The efficacy of the adipose tissue derived mesenchymal stem cells in the treatment of the lead acetate-induced nephrotoxicity in the adult male albino rats

Original Article

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ABSTRACT

Background and Objectives: Lead acetate is one of the heavy metals that causes food, water and air pollution. Exposure to lead acetate causes toxic effects on several body organs including the kidney. The adipose tissue contains multipotent mesenchymal stem cells that could be isolated and cultured on suitable media to proliferate and give large number of stem cells. When injected into a living animal body, these stem cells have the ability to migrate to the sites of the lesions to replace the destroyed cells. The aim of the present study was to evaluate the possibility of the adipose tissue derived mesenchymal stem cells (Ad-MSCs) to ameliorate the nephrotoxic effects of lead acetate.

Materials and Methods: Fifty adult male albino rats were used in this work. Ten rats were used as a source of the Ad-MSCs and the other forty rats were used in the experimental study. These forty rats were randomly divided into four groups; group 1 (control group), group 2 (lead acetate-treated group) received lead acetate to induce nephrotoxicity, group 3 (recovery group) received lead acetate to induce nephrotoxicity then left for spontaneous recovery. Group 4 (Ad-MSCs-treated group) received lead acetate to induce nephrotoxicity then, receive the Ad-MSCs to induce renal recovery.

Results: Lead acetate administration caused impaired renal function, disturbed renal oxidant/antioxidant status and histological renal destruction with insignificant spontaneous recovery. Administration of Ad-MSCs alleviated the biochemical and histological nephrotoxic effects of lead acetate.

Conclusion: The Ad-MSCs have curative properties against the nephrotoxic effects caused by lead acetate.

Key Words: Lead acetate, nephrotoxicity, stem cells.

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INTRODUCTION

Lead acetate is one of the heavy metals widespread in the environment. It is used commonly in industry for manufacturing several products of a daily human use such as paints, dray cell batteries, pigments and varnishes. Exposure of the human body to lead acetate in large doses causes lead acetate toxicity with several bad effects on the human health. Lead acetate toxicity occurs through contaminating the food, drinking water and air as it is easily absorbed from the intestinal and respiratory mucosae however, it is difficultly and slowly excreted from the body because it is non-degradable and has a long half-life. It is stored for several years in the different body organs including the kidney causing nephrotoxic effects[1].

Lead acetate produces its toxic effects on the human body through its capacity to enhance apoptosis and increase the level of the apoptosis-related proteins in addition to suppression of the rate of the cell proliferation and regeneration. It accelerates the rate of apoptosis by inducing oxidative stress-mediated DNA and mitochondrial damage with subsequent disturbance of the energy/metabolism balance[2].

The kidney is a vital organ responsible for removal of the waste products and excess water from the body. It is important in the control and regulation of the body fluids volume and osmolarity, electrolyte balance and acid-base balance. From superficial to deep, the kidney consists of 3 layers; cortex, medulla and sinus that consists of the renal pelvis and calyces[3]. Histologically, the cortex consists of about one million of nephrons that are responsible for filtration of the urine from the blood while the medulla consists of collecting tubules that transmit the urine from the nephrons to the calyces. The nephrons of the cortex consist of Bowman's capsules, proximal convoluted tubules, loops of Henle and distal convoluted tubules that open into the collecting tubules of the medulla. The Bowman's capsules are invaginated by loops of capillaries called renal glomeruli to form the renal corpuscles[4]. The Bowman's capsules extract the water and the small molecules from the blood running in the glomeruli then, the essential molecules and the greater part of water are reabsorbed from the renal tubules and loops of Henle into the peritubular capillaries[4].

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Stem cells (SCs) are undifferentiated cells present in the different body tissues. They are derived from the different layers of the embryonic disc; ectoderm, mesoderm and endoderm. The SCs can be classified into totipotent, pluripotent, multipotent, oligopotent and unipotent types. The totipotent SCs can differentiate to give the whole body and the extraembryonic membranes while the pluripotent SCs can differentiate to give the whole body tissues but without extraembryonic membranes. The multipotent SCs can give numerous cell types, the oligopotent SCs can give few cell types; just two or three types of differentiated cells while the unipotent SCs can give only one type of specialized cells. SCs have the ability to indefinitely proliferate and differentiate to replace the damaged cells hence; they potentially can be used to provide a curative treatment hope for the untreatable diseases[5].

The fatty tissue contains abundant number of the Ad-MSCs are more abundant than the bone marrow derived stem cells. They are multipotent having the capacity to proliferate and differentiate into different types of cells when cultured on a suitable medium. They can differentiate into bone, cartilage, muscle, fibrous tissue and endothelial cells[6].

The aim of the current study was to isolate the Ad-MSCs from the anterior abdominal wall of the adult male albino rats then, to culture these cells on a suitable medium to allow them to proliferate to give a large number of the Ad-MSCs. Furthermore, the study aimed to evaluate the potential curative capacity of these Ad-MSCs to treat the nephrotoxicity induced by lead acetate in the rats.

MATERIALS AND METHODS

Site of the study:

1- The experiment was done at the animal house, Medical research center, Faculty of Medicine, Ain Shams University, Cairo.

2- The stem cells were isolated and cultured in the tissue culture unit, Medical research center, Faculty of Medicine, Ain Shams University, Cairo.

3- The haematoxylin and eosin and the immunohistochemical specimens were prepared and processed at the Histology department, Faculty of Medicine, Al-Azhar University, Cairo

Chemicals:

1- Lead acetate was purchased from Sigma Aldrich Company. It was dissolved in normal saline and injected intraperitoneally at a dose of 15 mg/kg daily.

2- All the instruments and the media used for the stem cell culture were purchased from Sigma Aldrich Company

Animals:

Fifty adult male albino rats were used in the present study. The rats were healthy, weighing 180 - 230 grams and of 6 months of age. Ten rats were used as a source for the Ad-MSCs while the remaining 40 rats were used for the experimental study. The rats of the experiment were housed in a suitable room with good ventilation and a temperature maintained at 26 ± 2°C and received a standard diet ad libitum and tap water. These 40 rats were left for one week to acclimate in the animal house then, divided randomly into 4 separate groups with 10 rats in each group. The animals were used according to the code of ethics of the experimental researches guidelines adopted by Ain Shams University.

Isolation and culturing of the Ad-MSCs:

The adipose tissue samples were obtained from the anterior abdominal wall of the rats. These samples were put in Collagenase type IV at room temperature and left for ten minutes to be digested by Collagenase that facilitates the separation of the cells from each others through destruction of the intercellular junctions. Then, more separation of the cells was carried out through centrifugation for 5 min at 500 g at 37 °C. The supernatant cells were decanted to form a pellet then, cultured on MesenproRS medium enriched with 2% FBS and seeded in petri dishes at a density of 14×10^4 cells/cm2. Then the cultured stem cells were viewed and photographed every 3 days to evaluate the rate of proliferation[7, 8].

Characterization of the Ad-MSCs:

The Ad-MSCs were characterized and identified by the stem cells specific immunocytochemical markers nestin and CD44. Nestin is a type of the intracellular intermediate filaments that provide a good mechanical cellular support and necessary for cell proliferation and tissue regeneration. CD44 is a transmembrane glycoprotein playing an important role in the cell adhesion to the extracellular matrix through binding to hyaluronic acid[9].

Experimental design:

Group A (Control group): Received only the standard diet and tap water.

Group B (Lead acetate-treated group): Received lead acetate at a dose of 15 mg/kg daily by intraperitoneal injection for successive 12 days for induction of nephrotoxicity[10] then, sacrificed immediately at the end of the 12 days.

Group C (Recovery group): Received lead acetate at a dose of 15 mg/kg daily for successive 12 days for induction of nephrotoxicity. The animals then left for 11 days to give a chance for a potential spontaneous renal recovery[10].

Group D (Lead acetate and stem cells-treated group): Received lead acetate at a dose of 15 mg/kg daily for successive 12 days for induction of nephrotoxicity[10]. The animals then received Ad-MSCs in a dose of ten millions injected into the brachial vein in the forearm then, left for 11 days to give a chance for a potential stem cells-induced renal recovery[11].

Biochemical analysis:

i- Kidney function indicators:

At the end of the experiment and before sacrificing the rats, they were completely anaesthetized by inhalation of 4% isoflurane then, blood samples were obtained from the retro-orbital venous plexus through the lateral orbital angle approach[12, 13] (Fig. 1). These blood samples were analyzed to detect the levels of urea and creatinine that are the indicators for the kidney function[14].

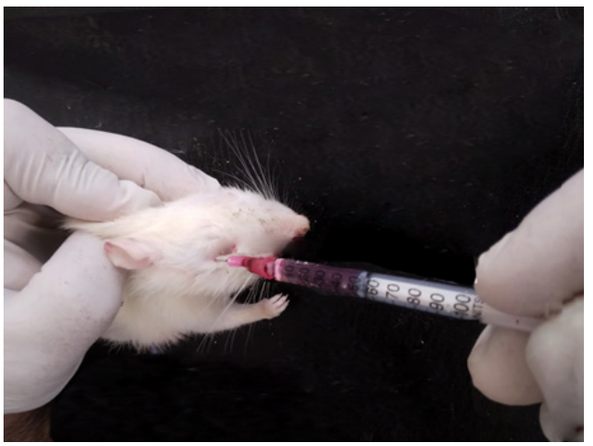

Fig. 1: The lateral angle approach to obtain the blood sample from the retro-orbital venous plexus.

ii- The oxidant/antioxidant status markers:

Samples of the renal tissue of the 4 groups were homogenized and assessed for malondialdehyde (MDA) and reduced glutathione (GSH) levels in addition to superoxide dismutase (SOD) and catalase (CAT) enzymes activity that are indicators for the oxidant/antioxidant status of the kidney[15].

Histological evaluation:

a- Haematoxyline and eosin staining:

The renal sections were stained with haematoxyline and eosin for morphological evaluation of the structure of the kidney[16].

b- Immunohistochemical staining:

Anti Bax antibodies were used to detect the renal tubular and glomerular lining cells undergoing apoptosis because Bax is one of the apoptotic proteins detected in the cytoplasm of the cells during apoptosis. Bax protein accumulation in the cytoplasm leads to mitochondrial membrane destruction with subsequent cell death[17]. The numbers of the Bax-positive cells were counted under the high power field lens in 10 non-overlapping fields in each section.

c- Immunofluorescence cell labeling:

The Ad-MSCs were labeled by PKH26 that is a red fluorescent dye before being injected into the rats. The renal sections of the Ad-MSCs treated rats were examined by the y fluorescent microscope to detect PKH26 positive fluorescent cells[18].

Morphometric study:

The Leica "Qwin 500C" image analyzer computer system (Cambridge, England) was used for counting the Bax immune positive cells in 10 non-overlapping fields in each specimen under the ×400 lens.

STATISTICAL ANALYSIS

The biochemical and the morphometric data were analyzed by the Statistical Package of Social Sciences (SPSS) with the assistant version 7.7. The means of the four groups were compared using the One-way analysis of variance (ANOVA). The differences were considered statistically significant when probability *(P) value* is < 0.05[19].

RESULTS

i- Ad-MSCs culture:

The Ad-MSCs showed rapid proliferation rate, formation of long cytoplasmic processes, formation of cell colonies and complete confluency was reached 12 days after seeding (Fig. 2).

ii- Identification and characterization of the Ad-MSCs:

The Ad-MSCs were identified by their positive nestin and CD44 reactions (Fig. 3).

iii- Biochemical results:

a- Results of the kidney function indicators:

The control group showed normal levels of urea and creatinine in the blood while the administration of lead acetate resulted in elevation of the levels of these

2 parameters when compared to the control group. The recovery group showed still elevated levels while the administration of the Ad-MSCs resulted in significant reduction of the urea and creatinine levels in comparison to the lead acetate-treated group (Table 1 and Fig. 4).

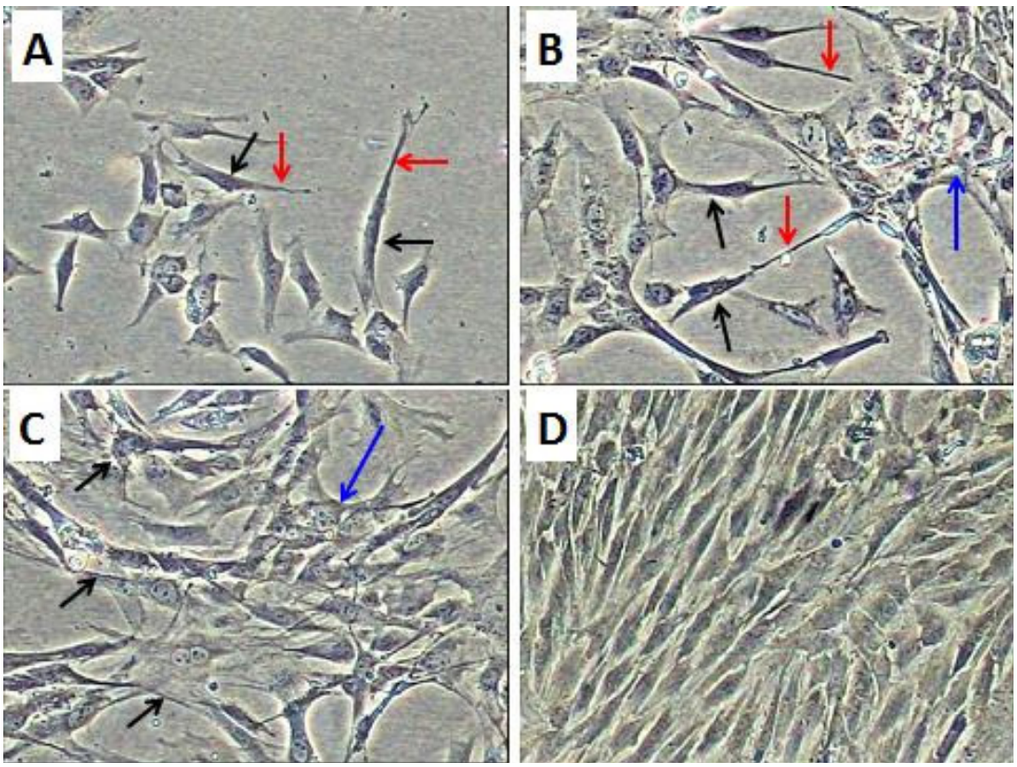

Fig. 2: Phase contrast photomicrographs [A, B, C and D] of cultured Ad-MSCs 3, 6, 9 and 12 days after seeding respectively. Photomicrograph [A] shows the proliferating Ad-MSCs (black arrows) with their cytoplasmic processes (red arrows) 3 days after seeding. Photomicrograph [B] shows more proliferation of the cells (black arrows), more elongation of their cytoplasmic processes (red arrows) and the formation of a colony (blue arrow) of the Ad-MSCs. Photomicrograph [C] shows more proliferation of the cells (black arrows) and a large cells colony (blue arrow). Photomicrograph [D] shows 100% confluency of the Ad-MSCs (X100).

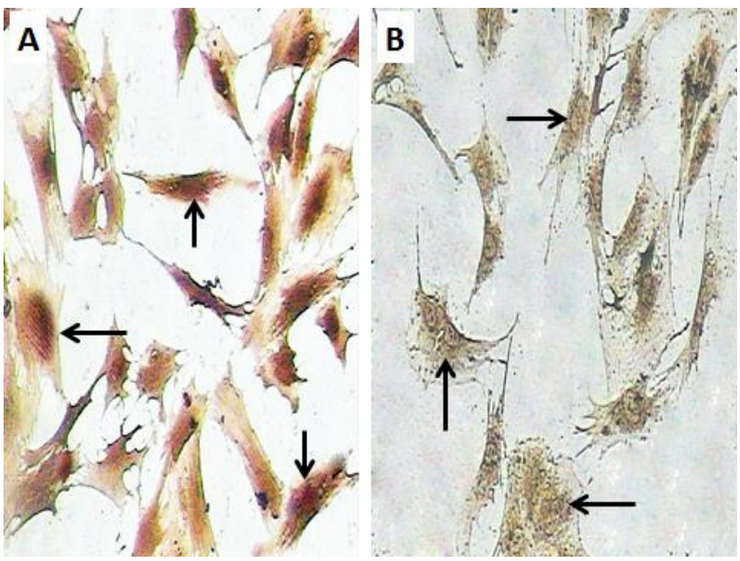

Fig. 3: Phase contrast photomicrographs of cultured Ad-MSCs. Photomicrograph [A] shows nestin surface antigen positive cells (arrows) (Nestin immunostain, X 100). Photomicrograph [B] shows CD44 surface antigen positive cells (arrows) (CD44 immunostain, X 100).

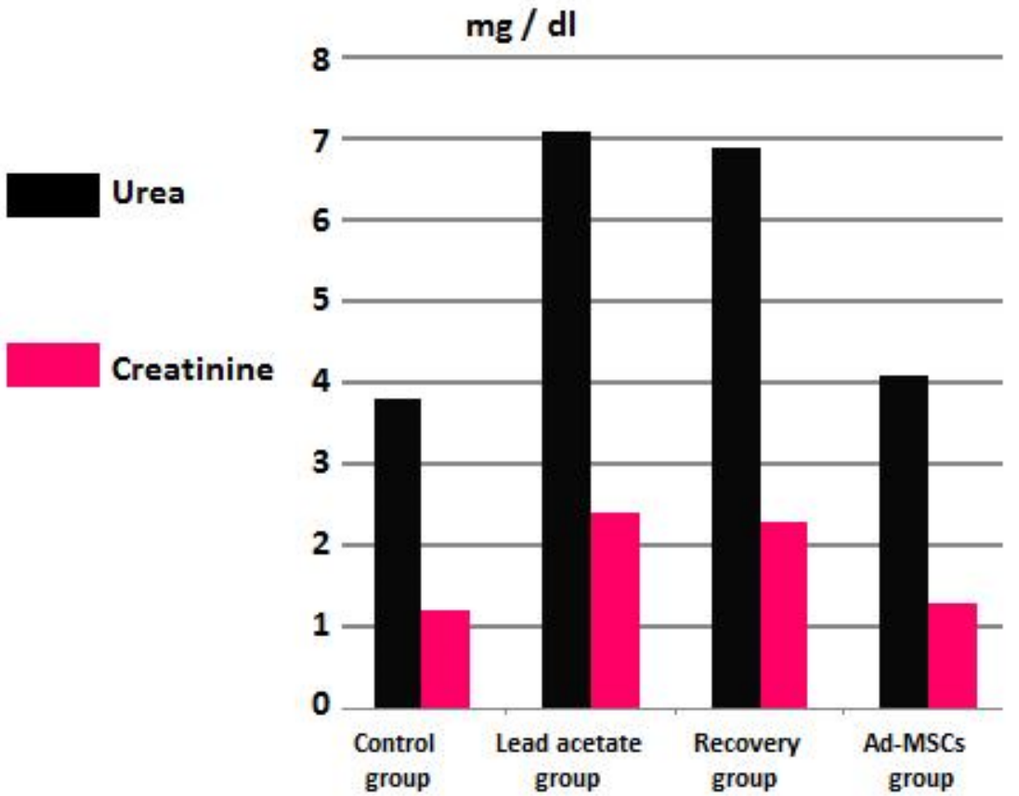

Fig. 4: The serum levels of urea and creatinine in the 4 groups

Table 1: The oxidant/antioxidant status markers in all groups:

Parameters	Control group	Lead acetate group	Recovery group	Ad-MSCs group
Urea ± SD mg / dl	3.84 ± 0.2	7.13 ± 0.5[a]	6.9 ± 0.2[ab]	4.1 ± 0.35[cd]
Creatinine ± SD mg / dl	1.26 ± 0.06	2.43 ± 0.04[a]	2.36 ± 0.04[ab]	1.37 ± 0.09 [cd]

Representation of the data was in mean ± SD (Standard deviation)
a = Significant *P-value* when compared to the control group ($P > 0.05$)
b = Insignificant *P-value* when compared to the lead acetate group ($P < 0.05$)
c = Significant *P-value* when compared to the lead acetate group ($P < 0.05$)
d = Insignificant *P-value* when compared to the control group ($P > 0.05$)

b- Results of the oxidant/antioxidant status markers:

The lead acetate and the recovery groups showed increased MDA level and decreased GSH, SO and CAT levels in comparison to the control group without significant differences between the recovery and the lead acetate groups. The Ad-MSCs group showed a significant reduction of the MDA level and a significant elevation of the GSH, SOD and CAT levels in comparison to the lead acetate and recovery groups. The levels of these parameters in the Ad-MSCs group showed insignificance differences when compared to the control group (Table 2 and Figures 5-8)

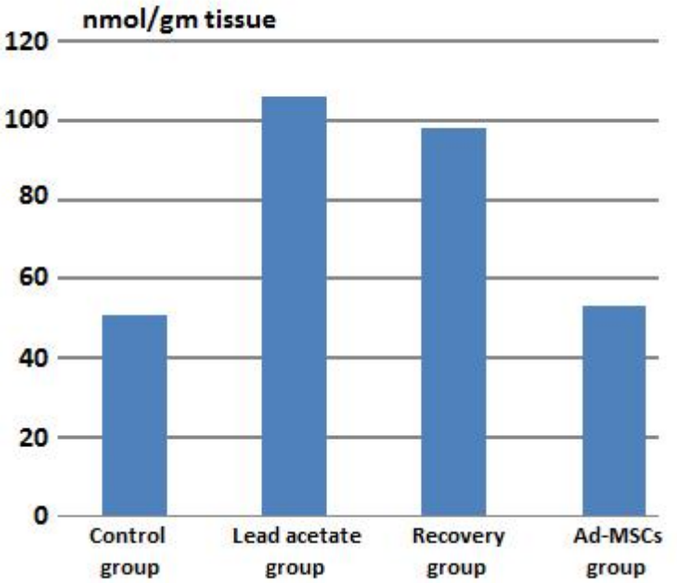

Fig. 5: The level of MDA in the renal tissue of the 4 groups

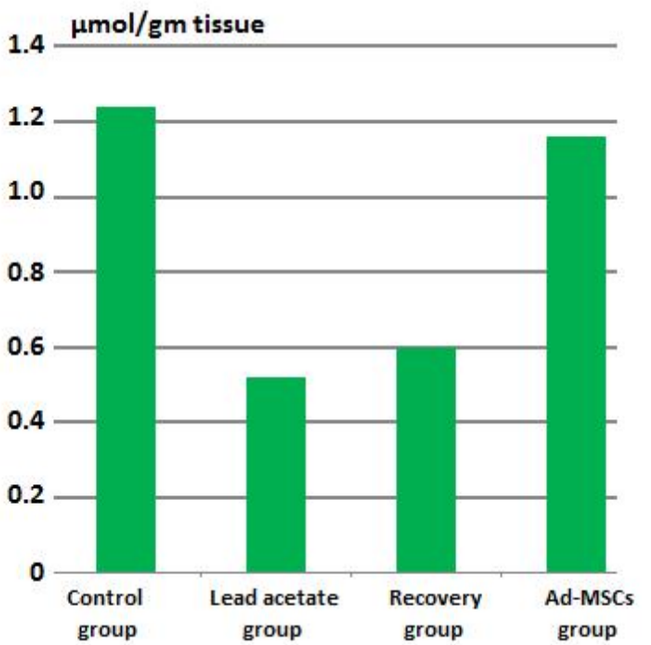

Fig. 6: The level of GSH in the renal tissue of the 4 groups

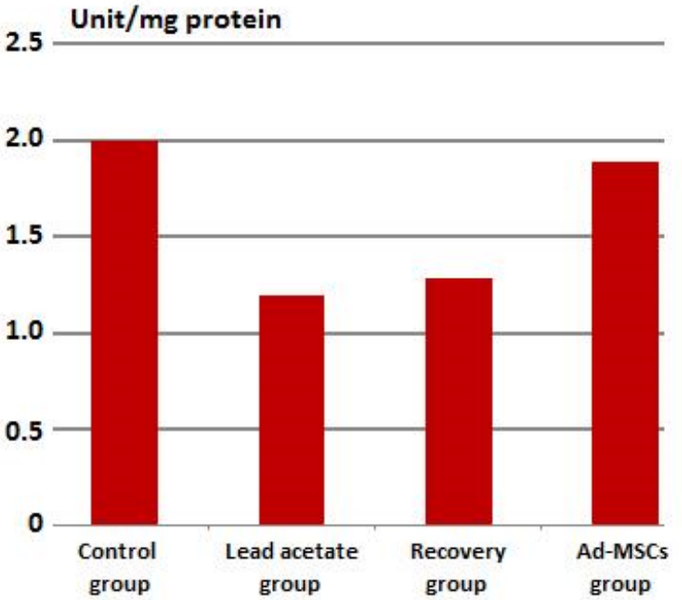

Fig. 7: The level of SOD activity in the renal tissue of the 4 groups

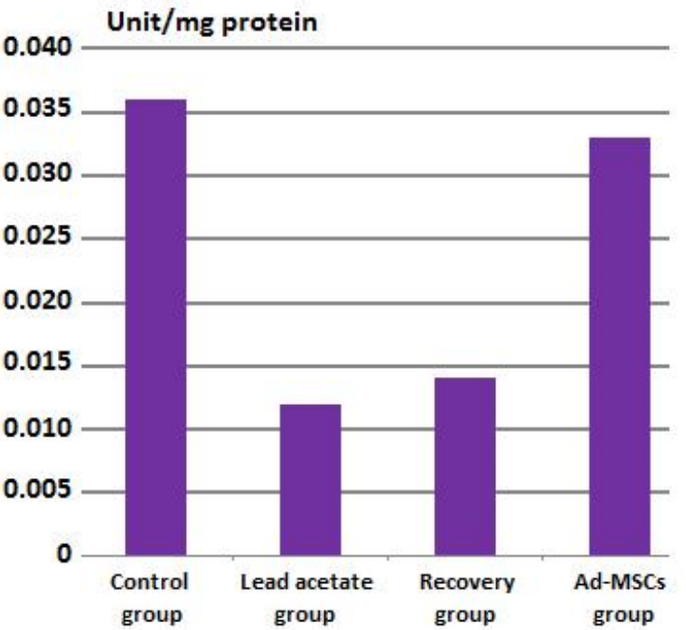

Fig. 8: The level of CAT activity in the renal tissue of the 4 groups

Table 2: The oxidant/antioxidant status markers in all groups:

Parameters	Control group	Lead acetate group	Recovery group	Ad-MSCs group
MDA ± SD nmol/gm tissue	51 ± 4	106 ± 3[a]	98 ± 3[ab]	53 ± 7[cd]
GSH ± SD µmol/gm tissue	0.62 ± 0.65	0.26 ± 0.21[a]	0.30 ± 0.57[ab]	0.58 ± 0.46[cd]
SOD ± SD Unit/mg protein	2.01 ± 0.23	1.19 ± 0.59[a]	1.28 ± 0.71[ab]	1.89 ± 0.35[cd]
CAT ± SD Unit/mg protein	0.036 ± 0.003	0.012 ± 0.005[a]	0.014 ± 0.005[ab]	0.033 ± 0.006[cd]

Representation of the data was in mean ± SD (Standard deviation)
a = Significant *P-value* when compared to the control group ($P > 0.05$)
b = Insignificant *P-value* when compared to the lead acetate group ($P < 0.05$)
c = Significant *P-value* when compared to the lead acetate group ($P < 0.05$)
d = Insignificant *P-value* when compared to the control group ($P > 0.05$)

iii- Histological results:

a- Haematoxyline and eosin staining results:

The renal sections of the control group showed the normal histological architecture with glomeruli, normal Bowman's spaces and renal tubules with intact lining cells. Administration of lead acetate resulted in degenerative changes in the form of atrophy of the glomeruli leading to widening of the Bowman's spaces in addition to degeneration of the lining cells of the renal tubules. The recovery group showed the same histological picture as in the lead acetate-treated group; atrophied glomeruli, wide Bowman's spaces and degenerated lining cells of the renal tubules. The Ad-MSCs treated group showed intact glomeruli, normal Bowman's spaces and renal tubules with intact lining cells (Fig. 9).

b- Immunohistochemical staining results:

The control and Ad-MSCs groups showed negative immunohistochemical reactions against the cytoplasmic Bax protein antigen while the lead acetate-treated and the recovery groups showed strong positive anti-Bax cytoplasmic reaction (Fig. 10).

The Bax positive cells in the four groups:

The lead acetate-treated and the recovery groups showed significantly increased number of the Bax positive cells in comparison to the control group. The administration of the Ad-MSCs in group 4 significantly reduced the number of these Bax positive cells when compared to the groups 2 and 3 (Table 3 and Fig. 11).

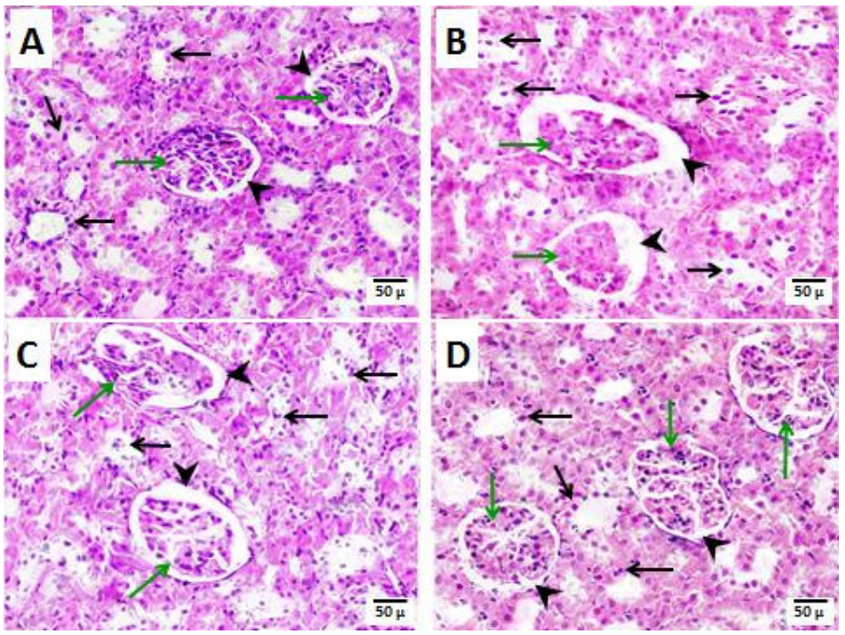

Fig. 9: Photomicrographs of the renal tissue of the 4 groups. The control group [A] shows glomeruli (Green arrows), normal Bowman's spaces (Arrow heads) and renal tubules with intact lining cells (Black arrows). The lead acetate-treated [B] and the recovery [C] groups showed atrophied glomeruli (Green arrows), wide Bowman's spaces (Arrow heads) and degenerated lining cells of the renal tubules. The Ad-MSCs treated group [D] showed intact glomeruli (Green arrows), normal Bowman's spaces (Arrow heads) and renal tubules with intact lining cells (Black arrows) (H & E, X 200).

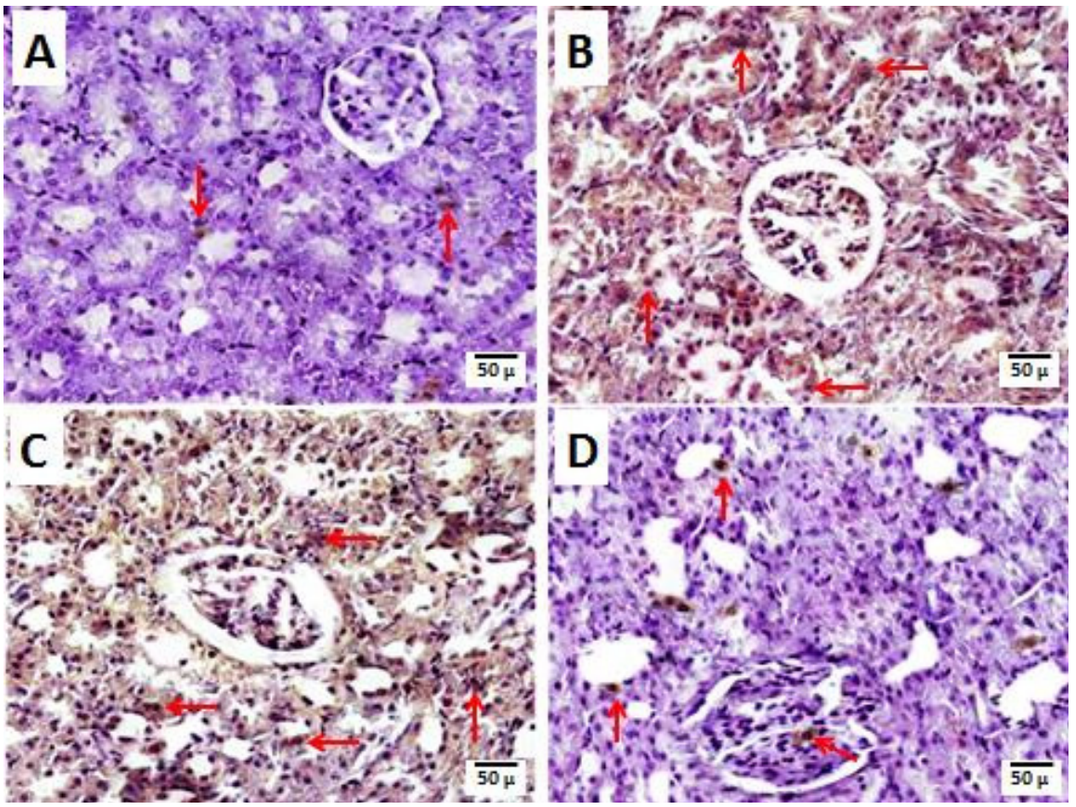

Fig. 10: Photomicrographs of the immunohistochemically stained renal tissue of the 4 groups. The control [A] and the Ad-MSCs treated [D] groups show few cells with positive anti-Bax protein cytoplasmic reaction (Arrows). The lead acetate-treated [B] and the recovery [C] groups show numerous positively stained cells (Arrows) (Bax immunohistochemical staining, X 200).

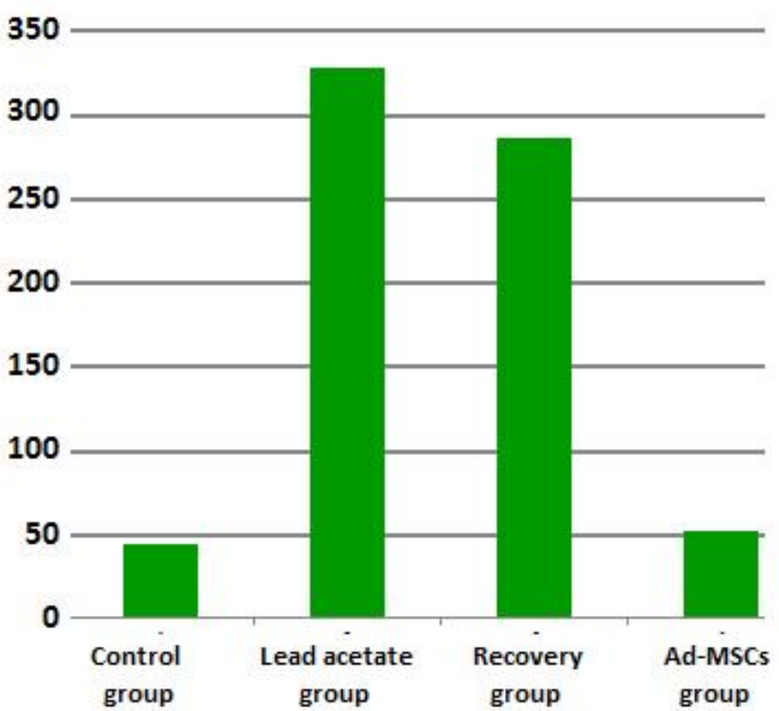

Fig. 11: The mean count of the Bax positive cells/high power field in the four groups.

Table 3: The mean count of the Bax positive cells/high power field in the four groups.

	Control	Lead acetate	Recovery	Ad-MSCs group
Bax positive cells number	44	328[a]	286[ab]	52[cd]

Representation of the data was in mean ± SD (Standard deviation)
a = Significant *P-value* when compared to the control group (*P > 0.05*)
b = Insignificant *P-value* when compared to the lead acetate group (*P < 0.05*)
c = Significant *P-value* when compared to the lead acetate group (*P < 0.05*)
d = Insignificant *P-value* when compared to the control group (*P > 0.05*)

c- Immunofluorescence cell labeling results:

The renal sections of the rats injected with the PKH26 immunofluorescent dye showed numerous PKH26 positive immunofluorescent cells. This indicated that, the PKH26-labelled Ad-MSCs when injected into the rats with lead acetate-induced nephrotoxicity, these Ad-MSCs reached the kidney and replaced the damaged renal cells (Fig. 12).

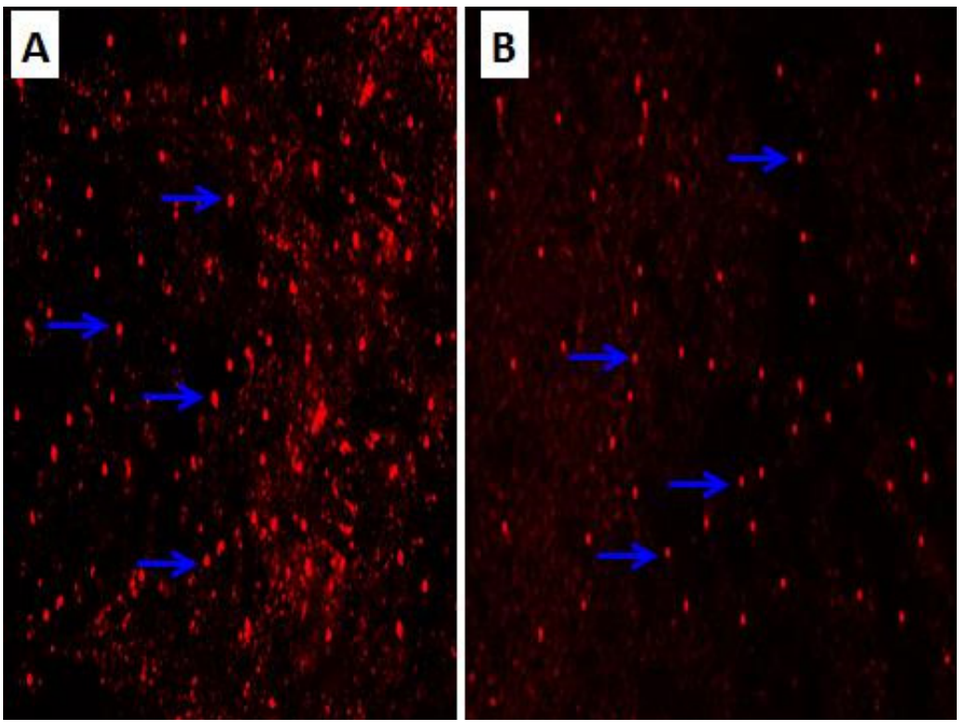

Fig. 12: PKH26 immunofluorescence micrograph of the renal sections of the Ad-MSCs-treated rats showing numerous PKH26 positive cells (arrows) (X 100**).**

DISCUSSION

The present study was carried out to isolate Ad-MSCs from the fatty tissue of the anterior abdominal wall of the rats. Then, these stem cells were assessed for their possible efficacy to induce recovery from the lead acetate-induced nephrotoxicity in rats. Our study proved that, the Ad-MSCs could be easily isolated from the anterior abdominal wall of the rats and proliferated at a good rate when cultured on MesenproRS medium and given a large number of cells. When injected into rats with lead acetate-induced nephrotoxicity, these cells migrated to the injured renal tubules and glomeruli and replaced the damaged cells.

In the present study, the Ad-MSCs were used because of the easy accessibility of the adipose tissue and has an abundant number of the MSCs with high multipotency and strong capacity for self-renewal so, the adipose tissue represents a suitable site to obtain large numbers of the MSCs without risk. Moreover, the Ad-MSCs have strong immunomodulatory and anti-inflammatory capacity through suppression of the proliferation and differentiation of the T-cells and dendritic cells[20]. In agreement with previous studies, the Ad-MSCs showed a good proliferation rate when cultured on MesenproRS medium that has been developed for culturing MSCs at greater than clonal densities[21].

Administration of lead acetate in the current study resulted in a disturbance of the renal functions indicated by high serum levels of urea and creatinine in addition to loss of the normal histological architecture of the kidney. The renal histological changes induced by lead acetate were in the form of atrophied glomeruli leading to widening of the Bowman's space in addition to destruction of the lining cells of the renal tubules. These biochemical and histological findings were in correlation with previous studies proved that, lead acetate enhances apoptosis in the renal tissue with subsequent glomerular and tubular destruction and renal functions disturbance[22, 23]. Moreover and in correlation with previous results, the present study detected that, lead acetate administration induced excessive formation of the Bax protein antigen in the cytoplasm of the renal cells. The Bax protein enhances and accelerates the apoptosis rate in the renal tissue by binding to and counteracting the apoptosis suppressor BCL2 and its adenovirus homolog E1B 19k protein[24].

In agreement with previous results, the present study proved that, lead acetate induced renal damage through induction of oxidative stress with loss of the antioxidant capacity and excessive production of the reactive oxygen species[10]. The lead acetate-induced oxidative stress was indicated by increased MDA and decreased GSH, CAT and SOD levels in the renal tissue. MDA is a sensitive oxidative stress indicator because it results from lipid peroxidation that is induced by the action of the ROS on the fatty acids of the cell membranes with subsequent cell damage[10]. GSH is a non-enzymatic strong antioxidant agent while CAT and SOD are strong antioxidant enzymes[10]. so, the lead acetate-induced reduction of the levels of GSH, CAT and SOD detected in the present study indicated depletion of the antioxidant capacity of the renal tissue.

In the current study, the Ad-MSCs produced curative effects against the lead acetate-induced nephrotoxicity. These curative effects were indicated by alleviation of the lead acetate-induced histological degenerative changes affecting the renal glomeruli and tubules, improvement of the renal function markers and alleviation of the disturbance of the oxidant/antioxidant status. Ad-MSCs administration increased the levels of GSH, CAT and SOD and decreased the level of MDA in the kidney tissue. These Ad-MSCs-induced renal protective effects were in agreement with previous results[25] and were attributable to the ability of the Ad-MSCs to migrate to the site of degeneration and replace the degenerated cells. Moreover, the Ad-MSCs ameliorate the renal inflammation and improves the renal function through inhibition of the release of the proinflammatory cytokines[25]. Also, the Ad-MSCs induce regeneration of the lining cells of the renal tubules through secreting a number of the regeneration promoting factors and by forming intercellular junctions between the mesenchymal stem cells and the tubular lining cells[26].

CONCLUSION

In conclusion, the present study proved that, Ad-MSCs can be easily isolated from the anterior abdominal wall subcutaneous fatty tissue. These cells have the capacity to proliferate to give a large number of cells when cultured on a suitable medium. When injected into the bodies of the rats with lead acetate-induced nephrotoxicity, these Ad-MSCs migrate to the injured kidney and replace the destroyed renal cells leading to improvement of the renal function.

CONFLICT OF INTEREST

There are no conflicts of interest.

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