Original Article

Comparative between the Effect of Bone Marrow-Derived Mesenchymal Stem Cells and Mobilized Bone Marrow-Derived Stem Cells on Experimentally-Induced Acute Kidney Injury in Adult Male Albino Rat (Histological, Immunohistochemical and Biochemical Study)

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ABSTRACT

Introduction : Cisplatin was used as a model of Acute Kidney Injury (AKI). Bone marrow-derived mesenchymal stem cells (BM-MSCs) were multipotent and capable of self-renewal. Granulocyte-colony stimulating factor (G-CSF) can move hematopoietic cells from the bone marrow into the circulation having therapeutic value in improving AKI.

Aim of the Work: Comparison between the effect of bone marrow-derived mesenchymal stem cells and mobilized bone marrow-derived stem cells on experimentally-induced acute kidney injury in adult male albino rat renal cortex using histological and immunohistochemical methods.

Materials and Methods: Five main groups of 42 adult male albino rats were established; Group 0; the source of BM-MSCs. Group I; Control including two subgroups IA (kept without treatment) & subgroup IB (animals received single dose of 0.5 ml of media into tail's vein). Group II: animals were injected intraperitoneally with cisplatin once in a dose of 10 mg/kg for induction of AKI. Group III: animals were injected by cisplatin as in group II and treated by BM-MSCs suspension 0.5 ml in a single dose into the rat tail vein 24 h after injection. Group IV: animals were injected by cisplatin as in group II and treated by 100 µgm / kg G-CSF subcutaneously every day for 5 days 24 h after injection. Blood samples were collected to measure blood urea nitrogen and serum creatinine. Animals were sacrificed at the 7th day of experiment except group II after 24 hours. Kidneys were dissected out to obtain renal cortex, processed for histological studies. Histological grading for the degree of AKI, statistical and biochemical analysis were done.

Results: BM-MSCs treated group III exhibited mild improvement while G-CSF treated group IV showed marked improvement in histological structure of renal cortex and renal functions.

Conclusion: G-CSF was more beneficial than BM-MSCs in alleviating cisplatin induced AKI.

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Key Words: AKI, G-CSF, MSCs, PCNA, Rat.

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INTRODUCTION

Acute Kidney Injury (AKI) is a condition of speedily deteriorating kidney function. It is highly common condition which has a great economic burden on the society. It has a mortality rate reaching from 30 to 80%. The usual standard techniques of therapy as dialysis and kidney replacement have no impact on mortality rate[1]. So, there is an intense need to develop new pharmacological therapies to overcome this problem.

Cisplatin induced AKI is a commonly used laboratory model. Cisplatin has two forms in the plasma; bound and unbound or free. The unbound cisplatin has a low molecular weight and passed easily through the glomerulus. It's concentration in the cells of proximal convoluted tubules is 5 times higher than in the serum causing its trapping in renal cortex leading to nephrotoxicity[2].

Bone marrow–derived mesenchymal stem cells (BM-MSCs) are multipotent cells which can distinguish to different cell kinds as osteocytes, chondrocytes, adipocytes, endothelial, myocardial, liver, renal and pulmonary epithelial cells. They have regenerative and immunomodulatory properties in addition to their easy accessibility and manipulation. They have many sources like adipose tissue, umbilical cord and amniotic fluid but it's main source is the bone marrow. Their role in improving AKI may be because of their immunomodulatory, anti-inflammatory effects and their capacity to differentiate into glomerular endothelium[3,4].

Bone marrow-derived stem cells (BMSCs) as hematopoietic stem cells can be agitated from the bone marrow by using granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) into peripheral blood and different organs to treat renal impairment, cardiac muscle necrosis and hematological malignancies[5].

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This work was carried out for comparison between the effects of mesenchymal stem cell either derived or mobilized from the bone marrow on experimentally-induced acute kidney injury in adult male albino rat renal cortex with histological, immunohistochemical and biochemical methods.

MATERIALS AND METHODS

Experimental design

Fourty two adult male albino rats their weight about 100–120 gram each. They were kept one week under adequate illumination and ventilation in plastic cages with mesh wire and allowed feed and water to acclimate, as recommended by The National Research Council of the National Academies (2011)[6]. The Research Ethical Committee of the Faculty of Medicine of Tanta University authorized all experimental methods.

The rats were separated into five main groups;

Group 0: Both tibias and femurs of two rats were used to obtain the bone marrow and served as the source of the stem cells.

Group I (Control group): were randomly separated into two equal subgroups (five rats each):

- Subgroup IA: animals didn't receive any treatment and used for histological study of the normal renal cortex.
- Subgroup IB: animals received 0.5 ml of media into tail's vein once then sacrificed on the 7th day.

Group II (Cisplatin model of AKI): includes 10 animals, each was injected by cisplatin 10mg /kg body weight intraperitoneally once for induction of AKI. Cisplatin was gained as liquid vial from Sigma Chemical Company, Cairo, Egypt. 1ml contained 100mg of cisplatin[7,8].

Group III (AKI-MSCs-treated group): includes 10 animals which were injected with cisplatin as in group II and after one day, each rat was injected with single dose of 2 x10^6 of BM-MSCs suspension in 0.5 ml PBS/rat into the rat's tail vein[9].

Group IV (AKI-Mobilized BMSCs--treated group): includes 10 animals and were injected with cisplatin as in group II and after one day, each rat was injected by 100 µgm / kg granulocyte-colony stimulating factor (G-CSF) subcutaneously once every day for 5 days[10,11]. G-CSF was purchased from Sedico pharmaceutics company, Egypt in a liquid 0.5 ml vial containing 300 microgram of recombinant human granulocyte-colony stimulating factor (rhG-CSF).

Twenty four hours after the induction of acute kidney injury, rats of groups II were sacrificed and blood samples were obtained directly from the hearts of this group and specimens to insure the induction of AKI in group II by measuring blood concentration of urea nitrogen and serum creatinine. Rats remaining in groups I, III and IV were anaesthetized and sacrificed on the 7th day of experiment and blood samples were collected for biochemical analysis. Specimens of kidney were obtained and processed for histological and immunohistochemical studies by light and electron microscopes[12,13,14].

Mesenchymal stem cells

They were selected and cultured from rats' bone marrow of long bones at Tissue Culture Lab in Histology and Cell Biology Department, Faculty of Medicine, Tanta University[15,16].

A. Histological and immunohistochemical studies of the renal cortex

Tissue specimens were fixed in 10% formalin for 24 hours then were embedded in paraffin wax according to standard procedure.

Five micrometers-thick sections[17] were stained with Hematoxylin and eosin stains (H&E)[18], Periodic Acid Schiff stain (PAS)[13] and immunohistochemically using Proliferating cell nuclear antigen (PCNA) monoclonal antibody from Lab Vision Company. The avidin-biotin complex technique was used. Negative control staining was done after omission of the primary antibody[12,19,20]. Tonsils served as a positive control for PCNA giving nuclear brownish reaction in positive cells.

Electron microscope

Kidney specimens were divided into 1 mm3 in size, were rapidly fixed in 2.5% phosphate buffered glutaraldehyde at 4˚C for 2 hours before being processed and embedded in epoxy resin according to standard procedure. Semithin sections were stained with toluidine blue and examined by light microscope to select suitable areas for ultrathin, their thickness were 1 µm. Sections of 80-100 nm in thickness were cut by LEICA ultramicrotome and examined by transmission electron microscope (TEM)[21].

B-Morphometric study and statistical analysis

Morphometric study

a. Quantitative analysis for histological changes in renal cortex[22].

b. The color density of PAS stain.

c. The mean PCNA positive cells number.

Statistical Analysis

The biochemical and morphometric measurements were expressed as means ± standard deviation (SD) and were analyzed using SPSS software version 13 (SPSS Inc., Chicago, IL, USA), then compared by one way analysis of variance (ANOVA) test then Tukey's test was done for comparison between different groups and control group. The difference was considered statistically significant if probability value (*P value*) <0.05, highly significant if *P value* <0.001 and non-significant if *P value* >0.05[23].

C- Biochemical study

The obtained blood samples from animals of each group through puncturing the heart apex of rats after anathesia were transferred to the EDTA-coated blood tube for up to 4 h and then, renal functions (blood urea and serum creatinine) were measured. Statistical analysis was done to compare between different groups.

RESULTS

General Observation

In the present work, all rats were survived along the periods of experiment. The histological, immunohistochemical and biochemical results of both subgroups of control group were similar.

I- In *vitro* studies

1- Morphological identification of unstained bone marrow-mesenchymal stem cells (BM-MSCs) culture using phase contrast inverted microscope:

On Day one, the cultured suspended cells were variable in size and shape and appeared rounded, floated and crowded (Figure 1A). On day twelve, the adherent cells reached about 80-90 % confluency and exhibited different shapes with multiple interdigitating processes, central vesicular nuclei and multiple nucleoli (Figure 1B). The cells were then harvested and injected in the required count in 0.5 ml media.

2- Morphological identification of BM-MSCs culture stained by Giemsa stain using phase contrast inverted microscope:

The adherent cultured cells had a star-shaped appearance with many interdigitating processes and blue vesicular nuclei with prominent nucleoli (Figure 1C).

II- Histological and immunohistochemical studies of the renal cortex

A. Light microscopic and immunohistochemical:

1. Hematoxylin and eosin stains (H&E)

Examined sections from group I (control) revealed normal histological architecture of the cortex consisting of renal corpuscles formed of tuft of glomerular capillaries surrounded by Bowman's space. Proximal convoluted tubules (PCT) appeared with narrow lumina lined by 4 to 6 large cubical cells with rounded nuclei and acidophilic cytoplasm. The distal convoluted tubules (DCT) had wide lumina and lined with 5-8 low cubical cells with less acidophilic cytoplasm (Figure 2A). Sections of group II (cisplatin model of AKI) showed disturbance of the normal histological architecture of the renal cortex in the form of focal loss of differentiation between PCTs & DCTs with flattening of their lining epithelium and detachment of tubular epithelial cells from their basement membrane with inflammatory cellular infiltration around dilated congested blood vessels were detected (Figure 2B). Group III (AKI-MSCs-treated group) showed improvement of histological structure of the cortex in the form of rounded Malpighian corpuscles lined by simple squamous epithelium in its parietal layer with glomerular capillaries and obvious Bowman's space. Some of PCT & DCTs were improved and appeared more or less as the control while others showed disruption of their apices with loss of parts of cytoplasm and mild congested blood vessels (Figure 2C). Sections of group IV (AKI-Mobilized BMSCs-treated group) revealed obvious improvement of the general histological structure of the renal cortex. Renal corpuscles, PCTs & DCTs appeared more or less like the control group except for few vacuolations of cytoplasm of some tubules (Figure 2D).

2. Toluidine blue stain

Examined semithin sections of group I revealed normal renal corpuscles with glomerular tuft of capillaries surrounded by Bowman's membrane. Proximal convoluted tubules with basal rounded pale stained nuclei and apical brush border and distal convoluted tubules with wide lumina and apical nuclei were also seen (Figure 3A). Semithin sections of group II showed dilatation of glomerular capillaries and loss of apical cytoplasm of certain tubular epithelial cells (Figure 3B). Semithin sections of group III revealed normal appearance of renal cortex except for disruption of the apical cytoplasm of some tubular epithelial cells and loss of brush border in others (Figure 3C). Semithin sections of group IV showed normal renal corpuscles, PCTs with intact brush border and DCTs more or less as control group excepting nuclear migration of certain cells of DCT (Figure 3D).

3. Periodic Acid Schiff stain (PAS)

Examined sections of group I showed PAS positive reaction in basement membranes of parietal layer of Bowman's capsule and of convoluted tubules as well as brush borders of proximal tubules (Figure 4A). Sections of group II revealed focal loss of PAS reaction in the basement membranes of parietal layer of Bowman's capsule and of convoluted tubules as well as brush borders of most proximal tubules (Figure 4B). Sections of group III showed that PAS reaction was less than normal in the brush border and basement membrane of few tubules (Figure 4C). Sections of group IV revealed that PAS positive reaction was more or less as the control group (Figure 4D).

4. Immunohistochemical study (PCNA)

Proliferating cell nuclear antigen (PCNA) immunostained sections of group I revealed positive nuclear reaction in some tubular cells, parietal epithelial cells, mesangial cells and interstitial cells (Figure 5A). Sections of group II revealed slight decrease in number of positive nuclei of some renal tubules, parietal epithelial cells, mesangial cells and interstitial cells (Figure 5B). Sections of group III showed an increase in number of positive nuclei of some renal tubules, parietal epithelial cells, mesangial cells and interstitial cells (Figure 5C). Sections of group IV revealed strong positive reaction in

most of nuclei of cells lining large number of renal tubules, parietal epithelial cells, mesangial cells and interstitial cells (Figure 5D).

B. Electron microscopic results:

Examined ultrathin sections of the renal cortex of group I (control group) revealed visceral layer of Bowman's space lined by podocyte with cell body sending cytoplasmic extensions forming primary processes and send secondary processes terminated by feet-like expansions on the basal lamina of the glomerular capillary walls which was lined by fenestrated endothelium. Cell body of podocyte contained large central euchromatic nucleus. Subpodocytic space was seen between the podocyte and GBM (Figure 6A). Ultrathin sections of the renal cortex of group II (cisplatin model of AKI) revealed podocyte with fusion of their feet processes at certain site and irregular nucleus in the center of the body (Figure 6B). Ultrathin sections of the renal cortex of group III (AKI-MSCs-treated group) revealed some improvement; the podocyte appeared more or less as the control except for focal fusion of feet processes (Figure 6C). Ultrathin sections of the renal cortex of group IV (AKI-Mobilized BMSCs-treated group) revealed marked improvement of the histological ultrastructure of renal cortex and appeared more or less similar to control group. Podocyte had large central euchromatic nucleus, primary processes, regularly spaced feet processes resting on uniform glomerular basement membrane (Figure 6D).

Proximal convoluted tubular cell (PCT) of group I appeared with single basal euchromatic nucleus and numerous basal invaginations with elongated mitochondria arranged in palisade manner and the characteristic brush border (Figure 7A). Group II showed PCT cell resting on thick basement membrane having basal oval euchromatic nucleus with prominent nucleolus, intact apical brush border, multiple cytoplasmic vacuoles and loss of basal palisade arrangement of mitochondria which appeared scattered, rounded and small (Figure 7B). Group III revealed PCT cell containing basal oval euchromatic nucleus with prominent nucleolus, intact apical brush border and small cytoplasmic vacuoles (Figure 7C). Group IV showed PCT cells more or less similar to control group (Figure 7D).

Distal convoluted tubular cells (DCT) of group I were resting on regular basement membrane. Each cell contains single slightly apical oval euchromatic nucleus, few short apical scattered microvilli and basal infoldings with basal longitudinally oriented mitochondria (Figure 8A). Group II revealed DCT cell containing cytoplasmic vacuole, small secondary lysosome and loss of basal infoldings and palisade arrangement of mitochondria and few short microvilli (Figure 8B). DCT cells of group III rested on regular basement membrane containing apically euchromatic nuclei with prominent nucleoli with basal vertically oriented mitochondria and apical few and short microvilli (Figure 8C). Most of DCTs cells of group IV appeared more or less similar to control group (Figure 8D).

III- Morphometric and Statistical results

1- Histological grading of Acute Kidney Injury (Table 1)

Statistical analysis of the data collected by image J analysis program revealed that 80% of group I (control group) belongs to grade 0 while grade 1 constitutes 20%. Grade 3 represents 58% of cisplatin model of AKI group II while grade 2, 1 and 4 represent 24%, 16% and 2% respectively. Grade 2 represents 60% of AKI-MSCs-treated group III while grade 1, 0 and 4 represent 26%, 10% and 4% respectively. Grade 1 represents 70% AKI-Mobilized BMSCs-treated group IV while grade 0 and 2 represent 20% and 10% respectively.

2- Colour density of PAS stain

The mean colour density of PAS stained sections (Table 2):

A highly significant decrease (*P value*<0.001) of the mean colour density in group II (cisplatin model of AKI group) was observed when compared to group I (control group). However, in group III (AKI-MSCs treated group), there were a significant increase in the mean colour density when compared to group II and a highly significant decrease when compared to groups I and IV (AKI-BMSCs treated group). Group IV showed a highly significant increase in the mean colour density compared with groups II & III and a significant increase (*P value*<0.05) when compared with group I.

3- Counting of PCNA positive cells

The mean number of PCNA positive cells (Table 3):

Statistical analysis of the data collected by image J analysis program showed a highly significant increase (*P value*<0.001) in the mean number of PCNA positive cells in group II as compared to group I. Group III showed a highly significant increase in the mean number of PCNA positive cells as compared to groups I & II and a highly significant decrease as compared to group IV. Group IV revealed a highly significant increase in the mean number of PCNA positive cells when compared with groups I, II and III.

4- Laboratory investigations (blood urea nitrogen and serum creatinine) (Table 4)

Statistical analysis of the collected data showed that there was a highly significant increase (*P value* <0.001) in the mean level of blood urea nitrogen and serum creatinine in group II when compared to group I. On the other hand, group III demonstrated a highly significant decrease when compared to group II and a highly significant increase when compared to groups I and IV. Group IV showed non significant change (*P*>0.05) when compared with group I and a highly significant decrease when compared with groups II and III.

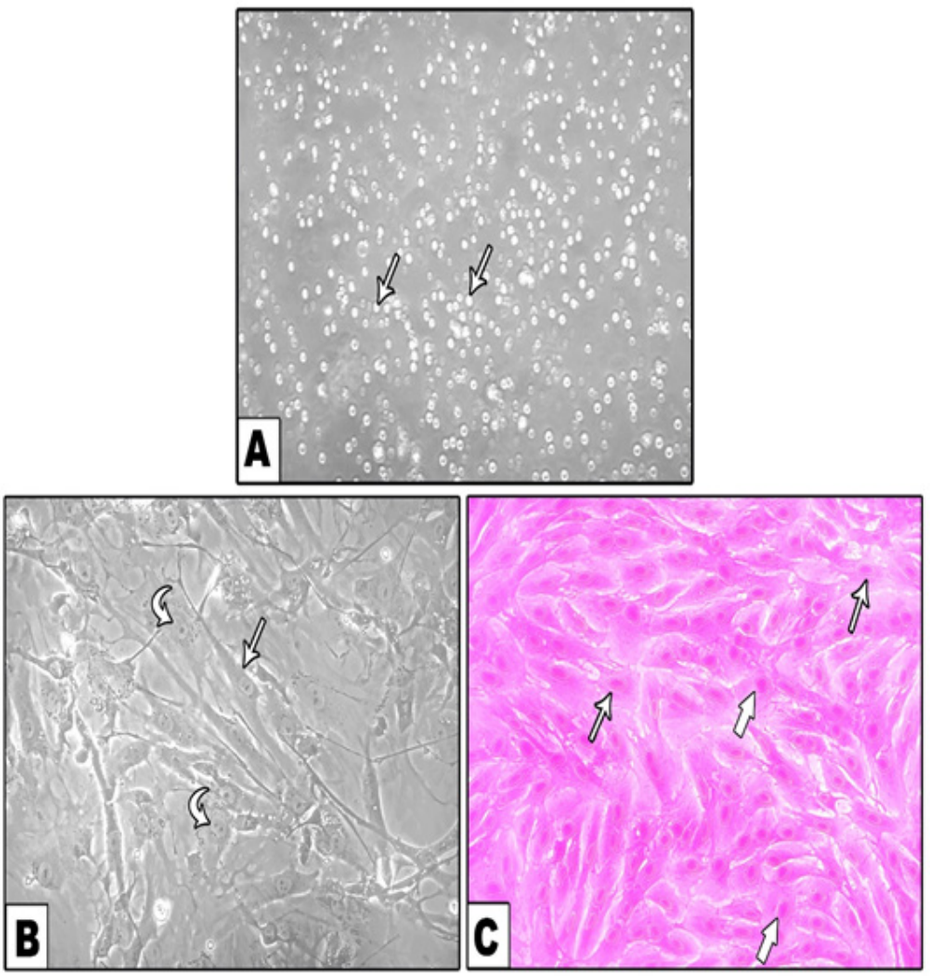

Fig. 1: A phase contrast micrograph of a primary culture of rat BM-MSCS shows the following results. 1A. on day one, cultured cells (thin arrows) appear rounded in shape, variable in size, crowded and floating. 1B. On day twelve, the adherent cells reach about 80-90 % confluency and exhibit different shapes with multiple interdigitating processes (thin arrows), central vesicular nuclei and multiple nucleoli (curved arrows). 1C. The adherent cells have a star-shaped appearance with many interdigitating processes (thin arrows) and blue vesicular nuclei with prominent nucleoli (thick arrows) (Giemsa x 200) (Inverted microscope, x 200)

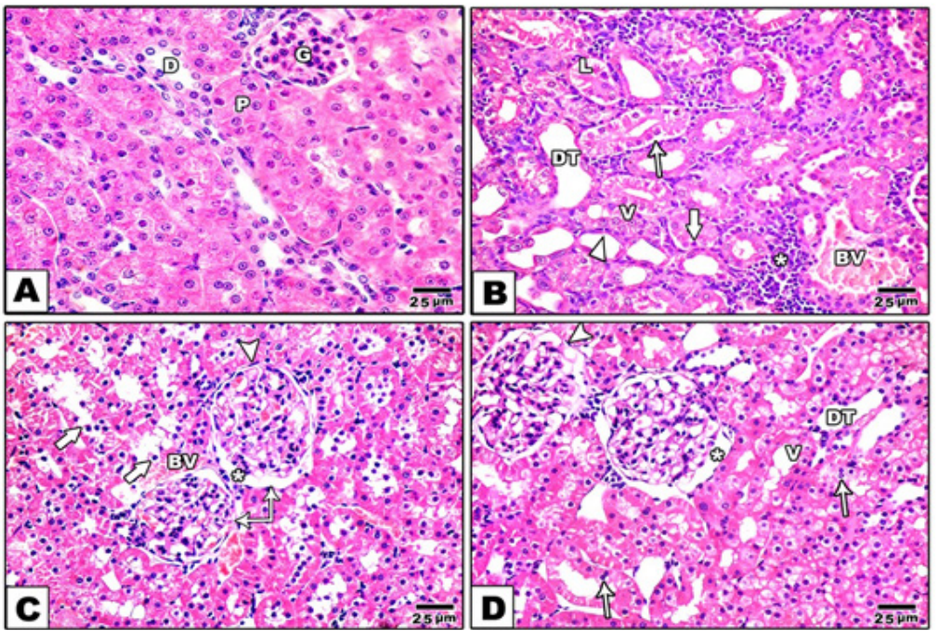

Fig. 2: A photomicrograph of the renal cortex of experimental groups: 2A. Control group I shows normal renal corpuscles with glomerular tuft of capillaries (G), proximal convoluted tubules (P), distal convoluted tubules (D). 2B. Cisplatin model of AKI group II shows focal loss of differentiation between proximal and distal convoluted tubules with irregularity, dilatation (DT) and flattening of their lining epithelium (►) with detachment of lining epithelial cells from their basement membrane (→) and vacuolations (V). Inflammatory cellular infiltration (*) around dilated congested blood vessels (BV) is seen. 2C. AKI-MSCs-treated group III shows renal corpuscles (→) lined by simple squamous parietal cell layer (►) with Bowman's space (*). Some of the convoluted tubules are improved while others show disruption of their apices with loss of parts of cyoplasm (➡) and mild congested blood vessles (BV). 2D. AKI-Mobilized BMSCs-treated group IV demonestrates rounded renal corpuscles with Bowman's space (*), simple squamous epithelium lining its parietal layer (►), mild dilatation (DT) and vacuolated cytoplasm (V) in some of convoluted tubules. Binucleation in few cells lining renal tubules is observed (→) (H&E X 400).

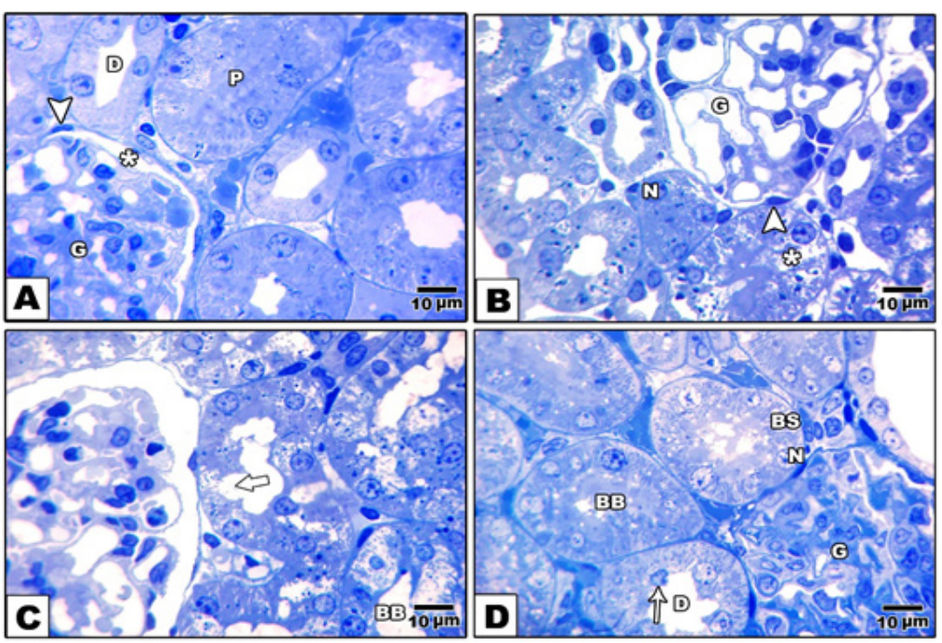

Fig. 3. A photomicrograph of semithin section of the renal cortex of different groups: 3A. control group I shows normal renal corpuscles with glomerular tuft of capillaries (G), Bowman's space (*) and simple squamous parietal cell layer (►), proximal convoluted tubules (P) and distal convoluted tubules (D). 3B. Cisplatin model of AKI group II shows dilated glomerular capillaries (G) and loss of apical cytoplasm of certain tubular epithelial cells (*), parietal layer of Bowman s capsule (►) and few tubular cells have spherical pale stained nuclei with prominent nucleoli (N). 3C. AKI-MSCs-treated group III demonestrates normal appearance of renal cortex except for disruption of the apical cytoplasm of some tubular epithelial cells ( ) and loss of ➡ brush border in others (BB). 3D. AKI-Mobilized BMSCs-treated group IV shows renal corpuscles (G), PCTs (P) with intact brush border (BB), basal striations (BS), spherical nucleus with prominent nucleolus(N) and DCTs (D) more or less similar to control group except for apical disruption and nuclear migration of certain cells of DCT (→) (Toluidine blue x 1000).

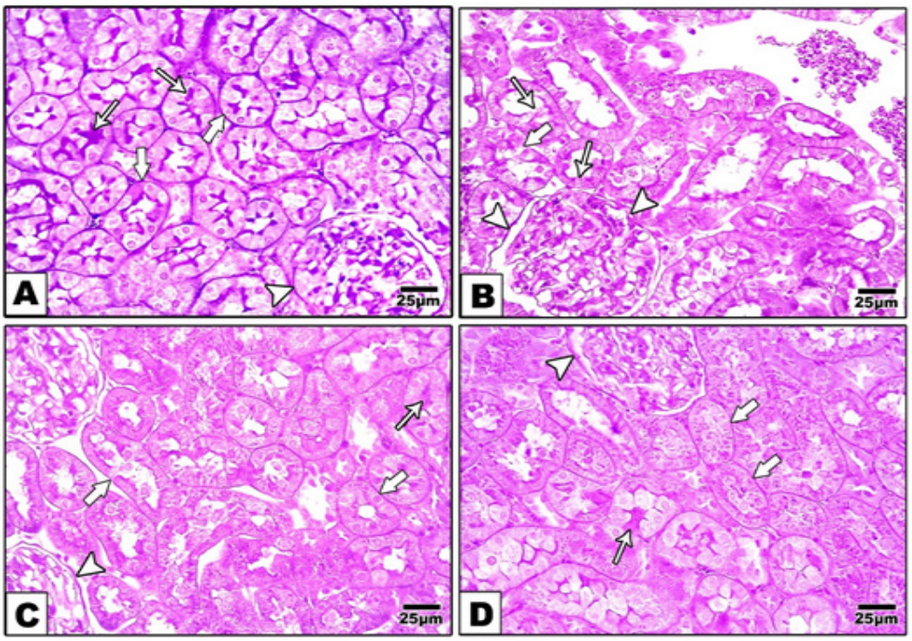

Fig. 4: A photomicrograph of section of the renal cortex. 4A. Control group I, PAS +ve reaction in the basement membranes of parietal layer of Bowman's capsule (►), convoluted tubules (➡) as well as brush borders of proximal tubules (→). 4B. Cisplatin model of AKI group II, focal loss of PAS reaction in basement membranes of parietal layer of Bowman's capsule (►), convoluted tubules (➡) as well as brush borders of most proximal tubules (→). 4C. AKI-MSCs-treated group III, PAS reaction was still less than normal basement membranes of parietal layer of Bowman's capsule (►), in the brush border (→) and basement membrane of few tubules appeared intense (➡). 4D. AKI-Mobilized BMSCs-treated group IV, PAS positive reaction was more or less similar to the control group in the basement membrane of the parietal layer of bowman capsule (►) , convoluted tubules (➡) and the brush border (→) (PAS x 400).

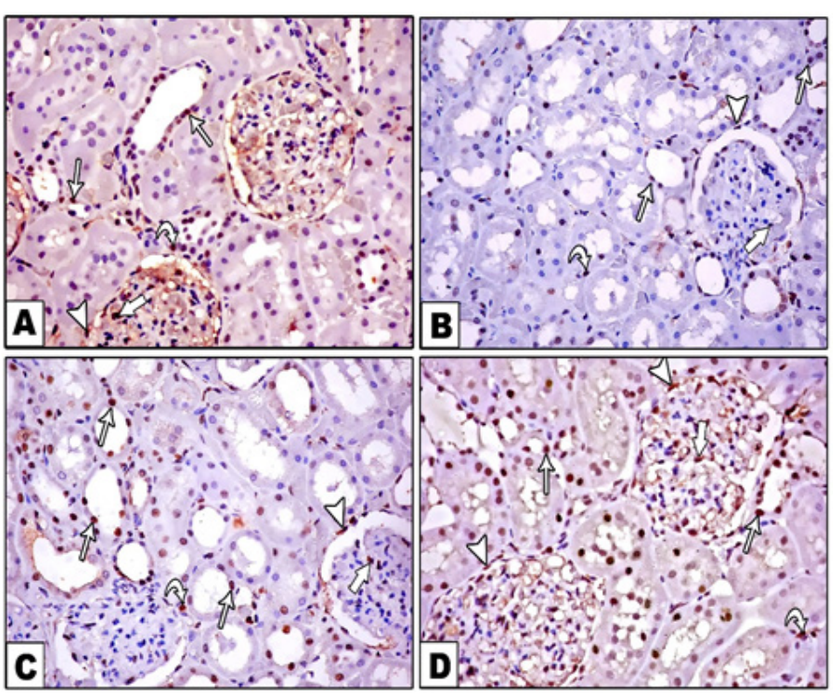

Fig. 5: A photomicrograph of the renal cortex of different groups: 5A. Control group I shows positive immunoreaction in some nuclei of renal tubules (→), parietal epithelial cells (►), mesangial cells (➡) and interstitial cells (↷). 5B. Cisplatin model of AKI group II demonestrates slight decrease in number of positive nuclei of some tubular cells (→), parietal epithelial cells (►), mesangial cells (➡) and interstitial cells (↷). 5C. AKI-MSCs-treated group III shows increase in number of positive nuclei of some tubular cells (→), parietal epithelial cells (►), mesangial cells (➡) and interstitial cells (↷). 5D. AKI-Mobilized BMSCs-treated group IV indicates strong positive reaction in most of nuclei of cells lining large number of renal tubules (→), parietal epithelial cells (►), mesangial cells (➡) and interstitial cells (↷) (PCNA immunoreaction counterstained with Hx x 400)

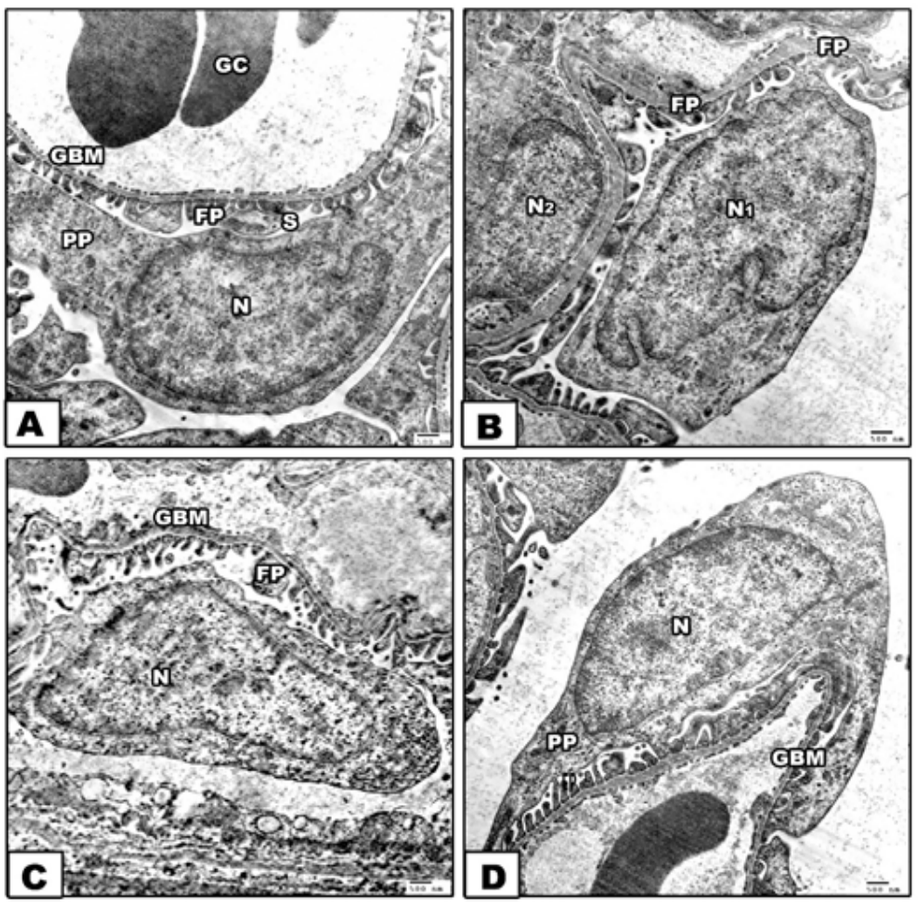

Fig. 6: An electron micrograph of ultrathin section of the renal cortex of different groups: 6A. Control group I shows Glomerular blood capillaries (GC), glomerular basement membrane with its trilamellar appearance (GBM), normal podocyte with euchromatic nucleus (N), primary process (PP) and foot processes (FP). Subpodocytic space is also seen (S). 6B. Cisplatin model of AKI group II reveals podocyte with large irregular shaped nucleus (N1), fusion of their feet processes (FP) at certain site. Notice: The nucleus of mesangial cell was seen (N2). 6C. AKI-MSCs-treated group III demonestrates podocyte containing large central irregular euchromatic nucleus (N), focal fused feet processes (FP) and uniform glomerular basement membrane (GBM). 6D. AKI-Mobilized BMSCs-treated group IV shows podocyte containing large central euchromatic nucleus (N) with its primary processes (PP) and glomerular basement membrane (GBM) (TEM x 3000).

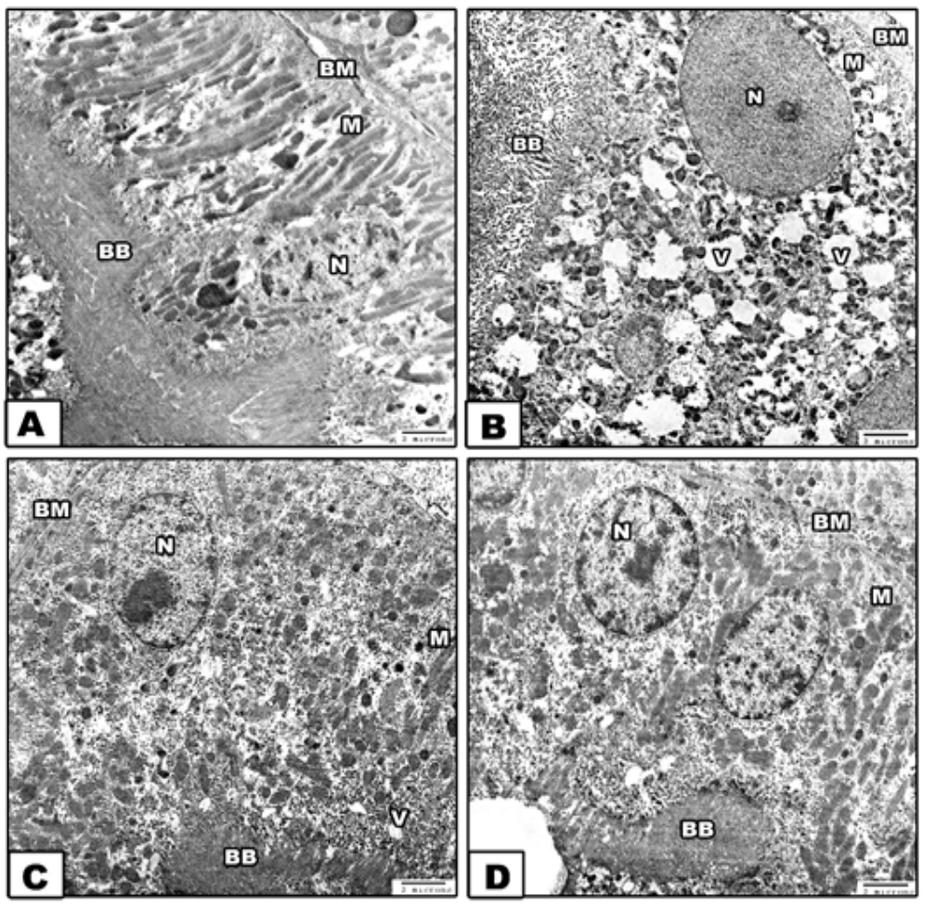

Fig. 7: An electron micrograph of ultrathin section of the renal cortex of different groups: 7A. Control group I reveals PCT cell with single slightly basal oval euchromatic nucleus (N) resting on basement membrane (BM), basal infoldings containing vertically oriented mitochondria (M) and apical thick brush border (BB). 7B. Cisplatin model of AKI group II shows PCT cell resting on thick basement membrane (BM) having basal oval euchromatic nucleus with prominent nucleolus (N), multiple cytoplasmic vacuoles (V), total loss of basal palisade arrangement of mitochondria (M) which appeared scattered, rounded and electron dense and intact apical brush border (BB). 7C. AKI-MSCs-treated group III demonestrates PCT resting on regular uniform basement membrane (BM) containing basal oval euchromatic nucleus (N) with prominent nucleolus and small cytoplasmic vacuole (V). Basal arrangement of mitochondria (M) has restored to more or less normal and intact apical brush border (BB). 7D. AKI-Mobilized BMSCs-treated group IV shows cells of PCTs resting on regular uniform basement membrane (BM) and containing basal rounded euchromatic nuclei (N) with prominent nucleoli, basal arrangement of mitochondria (M) and intact apical brush border (BB) (TEM x 1500).

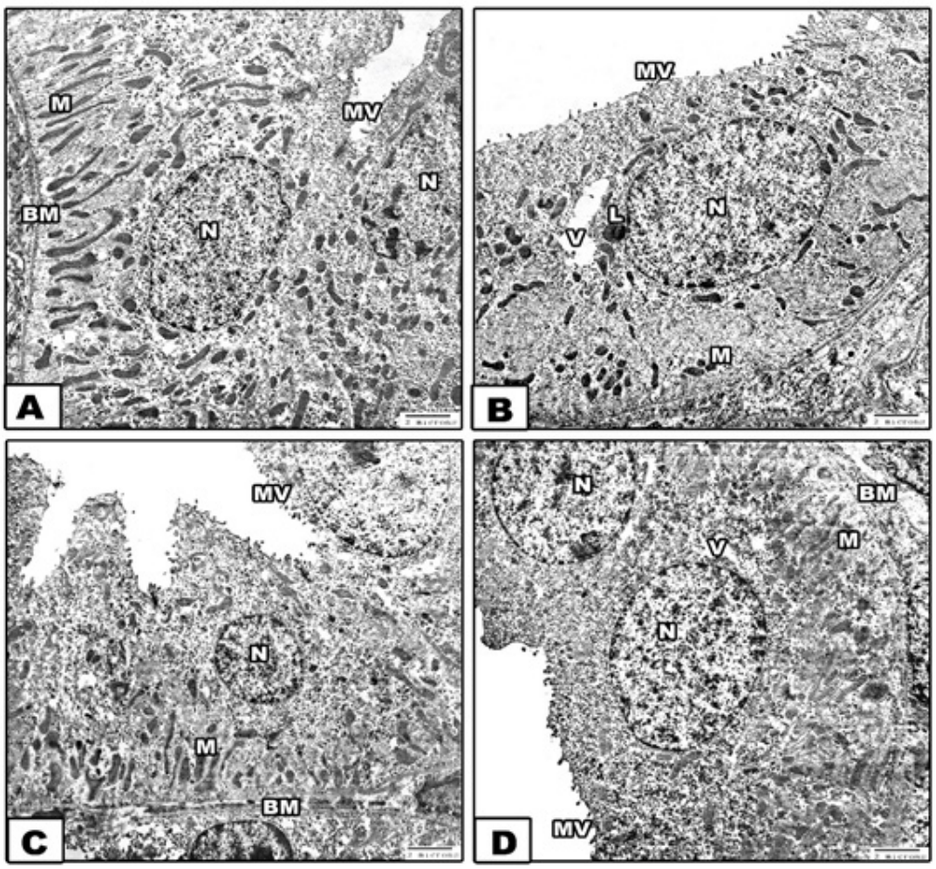

Fig. 8: An electron micrograph of ultrathin section of the renal cortex of different groups: 8A. Control group I shows two cells of DCTs resting on basement membrane (BM). Each cell contained single slightly apical oval euchromatic nucleus (N), basal palisade arrangement of mitochondria (M) and few, short, scattered apical microvilli (MV). 8B. Cisplatin model of AKI group II reveals DCT cell with apical rounded euchromatic nucleus (N), cytoplasmic vacuole (V), small secondary lysosome (L), loss of basal palisade arrangement of mitochondria (M) and few short apical microvilli (MV). 8C. AKI-MSCs-treated group III shows two DCT cells resting on regular basement membrane (BM) containing apical rounded euchromatic nuclei (N) with prominent nucleoli, basal vertically oriented mitochondria (M) and apical few and short microvilli (MV). 8D. AKI-Mobilized BMSCs-treated group IV indicates two DCT cells resting on regular basement membrane (BM) containing apical rounded euchromatic nucleus (N), cytoplasmic vacuole (V), basal vertically oriented mitochondria (M) and few short apical microvilli (MV) (TEM x 1500).

Table 1: Histological grading of different studied groups represented as percentage

Histological Grading		Group I (Control)	Group II (Cisplatin)	Group III (MSCs)	Group IV (Mobilized BMSCs)	Total
0	N	40	0	5	10	55
	%	80.0%	.0%	10.0%	20.0%	27.5%
1	N	10	8	13	35	66
	%	20.0%	16.0%	26.0%	70.0%	33%
2	N	0	12	30	5	47
	%	.0%	24.0%	60.0%	10.0%	23.5%
3	N	0	29	0	0	29
	%	.0%	58.0%	.0%	.0%	14.5%
4	N	0	1	2	0	3
	%	.0%	2.0%	4.0%	.0%	1.5%
Total	N	50	50	50	50	200
	%	100.0%	100.0%	100.0%	100.0%	100.0%

Table 2: The range and the mean colour density of PAS stain in different studied groups

PAS	Group I (control)	Group II (cisplatin)	Group III (MSCs)	Group IV (Mobilized BMSCs)
Range	30 – 60	19 – 35	19 – 43	25 – 55
Mean ± SD	44.44 ± 9.59	24.04 ± 4.48	28.96 ± 6.17	39.72 ± 7.73

Table 3: The range and the mean number of PCNA positive cells of the renal cortex in different studied groups

PCNA (N/400)	Group I (control)	Group II (cisplatin)	Group III (MSCs)	Group IV (Mobilized BMSCs)
Range	10 – 30	8 – 25	30 – 47	39 – 80
Mean ± SD	15.28 ± 5.19	13.76 ± 4.80	37.80 ± 5.58	62.48 ± 11.39

Table 4: The range and the mean value of blood urea nitrogen (BUN) and serum creatinine in different studied groups

BUN (mg/dl)	Group I (control)	Group II (cisplatin)	Group III (MSCs)	Group IV (Mobilized BMSCs)
Range	12 – 18	20.41 – 34	17.51 – 29	11.92 – 20
Mean ± SD	14.67 ± 1.71	26.41 ± 3.18	22.25 ± 2.67	14.89 ± 2.20
Serum Creatinine (ml/min)				
Range	0.61 – 1.35	2 – 4.81	1.33 – 2.52	1.01 – 1.63
Mean ± SD	1.14 ± 0.18	3.42 ± 0.72	1.75 ± 0.30	1.25 ± 0.15

DISCUSSION

Acute kidney injury (AKI) is a clinical condition recognized by unexpected decline of renal function and accumulated waste produces within renal tissue. Many factors were involved in its occurrence including direct damage of nephrons due to drug nephrotoxicity[24,25].

Cisplatin is one of the platinum-based medications used as chemotherapy to treat different types of cancer. Cisplatin prevents cancer cells from multiplying and subsequently inducing apoptosis through binding to DNA and inhibiting its replication. In this study, cisplatin was used as a model of AKI[26].

Previous studies showed that different possible mechanisms were implicated in cisplatin induced AKI. The unbound part of cisplatin in plasma has a low molecular weight permitting its filtration through the glomerulus and accumulation within the renal cortex leading to proximal tubular injury. It has a high concentration within the kidney which is 5 times more than those in blood. Moreover, it can be activated in the kidney and converted to a more powerful toxin in rats and mice[27,28].

This research tried to compare between the effect of mesenchymal stem cells either derived or mobilized bone marrow on induced acute kidney injury after 24 hours of cisplatin administration using different histological and immunohistochemical methods. Structural and functional changes were detected. These findings go in line with some researchers who found that receiving 10 mg/kg of cisplatin once intraperitoneally resulted in marked histological changes in the renal cortex[29-33].

Moreover, loss of differentiation between the convoluted tubules either proximal or distal and dilated renal tubules with flattening of their lining cells were in accordance with some researchers who mentioned that the intra-renal vasoconstriction associated with AKI result in deprivation of tubular epithelial cells from oxygen and nutrient leading to their flattening and atrophy[29,34,35].

Degenerative changes occurred in some renal tubular epithelial cells such as vacuolation of cytoplasm was coincided with some investigators who stated that cisplatin caused acute tubular necrosis and apoptosis through its binding with DNA. This binding forms intra and inter strands cross-links resulting in arrest of DNA synthesis and replication and the cells were unable to repair this damage so it underwent apoptosis[36]. Other investigators[37] revealed that tumor suppressor gene P53 activation and mitogen activated protein kinase P38 played a role in apoptosis in response to DNA damage.

The separation of epithelial cells lining renal tubules from the underlying basement membrane seen by light microscopic examination could be attributed to redistribution or alteration of integrin which anchor the tubular cells leading to sloughing and desquamation within the tubular lumen[29].

In the present study, the interstitial tissue inflammatory cellular infiltration occurred because cisplatin treatment triggers excretion of proinflammatory cytokines for example TNF-a and IFN-γ and can attract inflammatory cells[38]. In addition, cisplatin increases the amount of several proinflammatory cytokines for instance tumor necrosis factor (TNF)-α, ILs (1β, 6& 18), transforming growth factor-β1 (TGF-β1), and monocyte chemotactic

protein-1 (MCP-1). They also considered inflammatory infiltration guard against cisplatin toxic effect through rapid elimination of necrotic tissue. Also, the congestion observed in some blood vessels initiates inflammatory reaction and attracts cells to the site of congestion around the blood vessel[35].

The dilated congested blood vessels observed in this research was previously explained due to renal endothelial cells damage and dysfunction in cisplatin nephrotoxicity resulted in extravasation of RBCs, congestion of glomerular tuft and dilatation of peritubular capillaries[29,34].

In the present research, the basement membrane of parietal layer of Bowman's capsule and convoluted tubules of renal cortex showed weak PAS reaction with areas of focal damage of proximal convoluted tubules brush border. This could be attributed to reactive oxygen molecules (ROM) production that led to damage of cytoskeletal integrity[39].

In this study Proliferating Cell Nuclear Antigen (PCNA) was used as a marker for cell proliferation. It was reported that PCNA reaches its maximum level in the nucleus during late G1/S phase of cell cycle and this indicates the onset of DNA synthesis[40]. The significant increase in the mean number of PCNA positive cells in the renal tubules, parietal epithelial cells, mesangial cells and interstitial cells supported the findings of other researchers who stated that under normal conditions, a majority of renal tubular epithelial cells are quiescent and enter the cell cycle in response to ischemia and other injuries. This was triggered by motivation of mitogenic factors which motivate the CDK4/6 pathway to drive cell cycle progression from GO or G1 phase into S phase where DNA replication occurs[41].

Electron microscopic examination of podocytes showed irregular shaped nuclei and fusion of their feet processes in certain sites can be attributed to absence or decrease of nephrin in case of podocyte's injury resulted in foot processes fusion, effacement and proteinuria[42].

Other researchers stated that mitochondria were involved in a lot of mechanisms including control calcium levels inside the cells, cytoskeletal dynamics, apoptotic pathways and production of large amount of reactive oxygen species (ROS). Mitochondrial disorders were linked to disturbance of podocyte function. It is important in maintaining the large surface area of podocytes tertiary foot processes of by supplying ATP which maintains the actin cytoskeleton. So, mitochondrial DNA mutations and proteins resulted in interruption of the glomerular filtration barrier (GFB) due to loss of ATP[43,44].

Several elements are involved in occurrence of cisplatin-associated acute renal failure. The most important one is oxidative stress followed by the accumulation of ROS[45]. This resulted in increase lipid peroxidation products and decrease antioxidant enzyme expression. These products injured the podocytes through interference with slit diaphragm complex and its lipid rafts and with the actin cytoskeleton.

Podocytes have well developed cytoskeleton which accounts for their unique shape and maintenance of their processes. Any abnormality in the assembly of these microfilaments will disrupt foot processes and function of glomerular basement membrane[46].

In this study, cells of proximal and distal convoluted tubules revealed thickening of the tubular basement membrane, cytoplasmic vacuoles, secondary lysosomes, loss of apical microvilli, disruption of the basal membrane infoldings and loss of basal palisade arrangement of mitochondria. These findings were coincided with some people who studied the effect of cisplatin and the possible protective effect of vitamin c[33] on the kidney of albino rat.

Luminal debris and secondary lysosomes observed in this research were in agreement with two investigators who found necrotic debris in the tubular lumens with tubular damage, condensation of nuclear chromatin and appearance of secondary lysosomes indicating degenerative activity and onset of necrosis[47].

Moreover, decreased number or even loss of apical microvilli and loss of basal palisade arrangement of mitochondria observed in renal tubular cells supported results of other researchers[48]. Reactive oxygen species production observed during their study resulted in disturbance of cytoskeletal integrity leading to damage of brush-border , cell junctions and loss of polarity with mislocalization of adhesion molecules and other membrane proteins such as the Na+K+-ATPase and β-integrins.

Furthermore, cytoplasmic vacuoles of renal tubular cells observed in this work were referred to ATP depletion secondary to mitochondrial dysfunction that led to absence of selective permeability of the cell membranes and dilatation of cytoplasmic membranous components[49]. These vacuoles were also attributed to gathering of misfolded proteins in endoplasmic reticulum causing its dilatation after losing its ribosomes[28].

The biochemical results of the present research declared a statistically highly significant increase in the blood urea nitrogen (BUN) and serum creatinine in group II in relation to the control group. This was coincided with Barakat *et al*. (2021)[50] and McSweeney *et al*. (2021)[28] who mentioned that cisplatin increased the kidney parameters BUN and serum creatinine and cause rapid failure of renal excretory mechanisms, augmenting waste products accumulation formed as a result of protein metabolism (including urea, nitrogen, and creatinine).

In the present study, injecting bone marrow-mesenchymal stem cells (BM-MSCs) suspension one day after cisplatin injection slightly improved the histological and immunohistochemical changes occurred in the renal cortex.

Westenfelder & Togel (2011)[51], Furuichi *et al*. (2012)[52], Lee *et al*. (2012)[53] and Havakhah *et al*. (2018)[54] attributed the beneficial effect of BM-MSCs in AKI in rats to several mechanisms for instance transdifferentiation,

de-differentiation or homing of MSCs. The latter have paracrine effects through cytokines excretion and increased expression of growth factors like hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-I) that exert antiapoptotic, mitogenic, vasoprotective and angiogenic actions in AKI. Moreover, MSCs have immunomodulatory properties which might stimulate mitosis, proliferation and differentiation of intrinsic stem cells, impede fibrosis and apoptosis, and improve angiogenesis.

MSCs have a role in immune regulation through inhibition of the proliferation of immune cells such as T and B cells, inducing the differentiation of macrophages from pro-inflammatory phenotypes to anti-inflammatory phenotypes[55]. In addition, Wang *et al*. (2017)[56] explained that the immunomodulatory role of MSCs was due to their interaction with the immune cells which are T and B cells, natural killers (NK), monocytes/macrophages and dendritic cells (DCs) and even with neutrophils. Also, they had the ability to inactivate T cells production and reduce the reactive oxygen species (ROS) excretion by neutrophils.

Another mechanism of injection of MSCs suspension in dealing with AKI was the ability of the MSCs to modulate inflammation by secreting both interleukins-10 and 4 (IL-4&10) which facilitates tissue repair. It also had anti-oxidant effect improving the renal function, alleviating oxidative stress-induced cell senescence and increasing production of renal cells[57].

Certain studies reported that Mesenchymal stem cells could activate the regeneration of the damaged kidney and exogenous BM-MSCs could contrive into damaged tubules and transdifferentiate. In contrast with other studies, BM-MSCs can guard against acute tubular injury through a differentiation-independent process[58,59].

Most of MSCs were present in blood vessels or stick to the wall of blood vessel's in kidney and few MSCs fused to renal tissue and could differentiate, regenerate and/or protect tubular epithelial cells, endothelial cells, podocytes and mesangial cells[60].

Furthermore, Kraińska *et al*. (2021)[61] stated that MSCs help tissue regeneration and restoration through several mechanisms including contact between cells, excretion of mediators and extracellular vesicles (EVs), development of membrane nanotubes (TnTs) which carry the trophic factors (including messenger RNA (mRNA) and MicroRNAs (miRNAs) and mitochondria between cells to restore damaged organs. MSCs also secrete many bioactive factors that stimulate the angiogenesis and inflammation processes and regulate the immune system affecting the stem cells recruitment to the local organ injuries, stem cell survival, proliferation and differentiation.

BM-MSCs increase PCNA positive cells number in renal tubules in cisplatin-treated rats through regulation of Bax and Bcl-2 the expression. MSCs downregulate Bax (pro-apoptotic gene) and caspase-3[36].

In the present study, injecting granulocyte colony stimulating factor (G-CSF) improved the histological, immunohistochemical and statistical changes occurred in the renal cortex to be more or less as the control group. It has more superior benefit than mesenchymal stem cells in alleviating the cisplatin-induced acute kidney injury.

Previous studies showed that release of hematopoietic stem cells (HSCs) to peripheral circulation by G-CSF might enhance tubular renewal[62].

Melve *et al*. (2018)[63] and Gottschalk *et al*. (2021)[64] reported that G-CSF stimulates production of neutrophil from the bone marrow and maintains neutrophil survival, activation and function and can attenuate leukocyte infiltration in the kidney, promote regulatory T cell expansion and reduce proinflammatory cytokines. Treatment with G-CSF is commonly used for mobilization of peripheral blood stem cells in healthy donors and has significant effects on T cells which encouraged enrolment of naïve T cells, decreased expression of T cell activation markers as well as adhesion molecules and chemokine receptors.

It has been reported that using G-CSF can mobilize bone marrow derived stem cells into the peripheral blood. It could be a new plan for prevention of acute renal injury and saves the mice from chemotherapy-induced renal failure[65].

Pre-treatment with G-CSF for 2 days was enough to raise the number of CD133+ in the peripheral blood and in renal tissue 1 hour after injury and these CD133+ cells can quickly reach the injured tissue[66,67]. They also concluded that CD133+ cells possessed anti-inflammatory capacity in the peripheral blood and organs for example kidney. They found that these cells have the ability to migrate to renal tissue and accumulated in the area of glomeruli. This result indicated that CD133+ cells which reach the kidney could not differentiate into endothelial cells. Additional studies established that CD133+ cells achieve renal protection during early stages of AKI through their paracrine effects.

CD34-positive hematopoietic stem cells (HSCs) had the ability to differentiate to vascular endothelial progenitor cells also had anti-inflammatory potential. These cells have great ability to treat AKI together with different kinds of ischemic organ damage such as myocardial ischemia and stroke[67].

CONCLUSION

Based on the current study and from all previously mentioned results; it was discovered that mobilized bone marrow-derived stem cells by using Granulocyte- Colony Stimulating Factor (G-CSF) had a superior effect on bone marrow-derived mesenchymal stem cells in alleviating Acute Kidney Injury induced by cisplatin. This effect was confirmed by histological, immunohistochemical and biochemical methods.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

مقارنة بين تأثير الخلايا الجذعية الميزنشيمية المشتقة من نخاع العظم والخلايا الجذعية المستنفرة من نخاع العظم على إصابات الكلى الحادة المستحدثة تجريبياً فى ذكر الجرذ الأبيض البالغ (دراسة هستولوجية وهستوكيميائية مناعية وكيميائية)

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المقدمة: إصابات الكلى الحادة هي متلازمة تدهور وظائف الكلى بسرعة. تم استخدام سيسبلاتين لعمل نموذج لإصابات الكلى الحادة. نظرًا لقدرة الخلايا الجذعية الميزنشيمية المشتقة من نخاع العظم على التجديد الذاتي وتعدد القدرات وبسبب قدرة عامل تحفيز مستعمرات الخلايا المحببة على تحريك أعداد كبيرة من الخلايا المكونة للدم من نخاع العظم. يمكن أن يكون لهما قيمة علاجية محتملة في تحسين القصور الكلوي الحاد.

الهدف من العمل: الغرض من هذه الدراسة هو المقارنة بين تأثير الخلايا الجذعية الميزنشيمية المشتقة من نخاع العظم والخلايا الجذعية المستنفرة من نخاع العظم على إصابات الكلى الحادة المُحدثة تجريبياً في القشرة الكلوية لذكر الجرذ الأبيض البالغ باستخدام الطرق النسيجية والكيميائية المناعية.

المواد والطرق: أستخدم فى هذا البحث ٤٢ من ذكور الجرذان البيضاء البالغة قسمت إلى خمسة مجموعات رئيسية. المجموعة الصفرية بمثابة مصدرللخلايا الجذعية الميزنشيمية المشتقة من نخاع العظم. المجموعة الأولى كمجموعة ضابطة وتضمنت مجموعتان فرعيتان أ (تم الاحتفاظ بها بدون علاج) ومجموعة فرعية ب (تلقت الحيوانات جرعة واحدة من ٠,٥ مل من الوسسط في وريد الذيل). المجموعة الثانية: تم حقن الحيوانات بجرعة ١٠ مجم / كجم مرة واحدة من السيسبلاتين داخل التجويف البريتونى لإحداث إصابات الكلى الحادة. المجموعة الثالثة: حقنت الحيوانات بالسيسبلاتين مثل المجموعة الأولى وتركت ٢٤ ساعة ثم عولجت بجرعة واحدة .٥ مل (١ × ١٠٦) من معلق الخلايا الجذعية الميزنشيمية المشتقة من نخاع العظم في الوريد الذيلى. المجموعة الرابعة: حقنت الحيوانات بالسيسبلاتين مثل المجموعة الأولى وتركت ٢٤ ساعة ثم عولجت بعامل تحفيز مستعمرات المحببات تحت الجلد مرة واحدة يومياً لمدة ٥ أيام بجرعة ١٠٠ ميكروجرام / كجم. تم جمع عينات الدم لقياس اليوريا والكرياتينين في الدم. تم التضحية بجميع الفئران في اليوم السابع من التجربة باستثناء المجموعة الثانية تم التضحية بها بعد ٢٤ ساعة. تم تشريح الكلى للحصول على القشرة الكلوية ، ومعالجتها لإجراء دراسات نسيجية وكيميائية مناعية بواسطة المجهر الضوئي والإلكتروني. تم إجراء التصنيف النسيجي لمعرفة درجة إصابات الكلى الحادة كما تم عمل تحليل إحصائي وكيميائي.

النتائج: أظهرت المجموعة الثالثة التي عولجت بالخلايا الجذعية الميزنشيمية المشتقة من نخاع العظم تحسنًا ملحوظا بينما أظهرت المجموعة الرابعة التي أعطيت عامل تحفيز مستعمرات المحببات تحسنًا واضحا في التركيب النسيجي للقشرة الكلوية والوظائف الكلوية وذلك عند مقارنتهم المجموعة الثانية غير المعالجة.

الخلاصة: كان عامل تحفيز مستعمرات المحببات أكثر فائدة من الخلايا الجذعية الميزنشيمية المشتقة من نخاع العظم في تحسين التغيرات النسيجية في قشرة الكلى في إصابات الكلى الحادة المستحدثة تجريبياً بواسطة سيسبلاتين.