

Research Article

Histological and Histochemical Study of the Effect of Bone Marrow Derived Mesenchymal Stem Cell Therapy In Ischemia/Reperfusion Induced Acute Renal Injury

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Abstract

Background and objectives: Acute renal injury (ARI) is a critical clinical problem without established regimen for treatment. Mesenchymal stem cells (MSCs) are undifferentiated cells that can differentiate and give rise to other cell types. The aim of the present work is to study the effect of different routes of MSCs administration in their therapeutic role in the treatment of the deleterious changes occurred in renal tissue of adult male albino rat model of ischemia/reperfusion (I/R) ARI.

Material and methods: Forty adult male albino rats were used in this study. Animals were divided into four groups, 10 animals for each group. Group I is control sham-operated group. Three experimental groups were all subjected to I/R injury by clamping both renal pedicles for 40 min. These animals were further divided into three subgroups. Group II is non-MSC treated group. Group III (MSC treated group (local injection)): I/R animals received bromodeoxyuridine labeled BM-MSCs locally in renal cortex immediately after removal of the clamps and confirmation of reflow. Group IV (MSC treated group (systemic injection)): I/R animals that received single intravenous injection of bromodeoxyuridine labeled BM-MSCs in tail vein immediately after removal of the clamps and confirmation of reflow. Animals were sacrificed after 5 days of intervention. Serological measurements included serum urea and creatinine. Kidney specimens were processed for H&E, PAS, PCNA and Caspase-3. **Results:** Renal tissue exhibited marked improvement histologically and serologically after MSCs treatment. The improvement was the same in local and systemic treatment.

Conclusion: The present study shows the ability of MSCs to repair the structural and functional renal damage after I/R, both local and Systemic routes are beneficial in treatment of acute renal injury.

Keywords: Acute renal injury; Ischemia reperfusion; Mesenchymal stem cells; Proliferating cell nuclear antigen

Introduction

Acute kidney injury (AKI) is a common disease characterized by an abrupt deterioration in renal function that remains an important challenge in developed and developing countries^[1]. AKI is associated with increased short term mortality and long term risk of chronic kidney disease (CKD)^[2]. AKI causes a severe economic burden. Costs associated with AKI represent nearly 5% of overall hospital expenses^[3]. In many developed countries, more than 2-3% of the total annual health-care budget is directed for the care of patients with ESRD^[4].

AKI is mostly the end result of Ischemia-reperfusion (I/R) injury of the renal tissue^[5]. Ischemia/ reperfusion (I/R) injury is a common clinical problem associated with a high

morbidity and mortality^[6]. It is an inevitable consequence of kidney transplantation and also results from systemic hypoperfusion with subsequent circulatory resuscitation and local renal hypoperfusion following partial nephrectomy and aortic cross-clamping^[7]. (I/R) injury characterized by a marked decrease in glomerular filtration rate (GFR), and it is very common complication in hospitalized patients and especially in patients with multiorgan failure^[8]. It increases the risk of death by 10- to 15- fold^[9]. This is an urgent situation that insists development of new treatment modalities^[10].

Over the last couple of decades, the field of regenerative medicine emerged as a novel promising strategy to enhance recovery from AKI^[11]. Mesenchymal stem cells (MSCs) have

attracted the attention over other progenitor cells, due to their self-renewal capacity, multi-lineage differentiation, immunomodulatory properties that ameliorate inflammation and immune response. These properties facilitate their ability for tissue repair^[12]. MSCs are present in many adult tissues and can be easily extracted from different sources, including the bone marrow, adipose tissue, and umbilical cord, so they are an ideal candidate for cell-based therapy^[13].

However, the suitable route to deliver MSCs to renal tissue to gain their maximum benefits was not yet evaluated. Researchers assumed that systemic administration through intravenous (i.v.) injection resulted in improvement of renal injury, both morphologically and functionally^[14]. On the other side, local injection of MSCs in the injured kidney would ensure the delivery of more MSCs to the kidney^[15].

Aim of the present work is to study the possible reno-protective effects of BM-MSCs on induced acute kidney injury in a rat model of ischemia reperfusion, and to find out if there any difference in the outcome between local and systemic route of administration, using histological, immunohistochemical and biochemical methods.

Materials and Methods

Experimental animals

This study was conducted on 40 adult male spargue-dawley albino rats. They were 12 weeks old, weighing 150-200 g. The animals were housed in hygienic plastic cages and kept in clean well-ventilated room, with food and water ad-libitum. All animals' procedures were done according to the recommendation of El-Minia University Ethics committee for proper care and use of experimental animals. Animals were divided into four groups, each group has 10 animals as follows:

1) Group I (control sham-operated): 10 rats were subjected to sham operation, and then received intravenous saline.

2) Experimental group: 30 rats were subjected to renal ischemia reperfusion (I/R) injury by clamping both renal pedicles for 40 min. Rats were divided as follows:

A) Group II (untreated group): After removal of the clamps and visual confirmation of reflow.

The animals received intravenous saline injection, and they were sacrificed after 5 days.

B) Group III: 10 rats received single injection of bromodeoxyuridine labeled BM-MSCs locally in to renal cortex just after removal of the clamps and visual confirmation of reflow. They were sacrificed 5 days after removal of clamps.

C) Group IV (systemic treated group): 10 rats received single injection of MSCs in tail vein just after removal of the clamps and visual confirmation of reflow. They were sacrificed 5 days after MSCs injection.

Preparation of Bone Marrow Derived Mesenchymal Stem cells

The animals were anesthetized by halothane, and then the skin was sterilized with 70% ethyl alcohol before cutting the skin. The femurs and tibia were carefully dissected from adherent soft tissues. Then they were placed into sterilized beaker containing 70% ethyl alcohol for 1-2 min. The bones were put in Petri dish contain Phosphate buffer saline 1X PBS (Hyclone, USA) for wash. The bones were taken to laminar air flow (unilab biological safety cabinet class II, china) to extract the BM. The two ends of the bones were removed using sterile scissors. Bone marrow was collected by rapid infusion of the tibiae and femurs of 12 weeks adult male albino rats with Dulbecco's modified Eagles medium (DMEM) (lonza, Belgium) supplemented with 10% fetal bovine serum (FBS) (lonza, Belgium) and 1% Antibiotic, Antimicotic (penicillin streptomycin) (lonza, USA). To isolate the nucleated cells density gradient ficol/paqu (pharmacia fine chemicals) was used. The isolated cells were cultured in 20 ml complete media and incubated at 37°C in 5% humidified CO₂ incubator (shellab, USA) for 7-10 days as primary culture or upon formation of large colonies. MSCs in tissue culture are fusiform in shape and adhesive to the polystyrene dish. Adherent MSCs were washed with phosphate buffered saline and dislodged from the cultured dish using Trypsin/EDTA solution, then centrifuged and re-suspended in cultured media enriched with serum^[16,17].

Labeling of stem cells with bromodeoxyuridine dye

MSCs were harvested during the 2nd passage and were labeled with 10 µml 5-bromo-2

deoxyuridine fluorescent linker dye (BrdU; Sigma Aldrich, Taufkirchen, Germany) for 48h at 37°C to label more than 90% of incubated cells^[18].

Counting of cells

Washed cells were resuspended in 1 ml of appropriate media. From this cell suspension, 10 µl was removed for counting. Depending on the estimated (using a microscope) cell number, a dilution factor between two and ten was used to count cells. Test the cell viability 10 µl of cells was added to 10 µl of Trypan blue 0.4% (Lonza, USA) and mixed well. 10 µl of the mixture was taken and put on hemocytometer (Neubauer, Germany) and cells were counted under ordinary microscope (Olympus CX31, USA). Then use this equation:

NO of cells/ml = average of count cells × dilution factor × 10⁴

Injection of MSCs

Immediately after reflow, rats of group III received 0.5 ml labeled MSCs (2 × 10⁶) diluted with 1 ml of saline, loaded in a 1 ml sterile insulin syringe and injected 1 mm depth locally in renal cortex^[19]. Group IV received 2 × 10⁶ labeled MSCs diluted with 1 ml of normal saline, loaded in a 1 ml sterile syringe and injected systemically via tail vein for each rat^[20].

Induction of ischemia-reperfusion acute kidney injury

Animals were anesthetized and midline abdominal incision was done. Kidneys were exposed and renal pedicles were bilaterally clamped for 40 min to induce renal ischemia^[21]. The clamps were then removed to allow kidney reperfusion. Kidneys were observed to ensure reflow. The incision was closed followed by topical application of antibiotic cream.

Sham operation: Sham-operated animals are subjected to the same steps without applying the clamps^[22].

Laboratory investigations

Blood samples were collected from retro-orbital veins using capillary tubes. Urea and creatinine were measured for all rats, before and after the operation.

Histological examination

Light microscopic studies: At the end of the experiment, renal tissue specimens were cut

into small pieces 2 mm each, fixed in 10% formalin solution for 48 h, dehydrated in ascending grades of alcohol and embedded in paraffin. Serial sections of 5-6 µm thickness were cut, mounted on glass slides and subjected to the following techniques:

1. Examination of bromodeoxyuridine labelled MSCs using Fluorescent Microscope.
2. H&E staining for histological examination.
3. PAS staining for histochemical examination.
4. Immunohistochemical staining for:-
 - a) Mouse monoclonal antibody for PCNA (proliferating cell nuclear antigen).
 - b) Mouse monoclonal antibody for Caspase-3 (for detection of apoptosis).

Kidney sections were incubated with mouse monoclonal anti-PCNA antibody and Caspase 3 antibody using the avidin-biotin peroxidase complex technique. Sections were counter-stained with Meyer's hematoxylin^[23].

Positive cells for the anti-PCNA antibody showed brown nuclear reaction and the positive cells for the anti-caspase 3 antibody showed brown cytoplasmic reaction^[24].

Positive tissue control for PCNA: Sections of human tonsil were immunostained for PCNA positive cells. PCNA immunoreactivity appeared as brown nuclear reaction.

Positive tissue control for Caspase 3: Sections of human large intestine were immunostained for caspase 3 positive cells. Caspase 3 immunoreactivity appeared as brown cytoplasmic reaction.

Negative control: Additional specimens of kidney were processed in the same way but without the step of applying the primary antibody.

5. Examination of bromodeoxyuridine labelled MSCs using Fluorescent Microscope Leica Qwin 500 LTD (Cambridge, UK) in H and E stained sections.

Morphometric study: utilized a hardware consisting of a high-resolution color digital camera mounted on an Olympus BX51 microscope and connected to a computer. Five sections were examined from each animal in the different groups under 40 high power fields. The analysis has been performed on 10 different adjacent non overlapped fields for the same slide. Five different sections of the same animal were used. The number of PCNA and caspase 3 immunopositive cells were counted and analyzed. Statistical analysis was done by SPSS (IBM Corp. Released 2010. Windows, Version

21.0). The mean and standard deviation (SD) was calculated for the parameters of each group. Values were expressed as means ± SD. One-way analysis of variance (ANOVA) test was used for the detection of significant differences between groups followed by the use of Tukey-Kramer as a post hoc test.

Results

Results of laboratory investigations:

Measurements of serum urea and creatinine levels in the studied groups (Table 1, 2): On

the 5th day of intervention, mean serum urea and creatinine values for group II showed a significant increase (p < 0.05) when compared to the corresponding control. Mean serum urea and creatinine values for groups III and IV represented a significant decrease when compared to group II. Mean Serum urea values showed no significant difference between group III and IV. Mean Serum urea values showed no significant difference between group III and IV and the corresponding control.

Table 1:

parameter	Control	Untreated	Local treatment	Systemic treatment		
Urea 5 th day	27.6±5.9	102.4± 2.3	29.6± 2.7	28.6± 3.1		
P – value	0.001*					
	I vs. II	I vs. III	II vs. III	I vs. IV	II vs. IV	III vs. IV
	0.001*	0.5	0.001*	0.7	0.001*	0.6

*significant

Table 2:

parameter	Control	Untreated	Local treatment	Systemic treatment		
Creatinine 5 th day	0.7±0.2	4.6± 0.2	0.7± 0.2	0.6± 0.2		
P – value	0.001*					
	I vs. II	I vs. III	II vs. III	I vs. IV	II vs. IV	III vs. IV
	0.001*	0.8	0.001*	0.9	0.001*	0.4

*significant

Histological results

1-BM-MSCs localization into the renal tissue
Sections from treated groups examined with fluorescent microscopy showed

bromodeoxyuridine labelled cells homing into the renal cortex (Figure 1).

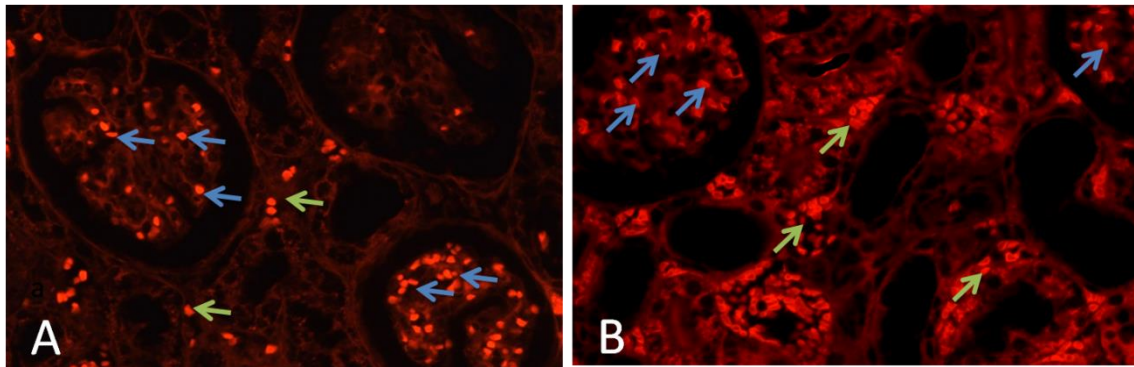


Figure 1: A photomicrograph of rat renal tissue of the treated groups showing: (A) group III MSCs labeled with bromodeoxyuridine are present within renal corpuscular cells (blue arrows) and among tubular lining cells (green arrows). (B) group IV more labeled MSCs are present within renal corpuscular cells (blue arrows) and tubular lining cells (green arrows) (fluorescent microscopy X400).

2-Hematoxylin and eosin-stained sections

In the present study, H&E stained sections of groups I (the control group) showed normal histological structure of renal tissue, with Malpighian corpuscles formed of tuft of glomeruli, Bowman's capsule with normal Bowman's space, proximal (PCT) and distal convoluted tubules (DCT). The lumina of the proximal convoluted tubules were narrow and were lined with pyramidal cells with apical brush border and basal pale nuclei. The DCTs showed wider lumina and were lined with cubical cells with rounded central nuclei. Examination of renal tissue of untreated group (II) showed marked degeneration of the renal corpuscles with loss of nearly all glomerular tufts, pyknotic nuclei and widening of Bowman's space. Marked degeneration of tubular lining cells with presence of pyknotic nuclei could be observed. Cytoplasmic vacuolations were noticed in tubular lining cells. Some tubular lumina contained

acidophilic hyaline casts and the others contain desquamated nuclei. Peritubular capillary dilatation and congestion were detected. Sections of the renal cortex treated with MSCs through local injection showed improvement in morphological changes that affect renal cortical tissue. Mild degeneration of renal corpuscle was noticed with decrease cytoplasmic vacuolations of the renal corpuscular cells. There was slight dilatation of Bowman's space. Tubular lining cells showed less vacuolated cytoplasm and less hyaline casts with decrease in peri-tubular capillary congestion. Sections from the renal tissue treated with MSCs through systemic injection showed marked improvement in tissue architecture represented by well-formed Malpighian corpuscles with normal glomerular tufts, and nearly normal Bowman's space. The tubules showed absence of acidophilic casts and epithelial shedding from their Lumina with decrease in peri-tubular capillary congestion (Figure 2).

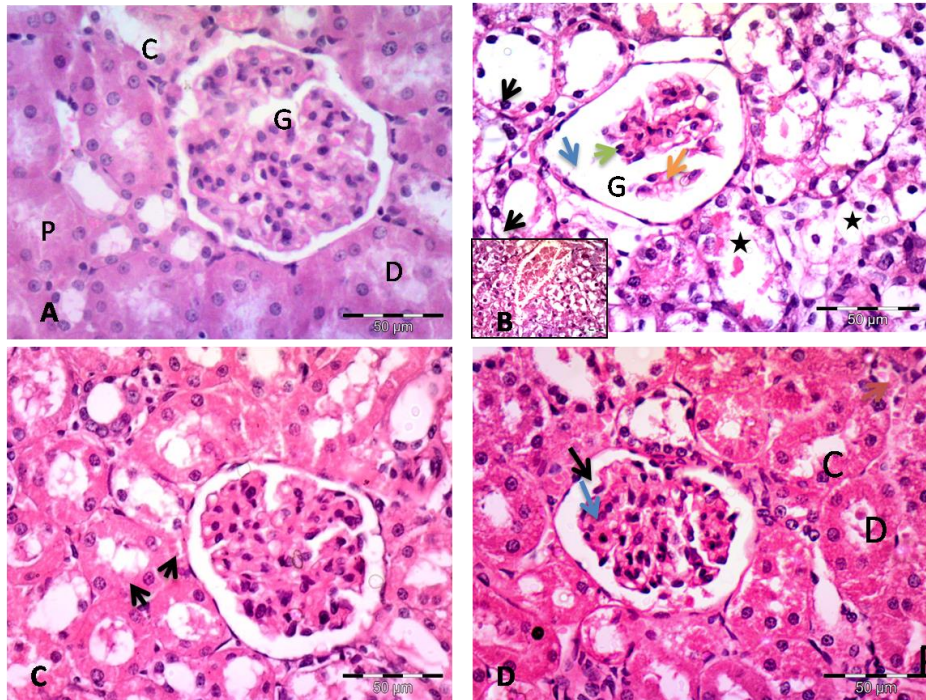


Figure 2: A photomicrograph of rat renal tissue showing: **A) Group I (control sham operated)** Malpighian renal corpuscle containing glomerulus (G) surrounded by Bowman's space (G). PCT (P) is lined with pyramidal cells having rounded basal nuclei. DCT (D) is lined with cubical cells having rounded central nuclei. Collecting tubule (C) is lined with low cubical cells having rounded central nuclei. **B) Group II** degenerated renal corpuscles with widening of Bowman's space (G). Vacuolated cytoplasm & darkly stained nuclei of tubular cells (arrow heads). C) Tubular Lumina with desquamated epithelial cells and acidophilic hyaline casts (stars). Notice peritubular capillary dilatation and congestion (insert). **C) Group III** improvement of renal histological structure. Well formed renal corpuscle & decrease vacuolations of the renal corpuscular cells (G), less cytoplasmic vacuolation of tubular lining cells (arrowhead). Notice: regression of intertubular congestion and inflammation. **D) Group IV** nearly normal renal cortical structure. (H&E X400 Scale bar 50 µm).

3- PAS stained sections:

Sections in the renal cortex of the control group showed positive PAS reactions in the BMs of glomerular blood capillaries, parietal layers of Bowman's capsules, PCT cells and DCT cells. It was obvious in the apical brush borders of PCT and DCT cells. Sections in the renal cortex of group II showed partial loss of BMs of glomerular blood capillaries and parietal layers of Bowman's capsules. Loss of BMs of PCT,

DCT and CT cells was observed. There was loss of brush borders of PCT and DCT cells. Some cortical tubules contained PAS positive deposits. Sections in the renal cortex of groups III and IV showed continuous BMs of glomerular blood capillaries, parietal layers of Bowman's capsule, PCT and DCT cells. Some PCT and DCT cells restored their brush borders in group III. While nearly all PCT and DCT cells restored their brush borders in group IV (Fig. 3).

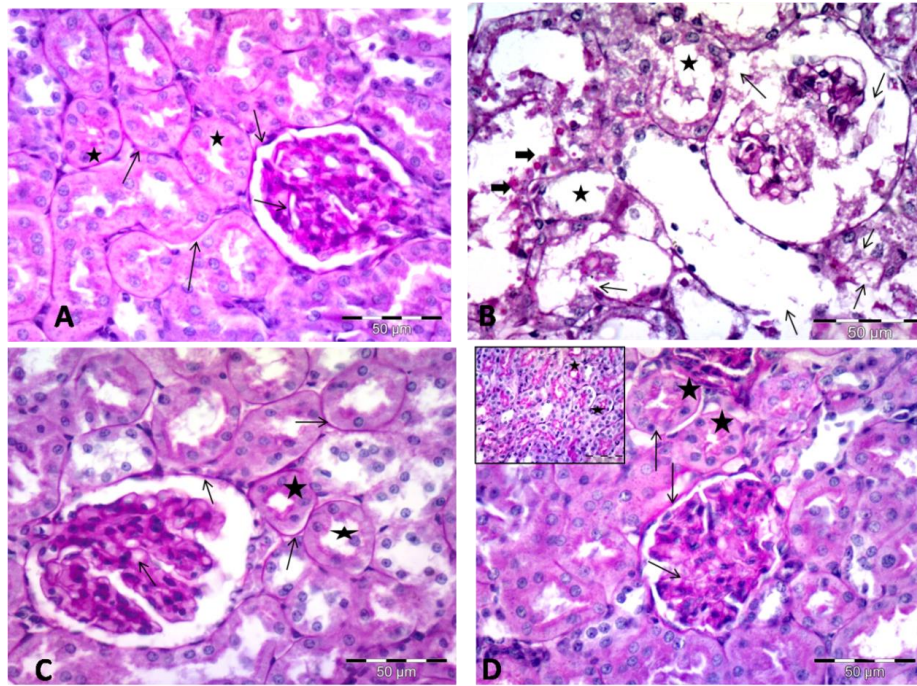


Figure 3: A photomicrograph of rat renal tissue showing: A) Group I positive PAS reactions in the BMs of glomerular blood capillaries, parietal layers of Bowman's capsules, PCT cells and DCT cells (arrows). Preserved apical brush borders of cortical tubules (stars). **B) Group II** partial loss of BMs of glomerular blood capillaries, parietal layers of Bowman's capsules, most of cortical tubules (arrows). Loss of brush borders of PCT and DCT cells (stars). PAS deposits observed (thick arrows). **C & D) Groups III and IV** continuous BMs of glomerular blood capillaries, parietal layers of Bowman's capsule, PCT and DCT cells (arrows). Some PCT and DCT cells restored their brush borders in group III. Most of PCT and DCT cells restored their brush borders in group IV (insert). (PAS X400 scale bar 50 µm)

4- Immune histochemistry results

A) PCNA

Immunohistochemistry for PCNA showed little immune-positive nuclei in normal renal tissue.

Group II sections showed few PCNA positive nuclei. Groups III and IV sections revealed marked increase in the number of PCNA positive nuclei (Figure 4).

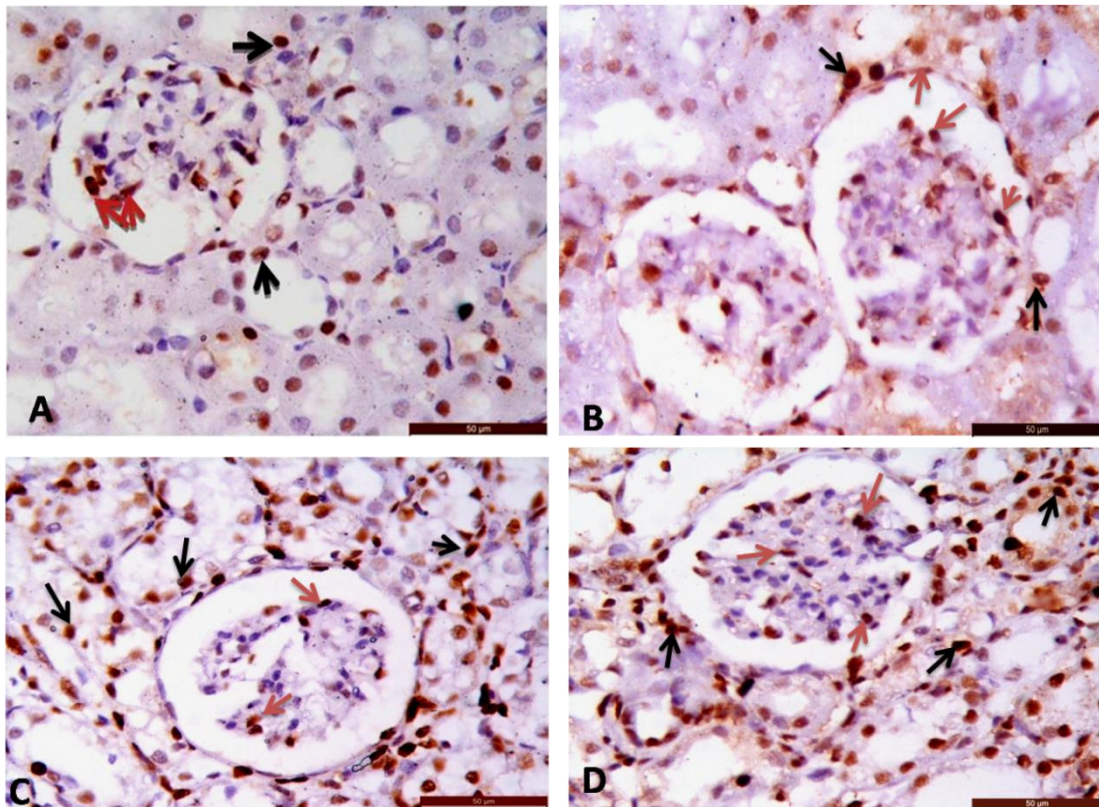


Figure 4: A photomicrograph of the rat renal tissue showing : **A) Group I** little positive PCNA expression in the renal corpuscular cells (red arrows) and among tubular lining cells (black arrows). **B) Group II** few PCNA immuno- reactive nuclei in the renal corpuscular cells (red arrows) and among the tubular lining cells in cortex and medulla (black arrows). **C & D)** Group III and IV multiple PCNA positive nuclei in renal corpuscles (red arrows) and among tubular lining cells (black arrows). (PCNA X400 Scale bar 50 µm).

B) Caspase-3

Immunohistochemistry for Caspase-3 showed negative expression in the control group. Group II showed strong expression for Caspase-3 in the cytoplasm and nuclei of cells in the renal

cortex. There was a significant decrease in Caspase-3 expression after local treatment with MSCs, while faint and nearly negative expressions were noticed in systemically treated group (Figure 5).

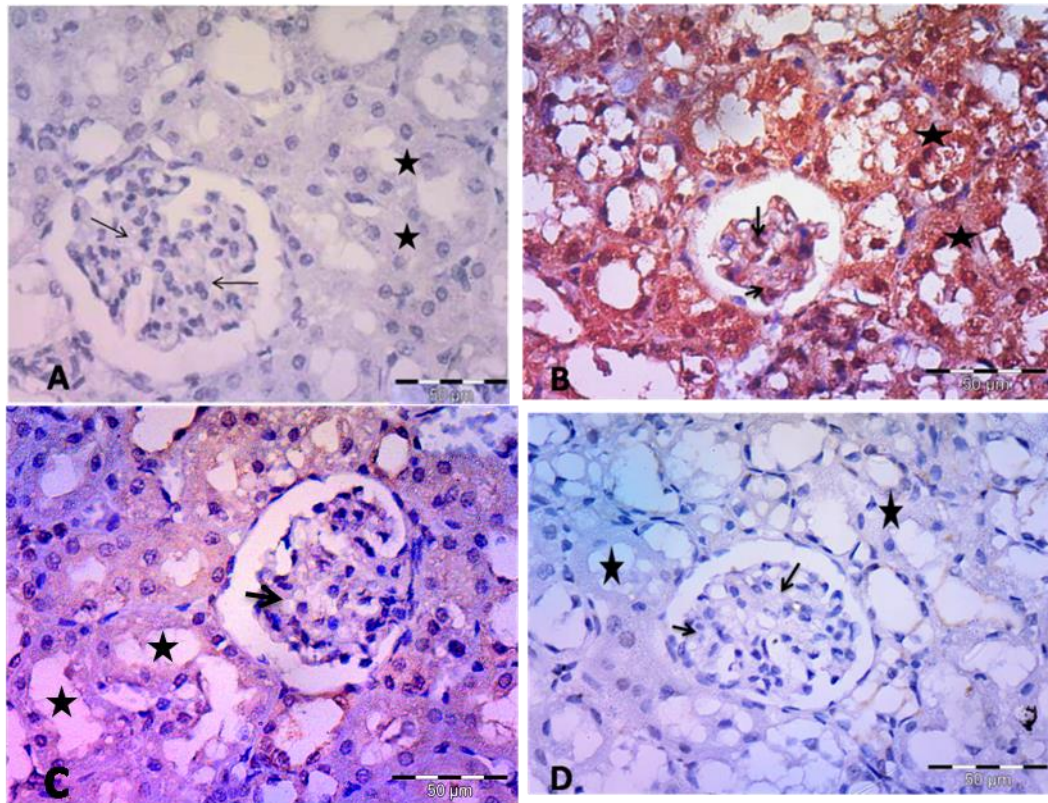


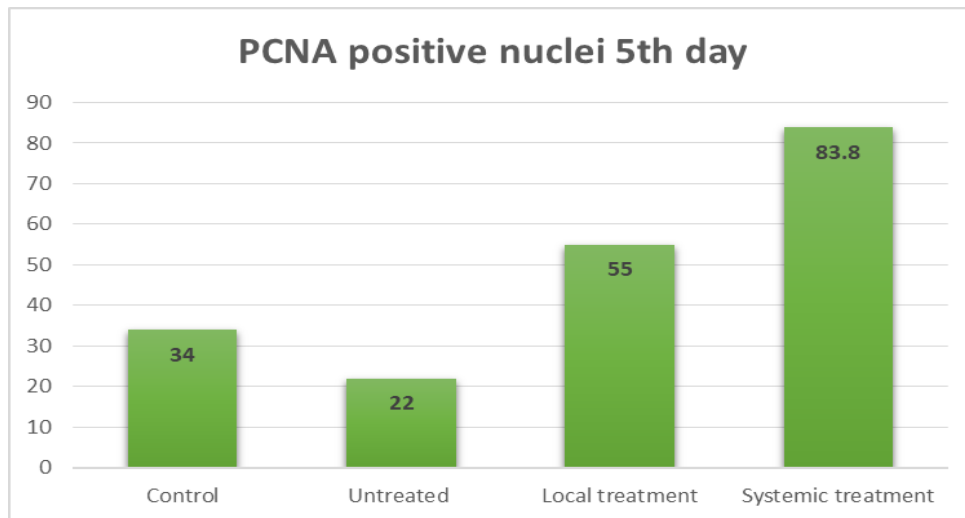
Figure 5: A photomicrograph of rat renal tissue showing: **A) Group I** negative Caspase-3 immunoreactivity in cytoplasm of the renal corpuscular cells (black arrows) and tubular lining cells (stars). **B) Group II** strong Caspase-3 immunoreactivity in cytoplasm and nuclei of the renal corpuscular cells (black arrows) and tubular lining cells (stars). **C) Group III** negative Caspase-3 immunoreactivity in the cytoplasm of renal corpuscular cells (black arrows) and in most of tubular lining cells (stars). **D) Group IV** negative Caspase-3 immunoreactivity in cytoplasm of the renal corpuscular cells (black arrows) and tubular lining cells (stars). (Caspase 3 X400 scale bar 50 µm)

Morphometric results:

Mean number of PCNA positive nuclei (± SD) in the studied groups in 5th day: Number of PCNA immunoreactive cells of renal tissue of group II showed a significant decrease when compared to groups I, III and IV. Groups III and IV showed a significant increase in number

of PCNA immunoreactive cells of when compared to control. The highest value for PCNA positive nuclei was recorded in group IV with high statistically significant increase in the number of PCNA positive nuclei in this group compared to group II & III (p<0.05) (Table 3 and Chart 1).

parameter	Control		Untreated		Local treatment	Systemic treatment
PCNA positive nuclei 5 th day	34 ± 1.9		22 ± 1.6		55 ± 2	83.8 ± 2.6
P – value	0.001*					
	I vs. II	I vs. III	II vs. III	I vs. IV	II vs. IV	III vs. IV
	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*

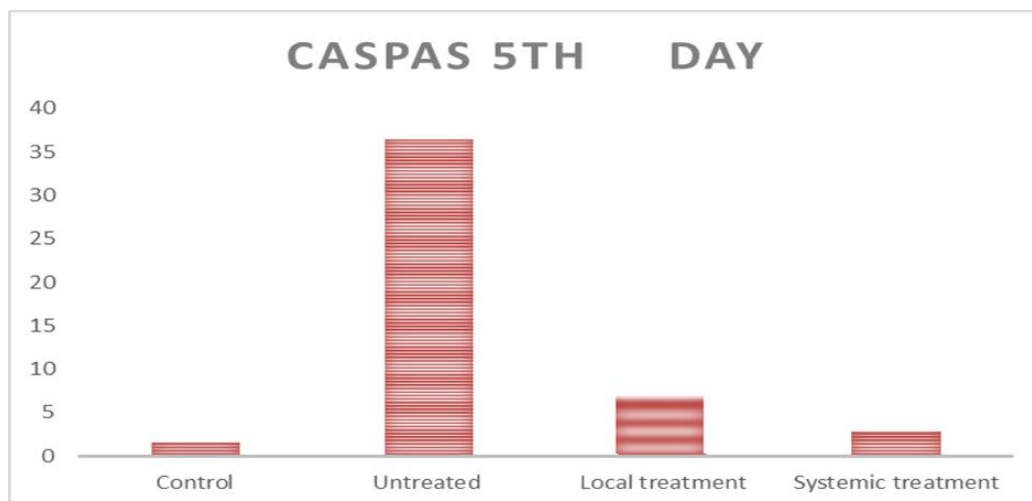


Mean area% of Caspase-3 positive cells (± SD) in the studied groups in 5th day:

Mean area% of Caspase-3 immuno-reactivity in tubular cells of group II showed a significant increase when compared to control. There was significant decrease in mean area% of Caspase-3 immunoreactivity in groups III and IV ($p < 0.05$). No significant difference between groups III and IV (Table 4, Chart 2).

Parameter	Control	Untreated	Local treatment	Systemic treatment		
Caspas 5 th day	1.6±1.1	36.4± 2.1	2.4± 1.1	2.8± 1.3		
P – value	0.001*					
	I vs. II	I vs. III	II vs. III	I vs. IV	II vs. IV	III vs. IV
	0.001*	0.3	0.001*	0.1	0.001*	0.6

*significant



Discussion

Stem cell-based approaches are promising for the development of future therapies. The use of stem cells has emerged in the past few years as an effective therapy for AKI^[25]. Recovery of the kidneys from AKI after injection with MSCs was attributed to the ability of MSCs to release growth factors and cytokines. MSCs are ideal vehicles for cellular gene transfer that enhance recovery and reduce renal tissue damage^[26]. Although the renoprotective effect of MSCs was evident in several studies^[27], few studies revealed the effect of different routes of MSCs administration on renal recovery^[28]. In the present study we used ischemic reperfusion animal model to induce acute renal injury, MSCs labeled with fluorescent bromodeoxyuridine was injected through two different routes (systemic and cortical injection) to evaluate their therapeutic effect biochemically and histologically.

Biochemical results of untreated group (II) showed marked deterioration of the renal function with high plasma level of urea and creatinine. Hx and E sections of the same group showed widening of the Bowman's space and shrinkage of glomerular tuft. Tubular cells showed vacuolated cytoplasm, pyknotic nuclei, and acidophilic hyaline casts. Exfoliated epithelial cells were observed in tubular lumina. Peritubular capillary dilatation and congestion were observed. PAS stained sections revealed interrupted BMs of the renal corpuscles and tubules. Partial and complete loss of brush border in most of the tubules was also evident in this group. PAS positive deposits were noticed in tubular lumina.

Acute renal injury is manifested by both serological and histological findings^[29]. Similar changes were previously reported by other researchers who demonstrated severe acute tubular damage in kidney sections of the I/R group^[30]. I/R injury cause cellular ATP depletion leading to cellular dysfunction and ultimately cell death^[31]. Desquamation of tubular epithelial cells occurs due to ischemia and anoxia which could lead to loss of cellular integrity^[32]. It was explained by investigators who stated that ischemia results in disruption of brush border and cytoskeletal integrity occurs as a result of loss of cell junctions with mislocalization of adhesion molecules and other membrane proteins such as the Na⁺K⁺-ATPase

and β -integrins^[33]. Acidophilic and PAS positive hyaline casts represent cellular debris that underwent molecular changes. Cells and their debris that detach from the tubular basement membrane combine with proteins present in the tubular lumina, such as Tamm-Horsfall protein and form gel that contributing to cast formation^[34]. Peritubular capillary dilatation and congestion occurred due to an inflammatory response of ischemia and shortage of oxygen supply^[35].

In our study homing of MSCs to the damaged renal tissue in the treated groups (III & IV) was confirmed by examination with the fluorescent microscopy, labeled cells appeared immediately in the cortical corpuscles and in the peri-tubular spaces. Sections in the renal cortex of group IV showed more MSCs than group III. In rat model of AKI through 40 min bilateral clamping of renal pedicle, fluorescence-labeled MSC were detected early after their systemic injection in glomeruli, and at microvasculature sites^[36]. In mice animal model of acute renal ischemia, MSCs transplanted into mice selectively grafted onto damaged area^[37]. The migration of MSCs to sites of injury might be attributed to release of chemokines at sites of tissue damage^[38]. Stromal-derived factor-1, platelet-derived growth factor and CD44 are candidates in the regulation of MSCs homing^[39].

In the present study, both MSCs treated groups showed marked improvement in renal function parameters. Serum level of urea and creatinine nearly returned back to normal 5 days after injection. Histological findings of renal damage showed marked improvement after MSCs injection. The histological improvement was the same in groups III and IV. Less tubular necrosis, less tubular casts and fewer desquamated cells within tubular lumen were observed. As regard PAS-stained sections, it was found that many cortical tubules exhibited preserved PAS positive brush border and continuous basement membrane was detected in nearly all the tubules. In cisplatin-treated rats MSCs infusion prevented occurrence of AKI through down regulation of tubular cell apoptosis and enhancing proliferation^[40]. The antioxidant activity of MSCs might be an important factor to set up an environment favoring proliferation of dedifferentiated epithelial cells surviving the injury or resident stem cells activity^[41]. The Peri-tubular dilatation and

congestion observed in the untreated group; showed improvement after MSCs administration.. The inflammatory cells as macrophages, neutrophils and T cells can enhance the development of AKI^[42,43]. MSCs move to sites of inflammation and release trophic growth factors to modulate the immune system, modulate macrophages activity and provide renal protection and repair^[44]. Restoration of PCT cells brush border in groups III and IV indicate the ability of MSCs to ameliorate tubular necrosis and suggesting its protective effect on cytoskeletal integrity of renal tubular cells^[45]. The paracrine activity of exogenous BM-MSCs might be also attributed to production of substances that stimulate endogenous renal stem cells leading to cellular recovery and renal injury repair^[46,47].

In the present study, PCNA immunostaining was used to evaluate the regenerative power of injured tubular epithelial. The highest value was recorded in group IV, there was significant increase when compared to all groups. Such result was in agreement with the finding of other investigators who reported that PCNA expression in I/R kidney increases from 9.4% on day 1 after I/R injury to 34% on day 2, thus it was time dependent. This might be explained by the fact that under normal circumstances, the tubular epithelial cells have a slow rate of proliferation. Such low rate of turnover changes dramatically after an ischemic or toxic insult, when there is a marked increase in cell death and a vigorous response to replace these cells^[48].

In our study, immunohistochemical staining for Caspase-3 as an apoptotic marker was done to detect apoptosis in renal cells. The results showed that the number of Caspase-3 positive cells in the kidney tubules in cortex and medulla markedly increased in group II while in group III and IV the infusion of MSCs significantly decrease the number of apoptotic cells.

Other studies stated that MSCs injection helps the recovery of renal function by stimulation of tissue proliferation^[49]. This proved by recent studies which explained the renal tubular protective effect of BM-MSCs by its potential ability to Trans-differentiate. MSCs release extracellular microvesicles (MSC-EVs) with small RNAs content, miRNA is expected to

have a reno-protective effect. The RNA is small non coding RNA of 21-25 nucleotides in length. This molecule is directed to the damaged cells and can regulate their gene expression pattern towards regeneration^[50].

The present study provided evidence that local and systemic infusion of BM-MSCs give nearly the same protective effect on the renal tissue after 5 days of administration. More experimental studies are still needed for better understanding of the different mechanisms through which MSCs induce their therapeutic effects on the damaged renal tissue. Controlled clinical trials should be done in parallel.

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