Physiology and Pharmacology

Physiol Pharmacol 24 (2020) 133-141 **www.phypha.ir/ppj**

Original Article

Therapeutic effects of adipose-derived mesenchymal stem cells on gentamicin-induced renal failure

Leila Hafazeh[1], Saeed Changizi-Ashtiyani[1], Houshang Najafi[2], Faezeh Ghasemi[3], Tina Rahjoo[1], Saeid Babaei[4], Majid Ramezani[5]*

1. Department of Physiology, Arak University of Medical Sciences, Arak, Iran
2. Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
3. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
4. Department of Anatomy, School of Medicine, Arak University of Medical Sciences, Arak, Iran
5. Department of Internal Medicine, School of Medicine, Bagiatallah University of Medical Sciences, Tehran, Iran

Abstract

Introduction: Considering the limitations of conventional therapeutic methods in renal failure, researchers are paying attention to the application of adipose-derived mesenchymal stem cells (AD-MSCs) and their protective effects against acute renal failure. This study aims to assess the therapeutic effects of AD-MSCs in gentamicin-induced renal failure in rats.

Methods: In this study, 40 male Wistar rats were studied in control, sham, gentamicin treated with and without receiving AD-MSCs. After 10 days, blood samples were collected and hemodynamic parameters, malondialdehyde and ferric reducing antioxidant power (FRAP) measured in the right and left kidneys underwent histologic examination.

Results: Gentamicin administration significantly increased plasma creatinine, blood urea nitrogen, oxidative stress parameters and histologic damages; while significantly reduced FRAP in the gentamicin-receiving group in comparison with the sham group. AD-MSCs treatment significantly improved renal function parameters, oxidative stress and histologic damages in comparison with the gentamicin receiving group.

Conclusion: Intravenous injection of AD-MSCs in gentamicin-induced renal failure improved renal function, oxidative stress parameters and histologic damages.

http://dx.doi.org/10.32598/ppj.24.2.30

Keywords:
Acute renal failure;
Gentamicin;
Mesenchymal stem cells;
Oxidative stress

*** Corresponding author:**
M. Ramezani
Email:
dr.ramezani145@gmail.com

Received 30 August 2019; Received in revised form 1 February 2020; Accepted 12 February 2020

Introduction

Recognition and treatment of kidney disease has a long history in medical history and Persian medical practitioners have gained a high reputation in this field (Ashtiyani et al., 2009; Shamsi et al., 2014). Drug-induced nephrotoxicity is a major cause of renal failure (Pfister et al., 2018). Although

gentamicin is a beneficial aminoglycoside antibiotic in the treatment of gram-negative bacterial infections, it may lead to renal failure in 10-20% of patients in the treatment course via increasing excretion rate in the proximal tubule (Silan et al., 2007; Ateşşahin et al., 2003). Gentamicin is a highly used aminoglycoside antibiotic whose usage has been limited due to nephrotoxicity side effect (Ali et al., 2011). Approximately 5% of gentamicin dosage remains in epithelial cells of segment 1 and 2 of proximal tubule after glomerular filtration. Higher concentrations are mainly observed in lysosomal vacuoles, Golgi body and endosome. This accumulation leads to functional and structural alterations and cytotoxicity of epithelial cells in kidney cortex, proximal and distal tubules and collecting ducts (Helal et al., 2018). Additionally, gentamicin binds to membrane phospholipids and results in phospholidosis via inhibition of phospholipase A1, A2 and C1 (Kuhad et al., 2006). In spite of the fact that the exact mechanism of gentamicin-induced renal failure is not clearly known, several studies have purposed different pathways including synthesis of reactive oxygen species (ROS) as well as reactive nitrogen species (RNS), suppression of antioxidant defense and activation of inflammatory processes which results in decreased renal blood flow, tubular necrosis, leukocyte infiltration, cell damage, reduced glomerular filtration rate (GFR) and renal dysfunction (Yarijani et al., 2016). Several evidences suggest the role of ROS in the pathogenesis of different renal diseases. ROS may cause cell damage by lipid peroxidation and DNA as well as protein destruction (Ateşşahin et al., 2003).

Conventional therapeutic methods for renal failure focus on removing the leading cause. However, these conventional methods are unable to treat the metabolic, endocrine and renal function disorders caused by cellular damage and cell loss (Pino et al., 2017). Despite using the most advanced techniques, renal transplant mortality is still high. Thus, it is vital to recognize definite causes of renal failure and develop novel therapeutic methods (Abedi et al., 2016). During the past decades, researchers eagerly tried to find new therapeutic approaches for the treatment of different kinds of renal failure. Mesenchymal stem cells (MSCs) therapy is a developing therapeutic method. Undifferentiated mesenchymal stem cells have self-renewal ability and may differentiate to different mesenchymal and non-mesenchymal cell lines including bone, cartilage and adipose tissue (Tabatabaei Qomi and Sheykhhasan, 2017). Although bone marrow is the most common source of MSCs, these stem cells can be extracted from both adipose tissue and umbilical cord blood, all of which demonstrated promising results in repairing renal injuries (Liu et al., 2018). Based on previous studies, MSCs recover renal failure through secretion of a variety of immunomodulators, anti-apoptotic and anti-inflammatory growth factors and cytokines (Bai et al., 2018).

Moreover, several studies have been conducted on cell therapy with extracted cells of different origins. Pengfei et al. study suggested that human umbilical cord blood mesenchymal stem cells show protective effects against gentamicin-induced renal failure through expression of IGF-1 (Liu et al., 2016). Also, Moghadasali et al. (2013) showed that MSCs repair renal tubules by cytokine secretion in gentamicin-induced nephrotoxicity. Despite previous studies, the answer to several questions, including the effectiveness of AD-MSCs, number of needed cells, optimal injection timing and the exact mechanism of gentamicin-induced nephrotoxicity is not yet clear. Thus, this study is conducted to assess the efficacy of AD-MSC transplant in gentamicin-induced renal failure.

Materials and methods

This experimental study was conducted on 40 male Wistar rats weighting 180-200g, purchased from laboratory animal center of Arak University of Medical Sciences. Current study is approved by research ethics committee of Arak University of Medical Sciences (Iran) with IR.ARAKMU.REC.1397.11 code and ethical codes of Health ministry were observed when working with laboratory animals. All animals were kept at 22-24°C temperature and 12 hours light and dark cycle with free access to standard food and water.

Experiment protocols and study groups

Rats in this study were divided into 4 groups (n=10), including control group: no interventions (injection, anesthesia induction, or surgery) were performed in this group; sham group: in this group rats received

intraperitoneal injection of 2ml normal saline for 7 days and received 0.5ml of normal saline from caudal vein 24 hours before the end of the course; gentamicin group: in this group rats received intraperitoneal injection of 100mg/kg gentamicin for 7 days and received 0.5ml of normal saline through caudal vein (Stojiljkovic et al., 2012) 24 hours before the end of the course; and gentamicin+AD-MSC group: which received intraperitoneal injection of 100mg/kg gentamicin for 7 days and an intravenous dose of 1×10^6 AD-MSCs 24 hours before the end of the course.

Isolation and recognition of AD-MSCs

Two rats were anesthetized by IP injection of 60mg/kg ketamine Hydrochloride (Rotex Media, Trittau, Germany) and 6mg/kg xylene hydrochloride (Alfasan, Woerden, Netherlands) and a small adipose specimen was excised for culture. Then, the adipose specimen was immediately put in tubes containing sterile phosphate-buffered saline (PBS), 100IU/ml penicillin (Sigma-Aldrich, St. Louis, MO), and 100mg/ml streptomycin (Sigma-Aldrich, St. Louis, MO). Afterward, rats were sacrificed by intra-cardiac injection of potassium chloride. Adipose specimens were precisely cut into very tiny parts and rinsed with sterile PBS. Later, adipose particles were shaked and incubated in PBS containing 2mg/ml collagenase (Sigma-Aldrich) for 90min at 37°C (Ghorbani et al., 2014). Purified tissue was centrifuged at 2000 rpm for 5min and adipose tissue layer was extracted. Stromal vascular cells were twice rinsed with PBS and then suspended in Dulbecco's Modified Eagle's medium (DMEM; Gibco Waltham, MA) which contained fetal bovine serum (FBS; Gibco), 100IU/ml penicillin and 100µg/ml streptomycin. Cells were placed in a 25 cm^2 flask and cultured in 5% CO_2 atmosphere at 37°C. Non-adherent cells were removed by culture medium exchange and adherent cells were distributed with 3 passages.

To determine the presence of AD-MSCs, the level of biomarkers was evaluated using fluorescence-activated cell sorting (FACS). These cells were incubated for 20min at 4°C in a dark room with the following antibodies: (NB 100-65543PE; No-vus Biologicals) CD 90-phycoerythrin (PE)T ،CD 29-fluorescein isothiocyanate (FITC,BD561796); BD-Biosciences, Franklin Lakes, CD 45-FIT (bs-4819 R; Bioss), CD34b-PE (ABIN671373) antibodies. Control isotype antibodies were purchased from Santa Cruz biotechnology (Dallas, TX). After being rinsed with PBS, cells were suspended again in PBS contains 500µl of 2% FBS and analyzed with flow cytometry analysis. A minimum of 1×10^4 stem cells were required for the analysis of each sample (Hoseini et al., 2017).

Measurement of biochemical parameters

At the end of 10-day course of experiment, rats were anesthetized for blood sampling to measure blood urea nitrogen (BUN) and plasma creatinine. For oxidative stress assessment, malondialdehyde (MDA) was measured to determine the level of phospholipids peroxidation in renal tissue. Moreover, total tissue anti-oxidant power was assessed by ferric reducing antioxidant power (FRAP) measurement (Azarkish et al., 2017; Moosavi et al., 2011). Because the FRAP assay is inexpensive, reagents are simple to prepare, results are highly reproducible and the procedure is straightforward and speedy, it is very useful and common test (Benzie and Strain, 1996). For this purpose, firstly FRAP solution containing 300mmol/l acetate buffer (pH=3.6), 10Mm 2,4,6-Tris(2-pyridyl)-s-triazine solution in hydrochloric acid (40mmlol/l), ferric chloride solution (2mmol/l) were prepared. Iron sulfate heptahydrate ($FeSO_4.7H_2O$) was used as standard curve. After preparation of solution, 1.5ml of the solution was added to test tube which reached 37°C after placing tissue sample or standard. Then, its absorption was measured at 593nm wavelength. FARP level was reported in µmol/l (Mahmoudzadeh et al., 2017). To determine MDA level, kidney tissue was homogenized in PBS. Then 20% acetic acid, 0.8% thiobarbituric and 8.1% sodium dodecyl sulfate were added to test tubes containing tissue sample or standard and heated for 60min at 95°C. Then, the pink complex was extracted by N-butanol and the optical absorbance was measured at 532nm of wavelength by spectrophotometer (Jane way model). Results were reported in nmol/l (Mahmoudzadeh et al., 2017).

Histologic examination method

For assessment of kidney histopathologic damages, hematoxylin-eosin stained sections were prepared and kidney cortex, external and internal medulla were separately examined using light microscopy. Histopathologic damages were graded in terms of bowman capsule enlargement, cellular necrosis,

vascular congestion and formation of protein casts in tubular lumens. Rats with highest bowmen capsule enlargement in comparison with sham group were considered as 100% and the bowman capsule enlargement of other rats were determined by comparison. It is noteworthy that in each section the bowman capsule of ten large glomeruli were measured with scaled eyepiece lens and the averaged values was considered as the size of bowman capsule of the rat. Other alterations such as cellular necrosis, vascular congestion and formation of intratubular protein casts were calculated as the percentage of the involved area. Cells whose nucleus and cytoplasm were separated with a definite border from the epithelium and shed to the tubules were considered as the necrotic cells. Then, these percentages were graded as follows: no damage, 0; 1-20% damage, 1; 21-40%, 2; 41-60% damage, 3; 61-80% damage, 4; and 81-100% damage, 5. Lastly, scale of all histopathologic damages was calculated by summing up all damage grades which was used for data analysis (Najafi et al., 2015).

Statistical analysis

SPSS version 18 was used for statistical data analysis. One-way ANOVA and Duncan post hoc tests were used to determine significance. Exact *P*-value was calculated by LSD test. Non-parametric data were analyzed using Kruskal-Wallis and Mann-Witney tests. Data were reported as mean±SEM and $P<0.05$ was considered as significant.

Results

Renal hemodynamic parameters including creatinine and BUN significantly increased after gentamicin injection for 7 consecutive days in comparison with sham group ($P<0.001$). AD-MSC treatment significantly reduced BUN compared with gentamicin group ($P<0.01$), yet a significant difference in comparison with sham group was observed ($P<0.001$). Although, AD-MSC treatment reduced plasma creatinine level in comparison with gentamicin group, the difference was not significant, while a significant difference was observed when compared to sham group (Table 1).

Table 1: Effect of AD-MSC administration on gentamicin induced changes in creatinine and BUN in different groups.

Parameter	Control	Sham	GM+NS	GM +AD-MSC
Plasma creatinine, (mg/dl)	0.52 ± 0.014	0.56 ±0.02	1.31 ± 0.11 ***	1.2 ± 0.05 ***
Blood Urea Nitrogen (mg/dl)	23.6 ± 1.65	25.4 ± 0.071	53.9 ± 3.04 ***	42.5 ± 3.28 ††***

***$P<0.001$ in comparison with the sham group.
††$P<0.01$ in comparison with the GM+NS group.

Table 2: Effect of AD-MSC administration on gentamicin induced histologic damages in different groups.

Histopathologic damages		**Study groups**			
		Control	sham	GM+NS	GM +AD-MSC
Cortex	Bowman capsule enlargement	0	0	0.15	0.11
	Proximal tubal damage	0	0	2.64	2.14
	Henle Ascending thick limb damage	0	0	2.16	1.11
External Medulla	Pars Recta damage	0	0	2.38	2
	Henle Ascending thick limb damage	0	0	2.83	1.98
	Vascular congestion	0	0	1.07	0.78
	Tubular protein cast	0	0	2.31	2.26
Internal Medulla	Vascular congestion	0	0	0.98	0.53
	Tubular protein cast	0	0	2.76	2.12
Total histopathologic damage degree		**0**	**0**	**17.28*** **	**13.03***† **

***$p<0.001$ in comparison with the sham group.
†$p<0.05$ in comparison with the GM+NS group.

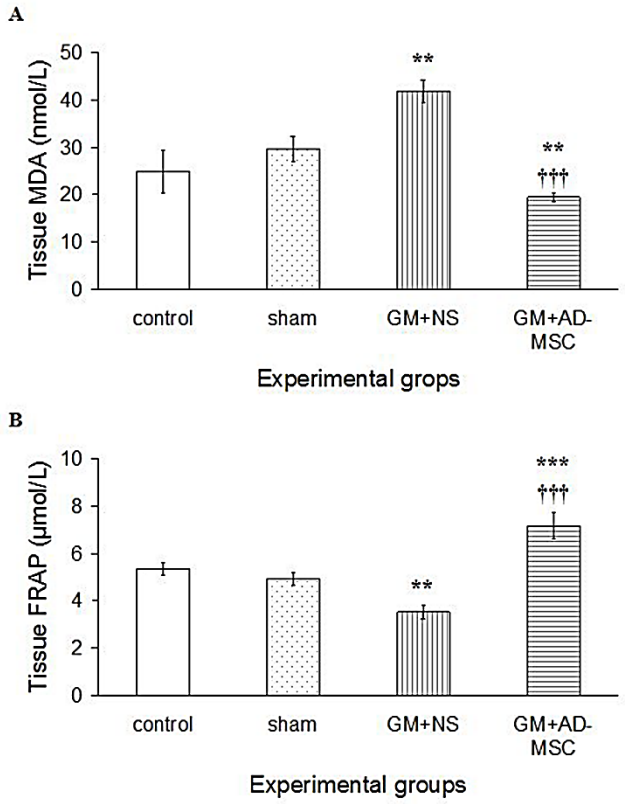

Fig.1. A) Renal tissue lipid peroxidation level (MDA) and B) total antioxidant capacity (FRAP) in different group. As it can be seen treatment by AD-MSC leads to decrease of oxidative stress (MDA) and increase of FRAP of renal tissue. $^{**}P<0.01$, $^{***}P<0.001$ in comparison with the sham group. $^{†††}P<0.001$ in comparison with the gentamicin group.

Moreover, gentamicin injection resulted in significant MDA level increment and FRAP level decrement in gentamicin group in comparison with sham one ($P<0.01$). After treatment with AD-MSC, MDA level decreased in comparison with gentamicin group ($P<0.001$) and FRAP increased ($P<0.001$). Yet, results showed a significant difference in MDA value ($P<0.01$) and FRAP value ($P<0.001$) in treated group compared with sham group (Fig. 1).

In this study, IP gentamicin injection caused damage to kidney medulla and cortex including bowman space enlargement, proximal tubule damage, thick ascending limb of Henle damage, vascular congestion and formation of intratubular casts in comparison with sham group (Fig. 2 and table 2). Generally, tissue damage grade was 17.28 in gentamicin receiving group which was significantly reduced to 13.03 following AD-MSC treatment (Table 2).

Discussion

Nephrotoxicity is among the common clinical complications of aminoglycosides. Current study evaluated the efficacy of AD-MSC treatment in gentamicin-induced nephrotoxicity. Results of present study suggested relative recovery of hemodynamic parameters, oxidative stress and histological damages in the group treated with AD-MSC in comparison with gentamicin receiving group.

Gentamicin is not metabolized in human body, yet it is excreted by glomerular filtration or reabsorbed by proximal tubule cells (Kuhad et al., 2006). Tubular damage caused by GM disturbs water and electrolyte reabsorption and increases their transportation to distal tubule stimulating tubuloglomerular feedback (TGF) which finally reduces GFR. Also, glomerular exposure to gentamicin induces contraction of mesenchymal cells and reduction of filtration coefficient (K_f) which leads to reduced GFR (Lopez-Novoa et al., 2011); consequently, plasma creatinine and BUN increases.

In this study, gentamicin administration for 7 consecutive days resulted in renal failure which was demonstrated with significant increment of creatinine and BUN in compression with sham group. Nevertheless, creatinine and BUN levels reduced after AD-MSC treatment compared to gentamicin group. Previous studies have proved that MSCs contain high levels of vasodilator factors. Thus, MSCs may have dilated renal vessels which increased GFR and thus reduced creatinine and BUN (Abedi et al., 2016). Liu et al. (2016) showed the effective role of umbilical cord blood derived mesenchymal stem cells in reducing creatinine and BUN in gentamicin-induced renal failure. They stated that stem cells may have exerted their effect in renal failure recovery by expression of IGF-1.

Oxidative stress is the other mechanism through which gentamicin induces nephrotoxicity. Gentamicin induces ROS including superoxide anion, hydrogen peroxide, hydroxyl radicals and RNS (Abdelrahman, 2018; Nasri and Rafieian-Kopaei, 2013). In addition, it is demonstrated that gentamicin induces the expression of inducible nitric oxide (NO) synthase which increases NO synthesis (Lopez-Novoa et al., 2011). Increased NO level reacts with superoxide anion which forms peroxynitrite leading to nitrosative stress and cytotoxicity (Lopez-Novoa et al., 2011).

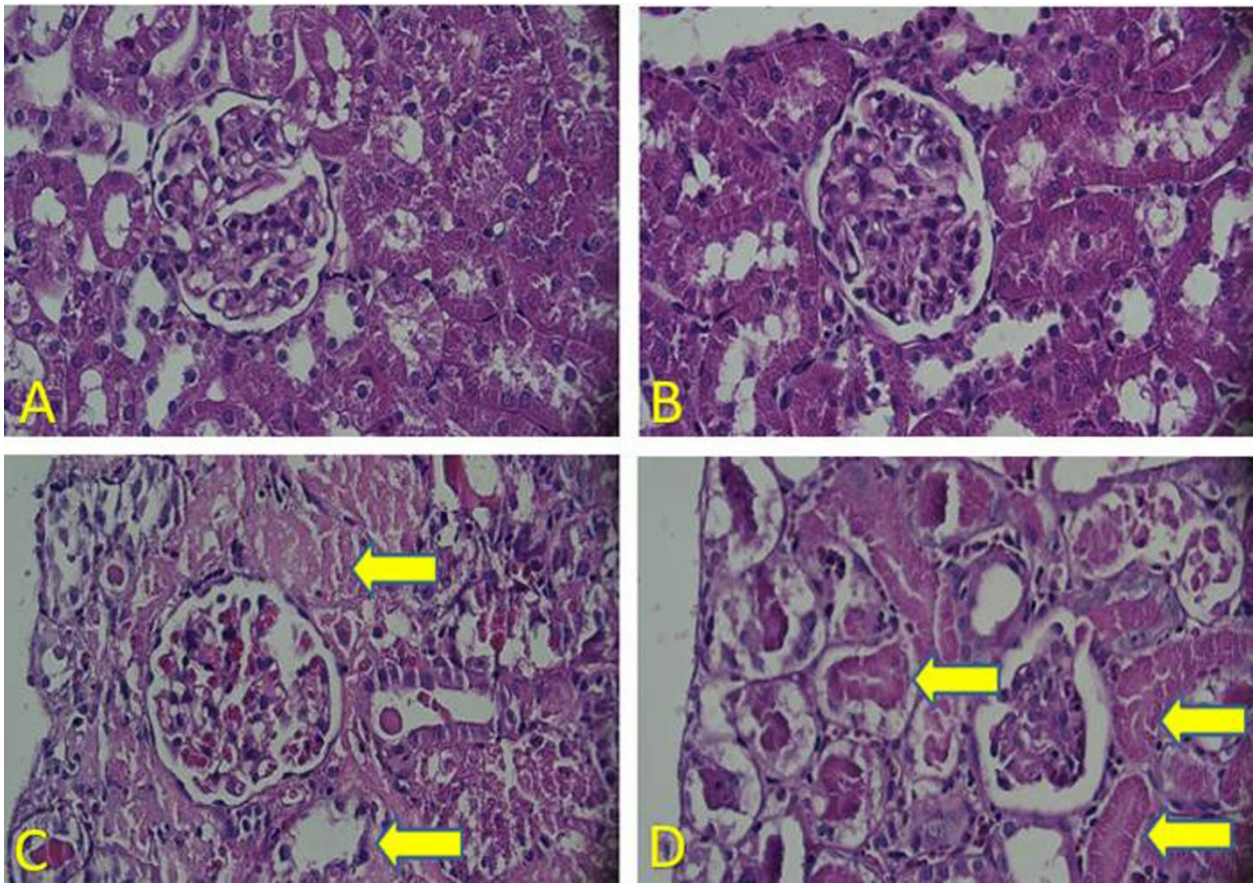

Fig.2. Representing histopathologic alterations in the kidney of rats following gentamicin administration and AD-MSC treatment. Cross-sectional view of the kidneys to indicate the Bowman's space (BS) enlargement and the necrosis of the cells (NS) in groups A) without any intervention (control), B) the normal saline received (sham), C) gentamicin and normal saline received, and D) gentamicin and adipose mesenchymal stem cells. The arrow points to formation of casts in tubules. (Haematoxylin-Eosin, 400 x magnification).

Oxidative stress leads to formation of numerous vasoconstrictor mediators which can directly affect renal function through renal vessels constriction and GFR reduction (Kuhad et al., 2006). MDA which is formed by membrane phospholipid peroxidation by reactive species is an indicator of oxidative stress. In this study, gentamicin administration significantly increased MDA in gentamicin receiving group in comparison with sham group; AD-MSCs injection reduced MDA level in treated group in comparison with gentamicin group. Mesenchymal stem cells are promising cellular resources for restorative treatments which are beneficial in the treatment of histological damages caused by oxidative stress (Benameur et al., 2015). Results of Valle-Prieto and Conget (2010) study indicated that MSCs are highly resistant to oxidative induced cell death which is attributed to low levels of ROS, continuous expression of enzymes required for oxidative stress management and high levels of glutathione peroxidase.

Furthermore, many studies have showed that role of MSCs in maintain antioxidant balance (Wojtas et al., 2017; Zhang et al., 2016). Vanella et al. (2012) showed that heme oxygenase-1 is expressed in MSCs structure which brings a strong antioxidant capacity through inhibition of superoxide anion synthesis. Also, FRAP assay is an easy technique to evaluate the total antioxidant status of biological samples (Golshan et al., 2017). Similarly, in this study, FRAP level of kidney tissue increased after AD-MSC treatment which is determined by increased FRAP level in treated group in comparison with gentamicin group.

In this study, gentamicin injection caused histologic damages such as bowman space enlargement, vascular congestion, proximal tubular damage and formation of intratubular proteinaceous casts in kidney cortex and medulla in gentamicin receiving group in comparison with sham group. In some

studies, it is observed that growth factor administration before and after renal damage leads to histological recovery in animal models which may occur due to their anti-apoptotic effects (Wise and Ricardo, 2012). Mesenchymal stem cells activate phosphatidylinositol protein kinase B pathway which phosphorylates and deactivates apoptotic factors and improve tubular epithelial cells recovery (Sanz et al., 2008). In the present study, AD-MSC treatment reduced histologic damages in the treated group compared with gentamicin group. Similarly, Abedi et al. (2016) showed the recovering role of MSCs on histologic damages in gentamicin-induced renal failure via expression of growth factors. Likewise, AD-MSCs may cause histologic damage recovery by secretion of multiple growth factors which further studies are required to fully understand their mechanism of action.

Conclusion

The results of present study showed that AD-MSCs treatment improved gentamicin-induced renal failure, oxidative stress and histologic damages in animal model. Probably, AD-MSCs are involved in renal tissue repair via paracrine pathways, which requires further studies.

Acknowledgments

The authors acknowledge the Research Council of Arak University, Arak, Iran, for the financial support of this study (grant No# 3060).

Conflict of interest

There is no conflict of interest.

References

Abdelrahman R. Protective effect of apocynin against gentamicin-induced nephrotoxicity in rats. Hum Exp Toxicol 2018; 37: 27-37. https://doi.org/10.1177/0960327116689716

Abedi A, Azarnia M, Jamali Zahvarehy M, Foroutan T, Golestani S. Effect of different times of intraperitoneal injections of human bone marrow mesenchymal stem cell conditioned medium on gentamicin-induced acute kidney injury. Urol J 2016; 13: 2707-16.

Ali BH, Al Za'abi M, Blunden G, Nemmar A. Experimental gentamicin nephrotoxicity and agents that modify it: a mini-review of recent research. Basic Clin Pharmacol Toxicol 2011; 109: 225-32. https://doi.org/10.1111/j.1742-7843.2011.00728.x

Ateşşahin A, Karahan I, Yilmaz S, Çeribaşi AO, Princci I. The effect of manganese chloride on gentamicin-induced nephrotoxicity in rats. Pharmacol Res 2003; 48: 637-42. https://doi.org/10.1016/S1043-6618(03)00227-5

Azarkish F, Hashemi K, Talebi A, Kamalinejad M, Soltani N, Pouladian N. Effect of the administration of solanum nigrum fruit on prevention of diabetic nephropathy in streptozotocin-induced diabetic rats. Pharmacognosy Res 2017; 9: 325-332. https://doi.org/10.4103/pr.pr_47_17

Bai M, Zhang L, Fu B, Bai J, Zhang Y, Cai G, et al. IL-17A improves the efficacy of mesenchymal stem cells in ischemic-reperfusion renal injury by increasing Treg percentages by the COX-2/PGE2 pathway. Kidney Int 2018; 93: 814-825. https://doi.org/10.1016/j.kint.2017.08.030

Benameur L, Charif N, Li Y, Stoltz JF, de Isla N. Toward an understanding of mechanism of aging-induced oxidative stress in human mesenchymal stem cells. Biomed Mater Eng 2015; 25: 41-6. https://doi.org/10.3233/BME-141247

Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996; 239: 70-6. https://doi.org/10.1006/abio.1996.0292

Changizi -Ashtiyani S, Zarei A, Elahipour M. Innovations and discoveries of jorjani in medicine. J Med Ethics Hist Med 2009; 2: 16.

Ghorbani A, Feizpour A, Hashemzahi M, Gholami L, Hosseini M, Soukhtanloo M, et al. The effect of adipose derived stromal cells on oxidative stress level, lung emphysema and white blood cells of guinea pigs model of chronic obstructive pulmonary disease. Daru 2014; 22: 26-38. https://doi.org/10.1186/2008-2231-22-26

Golshan A, Hayatdavoudi P, Hadjzadeh MA, Khajavi Rad A, Mohamadian Roshan N, Abbasnezhad A, et al. Kidney stone formation and antioxidant effects of Cynodon dactylon decoction in male Wistar rats. Avicenna J Phytomed 2017; 7: 180-190.

Helal MG, Zaki M, Said E. Nephroprotective effect of saxagliptin against gentamicin-induced nephrotoxicity, emphasis on anti-oxidant, anti-inflammatory and anti-apoptic effects. Life Sci 2018; 208: 64-71. https://doi.org/10.1016/j.lfs.2018.07.021

Hoseini SJ, Ghazavi H, Forouzanfar F, Mashkani B,

Ghorbani A, Mahdipour E, et al. Fibroblast growth factor 1-transfected adipose-derived mesenchymal stem cells promote angiogenic proliferation. DNA Cell Biol 2017; 36: 401-412. https://doi.org/10.1089/dna.2016.3546

Kuhad A, Tirkey N, Pilkhwal S, Chopra K. Effect of Spirulina, a blue green algae, on gentamicin-induced oxidative stress and renal dysfunction in rats. Fundam Clin Pharmacol 2006; 20: 121-8. https://doi.org/10.1111/j.1472-8206.2006.00396.x

Liu B, Ding F, Hu D, Zhou Y, Long C, Shen L, et al. Human umbilical cord mesenchymal stem cell conditioned medium attenuates renal fibrosis by reducing inflammation and epithelial-to-mesenchymal transition via the TLR4/NF-kB signaling pathway in vivo and in vitro. Stem Cell Res Ther 2018; 9: 7. https://doi.org/10.1186/s13287-017-0760-6

Liu P, Feng Y, Dong D, Liu X, Chen Y, Wang Y, Zhou Y. Enhanced renoprotective effect of IGF-1 modified human umbilical cord-derived mesenchymal stem cells on gentamicin-induced acute kidney injury. Sci Rep 2016; 6: 20287. https://doi.org/10.1038/srep20287

Lopez-Novoa JM, Quiros Y, Vicente L, Morales AI, Lopez-Hernandez FJ. New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. Kidney Int 2011; 79: 33-45. https://doi.org/10.1038/ki.2010.337

Mahmoudzadeh L, Najafi H, Ashtiyani SC, Yarijani ZM. Anti-inflammatory and protective effects of saffron extract in ischaemia/reperfusion-induced acute kidney injury. Nephrology 2017; 22: 748-754. https://doi.org/10.1111/nep.12849

Moghadasali R, Mutsaers HA, Azarnia M, Aghdami N, Baharvand H, Torensma tR, et al. Mesenchymal stem cell-conditioned medium accelerates regeneration of human renal proximal tubule epithelial cells after gentamicin toxicity. Exp Toxicol Pathol 2013; 65: 595-600. https://doi.org/10.1016/j.etp.2012.06.002

Moosavi SM, Changizi -Ashtiyani S, Hosseinkhani S. L-carnitine improves oxidative stress and suppressed energy metabolism but not renal dysfunction following release of acute unilateral ureteral obstruction in rat. Neurourol Urodyn 2011; 30: 480-7. https://doi.org/10.1002/nau.21035

Najafi H, Changizi -Ashtiyani S, Madani SH, Fakhri S, Yarijani ZM, Hazem M. Therapeutic effects of curcumin on renal tissue damages induced by ischemia reperfusion in rat. Koomesh 2015; 16: 273-281.

Nasri H, Rafieian-Kopaei M. Tubular kidney protection by antioxidants. Iran J Public Health 2013; 42: 1194-6.

Pfister F, Büttner-Herold M, Amann K. (Immuno) Pathology of drug side effects in the kidney. Pathologe 2018; 39: 576-582. https://doi.org/10.1007/s00292-018-0475-1

Pino CJ, Westover AJ, Buffington DA, Humes HD. Bioengineered renal cell therapy device for clinical translation. ASAIO J 2017; 63: 305-315. https://doi.org/10.1097/MAT.0000000000000485

Sanz AB, Santamaría B, Ruiz-Ortega M, Egido J, Ortiz A. Mechanisms of renal apoptosis in health and disease. J Am Soc Nephrol 2008; 19: 1634-42. https://doi.org/10.1681/ASN.2007121336

Silan C, Uzun O, Comunoglu NU, Gokçen S, Bedirhan S, Cengiz M. Gentamicin-induced nephrotoxicity in rats ameliorated and healing effects of resveratrol. Biol Pharm Bull 2007; 30: 79-83. https://doi.org/10.1248/bpb.30.79

Shamsi M, Haghverdi F, Changizi-Ashtiyani S. A brief review of Rhazes, Avicenna, and Jorjani's views on diagnosis of diseases through urine examination. Iran J Kidney Dis 2014; 8: 278-8.

Stojiljkovic N, Stoiljkovic M, Randjelovic P, Veljkovic S, Mihailovic D. Cytoprotective effect of vitamin C against gentamicin-induced acute kidney injury in rats. Exp Toxicol Pathol 2012; 64: 69-74. https://doi.org/10.1016/j.etp.2010.06.008

Tabatabaei Qomi R, Sheykhhasan M. Adipose-derived stromal cell in regenerative medicine: a review. World J Stem Cells 2017; 9: 107-117. https://doi.org/10.4252/wjsc.v9.i8.107

Valle-Prieto A, Conget PA. Human mesenchymal stem cells efficiently manage oxidative stress. Stem Cells Dev 2010; 19: 1885-93. https://doi.org/10.1089/scd.2010.0093

Vanella L, Sanford C, Kim DH, Abraham NG, Ebraheim N. Oxidative stress and heme oxygenase-1 regulated human mesenchymal stem cells differentiation. Int J Hypertens 2012; 2012: 890671. https://doi.org/10.1155/2012/890671

Wise AF, Ricardo SD. Mesenchymal stem cells in kidney inflammation and repair. Nephrology 2012; 17: 1-10. https://doi.org/10.1111/j.1440-1797.2011.01501.x

Wojtas E, Zachwieja A, Zwyrzykowska A, Kupczynski R, Marycz K. The application of mesenchymal progenitor stem cells for the reduction of oxidative stress in animals. Turk J Biol 2017; 41: 12-19. https://doi.org/10.3906/biy-1603-13

Yarijani ZM, Najafi H, Madani SH. Protective effect of crocin on gentamicin-induced nephrotoxicity in

rats. Iran J Basic Med Sci 2016; 19: 337-43.

Zhang G, Zou X, Huang Y, Wang F, Miao S, Liu G, et al. Mesenchymal stromal cell-derived extracellular vesicles protect against acute kidney injury through anti-oxidation by enhancing Nrf2/ARE activation in rats. Kidney Blood Press Res 2016; 41: 119-28. https://doi.org/10.1159/000443413