

Transplanted Adipose Derived Mesenchymal Stem Cells Attenuate The Acute Renal Injury Induced by Cisplatin in Rats

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Original
Article

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ABSTRACT

Background: Traditional therapeutic strategies used for the treatment of acute kidney injury (AKI) proved to be less effective in reducing the morbidity and mortality rate. Recently, stem cell therapy showed a promise for treatment of this complex disorder.

Aim: To investigate the therapeutic role of adipose-derived mesenchymal stem cells (AD-MSCs) in treatment of cisplatin-induced nephrotoxicity as a model of AKI.

Material and Methods: Twenty adult female Wister rats were divided equally into four groups. Group I was the control, the other three groups received a single intraperitoneal injection of cisplatin (5 mg /kg), where group II were sacrificed after one day from cisplatin injection, group III were sacrificed after seven days from cisplatin injection and group IV received adult male rat AD-MSCs (2x10⁶ cells/rat) in tail vein one day after cisplatin injection and were sacrificed seven days after cisplatin injection.

Results: The histopathological changes in the renal cortex were more obviously detected in group III than in group II. These changes include congested and shrunken glomeruli, dilated Bowman's space and loss of proximal convoluted tubules brush borders. Moreover, distal tubular cells showed cytoplasmic vacuolization, with pyknotic nuclei and presence of intraluminal hyaline casts. Interstitial collagen deposition was also noticed. In group IV, AD-MSCs administration almost restores the renal histological architecture. Increased tubular cell proliferation with marked reduction of the interstitial inflammation and fibrosis were also detected. However, some renal glomeruli and tubules showed degenerative changes. Male rat derived-stem cells were detected in the female kidney tissue by Y chromosome PCR technique.

Conclusion: Administration of AD-MSCs had a potential regenerative effect for the management of AKI.

Keywords: Acute kidney injury, adipose-derived mesenchymal stem cells, cisplatin, Y chromosome

Revised: 19 February 2017 , **Accepted:** 06 July 2017

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ISSN: 1110-0559, 2017, Vol 40. , No. 2

INTRODUCTION

Acute kidney injury (AKI) is one of the most challenging clinical conditions. It is characterized by rapidly deteriorating renal function caused by many different insults and associated with a high morbidity and mortality rate^[38]. Recent dialysis techniques, continuous renal enhancement therapy, and others have no significant impact on overall mortality. Furthermore, the efforts to perform kidney transplantation are strictly limited owing to difficulty to find organ donors and risks of graft versus host reaction^[25].

Cisplatin, a potent anti-neoplastic drug, used in the treatment of many human neoplasms such as testicular, ovarian and bladder tumors^[16]. Despite its anti-tumor actions, it has major side effects particularly nephrotoxicity with subsequent AKI, thus limiting its clinical use^[3].

The exact mechanism of cisplatin-induced nephrotoxicity is not yet fully understood. It might be due to direct toxicity and apoptosis of renal tubular epithelial cells especially proximal convoluted tubules^[9], oxidative damage and increased lipid peroxidation^[22] or activation of p53 tumor suppressor proteins^[41].

Over the last ten years, stem cell-based therapeutic approaches have been introduced in the treatment of complex disorders such as AKI. The goal of the regenerative medicine is to repair or regenerate the kidney tissues damaged by either drugs or diseases^[5]. Previous studies suggested that stem cell therapy may repair AKI induced by cisplatin, glycerol and ischemia-reperfusion injury^[6, 36].

Currently, a rich source of multipotent adult stem cells has been recognized in fat tissue known as Adipose-Derived Mesenchymal Stem Cells (AD-MSCs)^[15].

AD-MSCs have several advantages compared to any other stem cell isolated from other sources. They can be easily harvested from adipose tissue through simple and minimally invasive liposuction procedure^[35]. Furthermore, it was recorded that the proliferation rate of AD-MSCs seems to be greater than that of bone marrow-derived MSCs (BM-MSCs) as much as 40-fold^[20]. Indeed, AD-MSCs are like stem cells isolated from bone marrow in morphology, the ability to undergo multilineage differentiation and cell surface phenotype both *in vitro* and *in vivo*^[47].

AIM OF THE WORK

We designed this research to assess the possible therapeutic effect of AD-MSCs in reversing cisplatin induced nephrotoxicity using histological studies and immunohistochemical techniques. Homing of male rat derived-stem cell (Y-chromosome) in the female kidney tissue was detected by PCR technique. Morphometric studies and statistical analysis were performed for all obtained data.

MATERIALS AND METHODS

Isolation and culture of AD-MSCs:

Culture methods were based on the method of Park *et al.*^[29]. Inguinal pads of fat harvested from five young male rats (weighing 70-80 gm) were washed with sterile phosphate-buffered saline (PBS; Lonza Company, Swiss) three times to remove the contaminating debris and red blood cells. The washed tissues were cut into small pieces 1mm³ and were digested by 0.1% collagenase type-I (Sigma-Aldrich, St. Louis, MO, USA) in PBS for 45-60 minutes at 37°C with intermittent shaking every 10min. The enzyme activity was neutralized with 5 ml complete medium (Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% Penicillin /streptomycin mixture). The previous reagents were purchased from Lonza Company, Swiss. The suspension was filtered by 100 µm nylon mesh to remove undigested tissues. The filtered suspension was centrifuged at 1800 rpm for 10 min at 25°C. The supernatant containing mature adipocytes was discarded. The pellets containing cells of stromal vascular fraction (SVF) were resuspended in complete medium. Then plated in cell culture flasks 75ml at a density of 8000-10000 cells/cm² and incubated at 37°C with 5% CO₂. After three days of incubation, the media were removed and replaced by fresh ones. The medium was changed every 3 days, and the cells were passaged when the cells reached >70% confluence and denoted as first passaged cells (P1). Cells from the P3-P4 were used in this study.

Immunohistochemical characterization of cultured AD-MSCs:

The cultured AD-MSCs were characterized by using streptavidin-biotin immunoperoxidase technique for the primary antibodies: CD44, CD105, and CD34

monoclonal mouse anti-human antibodies (Thermo Fisher Scientific, Lab vision, USA). The third passaged cells were fixed by pre-cooled mixture of acetone and methanol (1: 1, v: v) for 15 min. After removing the fixative, the cells were washed twice with PBS. The Petri-dishes were covered by H₂O₂ (10%) for 15 minutes to block the endogenous peroxidase. Then the primary antibody was added (ready- to- use formulation) for one hour at 37°C. The cells were washed three times with PBS to be incubated with the secondary antibody (biotinylated goat anti-mouse) diluted in PBS 1:400 for one hour at 37°C. The cells were washed three times with PBS. The site of immunostaining was detected by adding 80-100 ml of Streptavidin horseradish peroxidase conjugate in a dilution of 1:300 for 15 minutes, then washing twice with PBS. Color was developed using 1-2 ml of freshly prepared diaminobenzidine (DAB) for 10 minutes. Then, the cells were washed well with distilled water. Positive nuclear reactions appeared brown in color. Negative control was performed using the 2nd antibody only to check for any cross reaction^[23].

Animals:

Twenty adult female Wister rats were used in the current study, each weighing between 150 – 180 gm. The animals were purchased and raised in the Medical Research Center, Faculty of Medicine, Ain Shams University, Egypt. The animals were housed in plastic cages with mesh wire covers and were given food and water ad libitum. During the experiments, the rats were housed under standard conditions with controlled light- dark cycle and temperature 22-25°C. Use of laboratory animals was performed in accordance to the guidelines for care, and approved by ethical committee of Ain Shams University, Egypt. The rats were divided into four equal groups:

Group I (control group): was further subdivided into: subgroup IA: in which each rat received a single intraperitoneal injection of equivalent volume of 0.9 % saline. Subgroup IB: in which each rat received a single dose of 1ml phosphate buffered saline (PBS) in tail vein.

Group II (cisplatin- treated group): each rat received a single intraperitoneal injection of cisplatin (5 mg/kg body weight)^[21]. Cisplatin was purchased in the form of UniStin vials, 10 mg/10 ml, manufactured by EIMC United Pharmaceuticals, Badr city, Cairo, Egypt. The animals of this group were sacrificed one day after cisplatin injection.

Group III (spontaneous recovery group): rats of this group received cisplatin at the same dose and route as group II. They were sacrificed after seven days from cisplatin injection (8th day of the experiment).

Group IV (AD-MSCs-treated group): in which each rat was administrated AD-MSCs (2x10⁶ cells / rat) in tail vein one day after cisplatin injection. All rats were

sacrificed seven days after cisplatin injection (8th day of the experiment).

Renal histology :

All animals were sacrificed after anesthesia with ether inhalation. Both kidneys were dissected out. The specimens of the right kidneys were fixed in 10% formalin, and processed to obtain paraffin blocks. Five μ m-thick sections were cut and stained with Haematoxylin and Eosin stain (H&E), Periodic acid Schiff's reaction (PAS) and Modified Masson's trichrome stain.

Tubular injury scoring:

Tubular damage in H&E stained sections was scored by grading the percentage of affected tubules under ten non-overlapping, randomly chosen fields per slide (HPF, $\times 400$) as follows: grade 0, 0%; grade 1, < 10%; grade 2, 10-25%; grade 3, 26-45%; grade 4, 46-75%; and grade 5, 76-100%. The grading percentage of the tubular injury was calculated in each field using the following equation: injury score (%) = (numbers of injured tubules/ number of whole tubules) $\times 100$ [21].

Proliferation assay by immunohistochemistry:

The number of proliferating tubular cells was determined by the expression of proliferating cell nuclear antigen (PCNA) immunohistochemically. Paraffin-embedded kidney sections were deparaffinized with xylene and rehydrated in descending grades of alcohol down to distilled water. In order to block endogenous peroxidase, the slides were immersed in 10 % hydrogen peroxide (H_2O_2) for 10 minutes. Unmasking of antigenic sites was carried out by transmitting sections into 0.01 M citrate buffer (pH= 6) for 10 minutes. Then, slides were incubated with PCNA antibody (Thermo Fisher Scientific, USA) at 37°C for 1hour. Thereafter, washed with phosphate buffer three times and incubated in biotinylated rabbit anti mouse IgG solution (1/400) for 20 minutes followed by streptavidin-biotin solution for 30 minutes. The antibody binding sites in the kidney sections were visualized by diaminobenzidine (DAB) and were counterstained with Mayer's hematoxylin, dehydrated, cleared and mounted [28].

Morphometric analysis:

1- Area percentage of collagen fibers (the green area) between renal tubules was measured using high power field (HPF $\times 400$) in Masson's trichrome stained sections of all groups.

2- The mean thickness of basement membranes of renal tubular cells is measured in PAS stained sections of all groups (HPF $\times 400$).

3- To perform scoring for PCNA-positive cells, the positive tubular nuclei in renal cortex were counted in at least 15 HPF ($\times 400$) for each slide.

The measurements were performed using the image analyzer Leica Q500 MC program at the Department of Histology, Faculty of Medicine, Ain Shams University, Egypt. The PC was connected to an Olympus XB microscope- Japan.

Detection of homing of male-derived MSCs (Y chromosome) in the female kidney tissue by PCR

The presence or absence of the male-specific sex determining region of Y chromosome (SRY) gene in the left kidneys of recipient female rats was assessed by PCR. Genomic DNA was extracted from left kidney tissue homogenate using Wizard Genomic DNA purification kit (Promega, Madison, WI, USA). The oligonucleotide primers for (SRY) gene were purchased from (Sigma-USA):

The forward primer had the following sequence:
5'-CAT CGA AGG GTT AAA GTG CCA-3'

The reverse primer had the following sequence:
5'-ATA GTG TGT AGG TTG TTG TCC-3'

The DNA was amplified using the primers as follows: For each sample, the master mix contained: 10 x PCR (5 μ l), the 2 primers (50 Pmol for each) (1 μ l), 10 mM dNTPs (1 μ l), Taq polymerase (1 μ l) and water (37 μ l). The thermal profile was adjusted as followed: denaturation (about 5 min at 94°C) was done followed by annealing 35 cycles of incubation at 94°C for 30 sec, 60°C for 45 sec, and 72°C for 2 min. Extension at a final incubation at 72°C for 7 min was performed. PCR products were separated using 2% Agarose gel electrophoresis and stained with Ethidium bromide for visualization by U.V. trans-illuminator. Positive (male Wistar rat genomic DNA) and negative (female Wistar rat genomic DNA) controls were included in each assay. In U.V. Trans-illuminated agarose gel of PCR products of SRY gene, Y chromosome marker detected as Trans-illuminated line [41].

Statistical analysis

The statistical analysis of morphometric results was carried out using SPSS program version 17 IBM Corporation, Route 100 Somers, NY 10589. Data were evaluated using the One-way analysis of variance test (ANOVA). Values were presented as mean \pm standard error of mean (SEM). Regarding the probability, the significance of data was determined by the p value where: $P > 0.05$ was non-significant and $P < 0.05$ was significant.

RESULTS

Morphology and immunophenotype of AD-MSCs

The AD-MSCs were isolated from male rats and expanded in culture for five to six passages. The cultured cells were mostly confluent on day 7 after primary culture forming monolayer of adherent cells. They attained fibroblastic appearance connected with each other by cytoplasmic processes (Fig. 1A). The third passaged

cells were characterized immunohistochemically to verify their purity and the identity of the cells as AD-MSCs. Almost all the cultured cells expressed the MSCs-specific markers CD105 and CD44 (Figs. 1 B and C respectively), but they did not express the hematopoietic cell marker CD34 (Fig. 1D).

Transplanted AD-MSCs improved renal histological structure and morphometric parameters after cisplatin-induced renal damage

In comparison with the control group (Fig. 2A), Hx. and E stained sections of the kidney of group II that received cisplatin for one day showed congestion of both glomerular and peritubular capillaries. Some tubular cells showed degenerative changes in the form of cytoplasmic vacuolization and others were collapsed forming spherical acidophilic masses. The renal interstitium showed mononuclear cellular infiltrate (Fig. 2B). These histopathological changes were apparently aggravated in animals of group III that were left without treatment for spontaneous recovery by intrinsic regenerative capacity of the kidney. Atrophy of the renal glomeruli with widening of Bowman's space was detected. Some renal tubules showed shedding of their epithelial cells in the lumen. Intraluminal homogenous acidophilic hyaline casts were detected. Moreover, pyknotic and karyolytic nuclei were seen in some tubular epithelial cells (Fig. 2C). Injection of AD-MSCs almost induced regaining of the renal histological architecture nearly as that seen in control group. However, some glomeruli and tubules still showed some degenerative changes (Fig. 2D).

To quantify the injury of the kidney tubules, histopathological score for renal tubular injury was done which obviously revealed significant increase in the number of injured tubules in group III 75.6% vs. 33.6% in group II. AD-MSCs treated group IV markedly attenuated tubular injury induced by cisplatin and decreased the score to 19.3% (Table 1 and Histogram 1).

PAS stained sections, in comparison with the control group (Fig. 3A), revealed thickened basement membranes of parietal layer of Bowman's capsule and basement membranes of some tubules. Focal loss of the apical brush border of some PCTs was reported (Fig. 3B). In the spontaneous recovery group, marked increase in PAS positive reaction of parietal layer of Bowman's capsule and basement membranes of glomerular capillaries were observed. The basement membrane of some renal tubules showed an increase in PAS positive reaction while, it appeared disrupted in others. Loss of the PAS positive apical brush border of most PCTs and hyaline materials in the tubular lumen were also seen (Fig. 3C). The administered AD-MSCs apparently retained the thickness of the basement

membranes of glomerular capillaries, parietal layer of Bowman's capsule and basement membranes of the renal tubules as compared to the control group. The brush borders of the PCTs were also restored (Fig. 3D).

These changes in thickness of the tubular basement membranes were quantified. There was a significant increase ($P < 0.05$) in the mean thickness of basement membrane in animals of both groups II and III (cisplatin injected groups) when compared with group I (the control group). However, the mean thickness of basement membrane in animals of group IV (that received AD-MSCs) showed non-significant difference ($P > 0.05$) as compared with group I (Table 1 and Histogram 2).

Masson's trichrome stained sections revealed mild increase in collagen fibers around the renal corpuscles and the renal tubules in group II as well as increased collagen fibers between glomerular capillaries (Fig. 4B) as compared to the control group (Fig. 4A). In the spontaneous recovery group, there was an obvious increase in the interstitial fibrous tissue (Fig. 4C). AD-MSCs prevented the deposition of the fibrous tissue around renal tubules and the glomeruli as comparable with the control group (Fig. 4D).

These findings were confirmed statistically. There was a significant increase ($P < 0.05$) in the mean area percentage of collagen in animals of groups II and III (cisplatin injected groups) when compared with group I (the control group). But the mean area percentage of collagen in animals of group III showed significant increase ($p < 0.05$) as compared with group II. In the AD-MSCs group IV, there was non-significant difference in mean area percentage of collagen ($P > 0.05$) as compared with group I (Table 1 and Histogram 3).

AD-MSCs promoted tubular cells proliferation as indicated by immunohistochemical staining for PCNA

The number of PCNA-positive cells in cisplatin groups (Figs. 5B and C) especially group III apparently increased as compared to the control group (Fig. 5A). However, there was marked increase in the number of the positive immune-stained nuclei for PCNA in the AD-MSCs injected group as compared with the cisplatin-treated groups (Fig. 5D).

Quantification of the PCNA positive cells was done. There was a significant increase ($P < 0.05$) in the mean number of PCNA positive cells in group III as compared with group II and the control group. While in the AD-MSCs treated group, the mean number of PCNA positive cells showed a highly significant increase ($P < 0.001$) as compared with group II and group III (Table 1 and Histogram 4).

Administrated AD-MSCs were detected in the kidney specimens by Y chromosome PCR.

Homing of the injected male derived AD-MSCs were detected in the kidney specimens of the female rates

with cisplatin induced nephrotoxicity by Y-chromosome-specific primers. PCR products of SRY gene was expressed only in group IV that received male derived AD-MSCs (Fig. 6).

Table 1: Mean± SD of the tubular injury scoring (%), mean± SD of the thickness of tubular basement membranes (μm), mean± SD of the area percentage of collagen fibers and mean± SD of the number of PCNA positive cells of different groups at the end of experiment

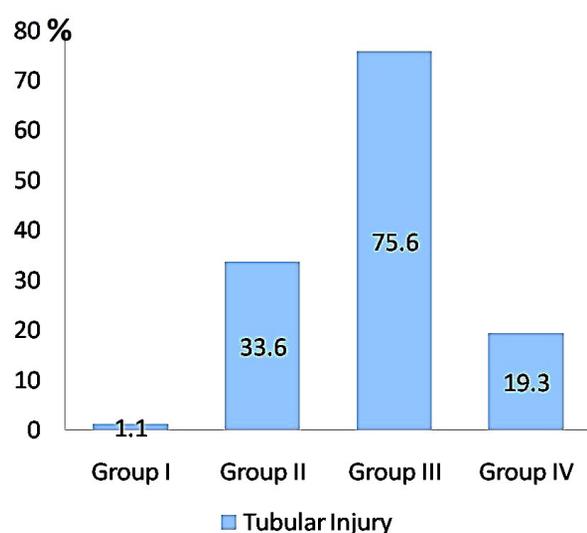
Groups	Tubular injury scoring (%)	Mean thickness of tubular basement membranes (μm)	Mean area percentage of collagen	Mean number of PCNA positive cells
Group I	1.1±0.88	0.52±0.05	3.1 ±0.51	2.1±0.31
Group II	33.6±11.27a	1.21±0.10a	15.4 ±0.8a	6.8±1.30a
Group III	75.6±13.51ab	1.52±0.14a	24.9 ±1.7ab	8.1±1.20a
Group IV	19.3±5.71abc	0.77±0.04bc	7.2 ±0.70bc	17.9±0.92abc

- Values are expressed as means ± SD.

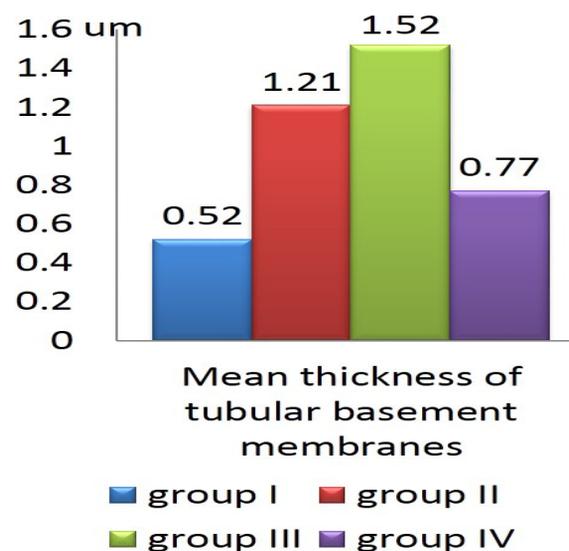
- a = Significant difference from the control group I at $P < 0.05$.

- b = Significant difference from group II at $P < 0.05$.

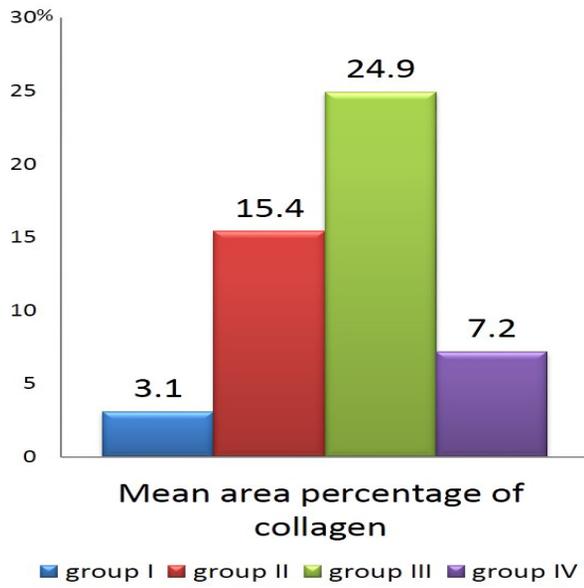
- c = Significant difference from group III at $P < 0.05$.



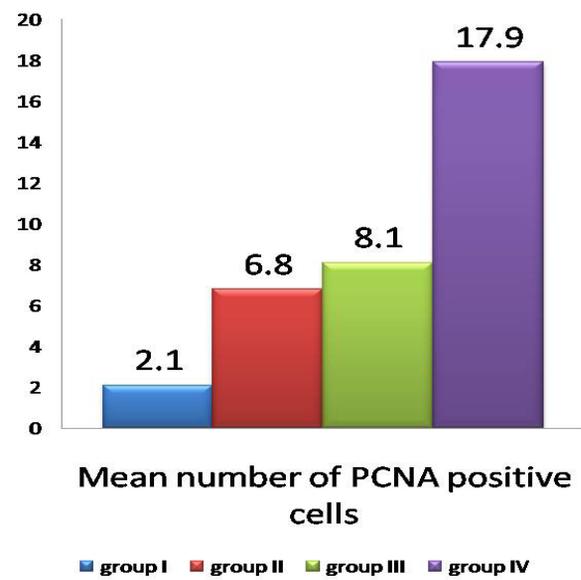
Histogram 1: Showing the tubular injury scoring different groups.



Histogram 2: Showing mean thickness of tubular basement membranes of different groups.



Histogram 3: Showing mean area percentage of collagen of different groups.



Histogram 4: Showing mean number of PCNA positive cells of different groups.

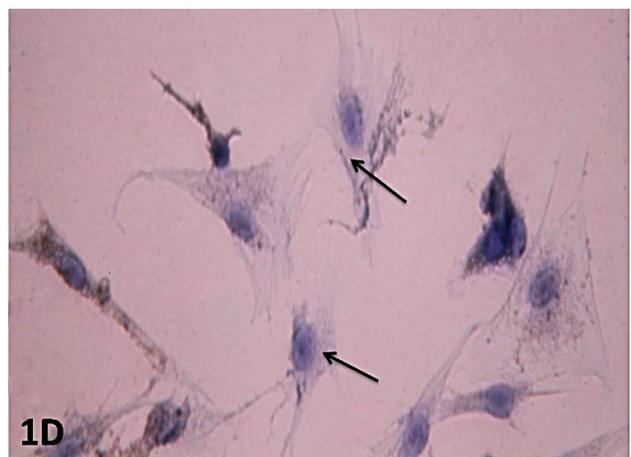
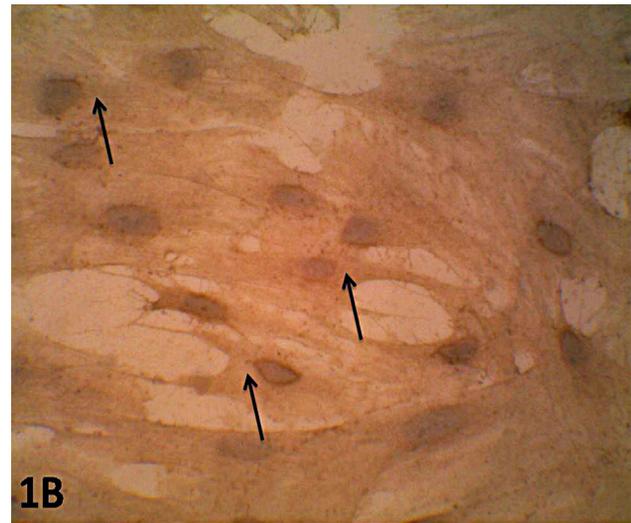
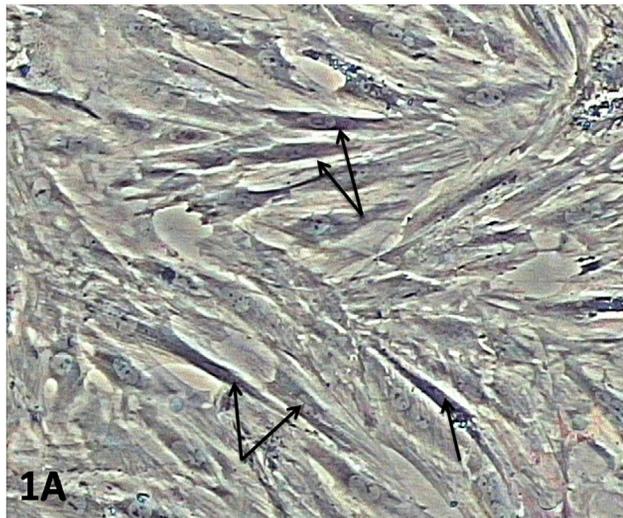


Fig. 1: Morphology and characterization of rat adipose-derived mesenchymal stem cells (AD-MSCs). (A) Phase contrast micrograph of AD-MSCs 7 days after primary culture. Most cells are spindle in shape with granular cytoplasm and vesicular nuclei (↑) (× 200). (B) & (C) Phase contrast images with positive brownish immune reaction for CD105 and CD44 in the cytoplasm of cultured cells (↑), respectively. (D) Phase contrast image shows the cultured cells express negative immune staining for the hematopoietic cell marker CD34 (↑). 1B, 1C and 1D, streptavidin-biotin peroxidase × 200.

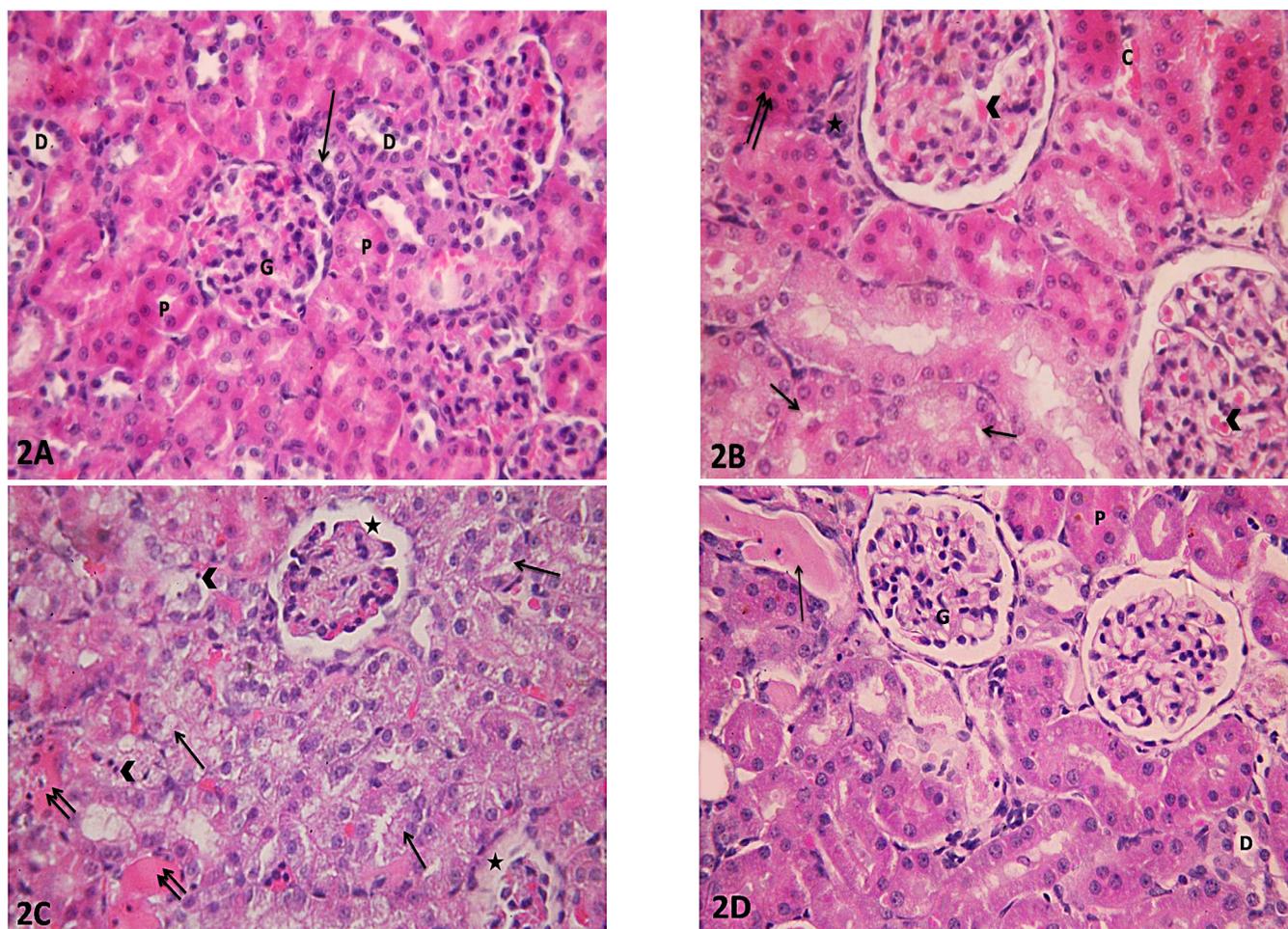
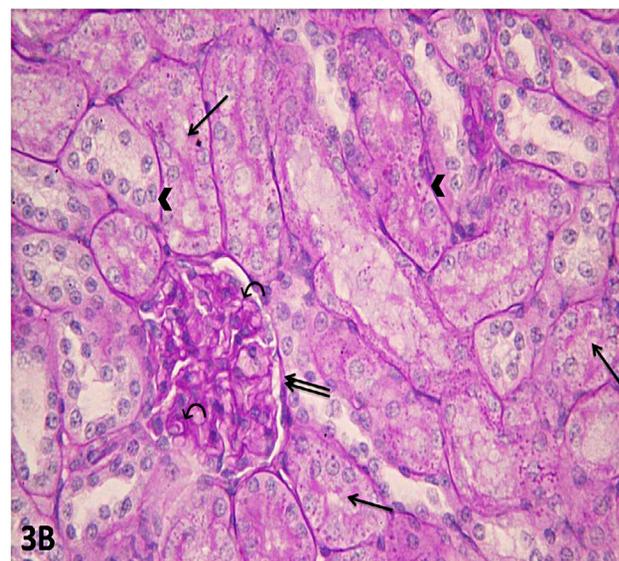
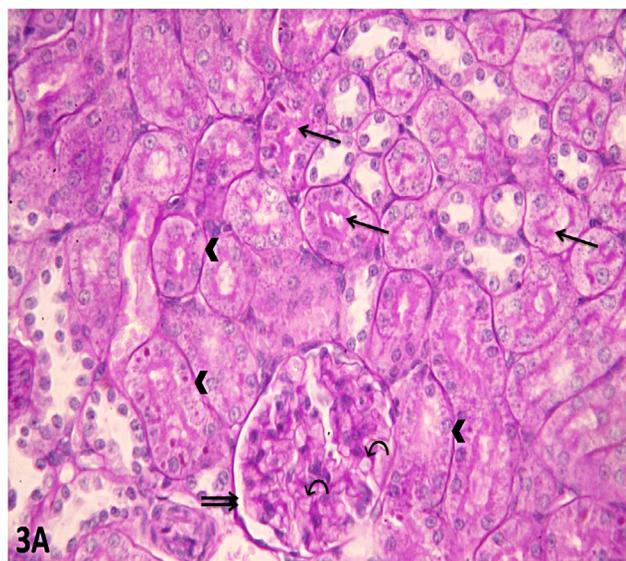


Fig. 2: (A) Renal corpuscles of group I are formed of glomeruli surrounded by Bowman's capsule (G). The PCTs have narrow lumen and are lined with cubical cells with dark acidophilic cytoplasm (P). The DCTs have wider lumen and are lined with cubical cells with pale acidophilic cytoplasm (D). Macula densa is clearly seen in this section (↑). (B) In group II, the glomerular (arrowheads) and peritubular capillaries (c) are congested. The cells of DCTs show vacuolated cytoplasm (↑). PCTs are forming spherical acidophilic masses (↑↑). Mononuclear cells infiltration is observed (*). (C) The glomeruli of group III are congested and shrunken with widening of Bowman's space (*). Intraluminal shedding of tubular epithelial cells inside the lumen (↑) with hyaline casts (↑↑) are noticed. Pyknotic nuclei are also observed in tubular cells (arrowheads). (D) With AD-MSCs treatment (group IV), renal glomeruli preserved its histological structure as compared to the control group (G). PCTs (P) and DCTs (D) are also comparable to the control group. However, some tubules still show intraluminal hyaline casts (↑).
Hx&E ×400.



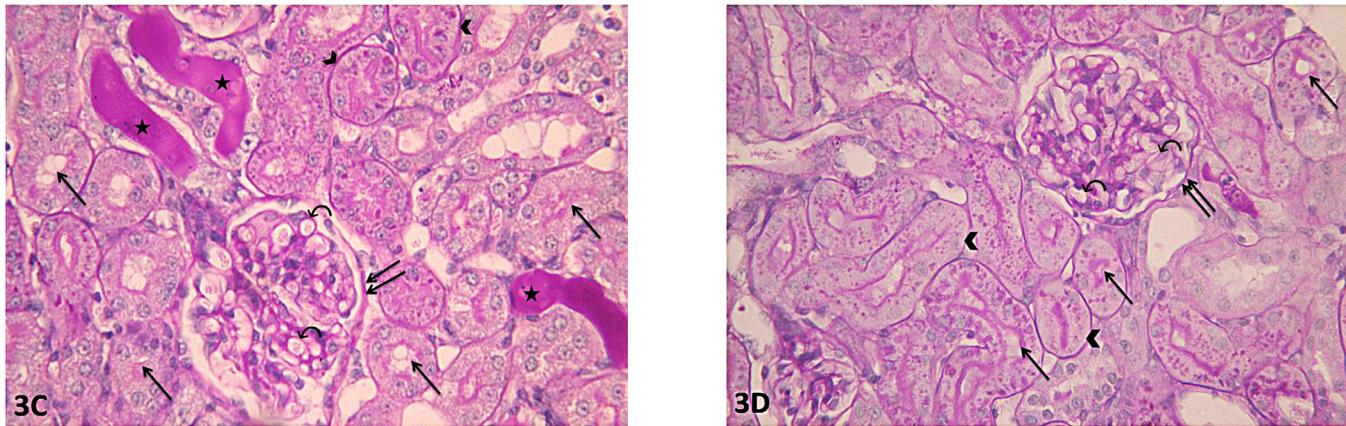


Fig. 3: (A) The PAS positive brush borders of the cells lining the PCTs are clearly seen in group I (↑). The basement membranes of the glomerular capillaries (curved arrows), parietal layer of Bowman’s capsule (↑↑) and renal tubules (arrowheads) are PAS positive. (B) Thickening of the basement membranes of glomerular capillaries (curved arrows) and parietal layer of Bowman’s capsule can be seen in group II (↑↑). The basement membranes of the PCTs & DCTs are also thickened (arrowheads) and the brush borders of the PCTs are lost (↑). (C) The basement membranes of glomerular capillaries (curved arrows), parietal layer of Bowman’s membrane and tubular membranes (arrowheads) are markedly thickened in group III (↑↑). The brush borders of the PCTs are lost (↑) with PAS positive hyaline casts in the tubular lumens (*). (D) The AD-MSCs treated group IV display restoration of histological architecture of the basement membranes of the parietal layer surrounding renal corpuscles (↑↑), glomerular capillaries (curved arrows) and many tubules (arrowheads) in comparison with the control group. PCTs reveal PAS positive apical brush borders (↑). PAS, ×400.

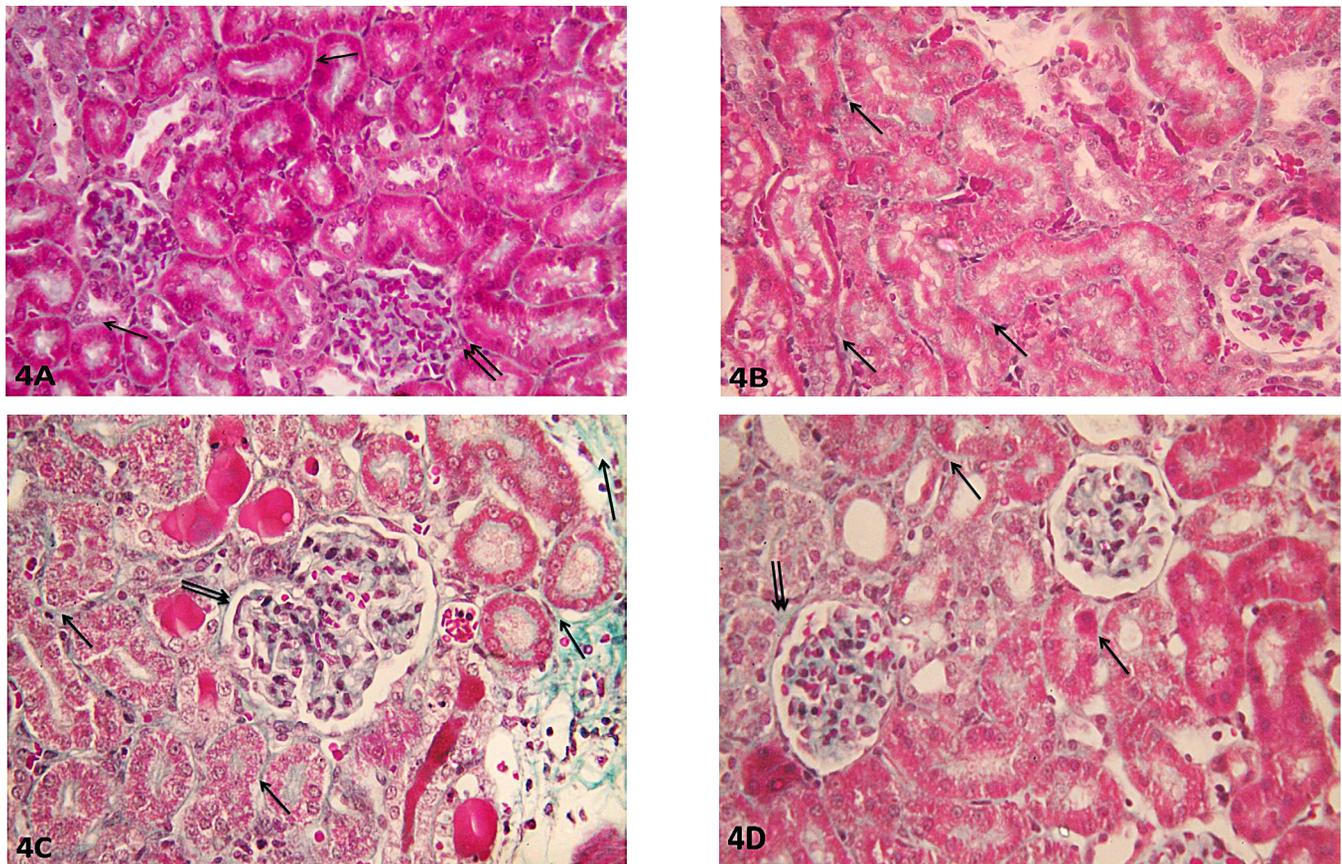


Fig. 4: (A) Fine and scattered collagen fibers around the renal corpuscle (↑↑) and the renal tubules (↑) can be hardly seen in the control group I. (B) In comparison with the control group there is few collagen fibers around the renal tubules of group II (↑). (C) In the spontaneous recovery group III, there is marked increase in collagen content around glomeruli (↑↑) and the tubules (↑). (D) The stem cells treated group IV display few collagen fibers around glomeruli (↑↑) and the tubules (↑) as present in the control group. Masson's trichrome, ×400.

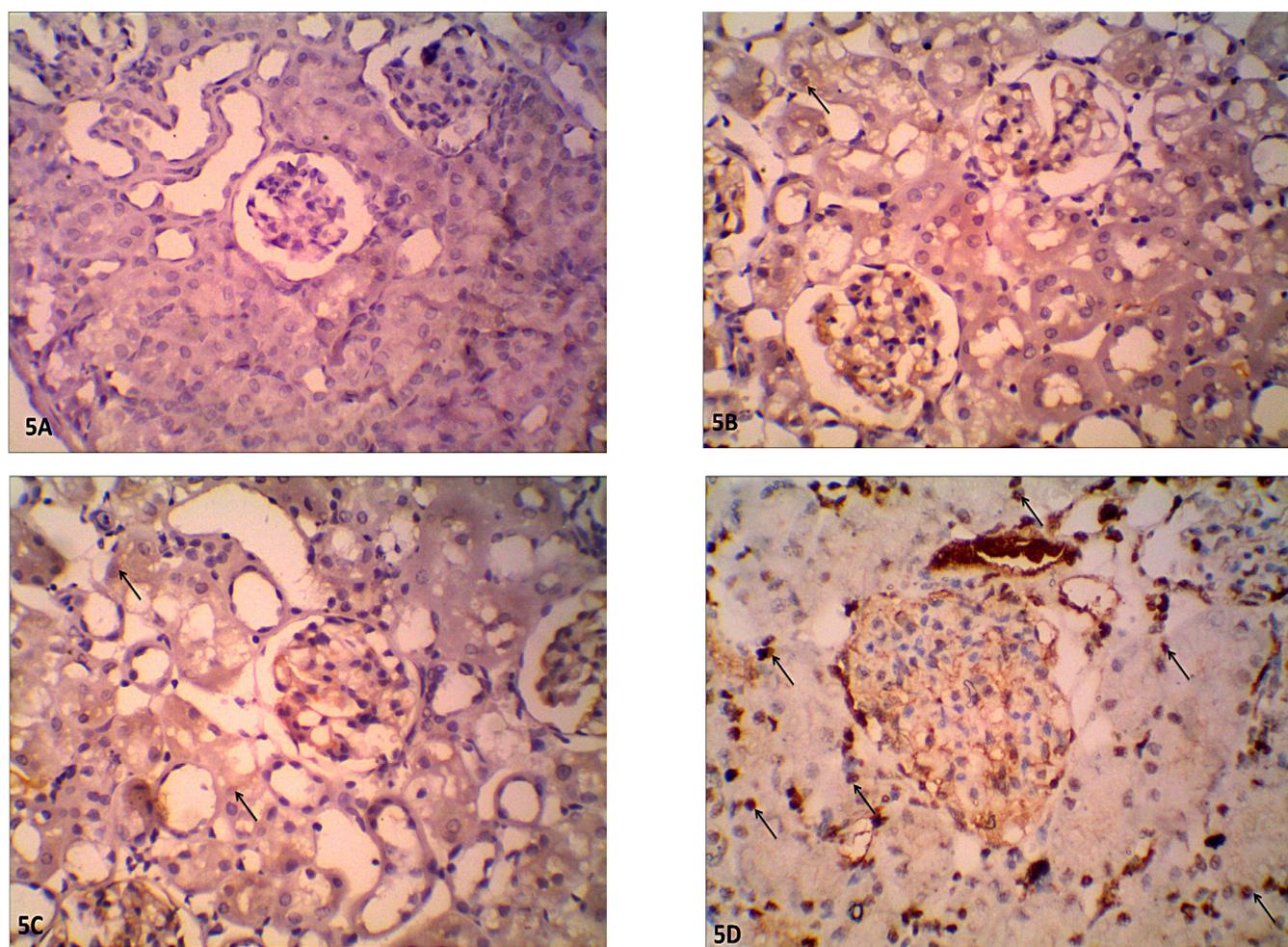


Fig. 5: (D) AD-MSCs significantly promote proliferation of the tubular cells in cisplatin-induced kidney injury as shown by the apparent increased number of the positive PCNA nuclei of the tubular cells (↑) in comparison with the control group (A) and cisplatin injected groups (B&C) PCNA, ×400.

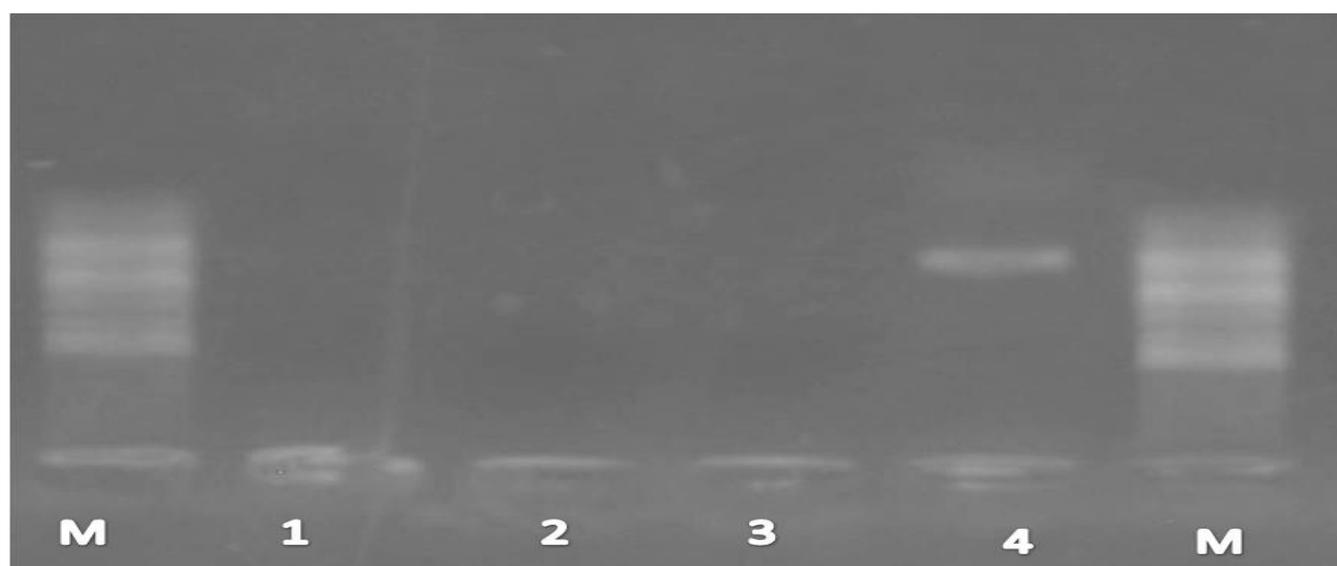


Fig. 6: PCR products of SRY gene expression of Y chromosome in female rat kidney specimens of all studied groups. Lane M: A 100 bp DNA-marker . Lanes 1, 2 and 3: No PCR products of SRY gene expression in the control group, groups II and III (cisplatin injected groups), respectively. Lane 4: PCR products of SRY gene is expressed in group IV that received male derived AD-MSCs.

DISCUSSION

The present study was designed to evaluate the nephrotoxic effect of a single injection of cisplatin (5mg/kg IP) for one day as a model of AKI (group II). The possible spontaneous regeneration capacity of the kidney if left without treatment for seven days (group III) and the role of exogenous AD-MSCs injection in repair of the cisplatin-induced nephrotoxicity (group IV) were also investigated.

The alterations in the histological architecture of the kidney were more aggravated in group III than in group II. As evident by hematoxylin and eosin stained sections, the glomerular capillaries were congested one day after cisplatin injection, whereas in group III the glomeruli were atrophied and shrunken with subsequent widening of Bowman's space. This was coincident with other researchers who observed that cisplatin injection for one day caused marked congestion of the glomeruli^[34]. On the other hand, some investigators reported that no obvious morphological changes in the glomeruli^[39].

Moreover, our results revealed that cisplatin-induced histopathological changes appeared significantly in the renal tubules. PCTs were collapsed forming acidophilic masses, while DCTs showed cytoplasmic vacuolations in group II. In group III, pyknosis of the nuclei were obviously noticed in cells of the PCTs indicating tubular cell apoptosis resulting in sloughing of the entire tubular epithelial. These changes could be explained by leakage of the fluid from damaged tubules into interstitium resulting in collapse of some tubules^[21, 31].

Notably, the pathological changes were more obvious in PCTs because the concentration of cisplatin in PCTs was about five times the plasma concentration followed by DCTs. Cisplatin enters the cells of the PCTs through passive as well as facilitated diffusion by means of transporter-mediated process. So, this contributed to cisplatin induced nephrotoxicity^[44].

The intraluminal acidophilic hyaline casts noticed in this study could be due to combination of the sloughed tubular cells with Tamm-Horsfall protein present in the lumen of the tubules. In addition, the increase in sodium concentration in the lumen, due to impairment of its reabsorption by the damaged tubular cells, resulted in polymerization of Tamm-Horsfall protein forming a gel-like material thus contributing in cast formation^[1].

Furthermore, the presence of apoptotic nuclei was more evident in group III. This was explained by cisplatin-induced apoptosis was involved with p53. Cisplatin activated P53 in renal tubular cells promoting apoptosis by upregulating the proapoptotic genes resulting in increase the permeability of the outer mitochondrial membrane with subsequent release of apoptogenic factors^[33, 41].

The inflammatory cell infiltration detected may be attributed to the ability of cisplatin to induce inflammatory reactions as well as its ability to increase the expression of

inflammatory cytokines and chemokines with subsequent increase in neutrophils, macrophages and lymphocytes number in renal interstitium^[45].

In the current study, in comparison to group II, PAS stain of group III revealed apparent thickening of basement membranes of the glomerular capillaries as well as some tubules. This might be due to up-regulation of integrins, laminin and fibronectin with subsequent increase in basement membrane thickness^[14]. However, some authors found no changes in basement membrane in cisplatin induced renal injury^[45]. Furthermore, there was loss of the brush border of the PCTs and PAS positive materials in the tubular lumina were observed in group III more significant than in group II. Similarly, some investigators detected loss of the brush border, cell polarity, and adhesion between cells and the basement membrane and they explained these observations by the fact that necrosis and apoptosis can all lead to the detachment of tubular cells from the basement membrane, leaving behind areas of denuded basement membrane^[12].

There was significant increase in collagen fibers in renal interstitium, around renal tubules and in between glomerular capillaries especially in group III as demonstrated by Masson's trichrome stained sections. The increase in collagen fibers content in between the glomerular capillaries was most probably due to increased activity of mesangial cells^[8]. Moreover, necrosis and apoptosis of tubular cells led to aggregation of macrophage which played an essential role in renal interstitial fibrosis via production of tumor necrosis factor α (TNF- α) and transforming growth factor β (TGF- β) (10). TGF- β was expressed in the cytoplasm of affected tubular cells and could induce transformation of tubular epithelial cells to myofibroblast through epithelial-mesenchymal transition (EMT). Myofibroblast was the major source of extracellular matrix secretion. Renal tubular cells lost their epithelial phenotype and acquired new characteristic features of mesenchyme^[11].

The present study demonstrated significant increase in number of PCNA-positive nuclei in group III as compared to group II. This could be attributed to activation of intrinsic capacity of the kidney to regenerate the injured tubular cells whereas the stem cell niches in the kidney are present. The renal stem cells are located around renal tubules, especially PCTs and under the urothelium of the renal papilla. These cells could differentiate into epithelial, neuronal and other cells. Induction of ischemic renal failure resulted in increased proliferation of these renal papillary stem cells. Also, injection of papillary stem cells under the renal capsule led to their incorporation into various tubule segments^[4]. Moreover, other niches had been detected in the Bowman's capsule, the cortical and medullary interstitium, the renal papilla and the hilar regions^[13].

It was observed from the present study that cisplatin caused direct tubular injury through multiple mechanisms. Significant interactions among the various pathways might

occur during the injury. Furthermore, the untreated group III showed aggravation rather than spontaneous improvement in the histological architecture of the kidneys, except for some signs of regeneration which still beyond the amount of insult.

Therefore, administration of AD-MSCs 24 hours after cisplatin injection group IV revealed marked improvement in the histological structure of the kidney. Examination of group IV sections revealed restoration of the architecture of renal corpuscles, PCTs and DCTs as compared to the control group. The regenerative findings detected in the present work coincide with the results of a previous study whereas the kidneys of BM-MSC treated mice after cisplatin administration exhibited low degrees of proximal tubular cell damage, cast formation, and focal cell loss^[27, 32].

The results of this study revealed that AD-MSCs also showed significant decrease in the interstitial inflammation attributed to the anti-inflammatory effect of MSCs on T cells. When MSCs co-cultured with either T helper 1 (Th1), T helper 2 (Th2), or natural killer (NK) cells, they decreased their secretion of pro-inflammatory cytokines such as TNF- α , interferon- γ (IFN- γ) and increased their secretion of the suppressive cytokine IL-10 (2). Furthermore, MSCs could suppress B lymphocyte proliferation as well as antibody production and possibly stimulate regulatory T cells^[26].

By PAS stain, AD-MSCs had markedly preserved the normal thickness of basement membranes surrounding renal corpuscles, glomerular capillaries and many tubules. PCTs restored their PAS positive apical brush borders. Similar findings were obtained when BM-MSCs infused in cisplatin-induced nephrotoxicity^[43].

In the present work, Masson's trichrome stained sections showed that AD-MSCs markedly decreased collagen fibers deposition around the renal corpuscles and the renal tubules. As reported before that MSCs reduced the production of TNF- α which mediated the deposition of extracellular matrix in the renal interstitium by the myofibroblasts^[10, 19].

The administration of AD-MSCs strongly induced tubular cells proliferation as indicated by the marked increase in the number of PCNA-positive cells in group IV in comparison to other groups. Similarly, BM-MSCs promoted PCNA expression by the tubular cells which was an index of renal regeneration as well as inhibiting cell apoptosis induced by cisplatin^[31]. Furthermore, injection of human cord blood-MSCs activated the pro-survival factor Akt in the PCTs inducing their proliferation as evidenced by the high number of cells positive for PCNA staining^[28].

In the present study, the presence of male derived AD-MSCs in renal tissue of female rats was evaluated by using the chromosome Y localization strategy. It was detected in group IV, indicating that AD-MSCs did migrate to the local site of injury in the kidney tissue. Different factors have been described in controlling MSC homing to injured

kidney, including platelet-derived growth factor (PDGF), chemokine receptor 4-stromal derived factor (CXCR4-SDF)^[36]. CD44 and hyaluronic acid were also shown to recruit exogenous MSCs to injured renal tissue and to enhance renal regeneration^[17]. Other methods were used to detect MSCs homing such as PKH-26 however it was more specific to stem cells of haematopoietic origin^[46].

Thus, it was well known that injected MSCs migrated by different homing mechanisms to injured kidney tissue and they improved the kidney structure. However, their exact mechanisms in repair of the injured kidney might be through trans-differentiation into tubular cells versus through a paracrine process.

In 2008, Lin observed that the improvement in renal structure after MSCs transplantation was not only due to significant increase in tubular cell proliferation but also MSCs showed differentiation into tubular epithelial cells^[24]. Besides, Wang et al. stated that replacement of damaged cells by MSCs through trans-differentiation might be only a small part of the mechanism by which, MSCs could exert their therapeutic effect^[40].

However, other investigators explained that the tubular cell repair in acute kidney injury treated with MSCs might be attributed to paracrine mechanisms through the production of different growth factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (b-FGF), monocyte-chemoattractant protein-1, insulin growth factor-1 (IGF-1), and hepatocyte growth factor (HGF). The paracrine effect of MSCs was mediated through production of protein or RNA (micro RNA) carried by the micro vesicles^[7, 37]. Moreover, direct contact of MSCs with the cellular microenvironment could stimulate asymmetric divisions of stem cells in renal niches and their differentiation into tubular cells^[18, 30].

CONCLUSION

Injection of AD-MSCs was effective in cisplatin-induced acute kidney injury in adult albino rats. The cell to cell contact was one of the mechanisms of AD-MSCs therapeutic effect with increased the proliferative ability of the tubular cells.

CONFLICT OF INTEREST

There are no conflicts of interest.

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الملخص العربي

حقن خلايا النسيج الأوسط الجذعية المشتقة من النسيج الدهنى أضعفت إصابة الكلى الحاد الناتج عن عقار السييسبلاتين فى الجرذان

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المقدمة والهدف من البحث: لقد أثبت استخدام الوسائل العلاجية التقليدية فى علاج إصابة الكلى الحادة أنه أقل قدرة فى تخفيض نسب الإصابة والوفاة. وقد ظهر حديثاً العلاج بالخلايا الجذعية كوسيلة واعدة لعلاج هذا الإعتلال المركب. وتهدف هذه الدراسة لتقييم الدور العلاجى لخلايا النسيج الأوسط الجذعية المشتقة من النسيج الدهنى فى تقليل إصابة الكلى الناتج عن عقار السييسبلاتين كنموذج لإصابة الكلى الحادة.

المواد والطرق: تم تقسيم عشرين جرذاً من الإناث البيضاء البالغة إلى أربعة مجموعات متساوية. المجموعة الأولى كانت المجموعة الضابطة، وقد تم حقن الثلاث مجموعات الأخرى بجرعة واحدة من السييسبلاتين (5 مجم/كجم) عن طريق الغشاء البريتونى، وتم التضحية بجرذان المجموعة الثانية بعد يوم واحد من حقن السييسبلاتين، وتم التضحية بجرذان المجموعة الثالثة بعد سبعة أيام من حقن السييسبلاتين، أما المجموعة الرابعة فقد تم حقنها أيضاً بالخلايا الجذعية المشتقة من النسيج الدهنى بجرعة 6×10^2 (خلية لكل جرذ) فى وريد الذيل، وتم التضحية بجرذان هذه المجموعة بعد سبعة أيام من حقن السييسبلاتين.

النتائج: وقد أظهرت النتائج أن التغيرات الهستوباثولوجية فى القشرة الكلوية حدثت بشكل واضح فى المجموعة الثالثة أكثر منها فى المجموعة الثانية، وذلك فى صورة احتقان وضمور فى الكبيبات الكلوية واتساع فى حيز بومان، وفقد حدود الفرشاة للأنايببيبات الملتوية القريبة. كما أظهرت خلايا الأنايببيبات الملتوية البعيدة تجاوزت فى السيتوبلازم، ووجود خلايا الموت المبرمج ذات أنويه داكنة، كما وجد ترسيبات زجاجية داخل تجويف الأنايببيبات الكلوية. وقد لوحظ زيادة فى ترسيب الكولاجين بين الأنايببيبات الكلوية. وقد استطاعت خلايا النسيج الأوسط الجذعية التى تم حقنها فى المجموعة الرابعة من استعادة التركيب الهستولوجى لنسيج الكلى مماثلًا لما عليه فى المجموعة الضابطة. كما ساعدت على زيادة تكاثر خلايا الأنايببيبات الكلوية، مع الانخفاض الملحوظ فى الالتهاب والتليف الموجود بين الأنايببيبات الكلوية. ولكن بعض الكبيبات والأنايببيبات الكلوية مازالت تظهر بعض التغيرات المرضية. وقد تم إثبات وجود خلايا الجذعية لذكر الجرذ الأبيض داخل أنسجة الكلى لأنثى الجرذ الأبيض عن طريق وجود كروموزوم Y.

الاستنتاج: أن حقن خلايا النسيج الأوسط الجذعية المشتقة من النسيج الدهنى لها دور فى تجديد أنسجة الكلى فى حالة الإصابة الحادة بها.