Figure 5. A photomicrograph of a section of the renal cortex from group II showing distention and swelling of tubules. Some tubules showed dilatation of the basement membrane (arrow). Acidophilic casts fill the lumen of tubules (V). Notice the cells with many processes inside the lumen (arrow heads). Some tubules showed dilatation of the basement membrane (G), glomerular. H&E, x200.

Figure 6. A photomicrograph of a section of the renal cortex from subgroup III showing hypercellularity of the renal cortex. The glomeruli appear normal and most tubules have regained their epithelial lining. Note the presence of large cells with heterogeneous cytoplasm and deeply stained nuclei in the lumen of some tubules (arrow). G: glomerular, V: rounded vesicular nuclei. H&E, x200.

Figure 7. A photomicrograph of a section of the renal cortex from subgroup IIIb (12 weeks after being injected with mesenchymal stem cells) showing normal tubules with acidophilic granular cytoplasm and rounded vesicular nuclei (N). Some tubules show degenerative changes (arrow heads). G: glomerular arrows; lumen of some tubules arrow head; degenerative changes. H&E, x200.

Figure 8. A photomicrograph of a section of the renal cortex from group N (vitamin C only) showing glomerular and peritubular congestion (G) glomeruli with tubular dilatation. Some tubules have regained their normal epithelial lining. Other tubules have a distorted shape and a denuded basement membrane (arrow head). H&E, x200.
Figure 9. A photomicrograph of a section of the renal cortex from group V showing normal histological architecture for the renal tubules, proximal convoluted tubules (a) and distal convoluted tubules (b). Some tubules show minor degenerative changes with mild glomerular hypercellularity (G).

Figure 12. A photomicrograph of a section of the renal cortex from subgroup IIa showing positive Periodic acid-Schiff (PAS) reaction in many cortical tubules with preserved brush border (arrows). A few tubules showed interrupted basement membranes (arrows).

Figure 13. A photomicrograph of a section of the renal cortex from subgroup IIb showing many cortical tubules with preserved brush border (arrows). Continuous basement membrane (arrow heads) is detected in nearly most of the tubules.

Figure 14. A photomicrograph of a section of the renal cortex from group V showing malignant renal corpuscles with partial or complete loss of the brush border in most of the tubules. The basement membrane was also interrupted at some sites (arrows) arrows: Interrupted basement membrane.
Figure 15. A photomicrograph of a section of the renal cortex from group V showing many cortical tubules with preserved brush border (arrows). Continuous basement membranes (arrow heads) are detected in nearly all tubules. Arrows: shows the reaction. Arrow heads: Continuous basement membrane. ×400.

Figure 16. A photomicrograph of a section of the renal cortex from group I (the control group) showing some positive survivin immunoreactive nuclei (arrows) among the tubular lining cells and glomeruli. Arrows: immunoreactive nuclei. Arrow heads: show reaction. ×400.

Figure 17. A photomicrograph of a section of the renal cortex from group II showing considerable survivin immunopositivity in the cytoplasm and nuclei among the tubules and glomeruli (arrows). ×400.

Figure 18. A photomicrograph of a section of the renal cortex from subgroup IIIa showing decrease in survivin immunoreactivity in the tubular lining cells (arrows). Survivin. ×400.

Figure 19. A photomicrograph of a section of the renal cortex from subgroup IIIa showing marked decrease in survivin immunoreactivity (arrows) in the tubular lining cells (arrow) arrows: immunoreactivity. Survivin. ×400.

Figure 20. A photomicrograph of a section of the renal cortex from group V showing apparent increase in survivin immunoreactivity (arrows) in the tubular lining cells. Survivin. ×400.
**Discussion**

Renal injury, whether caused by shock or during surgery and kidney transplantation, is the most common cause of AKI, and is associated with a high mortality rate [34].

In the present study, we investigated the possible restorative effect of BMSCs and vitamin C on HgCl₂-induced renal tubular damage. These changes were verified with histological, histochemical, immunohistochemical, biochemical, and morphometric studies. We observed that the results of serological analysis were parallel to morphometric results in all groups.

A statistically significant elevation in the serum levels of urea and creatinine was reported in group II, whereas a significant decrease in both was reported in all other groups. Such measurements confirmed the occurrence of renal dysfunction in the HgCl₂ groups. 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
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In this study, HgCl₂ administration revealed many forms of infection and damage of renal tissue. The tubules were widely separated with loss of their normal architecture. Some tubules showed multiple vacuoles and darkly stained nuclei, whereas others revealed karyolytic nuclei and acidophilic casts filling the lumens of tubules. These findings were analogous to the results of other investigators [37], who stated that kidney intoxication resulted in obvious tubular damage. Similar changes were previously reported by other researchers who demonstrated severe acute tubular damage in kidney sections of the HgCl₂ group [36]. The invasion of neutrophils and lymphocytes during HgCl₂ administration initiated a cascade of chemokines, with the subsequent production of reactive oxygen species and nitric oxide, resulting in further tubular damage.

Furthermore, the acidophilic hyaline casts demonstrated in some tubular lumina of the HgCl₂ group might represent cellular debris that underwent molecular changes. Cells and their debris that detached from the tubular basement membrane combined with proteins present in the tubular lumina resulted in cast formation [38].

Loss of renal tubular epithelium associated with HgCl₂ was attributed to both apoptotic and necrotic cell death, which occurred as a result of overwhelming cellular ATP loss. This led to actin-cytoskeleton dysregulation, which was followed by cellular dysfunction and ultimately in cell death [39]. HgCl₂-induced renal epithelial damage including activation of the extrinsic pathway through death receptors, such as tumor necrosis factor receptors, or through the mitochondrial pathway, in which the production of reactive oxygen species in the cells caused mitochondrial dysfunction through the xanthine-xanthine oxidase system [40].

In addition, sections from the HgCl₂ group showed expanded and congested glomeruli. Some glomeruli showed separation of their capillaries, which became attached to the parietal layer of Bowman's capsules. Marked glomerular capillary distortion or complete loss is described as a picture of 'end-stage kidney' resulting from acute and severe renal injury [41].

In the present study, histological sections of the BMSC-treated group, 8 weeks after BMSC administration, showed hypercellularity in the renal cortex with relatively preserved glomeruli. The walls of the tubules were invaded with patchily stained spindle-shaped cells with pale acidophilic cytoplasm. The regenerative aspect due to BMSC treatment accounted for their homing at the sight of injury and their capability to differentiate into effective tubular cells. Moreover, appearance of cells resembling macrophages that were detected in the peritubular spaces is due to interleukins secreted by BMSCs.

Researchers showed that BMSCs can produce macrophages that adapt a regularity like an M2 phenotype, which is characterized by significantly reduced production of proinflammatory cytokines and high production of IL-10 secretion. Specimens taken 12 weeks after BMSC injection revealed a normal structure for the tubules. The epithelial lining revealed acidophilic granular cytoplasm and large rounded vesicular nuclei. Marked decrease in hypercellularity in the renal cortex was seen [37].

The previous findings were confirmed in the present study by using fluorescence microscopy, which showed homing of the injected BMSCs in the kidney tissues of all treated groups by using PKH26 fluorescent staining.

Our results were previously explained by some investigators, who reported that the migration of BMSCs to the site of injury they were present in between the tubules at first and sometimes underwent transdifferentiation into mature functional renal tubular cells [37].

This was further explained by the paracrine mechanism of BMSCs, which combined the production of different growth factors such as vascular endothelial growth factor, basic fibroblast growth factor, and others. The release of these growth factors suggested that BMSCs can lead to regeneration and repair of damaged tissue by accelerating angiogenesis and stimulating cell proliferation [42].

In parallel with scotological examination results, histological examination of renal sections after vitamin C therapy revealed persistent damage in the form of glomerular distortion and tubular dilatation due to lack of epithelial lining.

However, the role of vitamin C in the protection of renal tissue by interfering with the mechanism of oxidative injury was obviously not equivalent to the amount of damage produced by HgCl₂, thus explaining the insufficiency of the healing effect by endogenous stem cells.

Thus, some investigators recorded that endogenous stem cells alone were unable to achieve and increase the regenerative process unless being helped by exogenous cells through cellular supply [43].

The observed histological results of group V after both BMSCs and vitamin C therapy revealed normal histological architecture for both cortical renal corpuscles and tubules. Similar findings were reported by other researchers who demonstrated reversibility of cortical tubular renal damage after treatment of rats with MSGs and vitamin [44].

The results of the present study revealed negative PAS reaction in parts of the basal lamina of the glomeruli and in the cortical tubules and brush border of the renal tubules, with PAS-positive reaction in the tubular lumen in both group II and group IV, confirming the inadequacy of vitamin C when used as the only mode of treatment.

These results were similar to those of other investigators, who observed loss of brush border, cell polarity, adhesion cells, and basement membrane. They explained that
necrosis and apoptosis may lead to detachment of the basement membrane, leaving behind denuded areas [45].

In contrast, PAS-positive reaction in the brush border of the renal tubules in both group III and group V showed an increase in the mean area, and revealed more or less normal reactions compared with group II. This was previously explained by other researchers who reported the positive therapeutic effect of BMSCs, attributing it to the interaction between integrin and extracellular matrix proteins such as collagen IV [46]. In addition to the helping effect of vitamin C in endorsing the healing effect of BMSCs [42]. Some scientists explained that BMSCs can reduce apoptosis and increase cellular proliferation through secretion of several mediators [44].

In the present study, survivin immunostaining was used to evaluate the regenerative capacity of injured tubular epithelial cells. Increased survivin immunostaining was recorded in group II, representing a significant increase when compared with group I, group III, and group V. These results were hand in hand with the findings of other investigators who reported that survivin expression increases after HgCl₂-induced kidney injury and thus was time-dependent [47]

This might be explained by the fact that, under normal circumstances, tubular epithelial cells have a slow rate of proliferation. Such low rate of turnover changes dramatically after a toxic insult, when there is a marked increase in cell death by necrosis and apoptosis and a vigorous response to replace these cells [48].

However, group IV (vitamin C) showed a nonsignificant decrease in survivin immunoreactivity. This was consistent with previous studies that postulated that vitamin C acts as a free radical scavenger, resulting in partial improvement of tissue injury and thus restricting apoptosis [13,39].

From the present study, it could be assumed that administration of BMSCs and vitamin C together results in improvement in renal injury, both morphologically and functionally.

The therapeutic potential of BMSCs and vitamin C in the current study was proven at both morphological and functional levels.

Future studies should be directed at exploring whether BMSCs have the same regenerative effect in humans and should clarify the exact mechanism of vitamin C by which these cells improve degenerative changes.

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There are no conflicts of interest.

References


الملخص العربي
دور الخلايا الجذعية المشكّلة من نخاع العظام وفيتامين (ج) في علاج اعتال الكليه المستحدث في الجردان البضام البالغة: دراسة مستوياتية نوعية
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قسم الأنسجة وبيولوجيا الخلايا - كلية الطب - جامعة بنها
قسم الكيمياء الحيوية - كلية الطب - جامعة القاهرة

المقدمة: بسبع اعطال الكليه وفيات من 50-80% في الوقت الراهن، وقرارات العلاج لهذا المرض الذي يهدد حياة مميتة، ولهذا، فإن الخلايا الجذعية أحدثت طفرة في إمكانية علاج أكبر كثر من الأطباء.

الهدف: نهدف من الدراسة لمعرفة ما إذا كانت الخلايا الجذعية للنخاع العظام وفيتامين (ج) معملية أو وحدها، لها دور فعل في علاج اعطال الكلي المستحدث بكودرد الزرق في الجردان البضام.

مواد وطرق البحث: (15 فران لكل تجربة) تم إجراء أكثر من 50 تجربة في الجردان البضام، تمت إدخال الخلايا الجذعية في نخاع العظام. استخدمت الجمجمة كمصدر للخلايا الجذعية، وتماول حبوب زرق في الثدي. المجموعة الثانية من التهابات النخاع العظمي، وتماول بعض الزرق في الثدي. المجموعة الثالثة من النخاع العظمي، وتماول بعض الزرق في الثدي. المجموعة الرابعة من النخاع العظمي، وتماول بعض الزرق في الثدي.

وتم العلاج بالجدار بعد أسبوع واحد مع ملاحظات لقياس التغيرات الكيميائية وقياسات الكليه. تم تجريبها بالعمليات الكيميائية لدراسة التغيرات الهيستولوجية والكيميائية، كما تم عمل وقفتين كاستخدامات حساسية.

النتائج: وظائف الناتج حدوث تغيرات بشكل جيد من خلال العلاج حيث ارتفعت نسبة الوريا والكربونين، إزالة القولية ودورة المحمولية، وبدأت تجارب الخلايا الجذعية في تقبل كلية النخاع العظمي، وبدأت تجارب الخلايا الجذعية في تقبل كلية النخاع العظمي.

同時に: يشير استخدام الخلايا الجذعية وفيتامين (ج) بعد استخدامات الأنسجة الكالوريا بكودرد الزرق ذو فاعلية في تحسن الاصابة.