Original Article

Histological Study on the Effect of Human Umbilical Cord Mesenchymal Stem Cells Versus their Microvesicles in the Repairing of Acute Kidney Injury Following Ischemia Reperfusion in Adult Male Albino Rats

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ABSTRACT

Introduction: Renal ischemia/reperfusion (I/R) injury is the major cause of acute kidney injury (AKI); it is associated with severe morbidity and mortality in both developing and developed countries.

Aim of Work: To compare between the therapeutic potential of human umbilical cord–mesenchymal stem cells (hUC-MSCs) and their microvesicles (MVs) on I/R induced AKI in a rat model.

Materials and Methods: 42 adult male albino rats with an average body weight 180-200 grams are included in the study. The control group included twelve rats they were subjected to sham operation and divided into (subgroups Ia & Ib) six rats each. The remaining thirty rats were exposed to I/R injury via ligation of both renal pedicles for 40 minutes (ten rats are the experimental non- treated group, ten rats injected once with hUC-MSCs after reperfusion and the last ten rats injected once with MVs after reperfusion). Intravenous injection of Paul Karl Horan (PKH) 26 labelled hUC- MSCs and MVs was done for the treated groups only. The sacrification was done after 48 hours and 2 weeks. The kidney sections were stained with hematoxylin & eosin, PAS and immunohistochemical staining for proliferating cell nuclear antigen (PCNA). Blood samples, were taken to measure serum urea and Cr on Day 2 and Day 14 following I/R acute kidney injury.

Results: Both hUC- MSCs and their MVs exhibited protection against AKI manifested by the improvement of histological architecture, the reduction of serum urea and Cr and the increase in PCNA expression in renal tubules with no significant differences between both.

Conclusion: These results suggest the potential renoprotective capacity of both hUC-MSCs and their MVs.

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Key Words: Acute kidney injury, hUC- MSCs, ischemia / reperfusion, MVs, PCNA.

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INTRODUCTION

Kidney disease is a leading cause of morbidity and mortality in hospitalized patients. For the care of end-stage renal disease it costs more than $32 billion annually which represents more than a quarter of annual medicare expenditures[1].

Acute kidney injury (AKI) frequently occurs in hospitalized critically ill patients, with poor outcomes. And it is a complex clinical disorder[2].

The major cause of intrinsic acute renal failure is Ischemia/reperfusion (I/R) damage; intrinsic acute renal failure is the most common condition in hospitalized patients and can be progressed to acute tubular necrosis (ATN)[3].

The damaging effects of renal I/R injury include events sequenced to finally result in necrosis and apoptosis of the renal cells[4]. The loss of renal cells can't be substituted by the endogenous capacity of kidney repair[5].

The management of AKI has improved in the last years, but the specific therapy for improving renal function has not been developed yet[6]. Failure to substitute damaged cells produces tubulo-interstitial fibrosis and scarring, which increases the tendency to chronic renal injury[7].

The studies showed that stem cells (SCs) enhance repair of tissue by secreting factors that increase proliferation and stimulate differentiation of endogenous stem-like progenitors, decrease inflammatory and immune reactions and enhance regeneration of injured cells[8].

Many recent reports have focused on mesenchymal stem cells (MSCs) isolated from human umbilical cord (hUC) as they can be easily obtained and cultured with no ethical issues[9].

The limitations of MSC transplantation include immune rejection and malignancy conversion[10].

The conditioned medium (CM) contains exosomes- like MVs derived from the MSCs. The CM may be used instead of MSCs in treating AKI, to avoid cell transplantations[11,12].

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MVs are composed of membrane fragments enclosing bioactive lipids, cytoplasmic proteins and ribonucleic acids. In general, the content of MVs reflects the cells of their source[13].

These membranous structures transfer molecular contents that are bioactive, including micro-RNAs (miRNAs) sequences, mRNAs and proteins which protect AKI in I/R animal models[14,15].

Accordingly, this study was designed to compare between the therapeutic potential of hUC-MSCs and their MVs on I/R induced AKI in a rat model.

MATERIALS AND METHODS

Materials

Animals

42 adult male albino rats with an average body weight 180-200 grams are included in this study included. They were housed in hygienic cages according to the guidelines for animal research issued by the National Institute of Health and approved by Animal Ethics Committee, Cairo University. The rats received chow and water.

The experimental design

The rats were divided into the following groups

A. Group I (control sham-operated group): Twelve rats underwent sham operation. Rats injected with 1 ml saline intravenously (IV) via tail vein. They were divided into (subgroups Ia & Ib) six rats each, which were sacrificed with the experimental subgroups.

The remaining thirty rats had undergone renal ischemia reperfusion injury (IRI) via the ligation of both renal pedicles for 40 minutes. Rats were divided equally into three groups as follow;

B. Group II (I/R injury group): It included ten rats injected once with 1ml saline in the tail vein at once after reperfusion. The sacrification was done as follow:

- Subgroup IIa: 48 hours after reperfusion five rats had undergone sacrification.
- Subgroup IIb: 2 weeks after reperfusion five rats had undergone sacrification.

C. Group III (experimental hUC-derived -MSCs-treated group): It included ten rats injected once with hUC-MSCs after reperfusion. The sacrification was done as follow:

- Subgroup IIIa: 48 hours following hUC- MSCs treatment five rats had undergone sacrification[16].
- Subgroup IIIb: 2 weeks following hUC- MSCs treatment five rats had undergone scarification.

D. Group IV (experimental MVs-treated group): It included ten rats injected once with MVs derived from hUC- MSCs after reperfusion. The sacrification was done as follow:

- Subgroup IVa: 48 hours following MVs treatment five rats had undergone sacrification[17].
- Subgroup IVb: 2 weeks following MVs treatment five rats had undergone sacrification[17].

Methods

Preparation of hUC-MSCs

a. Umbilical Cord Isolation and Subculture: From informed, consenting mothers from gynecology and obstetrics department of Kasr Al-Ainy we collected fresh umbilical cords within 6 hours then washed in sterile phosphate buffered saline (PBS). The cord was minced into small pieces with a sterile scalpel, placed into 6 well plates, and grown in media containing: Dulbecco's Modified Eagle's Medium (DMEM, GIBCO/BRL), foetal calf serum (FCS) (10%) (GIBCO/BRL) and penicillin and streptomycin (P/S) (GIBCO/BRL). Tissue explants were removed after 21 days in culture. Medium was changed every 2-3 days and cells were maintained in a humidified atmosphere at 5% CO2 at 37°C[18].

b. Labeling with PKH26 dye: PKH26 is a molecule that is fluorescent it inserts into the bilayered cell membrane and has stable, clear red fluorescence in labeled cells[19].

Labelled cells are perfect for in *vivo* cell tracking, reproduction studies and in *vitro* labeling of cell; the labelled cells keep both biological and reproductive activity[20].

Preparation of MVs

a. MVs Isolation: HUC-MSCs were cultured in DMEM with no adding of fetal bovine serum (FBS) with the addition of 0.5% bovine serum albumin (BSA) (Sigma-Aldrich) overnight. The conditioned medium was collected and stored at - 80°C. The medium was centrifuged at 2,000 g for 20 minutes to remove debris, and then ultracentrifuged at 100,000 g in a SW41 swing rotor (Beckman Coulter, Fullerton, CA, USA) for one hour at 4°C. MVs were washed once with serum free M199 (Sigma-Aldrich) containing 25 mM HEPES (pH = 7.4) and submitted to a second ultracentrifugation in the same conditions[21].

b. Labeling with PKH26 dye: The dilution of MVs was made in 100 µl of diluent C. Then pipetted to 100 µl of a 15 µM solution of PKH26 (final 7.5 µM PKH26), then incubated at room temperature. Freeze and light is avoided[22].

Induction of ischemia-reperfusion Acute kidney injury

I/R was induced in ketamine anesthetized animals. Povidone iodine (Betadine) was used to disinfect the abdomen then mid abdominal laparotomy was performed. Kidneys were exposed and ligation of renal pedicles for 40 minutes was performed. Reperfusion was seen. Four ml saline was given intraperitoneal (IP) to avoid ileus. Garamycin cream was used topically following the closure of the wound to decrease hazard of infection[23].

Sham Operation

The sham-operated group underwent the same surgical procedure with no ligation of renal pedicles[24]. Just to be exposed to the same stress of the operation. With the same antiseptic precautions and way of wound closure. Recovery of rats was allowed with the supply of food and water.

Administration of hUC-MSCs

It was given in a dose of 1x106 hUC-MSCs, diluted in saline given intravenously into the vein of the tail to rats[25].

Administration of MVs

It was given in a dose of 100 µg MVs, diluted in saline given intravenously into the vein of the tail to rats[21].

Laboratory results

Blood samples, were taken from tail vein using heparinized test tubes to measure serum urea and Cr for all rats to estimate the kidney functions. These samples were drawn on Day 2 and Day 14 following I/R acute kidney injury.

Histological examination

Sacrification using Ketamine (100 mg/kg body weight) IP was done, immediate dissection of right kidney from each animal, fixation in 10 % buffered formalin solution for 48 hours, dehydration in ascending grades of ethanol and embedding in paraffin. Cutting of serial sections µm thickness was done, mounting on slides and perform the following:

Histological staining

Hematoxylin & Eosin (H&E) stain: to examine the histological structure of the kidney in different groups[26].

Histochemical staining

Periodic Acid Schiff Reaction (PAS) stain: to examine brush border and the basal lamina[26].

Immunohistochemical Staining: For PCNA

PCNA is a sign of cellular proliferation and division and. It is present in the nucleus and was involved in the synthesis of DNA[27]. There were little numbers of PCNA positive cells in tubular cells and glomeruli in the normal human kidney[28].

Incubation of kidney sections with mouse Monoclonal Antibody (Lab Vision Corporation laboratories, USA, catalogue number MS-106-R7). It was supplied as (7.0ml) of antibody (200ug/ml) pre diluted in 0.05mol/L Tris-HCl, pH 7.6 containing stabilizing protein and0.015mol/L sodium azide. It was stored at 2-8 ◦C.

PCNA positive cells showed nuclear brown reaction

Positive tissue control: Sections of human small intestine stained with Proliferating Cell Nuclear Antigen (PCNA). PCNA immunoreactivity appeared as brown nuclear reaction.

Negative control: extra kidney sections were handled in the same way but with omitting of primary antibody.

Quantitative Morphometric Study

Using "Leica Qwin-C 500" image analyzer computer system (England), the following parameters were measured:

1. The mean number of affected Malpighian renal corpuscles at a magnification of X 100.
2. The mean number of affected renal tubules at a magnification of X 100.
3. The mean area percent of PAS-positive material in the glomerular basement membrane and brush border, measured in ten random fields for each specimen at a magnification of ×400.
4. Number of PCNA immunostained positive cells using the image analyzer. The number of PCNA +ve cells was measured in ten non overlapping fields for each group at a magnification of x400.
5. Mean number of immunofluorescent cells using the image analyzer. The mean number of immunofluorescent cells was measured in ten non overlapping fields for each group at a magnification of x200.

Statistical Analysis

Tabulation and statistical analysis of data using statistical package SPSS version 16 (SPSS Inc., Chicago, USA) for estimation of the difference between the studied groups. Descriptive statistics were done in the form of means ± standard deviation (SD). Comparison between different groups was made using analysis of variance (ANOVA). The differences were considered statistically significant when "*p value*" was < 0.05.

RESULTS

No mortality occurred throughout the experimental period.

Laboratory results

(a) Serum Urea values in all Groups (Table 1)

After 48 hrs there was increase which is significant ($P < 0.05$) in the mean values of serum urea (mg/dL) in all groups in comparison to the control. However after two weeks the levels of subgroups IIIb and IVb decreased significantly ($P < 0.05$) in comparison to subgroups IIa, IIb, IIIa and IVa but not reached normal values.

(b) Serum Cr values in all Groups (Table 2)

After 48 hrs there was increase which is significant ($P < 0.05$) in the mean values of serum Cr (mg/dL) in all groups in comparison to the control. However after two weeks the levels of subgroups IIIb and IVb decreased significantly ($P < 0.05$) in comparison to subgroups IIa, IIb, IIIa and IVa but not reached normal values.

Histological results

(a) Hematoxylin and Eosin (H&E) staining (Plates 1,2)

Group I (control group): Examination of H&E-stained sections from control group showed normal architecture; with Malpighian renal corpuscle, proximal and distal convoluted tubules (Figure 1A).

Group II (I/R injury group): Sub group IIa (I/R injury, 48hrs): Some cortical sections of sub group IIa showed partial destruction of renal corpuscle and dilation of capsular space. Most of the cortical tubules lining cells showed marked cytoplasmic vacuolar degeneration & debris in the lumen and the nuclei were pyknotic. Congestion of blood vessels in between the tubules was noticed with exudate accumulation (Figure 1B). In other sections renal corpuscle was destructed completely. In the lumen of some cortical tubules hyaline protein casts were seen (Figure 1C).

Sub group IIb (I/R injury, 2 weeks): The cortical sections of sub group IIb showed slight dilation of capsular space. The cytoplasm of lining tubular cells showed vacuolar degeneration. The epithelial cells fell off into the lumen forming debris (Figure 1D).

Group III (experimental hUC- derived MSCs-treated group): Subgroup IIIa (hUC-MSCs, 48 hrs): The cortical sections of sub group IIIa showed renal corpuscle containing retracted glomerulus with marked dilation of capsular space. The cytoplasm of tubular cells showed vacuolar degeneration. Debris was present in tubular lumen (Figure 2A).

Subgroup IIIb (hUC-MSCs, 2 weeks): The cortical sections of sub group IIIb detected relatively normal histological appearance of renal corpuscle containing well-formed glomerular capillaries. Some tubular cells had pyknotic nuclei (Figure 2B).

Group IV (experimental MVs-treated group): Subgroup IVa (MVs, 48 hrs): The cortical sections of sub group IVa (MVs, 48hrs) showed renal corpuscle containing retracted glomerulus with marked dilation of capsular space. The tubular cells showed vacuolar degeneration, pyknotic nuclei with debris in the lumen (Figure 2C).

Subgroup IVb (MVs, 2 weeks): The cortical sections of sub group IVb (MVs, 2 weeks) detected relatively normal histological appearance of renal corpuscle containing well-formed glomerular capillaries Some cells of the tubules showed pyknotic nuclei (Figure 2D).

(b) PAS staining (Plate 3)

Group I (control group): The cortical sections of the control group showed various tubules with intact brush border and uninterrupted basal laminae (Figure 3A).

Group II (experimental non-treated group= I/R injury group): The cortical sections of subgroup IIa (I/R injury, 48 hrs) and subgroup IIb (I/R injury, 2 weeks) showed brush border partially or completely lost. Basal lamina was discontinuous in some parts of tubules (Figures 3B,C).

Group III (experimental hUC-MSCs-treated group): The cortical sections of subgroup III a (hUC -MSCs, 48 hrs) revealed brush border partially or completely lost. Basal lamina was discontinuous in some parts of tubules (Figure 3D). While subgroup IIIb (hUC -MSCs, 2 weeks) revealed intact brush border in most tubules. Uninterrupted basal lamina was present in most renal tubules (Figure 3E).

Group IV (experimental MVs-treated group): The cortical sections of subgroup IV a (MVs, 48 hrs) revealed brush border partially or completely lost. Basal lamina was discontinuous in some parts of tubules (Figure 3F). While subgroup IV b (MVs, 2 weeks) revealed intact brush border in most tubules. Uninterrupted basal lamina was present in most renal tubules (Figure 3G).

(c) PCNA staining (Plate 4)

Group I (control group): The renal cortex of the rats of this group revealed positive PCNA immune reaction in renal tubular cells and within Malpighian renal corpuscle (Figure 4A).

Group II (experimental non-treated group= I/R injury group): The renal cortex of the rats of subgroup IIa (I/R injury, 48 hrs) revealed some positive PCNA immune reaction in renal tubular cells and within Malpighian renal corpuscle (Figure 4B). While subgroup IIb (I/R injury, 2 weeks) revealed mild increase in the nuclei expressing PCNA in renal tubular cells and within Malpighian renal corpuscle, when compared to subgroup IIa (Figure 4C).

Group III (experimental hUC-MSCs-treated group): The renal cortex of the rats of subgroup IIIa (hUC -MSCs, 48 hrs) revealed some positive PCNA immune reaction in renal tubular cells and within Malpighian renal corpuscle (Figure 4D). While subgroup IIIb (hUC -MSCs, 2 weeks) revealed marked increase in the nuclei expressing PCNA in renal tubular cells and within Malpighian renal corpuscle (Figure 4E).

Group IV (experimental MVs-treated group): The renal cortex of the rats of subgroup IVa (MVs, 48 hrs) revealed some positive PCNA immune reaction in renal tubular cells and within Malpighian renal corpuscle (Figure 4F). While subgroup IVb (MVs, 2 weeks) revealed marked increase in PCNA immune reactivity (Figure 4G).

Immunofluorescent Results (Plate 5)

The renal cortex of the rats of subgroup IIIa (hUC-MSCs, 48 hrs) and IVa (MVs, 48 hrs) showed apparently many hUC-MSCs and MVs labelled with PKH26 (Figures 5A,C). While subgroup III b (hUC-MSCs, 2 weeks) and IV b (MVs, 2 weeks) showed apparently few hUC-MSCs and MVs labelled with PKH26 (Figures 5B,D).

Morphometric Results

Mean percentage of Affected Glomeruli (±SD) in all Groups (Histogram 1)

The experimental groups revealed a significant increase in the mean percentage of affected glomeruli in comparison to the control group. While the levels in subgroups IIIb and IVb decreased significantly in comparison to subgroups IIa, IIb, IIIa and IVa. No significant difference between subgroups (IIIa, IVa) & (IIIb, IVb).

Mean percentage of Affected Tubules (±SD) in all Groups (Histogram 2)

The experimental groups revealed a significant increase in the mean percentage of affected tubules in comparison to the control group. While the levels in subgroups IIIb and IVb decreased significantly in comparison to subgroups IIa, IIb, IIIa and IVa. No significant difference between subgroups (IIIa, IVa) & (IIIb, IVb).

Mean Area Percent of PAS Positive Material (±SD) in all Groups (Histogram 3)

The experimental groups revealed a significant increase in the mean area % of PAS positive material in comparison to the control group. While the levels in subgroups IIIb and IVb increased significantly as compared to the control groups ($P < 0.05$). No significant difference between subgroups (IIIa, IVa) & (IIIb, IVb).

Mean number of PCNA immunopositive Nuclei (±SD) in all Groups (Histogram 4)

The experimental groups revealed a significant increase in mean number of PCNA immunopositive nuclei in renal tubular cells and within Malpighian renal corpuscle in comparison to the control group. While the levels in subgroups IIIb and IVb increased significantly in comparison to subgroups IIa, IIb, IIIa and IVa. No significant difference between subgroups (IIIa, IVa) & (IIIb, IVb).

Mean number of immunofluorescent cells (±SD) in subgroups IIIa, IIIb, IVa and IVb (Histogram 5)

The mean number of immunofluorescent cells in subgroups IIIb and IVb was significantly decreased in comparison to subgroups IIIa and IVa respectively. There was no significant difference of number of immunofluorescent cells between IIIa, IVa or IIIb and IVb.

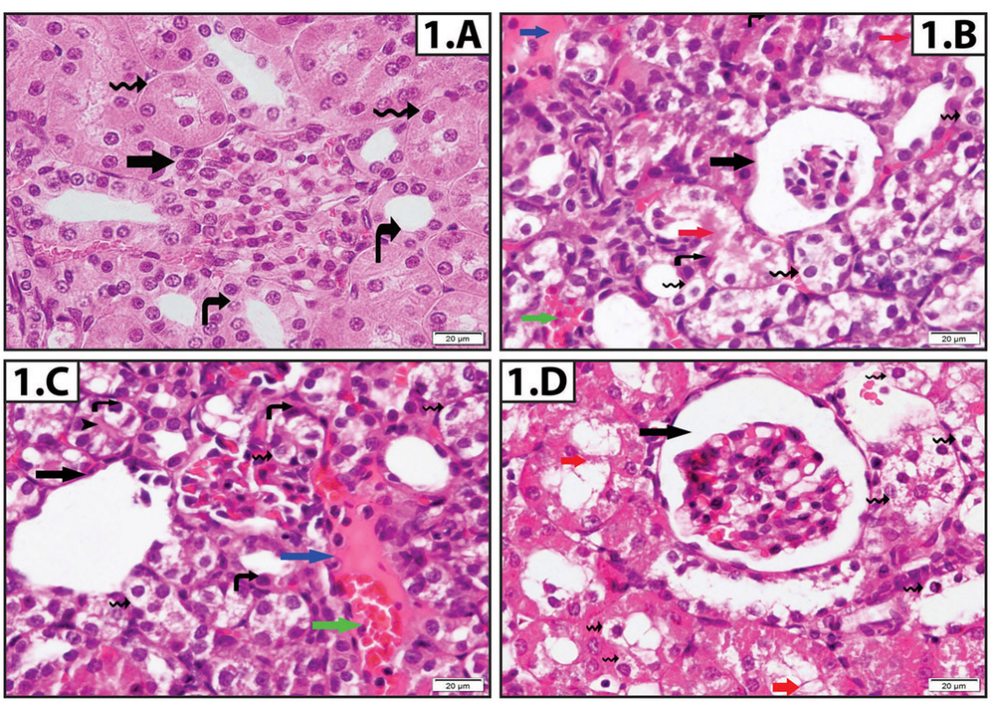


Plate 1: Photomicrographs of kidney cortical sections (H&Ex400) showing: Fig. (1A): group I (control group) showing normal appearance of renal corpuscle (black thick arrow), PCT (spiral arrows) and DCT (curved arrows). Fig. (1B): sub group IIa (I/R injury, 48hrs) showing partial destruction of renal corpuscle and dilation of capsular space (black arrow). The cortical tubular epithelial cells show marked cytoplasmic vacuolar degeneration (spiral arrows), pyknotic nuclei (curved arrows) and debris in lumen (red arrows). Congestion of blood vessels (green arrow) and exudate accumulation (blue arrow). Fig. (1C): sub group IIa (I/R injury, 48hrs) showing renal corpuscle completely destructed with absence of glomerular capillaries (black arrow). Cortical epithelial cells reveal marked cytoplasmic vacuolar degeneration (spiral arrows), pyknotic nuclei (curved arrows) and hyaline protein casts in lumen of tubules (arrow head). Congestion of blood vessels (green arrow) and exudate accumulation (blue arrow) in the interstitial tissue. Fig. (1D): sub group IIb (I/R injury, 2 weeks) showing slight dilation of capsular space (black arrow). The lining cells of the cortical tubules exhibit vacuolar degeneration (spiral arrows) and debris in lumen (red arrows).

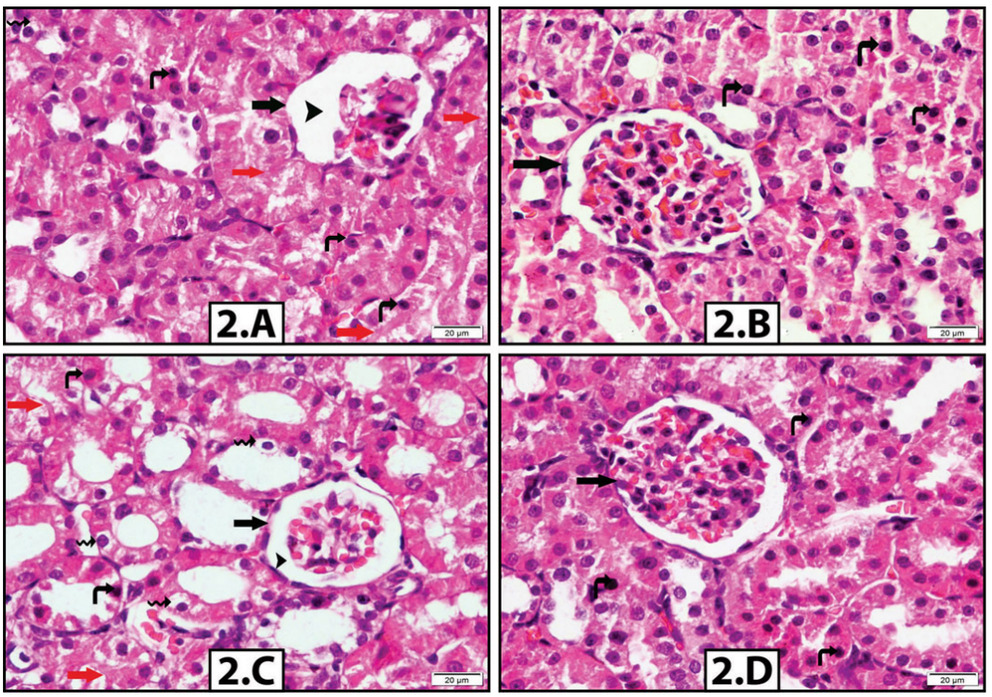


Plate 2: Photomicrographs of of kidney cortical sections (H&Ex400) showing: Fig. (2A): sub group III a (hUC-MSCs, 48 hrs) showing renal corpuscle with retracted glomerulus (black arrow) and marked dilation of capsular space (arrow head). The cortical tubular epithelial cells show cytoplasmic vacuolar degeneration (spiral arrow), pyknotic nuclei (curved arrows) and debris in lumen (red arrows). Fig. (2B): sub group III b (hUC-MSCs, 2 weeks) showing relatively normal appearance of renal corpuscle containing well-formed glomerular capillaries (black arrow). Some tubular cells show pyknotic nuclei (curved arrows). Fig. (2C): sub group IVa (MVs, 48 hrs) showing renal corpuscle with retracted glomerulus (black arrow) and dilation of capsular space (arrow head). The cortical tubular epithelial cells show cytoplasmic vacuolar degeneration (spiral arrows), pyknotic nuclei (curved arrows) and debris in the lumen of the tubules (red arrows). Fig. (2D): sub group IVb (MVs, 2 weeks) showing relatively normal histological appearance of renal corpuscle with well-formed glomerular capillaries (black arrow). Some cells of the cortical tubules show pyknotic nuclei (curved arrows).

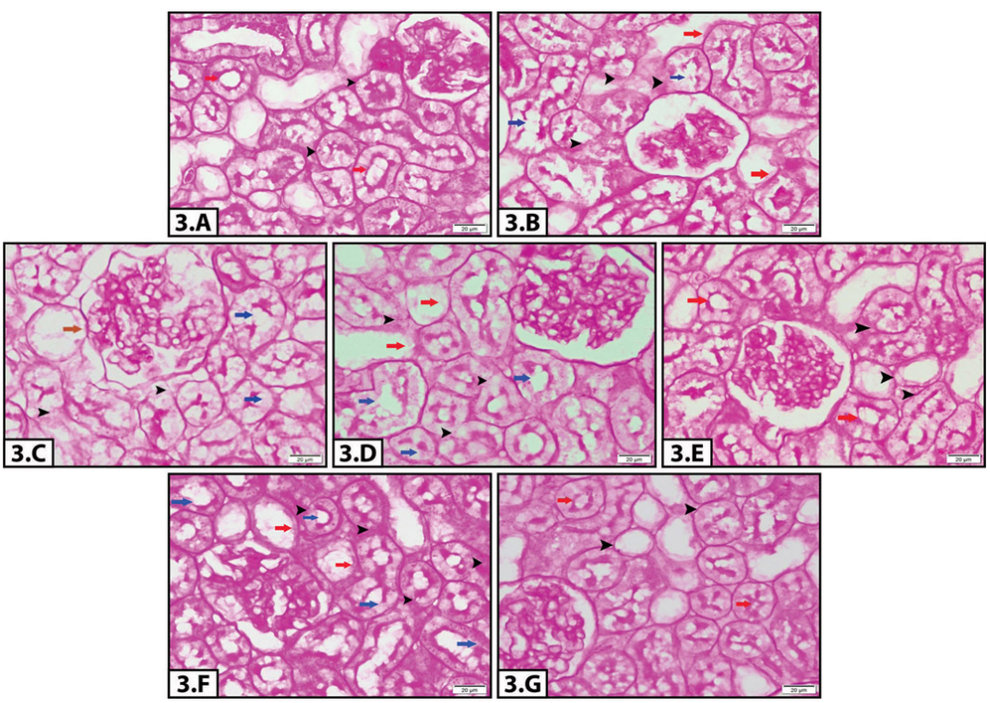


Plate 3: Photomicrograph of cortical sections (PAS reaction x400) showing: Fig. (3A): group I (control group) revealing various tubules with intact brush border (red arrows) and basal laminae (arrow heads). Figs. (3B, C, D &F): subgroup IIa (I/R injury, 48 hrs), subgroup IIb (I/R injury, 2 weeks), IIIa (hUC-MSCs, 48 hrs), subgroup IV a (MVs, 48 hrs) revealing brush border with partial loss (blue arrows) or complete loss (red arrows). Basal laminae are discontinuous in most tubules (arrow heads). Figs. (3E&G): subgroup IIIb (hUC-MSCs, 2 weeks) and subgroup IV b (MVs, 2 weeks) revealing many tubules with intact brush border (red arrows). Uninterrupted basal lamina is present in most renal tubules (arrow heads).

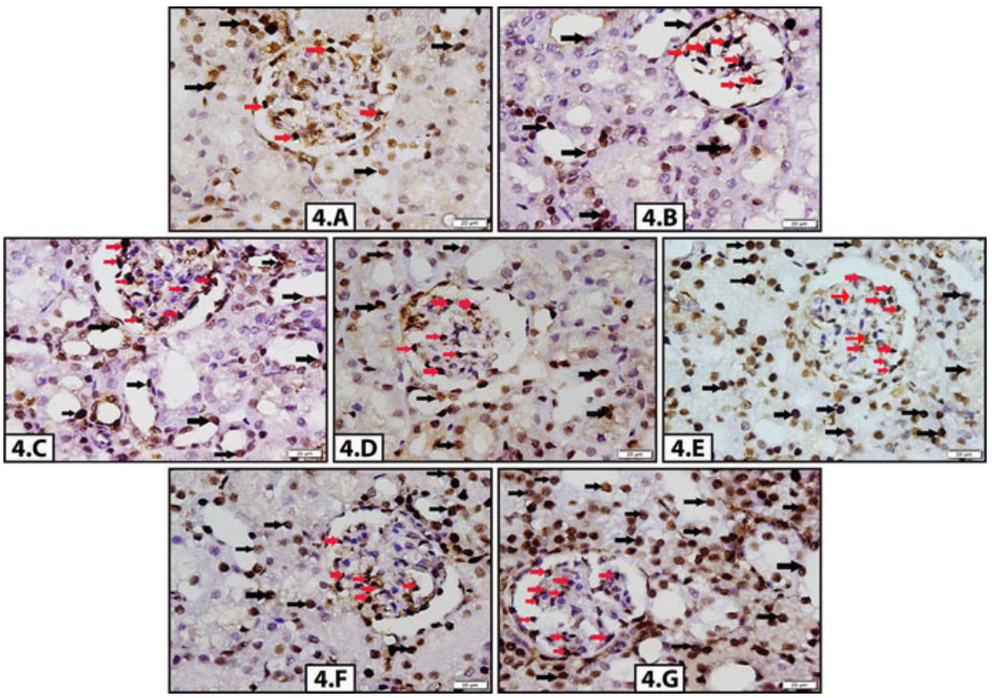


Plate 4: Photomicrographs of sections in the renal cortex (PCNAx400) showing: Fig. (4A): group I (control group) revealing positive PCNA immune reaction in renal tubular cells (black arrows) and within Malpighian renal corpuscle (red arrows). Figs. (4B, D& F): renal cortex of subgroup IIa (I/R injury, 48 hrs), subgroup III a (hUC-MSCs, 48h), subgroup IV a (MVs, 48h) revealing some positive PCNA immune reaction in renal tubular cells (black arrows) and within Malpighian renal corpuscle (red arrows). Fig. (4C) subgroup IIb (I/R injury, 2 weeks) revealing mild increase in the nuclei expressing PCNA in renal tubular cells (black arrows) and within Malpighian renal corpuscle (red arrows). Figs. (4E &G): revealing marked increase in the nuclei expressing PCNA in renal tubular cells (black arrows) and within Malpighian renal corpuscle (red arrows).

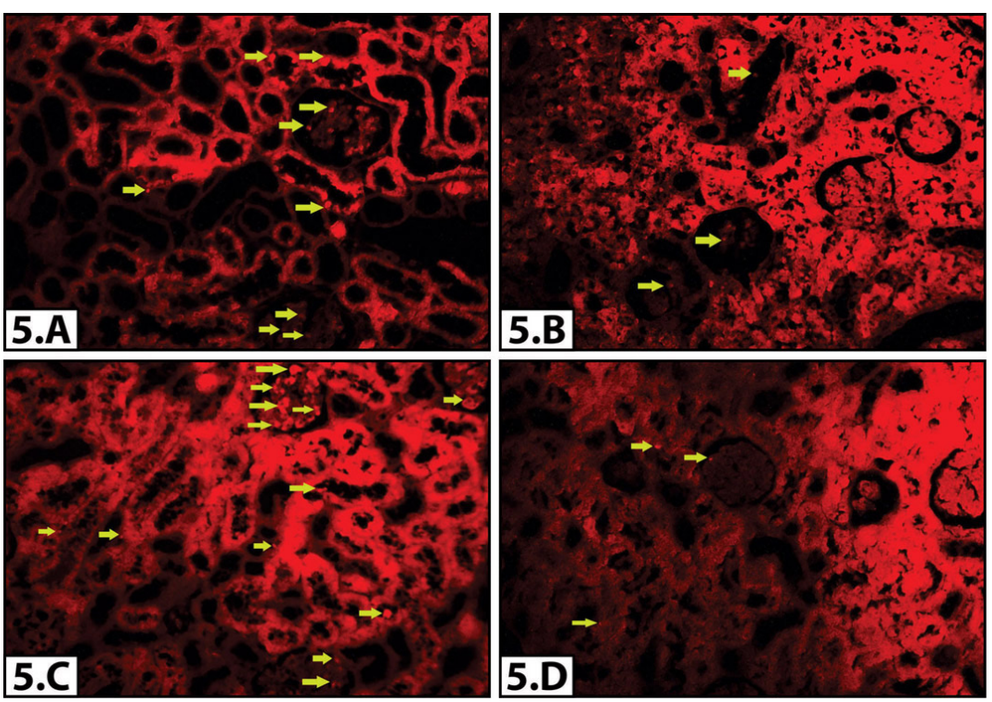


Plate 5: Photomicrographs of sections in the renal cortex (Fluorescent × 200) showing: Figs. (5A&C): subgroup IIIa (hUC-MSCs, 48 hrs), subgroup IVa (MVs, 48 hrs) showing many hUC-MSCs and MVs labelled with PKH26 involving the tubular epithelium and within the glomerular tuft (arrows). Fig. (5B &D): subgroup III b (hUC-MSCs, 2 weeks) and subgroup IV b (MVs, 2 weeks) showing few hUC-MSCs and MVs labelled with PKH26 involving the tubular epithelium and within the glomerular tuft (arrows).

Table 1: Comparison between the mean Serum Urea values (mg/dL) (±SD) for all groups

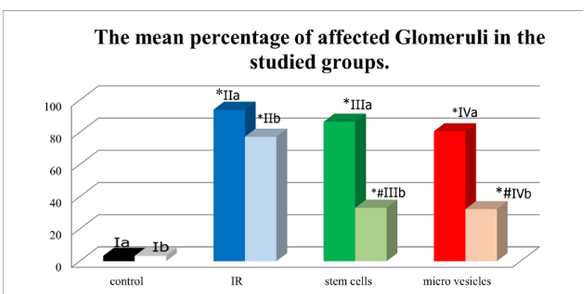
	control	I/R	hUC-MSCs	MVs
Urea 48 hrs	29.82±1.33	68.72±0.95*	67.64±0.99*	64.52±9.01*
Urea 2 weeks	29.82±1.33	70.1±1.52*	55.78±0.79*#	55.13±0.38*#

* Significant as compared to control group ($P < 0.05$).

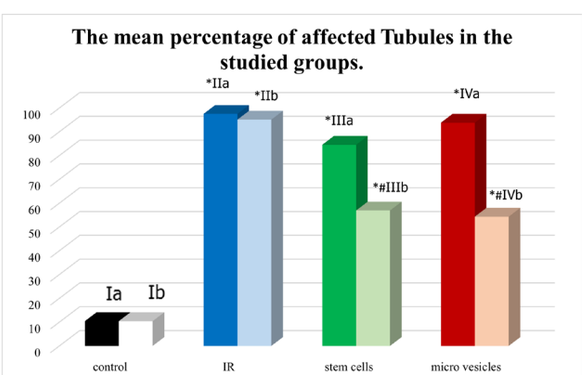
Table 2: Comparison between the mean Serum Cr values (mg/dL) (±SD) for all groups

	control	I/R	hUC-MSCs	MVs
Serum Cr 48 hrs	0.15±0.45	1.01±0.07*	0.98±0.47*	0.97±0.12*
Serum Cr 2 weeks	0.15±0.45	1.17±0.04*$	0.71±0.02*#	0.67±0.02*#

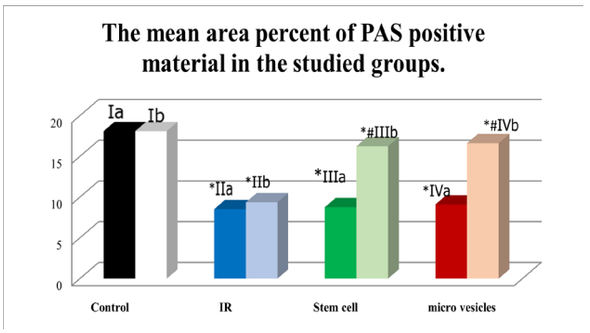
* Significant as compared to control group ($P < 0.05$).
$ Significant as compared to subgroup IIa ($P < 0.05$).
# Significant as compared to subgroups IIa, IIb, IIIa and IVa ($P < 0.05$).



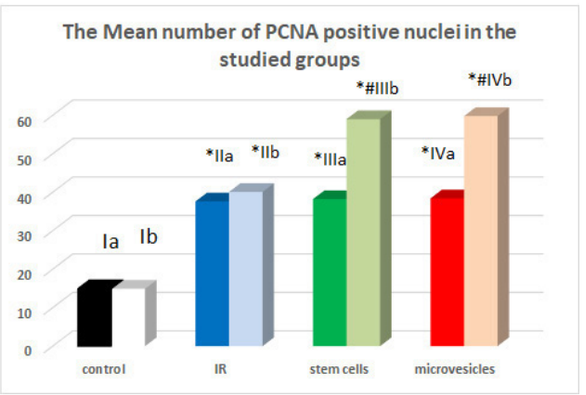
Histogram 1: Comparison between the mean percentage of affected Glomeruli in all groups.
* Significant as compared to control group ($P < 0.05$).
# Significant as compared to subgroups IIa, IIb, IIIa and IVa ($P < 0.05$).



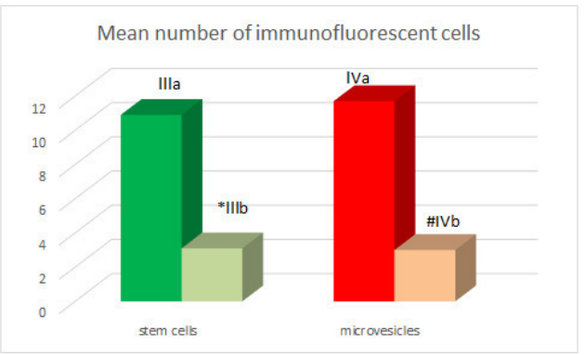
Histogram 2: Comparison between the mean percentage of affected Tubules in all groups.
* Significant as compared to control group ($P < 0.05$).
# Significant as compared to subgroups IIa, IIb, IIIa and IVa ($P < 0.05$).



Histogram 3: Comparison between the mean area percent of PAS positive material in all groups.
* Significant as compared to control group ($P < 0.05$).
# Significant as compared to subgroups IIa, IIb, IIIa and IVa ($P < 0.05$).



Histogram 4: Mean number of PCNA immunopositive nuclei in all groups.
* Significant as compared to control group ($P < 0.05$).
# Significant as compared to subgroups IIa, IIb, IIIa and IVa ($P < 0.05$).



Histogram 5: Mean number of immunofluorescent cells in subgroups IIIa, IIIb, IVa and IVb
* Significant decrease as compared to IIIa ($P < 0.05$).
# Significant decrease as compared to IVa ($P < 0.05$).

DISCUSSION

The main cause of acute kidney injury (AKI) is renal Ischemia/reperfusion (I/R) injury. It is linked to disease and death in both developing and developed countries[29].

There was elevation of serum urea and Cr in groups II & III, IV 48 hrs following I/R injury. This was in agreement with Weng *et al*., 2018[30] who demonstrated increase significantly in the concentrations of serum urea and Cr in animals following renal I/R.

The morphological findings detected in the renal sections of group II (I/R injury group) showed destructive changes in the renal tubules and renal corpuscles.

Regarding the Malpighian renal corpuscles, lesions ranged from partial destruction to complete destruction of renal corpuscles. Disappearance of glomeruli was in agreement with Zhang *et al*., 2017[31] who reported that I/R induced AKI caused changes, including loss of glomeruli, renal interstitium congestion and abnormal tubules due to swelling and atrophy of tubular epithelial cell.

Epithelial cells lining the tubules of the I/R injury group (48 hrs and 2 weeks) exhibited loss of the brush border, vacuolated cytoplasm and pyknotic nuclei. There was distortion in architecture where some tubules showed exfoliation and shedding of some epithelial tubular cells. Luminal tubular casts were also detected.

This was in accordance with the observation of Nakano and Nishiyama, 2016[32] who reported that AKI following renal I/R is identified by severe tubular damage, manifested by sloughing of tubular cell with cast formation.

Havasi and Borkan, 2011[33] postulated that following I/R cells either undergo apoptosis or necrosis or injured sub lethal or lethal. The injured cells with the debris and apical membrane material form cast in the lumen causing obstruction of the tubules.

Elmore, 2007[34] stated that, several biochemical regulated events occurs during apoptosis causing changes in morphology such as blebbing of plasma membrane, loss of cell membrane asymmetry, shrinkage of cell, detachment of cell and fragmentation of chromosomal DNA with condensation of the chromatin.

The renal cortex of I/R injury group showed congestion of the blood vessels in between the tubules with exudate accumulation. Similar observations were recorded by Peng *et al*., 2015[35] who stated that when rat kidney was subjected to ischemia, renal interstitial hyperemia and edema occurs.

Basile *et al*., 2011[36] explained the interstitial tissue exudation by the distension of endothelial cell, absence of glycocalyx and fall of cytoskeleton. All of these cause the loss of contact between the endothelial cells increasing the permeability causing fluid loss in the interstitium.

After two weeks (IIb) the percentage of affected glomeruli and tubules decreased. This could be clarified on the basis of Duffield *et al*., 2005[37] who reported the great regenerative capacity of tubular epithelial cells; thus injured cells are replaced within days of reflow.

In this work, PAS sections of I/R injury group (48 hrs and 2 weeks) showed brush border partially or completely lost. Similarly Weng *et al*., 2018[30] reported that PAS sections of I/R injury group showed loss of the brush border and vacuole degeneration.

In subgroups IIa, IIb PCNA staining increased in comparison to control. Similarly Li *et al*., 2017[38] reported that following I/R the number of PCNA-positive cells was increased significantly.

Serum urea and Cr values in subgroup IIIb (two weeks after hUC- MSCs therapy) decreased significantly in comparison to I/R injury group. These results were in accordance with Fahmy *et al*., 2017[39] who recorded that hUC- MSCs can reduce serum levels of Cr and urea.

Kidney sections of subgroup IIIa (48 hrs after hUC-MSC therapy) showed the persistence of damage in the glomeruli and tubules. While subgroup IIIb showed relatively normal histological appearance of Malpighian renal corpuscle and renal tubules in comparison to I/R injury group.

Most fields of the examined sections showed relatively normal architecture. The majority of the renal corpuscles exhibited well-formed capillary tufts. Most of the cortical tubules showed better preservation of structure. Minimal areas of peritubular congestion were noted. This was in accordance with Qiu *et al*., 2014[16] who stated that umbilical cord -MSC group showed slight tissue affection with minimal edema in interstitium and well arrangement of tubules compared with the I/R groups.

The PAS kidney sections of subgroup IIIb (two weeks following hUC-MSC treatment) showed intact brush border in most tubules and uninterrupted basal lamina in comparison to I/R injury group.

Jang *et al*., 2014[25] explained these findings by that umbilical cord-MSCs decrease the renal injury both structurally and functionally. So the percent of necrotic and damaged tubules are lower compared to control.

In the current work, the mean number of PCNA immunopositive nuclei in subgroup IIIb increased significantly in comparison to I/R injury group. These findings were in agreement with Abd Elwahab *et al*., 2017[40], who reported that the number of PCNA positive nuclei markedly increased following systemic MSCs therapy.

Cao *et al*., 2010[41] also reported that umbilical cord-MSCs treated animals showed more nuclei expressing PCNA. The percent of PCNA positive cells in umbilical cord-MSC treated group was higher as compared with sham groups.

In the present study sections in the renal cortex of subgroup III a (hUC-MSCs, 48 hrs) showed apparently

many hUC-MSC labelled with PKH26. Similar findings were recorded by Jang *et al*., 2014[25] who stated that following ischemia hUC-MSC therapy increases the number of PKH-26-labelled cells significantly.

While subgroup IIIb (hUC-MSCs, 2 weeks) showed apparently few hUC-MSCs labelled with PKH26. These results were in accordance with Torres Crigna *et al*., 2018[42] who reported that the cells were no longer observed 7 days after labelled stem cell treatment in animal model of AKI I/R.

In this study, rats given MV following I/R were monitored for kidney functions assessed by serum urea and Cr measurements. In subgroup IVb serum Cr level decreased significantly in comparison to I/R injury group. These findings were in agreement with Yun and Lee, 2019[43] who reported that MSC-derived EVs are safe and reinforce kidney function in AKI animal models.

Kidney sections of subgroup IVa (48 hrs after hUC-MV therapy) showed the persistence of damage in the glomeruli and tubules. While subgroup IVb (two weeks after hUC-MV therapy) showed relatively normal histological appearance of Malpighian renal corpuscle and renal tubules in comparison to I/R injury group.

These results were supported morphometrically as there was a decrease statistically significant in the mean percent of affected glomeruli and tubules in subgroup IVb when compared to I/R injury group. Zou *et al*., 2014[21] explained that when MVs are injected into tail vein after ischemic AKI it accumulate in the injured kidney and play therapeutic role by regulating the inflammation.

The PAS kidney sections of subgroup IVb (two weeks after hUC-MV therapy) showed intact brush border in most tubules and uninterrupted basal lamina in comparison to I/R injury group. These findings were supported by Zou *et al*., 2014[21] who mentioned that tubular affection clearly decreased in the MV group so human Wharton's Jelly (WJ) MSC-MVs have critical impact in AKI.

In the current work, subgroup IVb revealed an increase statistically significant in the mean number of PCNA immunopositive nuclei. These results were consistent with Zou *et al*., 2016[44] who reported that MV treatment attenuate renal cell apoptosis and enhance cellular proliferation.

Yun and Lee, 2019[43] explained that in renal I/R injury animal model, EVs have been integrated into the damaged tubular cells decreasing death and increasing cellular proliferation. Thus protecting against the metabolic stress.

In the present study subgroup IVa (hUC-MVs, 48 hrs) showed apparently many hUC-MVs labelled with PKH26. These results were consistent with Zou *et al*., 2016[44] who reported that when PKH26-labelled EVs were administrated intravenously they can be detected in renal tissues. However, the un labelled EVs could not be revealed. So the EVs play role in injured kidney.

Finally, the present work revealed that hUC-MSCs and their MVs have the same effect on I/R induced AKI which was detected biochemically, histologically and morphometrically. This was consistent with Zhang *et al*., 2014[45] who stated the similar effect of human WJ-MVs and cell therapy on I/R induced kidney injury recovery in rats.

But, Haga *et al*., 2017[46] reported the advantages of MSC- extracellular vesicles (EV) over MSC by avoiding unwanted effects of using cellular material. They added that the smaller size of MSC-EV (115±48 nm) than MSCs (~15–19 μm) gives further advantages than using MSCs which when injected IV become trapped in the lungs forming micro emboli.

CONCLUSION

In this rat model of ischemia reperfusion: hUC-MSCs and their MVs were proved to have a potential renoprotective capacity which is of considerable therapeutic promise in the management of AKI. Renoprotective potential of hUC-MSCs and their MVs in the current study was proved at both morphological and functional levels with no significant differences between both. HUC-MSCs and their MVs were capable of homing to injured kidney when injected intravenously soon after injury.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة نسيجية عن تأثير الخلايا الجذعية الوسيطة للحبل السرى الآدمى مقابل حويصلاتها الدقيقة فى اصلاح اصابة الكلى الحادة التالية لنقص التروية واعادتها فى ذكور الجرذان البيضاء البالغة

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الخلفية: نقص التروية الكلوية واعادتها هي السبب الرئيسى لإصابة الكلي الحاد ويرتبط بالمرض وزيادة الوفيات في كل من البلدان النامية والمتقدمة.

الهدف من العمل: للمقارنة بين الإمكانيات العلاجية للخلايا الجذعية الوسيطة المشتقة من الحبل السري وحويصلاتها الدقيقة في إصلاح اصابة الكلى الحادة المستحدثة في نموذج الجرذ من نقص التروية واعادتها.

المواد والأساليب: شملت هذه الدراسة ٤٢ من ذكور الجرذان البيضاءالبالغة وزنها من ١٨٠-٢٠٠ جرام. ضمت المجموعة الضابطة اثني عشر جرذا تعرضوا لعملية خادعة وقسمت إلى مجموعتين فرعيتين ، ستة جرذان لكل منهما (المجموعتان الفرعيتان ١أ و ١ب) . تعرضت الفئران الثلاثين المتبقية لضرر نقص التروية الكلوية واعادتها بواسطه الضغط علي العنق الكلوي لمدة ٤٠ دقيقة (عشرة جرذان هي المجموعة التجريبية غير المعالجة, تلقت عشرة جرذان حقنة واحدة من الخلايا الجذعية الوسيطة المشتقة من الحبل السري علي الفور بعد إزالة المشابك وتلقت الجرذان العشرة الأخيرة حقنة واحدة من الحويصلات الدقيقة مباشرة بعد إزالة المشابك) . تم الحقن الوريدي للخلايا الجذعية الوسيطة المشتقة من الحبل السري و الحويصلات الدقيقة المرقومة بال PKH٢٦ للمجموعات المعالجة فقط. تم التضحية بالجرذان بعد ٤٨ ساعة وإسبوعين. تم تجهيز عينات الكلى وصبغها بصبغة الهيماتوكسيلين والايوسين و صبغة بيرايوديك شيف الحامضي و الصبغ الهستوكيميائي المناعي لمولد المضاد التكاثري لنواة الخلية. تم أخذ عينات دم لقياس اليوريا والكرياتينين في اليوم الثاني واليوم الرابع عشر من إصابة الكلي الحاد الناتج من نقص التروية الكلوية واعادتها.

النتائج: أظهرت كلا من الخلايا الجذعية الوسيطة المشتقة من الحبل السري وحويصلاتها الدقيقة حمايه ضد إصابة الكلي الحاد تجلي في تحسين البناء النسيجى و إنخفاض معدل اليوريا والكرياتينين مع زياده مولد المضاد التكاثري لنواة الخلية في الأنابيب الكلوية وبدون اختلافات ذات دلالة احصائية بينهما.

الخلاصة: تشير هذه النتائج إلى القدرة المحتملة للحماية الكلوية لكلا من الخلايا الجذعية الوسطية المشتقة من الحبل السري و الحويصلات الدقيقة .