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Therapeutic effect of mesenchymal stem cells in acute kidney injury: implication of inflammatory and oxidative stress pathways

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Acute kidney injury (AKI) is a multi-factorial and multi-system disorder which is clinically associated with a rapid loss of renal functions. It is described as one of the major contributors to mortality worldwide. Modern dialysis techniques and pharmacological therapy had no significant impact on overall mortalities caused by AKI. Hence, it becomes important to seek for other therapeutic interventions for AKI treatment. Stem cell replacement therapy has a great promise for AKI as it provides an approach for repairment of deteriorated organs and tissues. The main target of this research was to appraise the therapeutic outcome of bone marrow mesenchymal stem cells (BM-MSCs) and adipose-derived mesenchymal stem cells (AD- MSCs) against AKI in rat model. Adult male *albino* rats were equally randomized into nine groups: (1) Control, (2) Cisplatin, (3) Cisplatin+losartan, (4) Cisplatin+BM-MSCs ($1x10^6$ cells), (5) Cisplatin+BM-MSCs ($2x10^6$ cells), (6): Cisplatin+BM-MSCs ($4x10^6$ cells), (7) Cisplatin+AD-MSCs ($1x10^6$ cells), (8) Cisplatin+AD-MSCs ($2x10^6$ cells) and (9) Cisplatin+AD-MSCs ($4x10^6$ cells). The data showed significant enhancement in serum creatinine, urea, kidney injury molecule-1, tumor necrosis factor-α, monocyte chemoattractant protein-1, interleukin-18, macrophage inflammatory protein-2 and malondialdehyde levels. While, significant inhibition of catalase and superoxide dismutase enzymes activity in serum of rats administrated cisplatin has been registered. On the contrary, MSCs transplantation could significantly recover renal functions, suppress the inflammatory markers, repress the oxidative stress marker and promote the anti-oxidative enzymes activity. The histopathological findings illustrated that MSCs have regenerative potentiality as manifested by minimizing the inflammatory cells infiltration and congested glomerular capillaries. Conclusively, MSCs therapy has a favorable impact in lessening kidney derangement after AKI through monitoring the inflammatory response and restoring oxidant/antioxidant homeostasis.

Keywords: AKI, Cisplatin, Inflammation, Oxidative stress, MSCs.

INTRODUCTION

Acute kidney injury (AKI) is a life- threatening condition as it is associated with high morbidity, mortality, and healthcare costs (Zuk and Bonventre, 2017). AKI mainly occurred in patients with advanced age, diabetes or vascular diseases (Tögel and Westenfelder, 2012). The impact of AKI has been estimated to expand dramatically

and to date no resolutive therapies exist. AKI is characterized by acute damage in renal tubules with a rapid aberration in kidney functions. Inflammation, oxidative stress and excessive deposition of extracellular matrix are considered as the molecular events that ultimately lead to the end stage renal disease (Bianchi et al., 2014).

Inflammation is thought to play a principle role in AKI pathophysiology (Du and Zhu, 2014). It is hypothesized that the initial insult in ischemia, sepsis and nephrotoxic AKI models results in morphological and/or functional changes in vascular endothelial cells and/or in tubular epithelium. Then, leukocytes infiltrate into the injured kidneys including neutrophils, macrophages, natural killer cells, and lymphocytes. This injury induces inflammatory mediators generation like cytokines and chemokines by tubules and endothelial cells which implicate to infiltration of leukocytes into the kidneys. Thus, inflammation plays a principle role in the initiation and extension phases of AKI (Akcay et al., 2009).

Oxidative stress represents a classical mechanism that is involved in early inflammation. Reactive oxygen (ROS) and nitrogen species (RNS) have been implicated in AKI pathogenesis. Superoxide anion ($O_2^{\bullet-}$), nitric oxide ($^{\bullet}NO$) and hydrogen peroxide (H_2O_2) are generated during kidney injury, the interaction between these species can generate peroxynitrite ($ONOO^-$), which is a key oxidant factor that is directly involved in protein oxidation and renal failure (Goligorsky et al., 2002).

Dopamine, furosemide, mannitol, calcium channel blockers, and several other hormones or pharmacologic therapy proved to be effective in experimental models but almost failed in clinical protocols (Grino, 1994). The current supportive therapy has some improvement but the mortality and morbidity rate as consequence of AKI is still high (Bianchi et al., 2014). Hence, searching for a substitutional therapy that is clinically effective and safe enough to improve survival outcomes for patients with AKI is deemed necessary.

Stem cells derived from the adult tissue were engaged in both repair and regeneration of organs. Circumstantial evidence from human and mice indicated that adult stem cells might trigger the regeneration of deteriorated tissue. Mesenchymal stem cells represent a population of self-renewing and multi-potent cells that can be isolated from adult tissues (Peired et al., 2016). After infusion of MSCs, they engraft in the damaged tissue and release key factors that enhance cell survival and tissue repair as the paracrine/endocrine secretion of bioactive molecules is the main mechanism of action of MSCs in tissue regeneration (Bianchi et al., 2014). This means that MSCs can migrate to the sites of inflammation and exert anti-inflammatory effects through cell and cell interactions between them and lymphocytes or through production of soluble factors (Zhao et al., 2014). This process was driven by the binding between the chemokines released by the injured sites and the receptors expressed by MSCs.

The oxidant/antioxidant homeostasis may be modulated by infusion of MSCs in post-ischemic kidneys. Zhuo et al., (2011) commented that MSCs infusion improved the activity of superoxide dismutase (SOD) significantly, a critical molecule responsible for reducing oxidative stress, and increased glutathione peroxidase (GSH-Px) expression, a potent antioxidant enzyme, in kidney. Treatment with MSCs also results in a significant reduction in malondialdehyde (MDA) levels, which is associated with renal injury. Moreover, de Almeida et al., (2013) noticed that MSCs can regulate the oxidant/antioxidant balance after renal injury *via* heme oxygenase-1 (HO-1) and erythropoietin (EPO), both of them contribute to lower oxidative stress and to functional renal recovery (de Almeida et al., 2013).

The purpose of this study was to elucidate the therapeutic effectiveness of BM-MSCs and AD-MSCs injected in different doses in antagonizing cisplatin- induced AKI in rats in order to facilitate the development of MSCs therapy in AKI patients. Also, the aim of the study was extended to gain better understanding of the anti-inflammatory and antioxidant capacity of MSCs in manipulating AKI.

MATERIALS AND METHODS

A- Preparation of bone marrow- MSCs (BM-MSCs)

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 re-suspended in complete culture medium supplemented with 1% penicillin–streptomycin (GIBCO/BRL). Cells were incubated at 37°C in 5% humidified CO_2 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; GIBCO/BRL) and the cells were trypsinized with 0.25% trypsin in 0.01% EDTA (GIBCO/BRL) for 5 min at 37°C. After centrifugation, cells were resuspended in serum supplemented medium and incubated in 50 cm^2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures (Abdel Aziz et al., 2007).

B- Preparation of adipose tissue derived MSCs (AD- MSCs)

Adipose tissue was excised from both the omentum (i.e., abdominal) and the inguinal fat pad (i.e., subcutaneous) of male *albino* rats under general anesthesia according to Tomiyama et al. (2008). The adipose tissue was resected and placed into a labeled sterile tube containing 15 mL of a phosphate-buffered saline (PBS; GIBCO/BRL). Enzymatic digestion was performed using 0.075% collagenase II (Serva Electrophoresis GmbH, Mannheim, Germany) in Hank's balanced salt solution for 60 min at 37°C with shaking. Digested tissue was filtered and centrifuged, and erythrocytes were removed by treatment with erythrocyte lysis buffer. The cells were transferred to tissue culture flasks with DMEM supplemented with 10% FBS and, after an attachment period of 24 hours, non-adherent cells were removed by a PBS wash. Attached cells were cultured in DMEM media supplemented with 10% FBS, 1% penicillin-streptomycin (GIBCO/BRL), and expanded *in vitro*. When large colonies of AD-MSCs developed (80-90% confluence), cultures were washed twice with PBS and the cells were trypsinized with 0.025% trypsin and 0.01% EDTA in PBS for 5 min at 37°C. After centrifugation, cells were resuspended with serum-supplemented medium and incubated in 50 cm^2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures (Alhadlaq and Mao, 2004).

C- Identification of mesenchymal stem cells

Microscopic follow up and photo-documentation

The MSCs in the culture were characterized by their adherence to the plate (plastic adherent ability) and spindle shapes (elongated shape).

Detection of gene expression of cell surface markers (CD29, CD166, CD34 and CD45)

RNA extraction from MSCs

In order to confirm that the isolated cells from both bone marrow and adipose tissues are MSCs, total RNA was extracted from cultured cells using RNeasy mini kit for purification of total RNA from cultured cells (Qiagen, Germany) according to the manufacturer's instructions.

Reverse Transcription

The reverse transcription was carried out using complementary DNA (cDNA) reverse transcription kit to convert total RNA into cDNA (Applied Biosystems, USA) according to the manufacturer's protocol.

Conventional PCR detection of cell surface markers (CD29, CD166, CD34 and CD45) gene expression

The conventional PCR reaction mixture for CD29, CD166, CD34 and CD45 was 12.5 µL of master mix (Qiagen, Germany), 1 µL of forward primer, 1 µL of reverse primer (Invitrogen, USA), 5 µL cDNA and 5.5 µL nuclease free water. PCR was performed using the thermal cycler (Biometra T professional, USA). The primer sequences for the target genes and the PCR conditions are illustrated in Table (1). The PCR product was separated by electrophoresis through 2% agarose gel, stained with ethidium bromide, and photographed using gel documentation apparatus.

D- Animals

Model creation and cell transplantation

Ninty adult male *albino* rats, with weights ranging from 220 g to 250 g, were obtained from a breeding stock maintained in the Animal House of the National Research Centre, Dokki, Giza, Egypt. The animals were housed in transparent plastic cages with wood shavings at a freely ventilated and naturally illuminated room at a constant temperature of 22 ± 0.5ºC. They were fed with standard rat diet and water provided *ad libitum*. The animals were allowed to acclimatize in their new environment for ten days before the commencement of the experiment. AKI was induced in the rats by intraperitoneal injection of cisplatin in a dose of 6 mg/Kg b.wt (Sigma Chemical Co.) (Nemmar et al., 2010). To investigate the therapeutic impact of BM-MSCs and AD-MSCs in animal model of AKI induced by cisplatin, the rats were assigned into nine groups (n=10): The control group; rats received a single intraperitoneal injection of saline. The cisplatin group; rats received a single intraperitoneal

injection of cisplatin. Cisplatin + losartan group; rats received intraperitoneal single dose of cisplatin and then they were treated with losartan (Sigma Chemical Co.) in a dose of 10 mg/kg orally for two months (Rastghalam et al., 2014). Cisplatin + BM-MSCs ($1x10^6$ cells/rat) group; rats received intraperitoneal single dose of cisplatin and then they were infused with BM-MSCs ($1x10^6$ cells/rat) by tail vein (Shaohua and Dongcheng, 2013). Cisplatin + BM-MSCs ($2x10^6$ cells/rat) group; rats received intraperitoneal single dose of cisplatin and then they were infused with BM-MSCs ($2x10^6$ cells/rat) by tail vein (Tögel et al., 2007). Cisplatin + BM-MSCs ($4x10^6$ cells/rat); rats received intraperitoneal single dose of cisplatin and then they were infused with BM-MSCs ($4x10^6$ cells/rat) by tail vein (Nakamura et al., 2000). Cisplatin + AD-MSC ($1x10^6$ cells/rat) group; rats received intraperitoneal single dose of cisplatin and then they were infused with AD-MSCs ($1x10^6$ cells/rat) by tail vein (Chen et al., 2011). Cisplatin + AD-MSCs ($2x10^6$ cells/rat) group; rats received intraperitoneal single dose of cisplatin and then they were infused with AD-MSCs ($2x10^6$ cells/rat) by tail vein (Yao et al., 2015). Cisplatin + AD-MSCs ($4x10^6$ cells/rat) group; rats received intraperitoneal single dose of cisplatin and then they were infused with AD-MSCs ($4x10^6$ cells/rat) by tail vein. All animals were sacrificed after two months of stem cells transplantation.

Urine, blood and tissue sampling

At the end of the intervention period, 24 hours urine samples were collected *via* metabolic cages. Then, the diets were withheld from the experimental rats for 12 hours and the blood samples were withdrawn using a retro-orbital puncture under diethyl ether anesthesia. Blood samples were centrifuged under cooling (4ºC) at 1800 ×*g* to separate sera. Serum samples were transferred to 1.5 mL of eppendorf tubes and stored at -20º C pending further analysis. After that, one kidney from each animal was harvested, rinsed with saline and fixed overnight in 10% formal saline for histological investigation.

E- Experimental setting

The experimental protocol was carried out according to the approval and guidelines given by the National Research Centre Ethical Committee which also conformed to the acceptable guidelines on the ethical use of animals in research.

F- Biochemical assays

Serum levels of creatinine, urea, catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) were quantified enzymatically using commercially available kits provided by Bio-Diagnostic Company, Giza, Egypt following the methods described by Tietz, (1995); Chaney and Marbach, (1962); Aebi (1984); Nishikimi et al., (1972) and Satoh (1978), respectively. Levels of urinary kidney injury molecule-1 (KIM-1), urinary interleukin 18 (IL-18), serum tumor necrosis factor (TNF)-α, serum monocyte chemoattractant protein (MCP)-1 and serum macrophage inflammatory protein (MIP)-2 were assessed by enzyme linked immunosorbent assay (ELISA) using commercially available kits provided by Glory Science Co. USA under the guidance of the manufacturer.

G- Histopathological procedures

The fixed kidney specimens were dehydrated in ascending grades of ethanol cleared in xylol and then embedded in molten paraffin wax. The paraffin blocks were cut into 5 µm thick slices using rotary microtome and then stained with hematoxylin and eosin (H&E). After staining, the slides were viewed with an Olympus CH (Japan) light microscope. The image capturing was performed with a Sony DSCOW 3 digial Camera (Japan) and photomicrograph calibration was done with image J (Abramoff et al., 2004).

H- Statistical analyses

All data were collected, tabulated and statistically analyzed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA). The experimental results were represented as arithmetic means with their standard deviations. Least significant difference (LSD) was used to compare significance between groups. Difference was considered highly significant when P-value was < 0.05.

RESULTS

Mesenchymal stem cells validation

Mesenchymal stem cells morphology

The microscopic images of BM-MSCs and AD-MSCs are represented in the following figures: Fig. (1) shows the spindle shape of BM- MSCs at the 14 day of isolation and culture. Fig. (2) shows the spindle shape of AD-MSCs at 14 days.

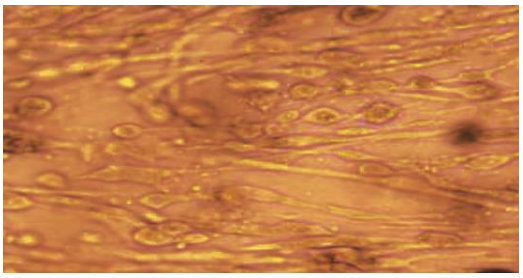

Figure. (1) Morphology of BM-MSCs.

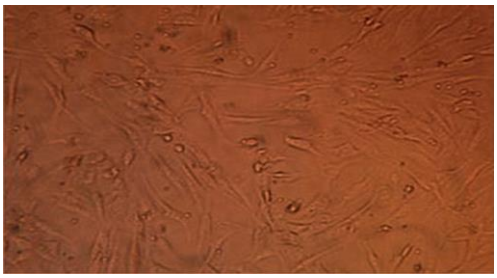

Figure. (2) Morphology of AD-MSCs.

Gene expression of cell surface markers

BM-MSCs

To investigate the expression of BM-MSCs surface markers (CD29, CD166, CD34 and CD45), RNA was isolated from the cultured cells and then cDNA was synthesized. Then, PCR was performed using the primers described in Material and Methods section. The agarose gel electrophoresis showed that the isolated BM-MSCs in the present study were positive for CD29 (Lane 2) and CD166 (Lane 3), and negative for CD34 (Lane 4) as well as CD45 (Lane 5) which are cell-surface markers associated with hematopoietic MSCs. Lane 1 represents ladder (100 bp). Lane 6 represents β-actin, Fig. (3).

AD-MSCs

The expression of AD-MSCs surface markers (CD29, CD166, CD34 and CD45) was detected through isolation of RNA from the cultured cells followed by cDNA synthesis. Then, PCR was conducted using the specific primers for each surface marker. The agarose gel electrophoresis showed that the AD-MSCs were positive for CD29 (Lane 2) and CD166 (Lane 3) genes expression. While negative for CD34 (Lane 4) and CD45 (Lane 5) genes expression. Lane 1 represents ladder (100 bp). Lane 6 represents β-actin, Fig. (4).

Biochemical findings

Table (2) listed the influence of BM-MSCs and AD-MSCs transplantation on the levels of serum creatinine and urea as well as urinary KIM-1 in AKI rat model. The results illustrated that creatinine, urea and KIM-1 levels are significantly ($P< 0.05$) elevated in cisplatin group as compared to control group. On the opposite side, all the treated groups (Losartan- treated group and MSCs- treated groups) experienced significant ($P< 0.05$) depletion in their levels versus cisplatin group. Of note, there is significant ($P< 0.05$) decline in creatinine, urea and KIM-1 levels in MSCs- treated groups (BM-MSCs and AD-MSCs) relative to losartan- treated group. Interestingly, the decline in serum creatinine and urinary KIM-1 levels in AD-MSCs- treated groups is more pronounced than BM-MSCs- treated groups. While the reduction in urea level is more prominent in AD-MSCs (4×10^6)- treated group than BM-MSCs (4×10^6)- treated group.

Table (3) depicted the results of the influence of BM-MSCs and AD-MSCs transplantation on the inflammatory markers in AKI rat model. TNF-α, IL-18, MCP-1 and MIP-2 levels revealed significant ($P< 0.05$) increase in cisplatin group with respect to the control group. Meanwhile, they are significantly ($P< 0.05$) decreased in all treated groups when compared with cisplatin group. Notably, the suppression of their levels in all MSCs- treated groups is more significant relative to losartan- treated group. Noteworthy, the depletion of urinary IL-18 level is more obvious in AD-MSCs- treated groups than in BM-MSCs-treated groups. While, the fall in serum MIP-2 level is more significant in AD-MSCs (1×10^6)-treated group than BM-MSCs- (1×10^6) treated group.

The data in **Table (4)** represented the influence of BM- MSCs and AD- MSCs transplantation on the oxidant/ antioxidant mediators in AKI rat model. Serum MDA level is significantly enhanced ($P<0.05$) in cisplatin group as compared to the control group. In contrast serum MDA level is significantly ($P<0.05$) blunted in all treated groups versus cisplatin group. There is also significant ($P< 0.05$) decline in serum MDA level in BM-MSCs and AD-MSCs- treated groups compared to losartan- treated group. It is relevant to note that serum MDA level is decreased more significantly in AD-MSCs- treated groups than BM-MSCs- treated groups. Serum CAT and SOD enzymes activity is significantly inhibited in cisplatin group in respect to control group. Conversely, they were significantly amplified in all treated groups when compared with cisplatin

group. There are also significant (P< 0.05) increase in CAT and SOD enzymes activity in BM-MSCs and AD-MSCs- treated groups versus losartan- treated group. It would be pertinent to mention that the activity of these enzymes are increased more significantly in AD-MSCs- treated groups than BM-MSCs treated groups.

To evaluate the favorable impact of BM-MSCs and AD-MSCs transplantation on the severity of acute kidney injury, histopathological examination based on the typical microscopic features of kidney tissue sections was adopted **(Fig.5).**

Histopathological observations

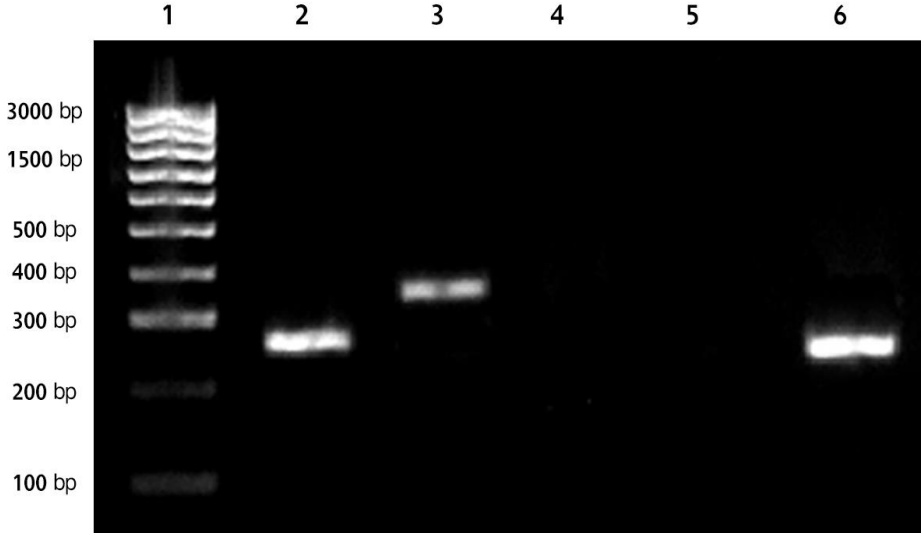

Figure. (3): RT-PCR analysis for CD29, CD166, CD34 and CD45 genes expression for BM-MSCs.

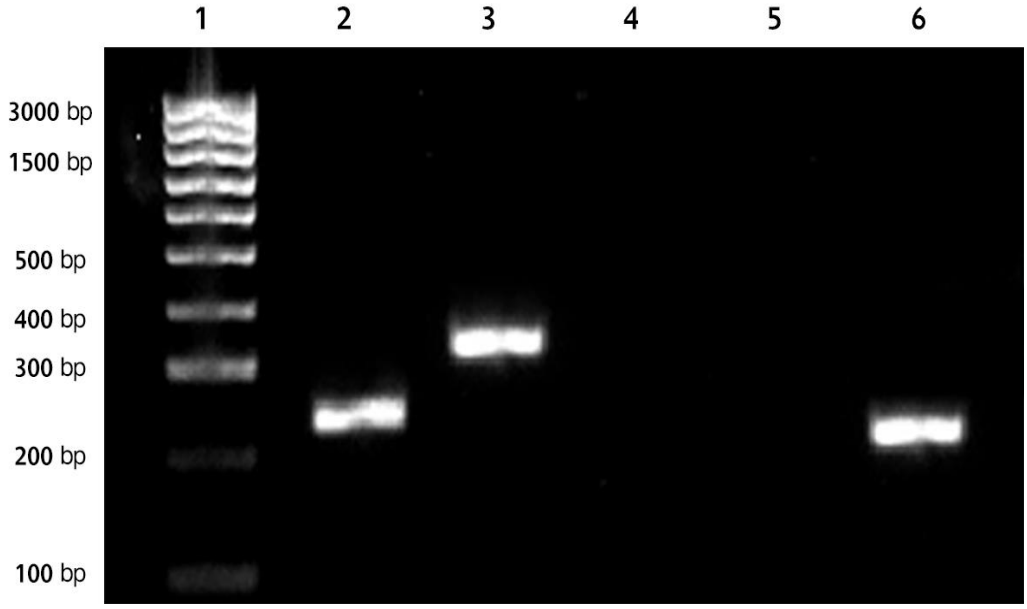

Figure. (4): RT-PCR analysis for CD29, CD166, CD34 and CD45 genes expression for AD-MSCs.

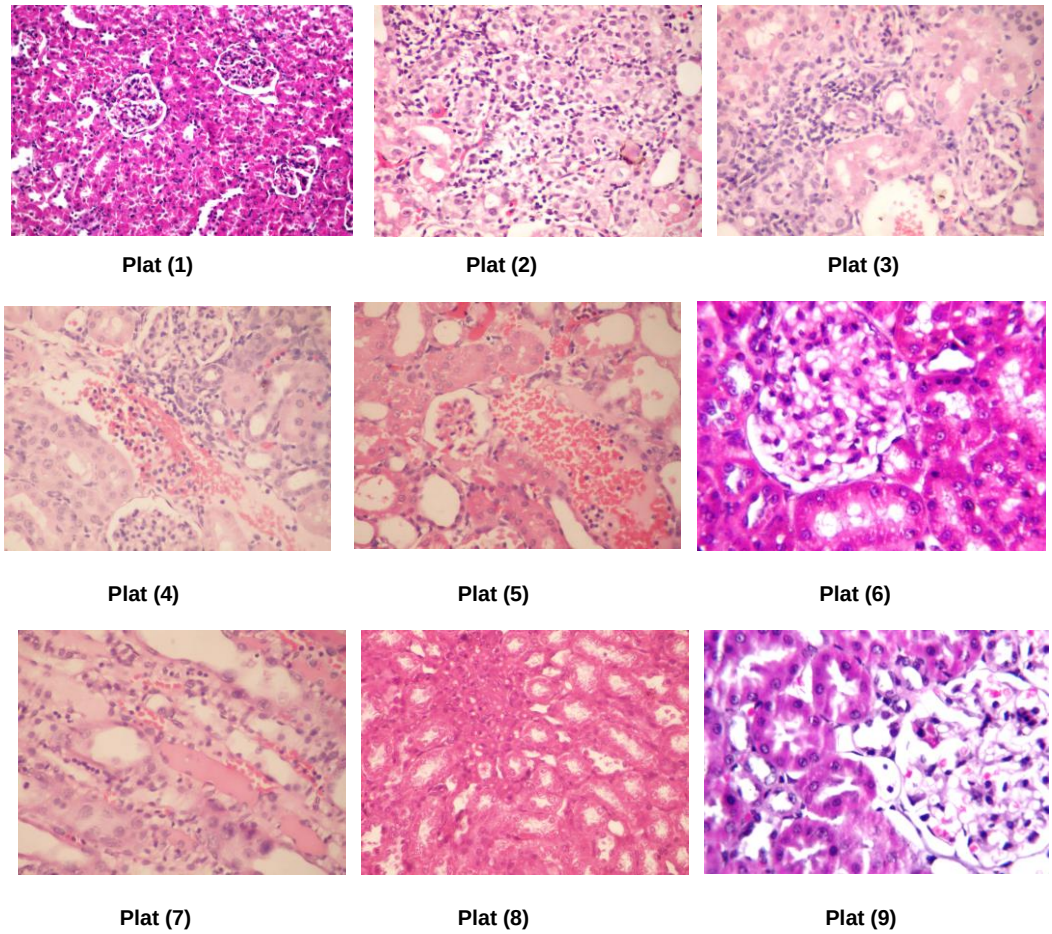

Fig. (5): Transverse section through the kidney demonstrating histopathological changes in all studied groups. Plat (1): Control group showing normal histological structure of the glomeruli and tubules (H&E ×40).Plat (2): Cisplatin group showing marked inflammatory infiltrate involving most of the renal tubules with interstitial exudates (H&E ×40). Plat (3): Cisplatin + Losartan group showing large number of mononuclear cells infiltrates many tubules with congested glomerular capillaries (H&E ×40). Plat (4): Cisplatin + BM- MSCs (1×10^6) group showing moderate inflammatory infiltrate involving group of tubules with the adjacent glomerulus, congested blood vessels associated with interstitial hemorrhage and exudates (H&E ×40). Plat (5): Cisplatin + BM-MSCs (2×10^6) group showing shrunken glomerulus with wide Bowman' space. Congested blood vessels, interstitial hemorrhage and exudate as well as few inflammatory cells (H&E ×40). Plat (6): Cisplatin + BM-MSCs (4×10^6) group showing no histological alterations (H&E ×40). Plat (7): Cisplatin + AD-MSCs (1×10^6) group showing congested blood vessels, hyaline casts and minimal inflammatory infiltrate (H&E ×40). Plat (8): Cisplatin + AD-MSCs (2×10^6) group showing localized collection of inflammatory cells and histiocytes surrounded by regenerated tubules (H&E ×40). Plat (9): Cisplatin + AD- MSCs (4×10^6) group showing well-formed glomeruli and tubules with no histological alteration (H&E ×40).

Table (1): Primer sequences of the target genes used for PCR and the PCR conditions

Genes	Primer sequences	PCR conditions	References
CD29	**F:** AATGTTTCAGTGCAGAGC **R:**TTGGGATGATGTCGGGAC	94°C for 30 s, 35 cycles at 57°C, 72ºC for 30 s	Wang et al., (2004)
CD166	**F:**GCTCCCCAGTATTTATTGCCTTC **R:**GTAGCACCT TTCCATTCCTGTA	94°C for 30 s, 35 cycles at 58°C, 72ºC for 30 s	Tan et al., (2013)
CD34	**F:** GCCCAGTCTGAGGTTAGGCC **R:**ATTGGCCTTTCCCTGAGTCT	94°C for 30 s, 35 cycles at 55°C, 72ºC for 30 s	Qin et al., (2011)
CD45	**F:**ACCAGGGGTTGAAAAGTTTCAG **R:**GGGATTCCAGGTAATTACTCC	94°C for 30 s, 35 cycles at 57°C, 72ºC for 30 s	Muñoz-Fernández et al., (2006)
β-actin	**F:** AGACCTTCAACACCCCAG **R:** CACGATTTCCCTCTCAGC	94°C for 30 s, 35 cycles at 56°C, 72ºC for 30 s	Qu et al., (2016)

Table (2): Influence of BM-MSCs and AD-MSCs transplantation on serum creatinine, urea and urinary KIM-1 levels in AKI rat model. Data were represented as Mean ± S.D of 10 rats/group.

Groups	Creatinine (mg/dL)	Urea (mg/dL)	KIM-1 (ng/L)
Control group	0.50 ± 0.02^{c}	43.14 ± 2.34 ^{c}	68.15 ± 2.73 ^{c}
Cisplatin group	2 ± 0.11^{d}	77.09 ± 3.96 ^{d}	119.66 ± 4.90 ^{d}
Cisplatin + losartan group	0.94 ± 0.02	64.94 ± 2.78	92.61 ± 4.40
Cisplatin + BM-MSCs (1×10^6) group	0.86 ± 0.019 ^{abe}	55.18 ± 1.2 ^{ab}	87.98 ± 2.00 ^{abe}
Cisplatin + BM-MSCs (2×10^6) group	0.77 ± 0.03 ^{abf}	48.9 ± 2.8 ^{ab}	84.17 ± 3.70 ^{abf}
Cisplatin + BM-MSCs (4×10^6) group	0.64 ± 0.016 ^{abg}	46.12 ± 3.14 ^{abg}	77.53 ± 2.36 ^{abg}
Cisplatin + AD-MSCs (1×10^6) group	0.78 ± 0.019 ^{ab}	51.42 ± 1.75 ^{ab}	84.88 ± 4.15 ^{ab}
Cisplatin + AD-MSCs (2×10^6) group	0.69 ± 0.020 ^{ab}	47.72 ± 2.05 ^{ab}	80.61 ± 2.88 ^{ab}
Cisplatin + AD-MSCs (4×10^6) group	0.56 ± 0.02 ^{ab}	44.90 ± 1.5 ^{ab}	72.66 ± 2.17 ^{ab}

a: Significant difference between each group and cisplatin group, **b**: Significant difference between each group and cisplatin + losartan group, **c**: Significant difference between control group and cisplatin group, **d**: Significant difference between cisplatin group and cisplatin + losartan group, **e**: Significant difference between cisplatin+ BM-MSCs (1×10^6) group and cisplatin+ AD-MSCs (1×10^6) group, **f** : Significant difference between cisplatin+ BM-MSCs (2×10^6) group and cisplatin+ AD-MSCs (2×10^6) group, **g**: Significant difference between cisplatin+ BM-MSCs (4×10^6) group and cisplatin+ AD-MSCs (4×10^6) group.

Table (3): Influence of BM-MSCs and AD-MSCs transplantation on the inflammatory markers (TNF-α, IL-18, MCP-1 and MIP-2) in AKI rat model. Data were represented as Mean ± S.D of 10 rats/group.

Groups	TNF-α (pg/mL)	IL-18 (pg/mL)	MCP-1 (pg/mL)	MIP-2 (pg/mL)
Control group	44.10 ± 2.81 [c]	63.71 ± 1.74 [c]	46.65 ± 4.16 [c]	6.71 ± 0.32 [c]
Cisplatin group	90.04 ± 3.81 [d]	124.33 ± 3.33 [d]	93.99 ± 2.95 [d]	24.36 ± 1.21 [d]
Cisplatin + losartan group	56.75 ± 1.99	89.50 ± 2.91	58.30 ± 1.72	15.92 ± 1.73
Cisplatin + BM-MSCs (1×10^6) group	54.43 ± 1.75 [ab]	78.56 ± 1.93[abe]	55.98 ± 1.74 [a]	11.90 ± 0.55 [abe]
Cisplatin + BM-MSCs (2×10^6) group	51.23 ± 1.97 [ab]	75.01 ± 2.24 [abf]	52.88 ± 2.59 [ab]	10.49 ± 0.47 [ab]
Cisplatin + BM-MSCs (4×10^6) group	48.55 ± 2.17 [ab]	72.14 ± 3.17 [abg]	49.93 ± 2.41 [ab]	8.89 ± 0.54 [ab]
Cisplatin + AD-MSCs (1×10^6) group	53.50 ± 2.29 [ab]	75.70 ± 1.33 [ab]	54.85 ± 2.48 [ab]	10.99 ± 0.54 [ab]
Cisplatin + AD-MSCs (2×10^6) group	49.70 ± 2.06 [ab]	71.01 ± 1.08 [ab]	51.45 ± 2.63 [ab]	9.94 ± 0.30 [ab]
Cisplatin + AD-MSCs (4×10^6) group	47.53 ± 2.05 [ab]	68.03 ± 1.29 [ab]	48.83 ± 2.42 [ab]	8.21 ± 0.38 [ab]

a: Significant difference between each group and cisplatin group, **b**: Significant difference between each group and cisplatin + losartan group, **c**: Significant difference between control group and cisplatin group, **d**: Significant difference between cisplatin group and cisplatin + losartan group, **e**: Significant difference between cisplatin+ BM-MSCs (1×10^6) group and cisplatin+ AD-MSCs (1×10^6) group, **f** : Significant difference between cisplatin+ BM-MSCs (2×10^6) group and cisplatin+ AD-MSCs (2×10^6) group, **g**: Significant difference between cisplatin+ BM-MSCs (4×10^6) group and cisplatin+ AD-MSCs (4×10^6) group.

Table (4): Influence of BM-MSCs and AD-MSCs transplantation on the oxidant/ antioxidant mediators in AKI rat model. Data were represented as Mean ± S.D of 10 rats/group.

Groups	MDA (nmoL/L)	CAT (U/mL)	SOD (U/mL)
Control group	5.56 ± 0.40 [c]	7.15 ± 0.50 [c]	3.38 ± 0.29 [c]
Cisplatin group	9.96 ± 0.28 [d]	2.99 ± 0.16 [d]	1.60 ± 0.13 [d]
Cisplatin + losartan group	7.20 ± 0.22	5.22 ± 0.29	2.03 ± 0.13
Cisplatin + BM-MSCs (1×10^6) group	7.05 ± 0.18 [ae]	5.40 ± 0.24 [ae]	2.12 ± 0.14 [ae]
Cisplatin + BM-MSCs (2×10^6) group	6.80 ± 0.16 [abf]	6.06 ± 0.31 [abf]	2.31 ± 0.13 [abf]
Cisplatin + BM-MSCs (4×10^6) group	6.54 ± 0.14 [abg]	6.26 ± 0.28 [abg]	2.82 ± 0.13 [abg]
Cisplatin + AD-MSCs (1×10^6) group	6.66 ± 0.21 [ab]	6.07 ± 0.22 [ab]	2.31 ± 0.15 [ab]
Cisplatin + AD-MSCs (2×10^6) group	6.26 ± 0.28 [ab]	6.43 ± 0.13 [ab]	2.62 ± 0.12 [ab]
Cisplatin + AD-MSCs (4×10^6) group	5.74 ± 0.36 [ab]	6.80 ± 0.16 [ab]	2.98 ± 0.15 [ab]

a: Significant difference between each group and cisplatin group, **b**: Significant difference between each group and cisplatin + losartan group, **c**: Significant difference between control group and cisplatin group, **d**: Significant difference between cisplatin group and cisplatin + losartan group, **e**: Significant difference between cisplatin+ BM-MSCs (1×10^6) group and cisplatin+ AD-MSCs (1×10^6) group, **f** : Significant difference between cisplatin+ BM-MSCs (2×10^6) group and cisplatin+ AD-MSCs (2×10^6) group, **g**: Significant difference between cisplatin+ BM-MSCs (4×10^6) group and cisplatin+ AD-MSCs (4×10^6) group.

DISCUSSION

Due to more people being affected and the accelerating importance of AKI, revealing the underlying mechanisms and developing efficient therapeutic approaches are essential. The target of the present research work was to investigate the potency of MSCs (BM-MSCs and AD-MSCs) in mitigating AKI induced by cisplatin in rats and to spotlight on the implication of the anti-inflammatory and anti-oxidative properties in the therapeutic action of MSCs.

In the present approach, significant elevation in serum creatinine level has been found in cisplatin group. The studies of Kawai et al. (2005) and Filipski et al., (2009) showed parallelism with our study. Cisplatin confers nephrotoxicity by decreasing GFR basically through damaging the S3 segment of the proximal tubules and the distal nephron (Arany and Safirstein, 2003). So as GFR decreases, serum creatinine level increases where serum creatinine concentration is well known to have inverse relationship with GFR (Lew and Bosch, 1991).

According to the results of our study, the losartan- treated group showed significant depression in serum creatinine level. The inhibitory effect of losartan on creatinine level observed in this study is in concert with the report of Kontogiannis and Burns (1998). Losartan belongs to angiotensin II blocker family that promotes dilatation of the efferent arterioles more selectively than the afferent arterioles and inhibits the degradation of bradykinin, which cause dilation of the efferent arterioles. This action of losartan leads to the decrease in intra-glomerular pressure and the improvement of glomerular

hyperfiltration (Kobori et al., 2013) which ultimately resulting in the enhancement of GFR and the reduction of serum creatinine level.

In the current investigation, mesenchymal stem cells- treated groups (BM-MSCs and AD-MSCs) experienced significant decline in serum creatinine level. This finding is in harmony with that of Qi and Wu (2013). The favorable impact of MSCs on serum creatinine level could be attributed to the amplification of GFR *via* insulin-like growth factor (IGF-1) secretion. IGF-1 has been found to increase renal blood flow and GFR (Bancu et al., 2016) which lead to the reduction of serum creatinine level as observed in the current results.

The tabulated results revealed significant increase in serum urea level in cisplatin group. This aberration in serum urea level as a consequence of cisplatin administration is comparable to the findings of Filipski et al., (2009) and Qi and Wu (2013). The transport of urea from blood is primarily done through five types of urea transporters (UTs) which enhance kidney permeability to the highly polar molecules like urea (Sands, 2003; yang and Bankir, 2005). It has been found that during severe inflammation as in the case of AKI, the down regulation of UTs has occurred. This is owing to the pro-inflammatory cytokines mainly TNF-α (Bucher and Taeger, 2002; Bucher et al., 2003). Cisplatin has been reported to trigger the production of TNF-α (Liu et al., 2006) which may down regulate UTs and hence inhibit urea transportation from the blood leading to increasing serum urea level.

Losartan treatment in the present investigation brought about significant reduction in serum urea level. This goes hand in hand with the results of Milan et al., (2016). Those investigators mentioned that losartan treatment aggravates renal blood flow and inhibits renal vascular resistance as well as increases urea clearance and so decreases serum urea level.

MSCs transplantation in the current research produces significant decline in serum urea level. This observation is in keeping with the previous findings of Filipski et al. (2009) and Kawai et al., (2006). MSCs infusion has been found to reduce TNF-α production (Sherif et al., 2015) which down regulates urea transporters. This leads to up regulation of urea transporters with consequent increase in renal urea excretion and a decrease in serum urea level.

The current results indicated that urinary KIM-1 level showed significant elevation in cisplatin group. This result is concordant with that of Tekce et al., (2015). Structurally, KIM-1 has a single transmembrane domain and undergoes membrane cleavage in proximal renal tubules leading to the release of soluble KIM-1 ectodomain into the urine. Urinary KIM-1 ectodomain is a sensitive and specific biomarker for AKI in humans (Zhang et al., 2007). KIM-1 cleavage is mediated by ERK activation, and that cleavage is enhanced by p38 MAPK. MAPKs have also been shown to motivate the expression and activation of many types of metalloproteinases (1, 2, 3, 9, 10, and 13), that also mediate KIM-1 ectodomain shedding in urine (Sylvester et al., 2004). It has been found that p38, MAPK and ERK are up regulated in AKI induced by cisplatin (Jo et al., 2005; Ramesh and Reeves, 2005). So, cisplatin causes urinary KIM-1 shedding *via* MAPK pathway.

In this study, losartan- treated group experienced significant suppression in urinary KIM-1 level. This finding is comparable to that of Waanders et al., (2009). MAPKs (p38- MAPK, c-Jun NH2-terminal kinase [JNK-1 and JNK-2], and extracellular signal- regulated kinases [ERK-1 and ERK-2]) have been reported to be incorporated in angiotensin II (AII) -induced kidney injury (Kumar et al., 2003). AII, a multifunctional cytokine of the renin-angiotensin system, acts on two main receptor subtypes (AT1 and AT2) to exert its physiologic effects (Assender et al., 1997; Campbell-Boswell and Robertson, 1981). Both AT1 and AT2 receptor subtypes were found to be the main actors in MAPKs activation (Alpert et al., 1992) which increase urinary KIM-1 shedding. Thus, the inhibition of AII by AII antagonist such as losartan leads to the inhibition of MAPK with consequent reduction in urinary KIM-1 level.

The present results indicated a significant decline in urinary KIM-1 level by MSCs transplantation. This result agrees with the study of Abouelkheir et al. (2016). It has been reported that infusion of MSCs results in a reduction of ERK phosphorylation and p38 phosphorylation (Qi and Wu, 2013). This leads to a decline in KIM-1 shedding in urine as shown in the present data. The findings of the current work revealed that urinary IL-18 level showed significant elevation in cisplatin group. This coincides with the studies done by Nozaki et al. (2012). IL-18 is a pro-inflammatory cytokine that is increased after ischemia-reperfusion (IR) injury, glycerol injection, and cisplatin-induced kidney injury (Homsi et al., 2006; Wu et al., 2008). Following stimulation of toll like receptor 4 (TLR4) by cisplatin (Arumugam et al., 2009), the activation of inflammasome leads

to the cleavage of pro-caspase 1 to caspase-1. This in turn leads to the cleavage of pro-IL-18 into the active IL-18 molecule (Charlton et al., 2014).

The data of this investigation recorded significant blunting in urinary IL-18 level in the group of rats treated with losartan. These results agree with the result of Marghani et al., (2017). The study of Sahar et al., (2005) mentioned that AII directly up regulate mRNA and amplified protein level of IL-18. Moreover, AII contributes significantly in renal diseases, *via* AT1R, by promoting inflammatory response (Benigni et al., 2010). AII can also act through AT2R, which has counter-regulatory action on AT1R (Ohkubo et al., 1997; Steckelings et al., 2005). The blockade of AT1R by angiotensin receptor blockers (ARBs), such as losartan, leads to a feedback loop resulting in an increase in free angiotensin II and stimulation of AT2R with consequent regression of the inflammatory response (Naito et al., 2010; Habashi et al., 2011). Therefore, the blockade of AT2R by losartan is accounted as a probable mechanism by which losartan could induce the decline of urinary IL-18 level. The present results registered significant drop in urinary level of IL-18 in the groups of rats subjected to MSCs transplantation. These results match those reported by Tögel et al., (2005) and Sherif et al. (2015). MSCs have the ability to significantly down regulate the expression of the pro-inflammatory cytokines like IL- 1β, TNF-α, IFN-γ and iNOS. Also, they are capable to up regulate the expression of the anti-inflammatory cytokines such as IL-10, bFGF and TGF-β in the kidney. During injury, MSCs migrate to the damaged site and this homing capability is driven by chemokines (stromal cell-derived factor-1/CXCR4 and CD44) released from the damaged site as well as from MSCs themselves (Zhu et al., 2006). At the site of injury, MSCs have the capacity to reprogram monocytes and macrophages to shift from a pro-inflammatory state to anti-inflammatory one. This shift is paralleled by the decreased levels of TNF-α, IL-1β, and IFN-γ (pro-inflammatory cytokines) which in turn reduce the inflammatory response. MSCs also can release a number of soluble factors such as IL-1, IL-10 and PGE2 to enhance the anti-inflammatory activity and decrease the pro-inflammatory action (Tögel et al., 2005).

The results of the current experiment showed significant elevation in serum TNF-α, MCP-1 and MIP-2 levels in cisplatin group. These findings fit similar to findings reported by Ueki et al. (2013). Cisplatin has been found to increase the degradation of IκB and enhance NF-κB binding activity. This effect of cisplatin on these critical factors leads to an increase in serum TNF-α level. TNF-α in turn stimulates the release of other cytokines and chemokines, such as MCP-1 and MIP-2 (Banas et al., 1999).

On the other hand, there were a significant decline in TNF-α, MCP-1 and MIP-2 in losartan-treated group. This agrees with the result of Amin et al., (2017). AII has a pro-inflammatory effect at the renal interstitium. Where AII stimulates inflammatory cells such as lymphocytes (Nataraj et al., 1999) and activates NF-κB in monocytes (Ruiz-Ortega et al., 1998). In kidney, AII stimulates secretion of chemokines and growth factors such as MCP-1 (Ruiz-Ortega et al., 1998). AII exerts a pro-inflammatory effect *via* AT1R (Benigni et al., 2010). AT1R is responsible for most of the physiological and pathological actions of AII, angiotensin II can also act through AT2R, which has counter-regulatory actions to AT1R (Steckelings et al., 2005). AT1R blockade by ARBs such as losartan might lead to a feedback loop that increases free AII, consequently resulting in the stimulation of AT2R and resulting in anti-inflammatory effects (Naito et al., 2010; Habashi et al., 2011). So, losartan may have a beneficial effect in inflammatory effects produced by cisplatin in AKI.

A significant decline in serum TNF-α, MCP-1 and MIP-2 levels has been registered in the groups of rats submitted to MSCs transplantation. MSCs exert immunomodulatory effect on immune cells through cell-to-cell contacts and *via* the secretion of cytokines, chemokines and growth factors (Bassi et al., 2012; Wang et al., 2012). Also, MSCs can inhibit different types of inflammatory cells, such as CD4+, CD8+, NK, B cells, macrophages, and dendritic cells, while they can stimulate other cell types like regulatory T cells to further reduce AKI-associated inflammation and restore renal functions (Bassi et al., 2012). The therapeutic impact of MSCs on kidney was confirmed by down regulation of pro-inflammatory molecules (TNF-α, IL-1α, IL-1β, IFN-γ and IL-6), adhesion molecules (ICAM-1) and chemokines (CXCL-2, MIP-2, G-CSF, GM-CSF, KC, MCP-1, MIP-3α, NGF-β and MSP) and up regulation of the anti- inflammatory mediators (IL-1 and IL-10) (Chen et al., 2011).

The data of the present experimental setting revealed significant increase in serum MDA level in cisplatin group as a final product of lipid peroxidation. This observation is in congruent with that of Khattab et al., (2004). Within the cell,

cisplatin is transformed into a highly active form that rapidly reacts with thiol-containing antioxidant molecules such as glutathione (Siddik, 2003). The depletion of glutathione leads to increased oxidative stress within the cells. Cisplatin may also cause mitochondrial dysfunction and increase ROS production through an impaired respiratory chain (Kruidering et al., 1997). Finally, cisplatin may induce ROS formation *via* the cytochrome P450 (CYP) system (Baliga et al., 1998).

Treatment with losartan in the present study leads to significant drop in serum MDA level. This finding is in parallel to that obtained previously by Khattab et al., (2004) and Milan et al., (2016). Where administration of losartan as an antioxidant could ameliorate this effect. It is documented that losartan prevents lipid peroxidation in renal tubules (Khattab et al., 2004). So it can reduce the production of oxygen free radicals.

Transplantation of MSCs in AKI rat model evoked significant reduction in serum level of MDA. This result correlates well with that of Zhuo et al., (2011). MSCs have the ability to secrete HO-1 and EPO, which are potent anti-oxidant candidates (Kim et al., 2010). In addition, molecules such as iNOS, eNOS and 8-OHdG which are associated with the release of free radicals, are significantly decreased after MSCs transplantation (Liu et al., 2012). Thus, the manipulation of these key events by MSCs transplantation is accountable for the regression of serum MDA level.

Serum antioxidant enzymes activity (SOD and CAT) recorded significant inhibition in cisplatin group as shown in the tabulated results. Cisplatin induces glucose-6-phosphate dehydrogenase and hexokinase activity, which in turn increase ROS production and decrease antioxidants activity (SOD and CAT) (Yilmaz et al., 2004). Cisplatin also increases intracellular Ca^{2+} level which activates NADPH oxidase activity that in turn stimulates ROS generation by damaged mitochondria (Kawai et al., 2006).

A significant stimulation of serum SOD and CAT activity has been observed in losartan-treated group in the current work. Ivanov et al. (2014) stated a positive effect of losartan on oxidative stress in the postischemic injured hypertensive kidney. AII is implicated in the generation of ROS. Also, the overproduction of AII during AKI (Kontogiannis and Burns, 1998) may up regulate the expression and activity of one of the major ROS generators (NADPH oxidase) (Rajagopalan et al., 1996). Therefore, blocking of AII by losartan leads to the reduction of lipid peroxidation and amplification of the antioxidative defense system (Milan et al., 2016).

MSCs transplantation in rats bearing AKI brought about significant stimulation of SOD and CAT activity in serum as registered in the current approach. This result agrees with the study of Zhuo et al. (2011). MSCs infusion has been found to significantly improve the activity of SOD, a key mediator responsible for reducing oxidative stress, and up regulate GSH-Px expression, a potent antioxidant enzyme, in renal tissues. MSCs transplantation reduces the molecules associated with the release of free radicals such as iNOS, eNOS and 8-OHdG (Zhuo et al., 2011; Liu et al., 2012). MSCs can also control the oxidant/antioxidant homeostasis after kidney injury, especially *via* HO-1 and EPO which are considered to be potent antioxidant molecules and contribute to lower oxidative stress (Vanella et al., 2012).

The extent of renal damage was confirmed by histopathologic examination of the kidney. Histological changes of the kidney in cisplatin group revealed acute tubular necrosis, marked inflammatory infiltrate involving most of the renal tubules and marked dilation of proximal convoluted tubules. The changes obtained in the present study run parallel with the studies documented by Goldstein and Mayor (1982); Borch (1987) and Silkemsen et al., (1997).

On the other side, losartan- treated group showed less damage as tubular dilatations were smaller and tubular necrosis was absent. While, still there was large number of mononuclear cells infiltrates many tubules and esinophilic casts in some tubules. These changes were in agreement with the reports of Zhibin et al., (2012) and Milan et al. (2016).

There was moderate inflammatory infiltrates, congestion of the glomuli and cast formation observed in cisplatin group treated by low dose of BM-MSCs ($1x10^6$). In group treated by moderate dose of BM-MSCs ($2x10^6$), there was congested blood vessels, few inflammatory cells and swelling of the tubular cells. While in cisplatin group treated with high dose of BM-MSCs ($4x10^6$), there were well-formed tubules. In group treated by low dose of AD-MSCs ($1x10^6$), congested blood vessels, hyaline casts and minimal inflammatory infiltrates were indicated. Moderate dose of AD-MSCs ($2x10^6$)showed inflammatory cells and swelling of the tubular epithelial cells. While, high dose of AD-MSCs ($4x10^6$) showed well-formed glomeruli and tubules with no histological

alterations. This comes in line with the results of Morigi et al., (2004, 2010).

CONCLUSION

Based on the aforementioned findings, it is reasonable to assume that mitigation of inflammatory response and oxidative stress may be the probable mechanisms by which MSCs can offer its therapeutic action against AKI in the experimental model. This suppression stems from the capacity of MSCs therapy to repress IL-18, TNF-α, MCP-1, MIP-2 and MDA levels as well as enhance the antioxidant enzymes activity (CAT and SOD). Also, the outcomes of the present study delivered an important insight into the superior effect of AD-MSCs than BM-MSCs in combating AKI in rats. The biological advantages of AD-MSCs are attributed to their ability to secrete growth factors, proliferate rapidly and modulate the immune response more than BM-MSCs. Finally, these pre-clinical findings justify the potential of MSCs against AKI and may be clinically beneficial to proceed for further clinical trials.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Dr. Rehab E. Selim participated in preparing the experiment, analyzing data and writing paper. Prof. Gilane M. Sabry participated in reading and criticizing the manuscript. Prof. Hanaa H. Ahmed participated actively in intervention of the scientific idea, conducting the necessary biochemical analyses, revising the paper critically. Prof. Somia H. Abd Allah accomplished this work in the part of stem cells. Dr. Rasha E. Hassan shared in editing and approving final version. Dr. Aziza B. Shalaby contributed in reading the paper and submission of manuscript to the journal. Dr. Nehal S. Abouhashem conducting the histopathological investigations.

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