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Therapeutic Role of Mesenchymal Stem Cells in Cisplatin Induced Renal Failure in Adult Male Rats

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Abstract: Acute renal failure (ARF) is the rapid loss of the ability of the kidneys to remove waste and toxins (urea and creatinine) from the blood that are normally excreted in urine. This syndrome is still associated with high mortality and morbidity rates. Routine treatment involves dialysis and usually ultimately kidney transplant. This disease continues for the remainder of the patient's life creating a large drain on the health care system. Stem cell therapy holds a great promise for the repair of injured tissues and organs, including the kidney. Stem cells behave the unique ability to multilineage differentiation and self-renewal. This study was aimed to assess the potential therapeutic effect of bone marrow derived mesenchymal stem cells (MSCs) in male rats with Cisplatin induced renal failure. We used induced ARF model following intraperitoneal injection of Cisplatin (5mg/kg, for 10 days) into male *Sprague Dawley* rats, kidney functions were assessed as a markers for induced ARF.. Bone marrow derived mesenchymal stem cells were isolated and characterized then labeled using PKH26 red fluorochrome for tacking of stem cells and imaging kidney section following stem cell doses infusions. Sera were collected, kidney function tests were assessed pre-and post stem cells infusion. Kidneys were fixed, paraffin sections prepared, sectioned and stained. Results showed that: Kidney of rats injected with Cisplatin showed inflammatory leucocytic infiltration, hypertrophied glomeruli, tubular necrosis and congestion in the renal blood vessels. Moreover, the levels of urea and creatinine were elevated. Treating rats with MSCs revealed that kidney tissue displayed an improvement in the histological structure. Levels of urea and creatinine decreased. In Conclusion: The results of this study showed a significant improvement in kidney functions as well as renal tissues. These results reflect the therapeutic potential of mesenchymal stem cells in the treatment of acute renal failure.

Key words: Mesenchymal Stem cells · Renal failure · Cisplatin

INTRODUCTION

Acute renal failure (ARF),characterized by sudden loss of the ability of the kidneys to excrete wastes, with or without oliguria, conserve electrolytes and maintain fluid balance. ARF is a frequent clinical problem, affects 5-7% to 7% of hospitalized patients and up to 30% of patients in intensive-care settings, where it is associated with a mortality of between 50% and 80% [1]. ARF causes both apoptosis and necrosis of renal tubular epithelial cells. Over time, the injured tubules regenerate through cell proliferation, although the source of cells that repopulate the injured nephron is not clear [2].

Cisplatin (cis-diaminedichloroplatinum II) is one of the most effective chemotherapeutic agents and plays a major role in the treatment of a variety of human solid tumors including those of the head, neck, testis, ovary and breast [3-5]. Cisplatin induced nephrotoxicity manifested in ARF [6]. Several studies have suggested that free radical like superoxide radical play an important role in cisplatin-induced nephrotoxicity [7]. It has also been proposed that increase in lipid peroxidation in the kidney is also associated with cisplatin-induced nephrotoxicity and hence renal tissues degeneration [8].

The natural mechanism of tissue regeneration may result from proliferation of surviving dedifferentiated cells, from renal stem cells that reside inside the kidney and migrate to the site of regeneration, or from bone marrow cells that gain access to the injured epithelium and differentiate into mature cells [9].

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Recently, mesenchymal stem cells (MSC) are the preferred stem cells for cellular therapy of AKI. This is because MSCs are able to differentiate into cells of mesenchymal and non mesenchymal origin; ease of culture and ex vivo expansion; and pursue paracrine, anti-inflammatory, antiapoptotic and immunosuppressive effects [10,11].

Therapeutic effects of MSC have been tested both in animal models of AKI and patients. There is increasing evidence that MSC facilitate regeneration by resident kidney cells predominantly by delivering small molecules to the site of kidney injury. It is unlikely that the benefit of MSC is through transdifferentiation into renal tubular cells [12]. Where mouse and rat models for AKF induced using Cisplatin. After infusion of of MSC at the time of injury facilitated kidney regeneration by both paracrine effects and direct cellular incorporation, resulting in functional improvement [13- 15]. In patients suffering from renal failure it was reported also that bone marrow stem cells can contribute to the formation of kidney cells, including mesangial cells [16,17] tubular epithelial cells and podocytes [18] which gave rise to the hypothesis that some renal stem cells are resident in and mobilized from bone marrow. Therefore many researchers initially tried to identify renal stem cells extrarenaly within bone marrow or the circulation and many experiments using bone marrow transplantation of marked donor cells were performed to trace their progenies.

The massive results of the investigators world wide confirmed the mechanistic possibilities for the therapeutic effects of stem cells include fusion with resident organ cells [19], immunomodulation [20] and paracrine mechanisms elicited through trophic mediators [21] that result in the inhibition of fibrosis and apoptosis, enhancement of angiogenesis, stimulation of mitosis and proliferation and differentiation of organ-intrinsic precursor or stem cells. Therefore, this study was designed to shed more light on the therapeutic potentials of mesenchymal stem cells in Cisplatin induced nephrotoxicity rats.

MATERIALS AND METHODS

Experimental Animals: The study was carried on 40 Sprague-Dawley male albino rats, of an average weight (170 – 200 g). Rats were bred and maintained in an air-conditioned animal house with specific pathogen free conditions and were subjected to a 12:12-h daylight/darkness and allowed unlimited access to standard laboratory diet and water. All the ethical protocols for animal treatment were followed and supervised by the Animal House Facility, Zoology Department, Faculty of Science and Al Azhar University. They were divided into 3 groups as follow: I. Control group: including 10 rats were fed with normal rat diet and water then sacrificed (by co2 narcosis) at the time of study termination. II. Cisplatin group: including 20 rats were injected intraperitoneal with 5mg/kg Cisplatin for 10 days. Ten rats of this group were sacrificed (by co2 narcosis) after 72 hours of induction of the acute renal failure to obtain renal tissue specimens and heart blood samples were drawn for assessment of Creatinine and Urea. III. MSCs Group: 10 rats; with induced ARF received MSCs via caudal vein, after 10 days. All rats of this group were sacrificed (by co2 narcosis) after 10-days of MSCs infusion to obtain renal tissue specimens and heart blood samples were drawn for assessment of Creatinine and Urea.

Preparation of BM-derived Mesenchymal Stem Cells from Rats: Bone marrow was harvested by flushing the tibiae and femurs of 6-week-old male white albino rats with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine serum (GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia)] and resuspended in complete culture medium supplemented with 1% penicillin–streptomycin (GIBCO/BRL). Cells were incubated at 37°C in 5% humidified CO2 for 12–14 days as primary culture or upon formation of large colonies. When large colonies developed (80–90% confluence), cultures were washed twice with phosphate buffer saline(PBS) and the cells were trypsinized with 0.25% trypsin in 1mM EDTA (GIBCO/BRL) for 5min at 37°C. After centrifugation, cells were re-suspended in serum supplemented medium and incubated in 50 cm2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures [22].

Cytometry:

In Situ Labeling of MSCs with PKH26: MSCs were harvested during the 4th passage and labelled with a fluorescent dye PKH26, which is a red fluorochrome. It has excitation (551nm) and emission (567 nm) characteristics compatible with Rhoda mine or phycoerythrin detection systems. This process allows qualitative analyses that indicates cell homing in renal tissues. The linkers are physiologically stable and show little to no toxic side-effects on cell systems. Labeled cells retain both biological and proliferating activity and are ideal for *in vitro* cell labeling, *in vitro* proliferation studies

and long, *in vivo* cell tracking. In the current work, MSCs were labeled with PKH26 from Sigma Company (Saint Louis, Missouri USA). Cells were centrifuged and washed twice in serum free medium. Cells were pelleted and suspended in dye solution. Cells were injected intravenously into rat coude vein. After 40 days, kidney was examined with a fluorescence microscope to detect and trace the cells. MSCs homing were detected in kidney tissue after its labeling with PKH26 dye by fluorescent microscope to detect its red fluorescence [23].

Flow Cytometry & Phenotypic Analysis of Bone Marrow-derived MSCs: Flow cytometry of a cell suspension carried out according to method of Yeung and his coworkers [24] while losing the relationship between cytochemistry and morphology, samples up to 1×10^7 cells, can measure multiple cellular constituents and activities [25,26] and enables correlation of these measurements with other cellular parameters, such as cell size, lineage and viability [27]. Passage 3-5 cells cultured cells were used for the analysis of cell surface molecule pattern. The cells were washed with PBS, detached with trypsin/EDTA, suspended in complete culture media and analyzed [28]. Controls as well as MSCs (3×10^5 per sample) were incubated with primary antibodies in 10x dilution on ice in the dark for 20 min. Cells were washed, resuspended in 50 ml of PBS and vital staining with Hoechst 33258 (Molecular Probes, Carlsbad, CA) was carried out at room temperature for 10 min. Immunophenotyping analysis was performed against the following antigens: CD29, CD34 and CD90.

Biochemical Analysis: Samples of blood sera were collected and kidney functions assay were assessed. Serum urea and creatinine were determined according to the method of Butler [29] While serum uric acid was determined according the method of Corcostegui [30].

Histopathological Examination: Examination of sectioned renal tissue, stained by Haematoxylin and Eosine (H/E) was carried out to evaluate the histological architecture changes in the kidney tissues of animals with induced ARF and those infused with stem cells in comparison with the control animals [31].

Statistical Analysis: Data are presented in tables as means ± standard deviation (S.D.). Values were statistically analyzed by using SPSS 12 software package. The *P* values <0.05 were considered significant [32].

RESULTS

MSCs Culture, Identification & Homing: The isolated undifferentiated mesenchymal stem cells (MSCs) were cultured and reached 70-80 % confluence at 14 days (Figure 1A, B, C&D). MSCs were identified by surface marker CD45 (-ve) & CD44 (+ve) detected by flow cytometry (Figure 2) and CD29 (+) by PCR respectively. MSCs labeled with PKH26 fluorescent dye was detected in the renal tissues confirming that these cells homed into the kidney tissue (Figure 3).

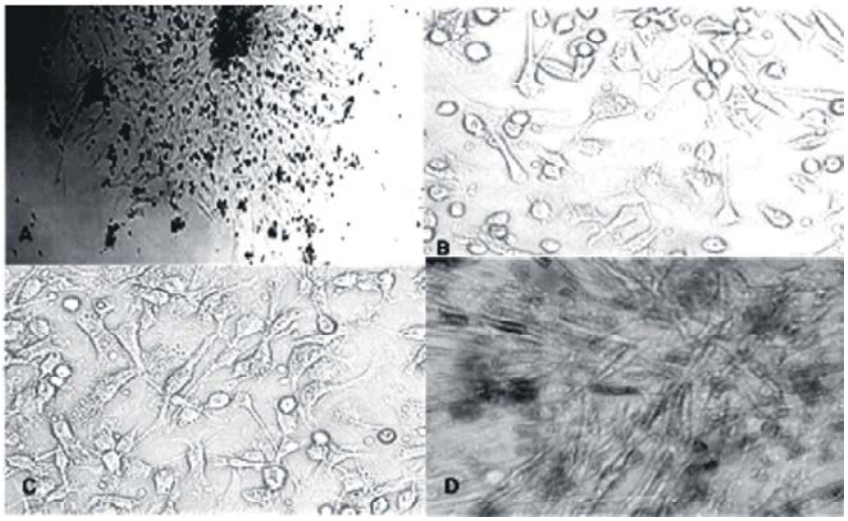

Fig. 1: Showing isolated mononuclear cells (A) (X200) (MSCs) cultured (B&C) (X400) and reached 70-80 % confluence at 14 days (D) (X400).

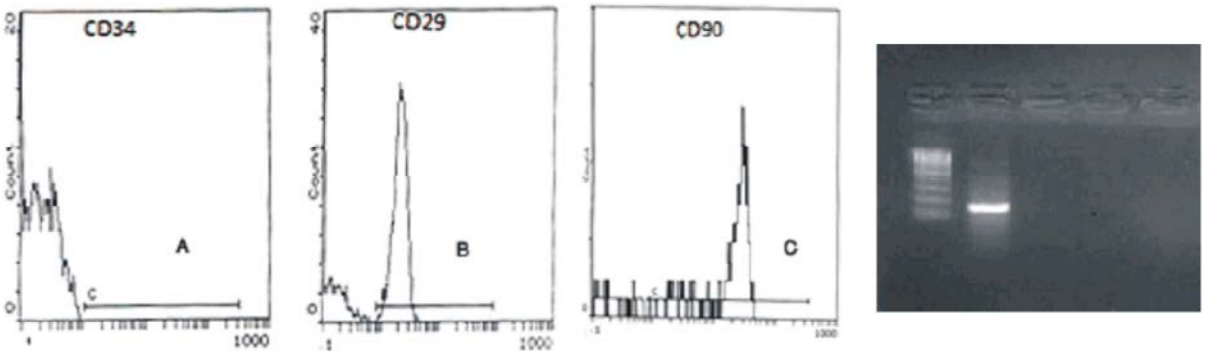

Fig. 2: Characteristics of BM-MSCs. Cells were stained with the CD34, CD29 & CD90 antibody and analyzed by flow cytometry. BM-MSCs are shown as the expression levels of CD34-ve (A), CD29 + ve (B) & CD90 + ve (C) of BM-MSCs are presented as a histogram. Surface marker characterizing BM-MSCs CD29 (+) detected by PCR.

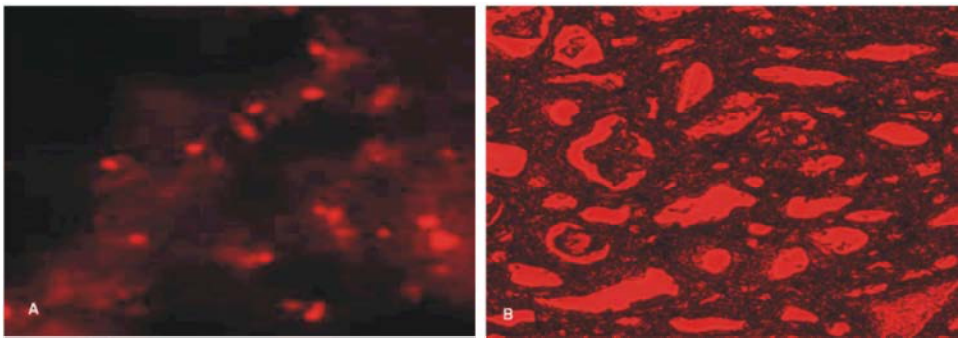

Fig. 3: MSCs labeled with PKH26 fluorescent dye was detected in the renal tissues confirming that these cells homed into the kidney tissue. PKH26 labelled cells showed strong red auto fluorescence after transplantation into rats. Thus, kidneys were sectioned and examined with fluorescent microscopy. (A) PKH26-stained BM-MSCs. (B) Representative image of a kidney transverse section showing PKH26-labeled MSCs clustered around the main branches renal vessels, renal corpuscles and renal tubules (X200).

Table 1: Showing kidney functions pre- and post- stem cell transplantation.

Kidney Functions	Control	Induced Acute Renal Failure	After Stem Cell Transplantation
Creatinine	0.65± 0.10	0.75 ± 0.10	0.78 ± 0.08
% change	--	+15%	+ 20%
Urea	17.85 ± 4.01	89 ± 15.94	47.22 ± 5.00
% Change	--	+ 398.50 %	164.53 %
Uric acid	1.60 ± 0.23	3.38 ± 0.30	2.17± 0.16 *
% Change	--	111.25 %	35.62 %

Data presented as means ± standard deviation (SD) & % = change from normal control.

Biochemical Analysis: Serum urea, creatinine and uric acid levels were measured in all study groups. In Cisplatin induced ARF group, urea, creatinine and uric acid levels were increased and the percentage of such increase reached (398.50%), (15.00%) and (111.25 %) respectively. While, after stem cell transplantation these parameters reached (165.53%), (20.00%) and (35.62 %) respectively. These changes were statistically significant ($P<0.05$) when compared with the control group. As shown in table (1).

Histopathological Changes: Histopathological examination of kidney tissue of kidneys from rats in control group, showed normal structure in the renal tubules, renal corpuscles as well as all renal cells and blood vessels as shown in plate (1 A&B).

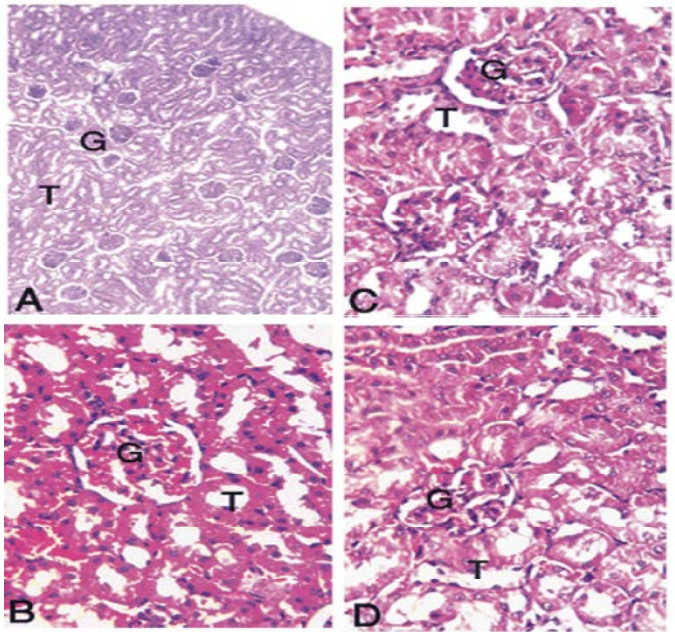

Plate 1: Showing kidney sections (A&B) from control rat group section shows normal renal architecture. While, (C&D) showing kidney sections from rats with ARF and injected with MSCs representing marked improvement in renal tissues which reflect the potential role of MSCs in tissue regeneration (x400). G: glomerulus, T: tubule. (A: X100; B,C&D: X400)

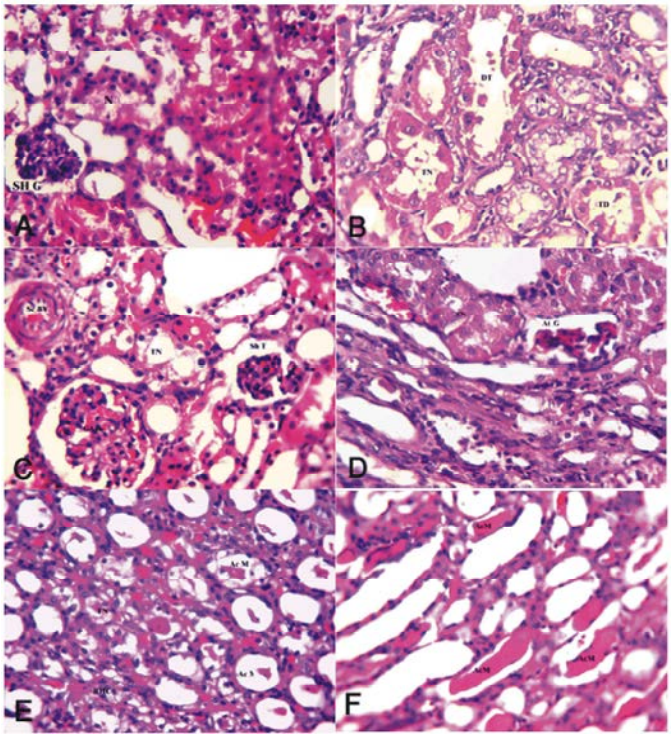

Plate 2: Showing kidney sections (A) from rats with ARF; sections represent necrosis (N) and shrinkage of glomeruli (SH G). (B) Tubular necrosis (TN) and tubular degeneration (DT). (C) Sclerotic blood vessel (Sc BV) and shrinkage tuft (Sh T). (D) Atrophied glomeruli (At G) and necrosis in the renal tissue (N). (E &F)Amyloidosis manifested by a cellular hyaline materials filled lumen of the tubules (AcM) and renal hypercelluarity and tubular necrosis. (X400).

While, examination of kidney tissue of Cisplatin induced ARF group showed necrotic changes and tubular degeneration in renal tubules, shrinkage glomeruli, lobulated tufts and glomerular atrophy. Referring to renal blood vessels appeared sclerotic with marked thickened wall. Amyloidosis appeared clearly in most of examined renal tissues manifested by a cellular hyaline materials and debris in the lumen of the renal tubules. Also, renal hypercelluarity and tubular necrosis were predominant as seen in plate (2 A, B, C, D and E&F). Referring to, kidney sections from rats with Cisplatin induced AKF then injected with MSCs and examined after 40 days. A significant improvement in the histological architecture of the renal tissues with decrease in the degenerative changes which reflects the homing potentials and transdifferentiation of the MSCs to repair the damaged renal epithelium as seen in plate (1 C&D).

DISCUSSION

Stem cell therapy holds a great promise for the repair of injured tissues and organs. Stem cells are undifferentiated cells that undergo both self-renewal and differentiation into one or more cell types. Stem cells have potential uses in therapies designed to repair and regenerate organs [33, 34]. For this purpose this study focused on using of BM-MSCs to repair renal tissues and functions renal repair following injury. However, the beneficial effects of MSCs occur through differentiation-independent pathways that include increased cell survival and proliferation, decreased inflammation and suppression of immune function [35].

In this study, rats were injected with Cisplatin resulted in ARF and showed discomfitures in the renal architecture with inflammatory leucocytic infiltration, hypertrophied glomeruli, tubular necrosis and congestion and sclerosis in the renal blood vessels. Moreover, the levels of urea uric acid and creatinine were elevated. Injecting rats with MSCs revealed that kidney tissue displayed an improvement in the histological structure. Levels of urea and creatinine were decreased.

The results of this study supported by several studies as this reported that MSCs are attractive candidates for renal repair, because nephrons are of mesenchymal origin and because stromal cells are of vital importance for signaling, leading to differentiation of both nephrons and collecting ducts [36]. In the present study, BM-MSCs were labelled and injected into rats to detect their possible anti-inflammatory, angiogenesis and homing potentials in amelioration of renal tissues and functions in experimental model, cells were at that time detected by fluorescent microscope. This result was in accordance to that reported by Morigi and his colleagues [37], who reported that BM-MSCs accelerate recovery of acute renal injury. BM-MSCs fused with renal cells and showed 50% replacement of proximal tubular cells with donor cells [38]. BM-MSCs may home to injured glomerular endothelium, differentiate into endothelial cells and participate in regeneration of the highly specialized glomerular microvasculature [39]. Also, bone-marrow-derived cells can replace injured mesangial cells [17,40]. These results are in agreement with our data, whereas BM-MSCs were homed into renal tissues of the rats as clearly showed by immunofluorescent examined kidney sections. Not only BM-MSCs transplantation improves both renal tissues and functions but also, adipose derived MSCs (AD-MSCs) are known for their anti-inflammatory properties and immune modulation. It also, proved their long-term safety and efficacy in treating kidney disease [41].

It well known that BM-MSCs homed into injured renal tissues after infusion via caudal vein [42], retaining MSCs in renal tissues could physically transdifferentiated to replace lost kidney cells [43]. MSCs express several growth and antiapoptotic factors such as VEGF, IGF-I, HGF and Bax protein all these factors known to improve renal function in renal failure models. One of the main therapeutic properties of MSCs is the paracrine action, via this action MSCs mediating their antiapoptotic, mitogenic and other cytokine actions, [43,44,45], which resulted in the renal down regulation of proinflammatory cytokines IL-1β, TNF-α and IFN-γ [46] and fibrogenic growth factors TGF-β [47] as well as iNOS. Also, it upregulation of anti-inflammatory, organ-protective IL-10 factors, as well as bFGF, TGF-α and Bcl- 2 [48]. In our study there were several histopathological lesions reported in the ARF rats and then after MSCs transplantation these lesions markedly improved. This finding agreed with Kawaida and Miller [49,50] who stated that HGF (Hepatocyte growth factor) prevents ARF and accelerates renal regeneration and recovery from acute ischemic renal injury in rats and mice, which also confirmed by results of Eirin and Lerman [51]. On clinical trials BM-MSCs are a promising therapeutic approach to ameliorate condition in chronic renal failure patients [52] and whom undergoing renal transplantation by lowering incidence of acute rejection, decreased risk of opportunistic infection and better estimated renal function at 1 year [53,54].

CONCLUSION

The results of this study showed a significant improvement in kidney functions as well as renal tissues. These results reflect the therapeutic potential of mesenchymal stem cells in the treatment of acute renal failure. Acute kidney injury one of the conditions that direct the attention of the scientist and although initial studies are promising, the long-term efficacy and safety of MSC infusion awaits further study to prove if this improvement transit or permanent.

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