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Role of adipose derived mesenchymal stem cells cultured in media containing either normal or pathogenic serum or renal homogenate in regenerating renal medulla of cisplatin-intoxicated male Sprague Dawley rats

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Abstract

Renal failure represents a public health problem in Egypt as a result of cancer therapy, misused of drugs and environmental toxins. There is a great need of mesenchymal stem cell therapy otherwise of renal transplation due to the high cost demand, continuous need to immunosuppressive drugs and incidence of rejection of transplanted organs. Cisplatin-intoxication represents a good model of inducing renal dysfunction male Sprague Dawley. Adipose derived mesenchymal stem cells (AD-MSCs) were derived form lipoaspirate of human adipose tissue and after further characterizations with CD markers; the AD-MSCs were cultured in media containing extra inclusions of either normal or pathogenic renal homogenate as well as normal or pathogenic serum. AD-MSCs was injected through tail vein at dose of 0.5 ml of culture media containing 5 $x10^6$ after 4 days of incubation. Six groups of male Sprague Dawley rats were used in the present work; G1 (control) and G2 (cisplatin-intoxicated group, single IV dose 7 mg/kg), G3 (ADMSCS in media containing normal renal homogenate), G4 (ADMSCS in media containing pathogenic renal homogenate), G5 (ADMSCS in media containing normal serum), G6 (ADMSCS in media containing pathogenic serum). After 4,7,11 and 30 days of treatment, rats were sacrificed. Body weight, serum creatinine, blood urea nitrogen and urine creatinine clearance as well as renal glutathione reductase, superoxide dismutase and maondialdhyde were determined. Histopathological investigations and score assessments were carried out. The present findings revealed that treatment with ADMSCS in media containing normal renal homogenate showed a more regenerated activity compared to that cultured in media containing normal serum explained by improvement of histological picture and decreased pathological score. However, treatment with ADMSCS cultured in media containing pathogenic serum or renal homogenate showed the least degree of improvement. Also, the urine creatinine clearance, blood urea nitrogen and GSH and SOD were increased, meanwhile serum creatinine and malondialdhyde were increased in G2, G4 & G6 compared to the control and G3 &5. The author finally concluded that cisplatin nephrotoxicity explained by direct depletion of the antioxidant defense of GSH and SOD initiating tubular epithelium damage, improved by ADMSCS cultured in media containing normal elements of serum and renal homogenate which increase antioxidant enzymes. These led to scavenge free radical and improve renal function tests and decrease apoptic markers explained by altered malondialdhyde level.

Keywords: Cisplatin, Renal damage, adipose derived mesenchymal stem cells, renal function tests, light microscopic investigation.

Introduction

Chronic kidney disease (CKD) is a significant medical problem resulted from progressive damage in renal tissues including glomeruli, tubules, interstitium and/or vasculature [1]. This chronic kidney disease

(CKD) is influenced by increased incidence of mortality and type 2 diabetes [2]. CKD is a progressive condition causing significant morbidity and mortality. Cisplatin (cis-dichlorodiamimine-platinum II) is an effective antineoplastic agent [3,4] having a great affinity to develop nephrotoxicity in both in vitro and in vivo animal models [5,6]. Cisplatin nephrotoxicity is widespread among 1/3rd of patient undergoing chemotherapeutic-treatment[7] and characterized by tubular cell death [8]. It may depend on the metabolic activation of metabolic pathways of γ- glutamyl transpeptidase (GGT) and cysteine-S-conjugate b-lyase [9].

Both proximal [10] and distal renal tubules [11] were highly susceptible to cisplatin- nephrotoxicity. Cisplatin was accumulated by the peritubular uptake in both the proximal and distal nephrons. The S3 segment of the proximal tubule accumulated the highest concentration of cisplatin more than that of the distal ones [12]. These were interfered with the functioning activities of different organelles such as mitochondria, lysosomes, endoplasmic reticulum, nuclei and cell membranes, causing inflammation and cell death [13].

It is well known that the kidney is important for restore of metabolic substrates, synthesis of glutathione, free-radical scavenging enzymes, gluconeogenesis, ammoniagenesis, hormones, growth factors, and the production and regulation of multiple cytokines critical to inflammation and immunological [14]. Acute renal failure (ARF) is greatly increased in Egypt reached approximately to 367 per million populations [15]. The Costs for dialysis and renal transplantation are still expensive, need more efficient [16]. Multipotent MSCs can be differentiated *in vitro* and *in vivo* into various cell types which have a great affinity to migrate to sites of tissue injury and to enhance repair by secreting anti-fibrotic and pro-angiogenic factors. In bilaterally ischaemic mice, MSC infusion resulted in the accelerated recovery of renal function at days 2–3 compared to control mice. Within 2 weeks, infused stem cells partially contributed to angiogenesis, vasculogenesis and endothelial repair [17]. Mesenchymal stem cells therapy of glomerulonephritis led to preserve damaged glomeruli and maintaining renal function through a long-term as well as partial mal-differentiation of intraglomerular MSC into adipocytes accompanied by glomerular sclerosis [18]. Bone marrow -derived MSCs of human origin exhibited apparent decrease of mice renal cell apoptosis, increase proliferation, and preserved the integrity of the tubular epithelium 19. Quimby et al. [20] reported improvement in GFR and a mild decrease in serum creatinine concentration in two cats with CKD that received AD-MSCs. Chronic renal failure patients received harvested stem cells through transfemoral catheter every other week for six months led to a statistically significant improvement in blood urea, creatinine levels and glomerular filtration [21].

Kidney dysfunction is characterized by impairment of regenerative ability. Recently, many investigators reported the potential effects of mesenchymal stem cell in regenerating kidney disorders in animal models [20, 22, 23].

The present study aims to illustrate the role of human adipose tissue-derived mesenchymal stem cells cultured in media containing normal and pathogenic serum as well as normal and pathogenic kidney homogenate in ameliorating cisplatin-induced nephrotoxicity in male Sprague Dawley (SD) rats.

Materials and Methods

Isolation of MSCs from human adipose tissue:

Processed lipoaspirate (PLA) cells were obtained from raw human lipoaspirates, washed thoroughly with phosphate-buffered saline (PBS) to remove contaminating debris and red blood cells and treated with 0.075% type IA collagenase (C- 2674; Sigma-Aldrich, St. Louis, MO) in PBS for 30 min at 37°C with gentle agitation. The digested tissue was centrifuged at 1,200 *g* for 5 min to obtain a cell pellet.

Cells were plated in medium at a density of 51106 nucleated cells/100-mm tissue culture dish [24]. The yield was determined by calculating the number of adherent cells per gram of tissue harvested. The MSCs of both tissue types are usually cultured in CO2 incubator (36°±1 C°) containing 5% regular media (DMEM, 10% % FBS and 1% penicillin/streptomycin, Sigma) freshly prepared media was carried out every week to remove non-sticky cells. Serum and renal homogenates were added to the culture medium according to table (1):

Table 1 Serum and renal homogenates in culture medium

Groups	FBS	Serum	Homogenate
II	10%	--	--
III	10%	--	0.5%
IV	10%	--	0.5%
V	5%	5%	--
VI	5%	5%	

The samples were centrifuged at 1100 rpm for 5 minutes and all the cells isolated were plated in six 35-mm Petri dishes containing low-glucose Dubelco modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA, USA) supplemented with 100 U/mL of penicillin, 0.1mg/mL of streptomycin, 10 ng/mL of basic fibroblast growth factor, 10 ng/mL of epidermal growth factor (all from Peprotech, Rocky Hill, NJ, USA), and 10% of fetal bovine serum (Invitrogen). The medium was renewed after incubation of the cells at 37 °C with 5% humidified carbon dioxide for 7 days and the nonadhering AF cells were removed. The medium was replaced weekly until the cells reached 70% confluence, when they treated with 0.25% trypsin and 1 mM EDTA (Invitrogen) for 3 minutes. The MSCs were released, collected and replated in a split ratio of 1:3 under the same culture conditions. After this time, the stem cells are collected and characterized. The viability of stem cells was checked by Trypan blue according to Maclimans *et al.*[25]. Adipose derived-mesenchymal stem cells (AD-MSCs) were used after 4 days of culturing. Culturing of mesenchymal cells was carried out in culturing media containing homogenate of either normal or cisplatin-intoxicated kidney as well normal or pathogenic serum.

Flow cytometry for cell surface antigen expressions:

Human adipose tissue derived stem cells was aspirated and lysed by trypsinization and analyzed by flow cytometry. In brief, 100 µL of cell suspension was mixed with 10 µL of the fluorescently labeled mAb and incubated in at room temperature for 30 min. After washing with PBS containing 2% Bovine serum albumin (BSA**)** , the pellet was resuspended in PBS and subjected to flow cytometric analysis. The mAbs were used in different combinations of fluorochromes; namely fluorescein isothiocyanate (FITC), phycoerythrin (PE) and phycoerythrin-cyanine 5 (PeCY5). Different combinations of mAb were used against various antigens CD 14, CD 90, CD 105, CD 13, CD 34 The immunophenotyping was performed on EPICS-XL flowcytometry (Coulter, Miami, and Fl). The cells were analyzed with the most appropriate gate using the combination of forward and side scatters.

Experimental Work:

Adult inbred male Sprague Dawley (SD) rats weighed approximately 180 ±30 gm body weights (Nalge co., Rochester, NY). The animals kept in standard environment with controlled temperature, light and aerated environment. Free access of food and water were allowed *ad Libitum*. One hundred and twenty rats will be divided into seven groups.

Group I (n = 20): Normal group IV injected with 0.5 ml of saline into the tail vein.
Group II (n = 20) (Cisplatin-treated) received IV injection of a single dose of cisplatin (7 mg/kg) in 1 ml saline. (Cisplatin, David Bull Laboratories 'DBL').
Group III (n = 20) composed of cisplatin + human AD-MSCs from media containing homogenate normal rat kidney. 0.5 ml of culture media containing 5 $x10^6$ MSCs was injected in tail vein.
Group IV (n = 20) composed of cisplatin + human adipose stem cells from media containing homogenate pathogenic rat kidney. 0.5 ml of culture media containing 5 $x10^6$ MSCs were injected in tail vein.
Group V (n = 20) composed of cisplatin + human adipose stem cells from media containing normal rat serum. 0.5 ml of culture media containing 5 $x10^6$ MSCs was injected in tail vein.
Group VI (n = 20) composed of cisplatin + human adipose stem cells from media containing serum of pathogenic rat kidney. 0.5 ml of culture media containing 5 $x10^6$ MSCs was injected in tail vein.

Normal renal tissues homogenate and serum were obtained from normal rat. Also, pathogenic kidney homogenate and pathogenic serum were obtained from cisplatin intoxicated rats.

Rats of the animal groups were examined at 4,7,11 and 30 days post-treatment. Their urine was collected in metabolic cages. Also at the mentioned intervals, rats were sacrificed by diethyl ether and blood was aspirated from heart puncture, centrifuged at 3000 rpm

for separating serum, and dissected for removing their kidneys. Known weight of renal tissues was homogenized in 50 mM Tris-HCL with 2 mM EDTA, pH 7.4.and kept at 4°C. The homogenate was centrifuged at 130000 rpm and supernatant separated and stored at -80° C and investigated as follows:

1. Body weight change:

Body weight change was recorded at intervals 4,7,11 & 30 days post -treatment.

2. Biochemical assays:

Creatinine was assayed in serum samples of the studied groups by using Diamond kits (Diamond Diagnostics, 333 Fiske Street, Holliston, MA). Serum creatinine and blood urea nitrogen were determined according to Walker et al. [26]. Urine creatinine clearance was determined according to Van Acker et al., [27]. The antioxidant enzymes reduced glutathione (GSH) and superoxide dismutase (SOD) and malondialdhyde were determined in renal tissues of the animal groups. SOD was determined according to Nishikimi et al. [28] and GSH by the method of Ellman et al. [29]. Malondialdhyde was determined according to Ohkawa [30].

3. Histopathological investigation & score assessments:

At intervals 4,7,11 &30 days post- AD-MSCs therapy, renal specimens were fixed in 10% formal saline, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and mounted in molten paraplast 58-62°C. 5µm histological sections were cut and stained with hematoxylin and eosin and examined under bright field light microscope and photographed.

For determination of the histopathological score, 4µm sagittal sections were prepared from renal tissues of studied animals and stained with hematoxylin-eosin. Changes found were scored as follows:

Necrotic Tubules: Counted per high power field. 1: 2 = (+), 3 : 5 = (++), 6 :10 = (+++), more than 10 = (++++). Mitotic Figures: Counted per 10 high power fields (10 HPFs).Regenerating interstitial solid sheets: Counted per high power field. 1: 2 = (+), 3 : 5 = (++) and more than 5 = (+++).Solid Tubules: Counted per high power field.

1: 2 = (+), 3 : 5 = (++) and more than 5 = (+++). The other findings were commented upon as present or absent. Scoring was done as follows: Necrotic tubules: They were counted as Number of necrotic tubules / HPF

1-3 necrotic tubules / HPFs ……..….1
4-5 necrotic tubules / HPFs ……..2
5-10 necrotic tubules / HPFs …….3
More than necrotic tubules / HPFs ……...4

Solid sheets of cells in the intertitium .They was counted as number / HPF
1-2 solid sheets / HPF……1
3-5 solid sheets / HPF…….2
More than 5 solid sheets / HPF……3

Regenerating tubules: They was counted as number / HPF

1:2 solid sheets / HPF…….1
3:5 solid sheets / HPF…… . .2
More than 5 solid sheets / HPF……..3

Mitotic figures: They are counted as Number /10HPFs

1-2 /10 HPFs…….……1
3-5 /10 HPFs……..…2
More than 5 /10 HPFs……3

Inflammatory cells: Focal: F
Diffuse D

In each case if inflammatory cells are only
1-3 rows in between the tubules, +
4-5 rows in between the tubules, ++
6 or more rows in between the tubules, +++

Interstitial fibrosis:

If occupies < 25% of the 100x field…….1
If occupies 25-50% of the 100x field……2
If occupies 5o-75 % of the 100x field……..…….3
If occupies 75% or more of the 100x field……4

Atrophic tubules; flat lining with casts & thick basement membranes: Counted as number / HPF :

1-5 Atrophic tubule / HPF…….1+
5-10 Atrophic tubule / HPF…….2++
More than 10 Atrophic tubule / HPF……3 +++

Statistical analysis

Data were expressed as the means ± SD. Statistical analysis was performed by SPSS 16 software. The Student's t-test was used to compare serum parameters. $P < 0.05$ was considered to indicate a statistically significant result.

Results

1. Harvesting and Culturing of MSC:

Mesenchymal stem cells (MSCs) were harvested from the human adipose tissue. AD-MSCs were separated and cultured. AD-MSCs were differentiated to form spindle-shaped morphology and form fibroblast like colonies (Fig. 1A). Flow-cytometric observations revealed that the obtained AD-MSCs possessed a specific surface protein expression positive CD13, CD29, CD105 and CD90 and negative for the endothelial and hematopoietic-specific markers CD34 and CD 14 (Fig.1B).

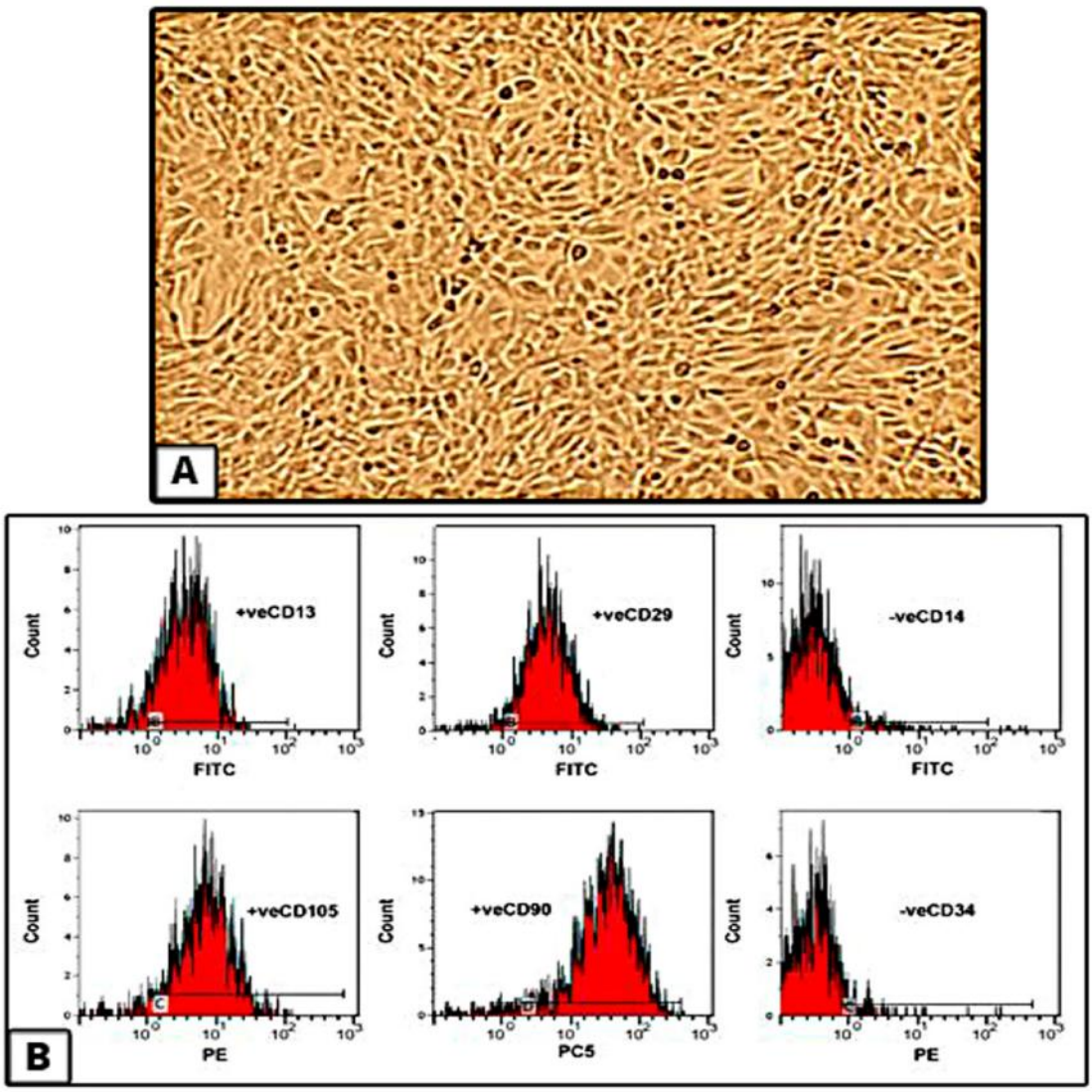

Figure (1). A. Photomicrographs of HAD-MSCs at 4 days post incubation showing small spindle-shaped fibroblastoid cells with oval-shaped structure. B. Flow cytometry of human AD-MSCs showing positive expression of surface protein markers CD13, 29, 90,105 and negative for CD 14 and 34.

2. Body weight & Biochemical Observations:

Body weight gain was markedly depleted in cisplatin-intoxicated group compared to the control. On the other hand, treatment with AD-MSCs cultured in media containing normal serum or renal homogenate improved body weight gaim more than that cultured in media containing pathogenic serum or renal homogenate. Both treatment showed less weight gain compared to the control (Fig. 2A).

Experimental group received cisplatin-treatment (single iv injection of 7 mg/kg body weight) exhibited significant increases in serum creatinine and BUN (G II), reached highest peak at 4 and 7 days, and then depleted at days 11 and stabilized at days 30 to values slightly higher than basekine. The urine creatinine clearance was markedly decreased. Intravenous injection of 5×10 6 human AD- MSCs (G III) to SD rats on day 1 after receiving cisplatin -treatment significantly depleted serum creatinine and BUN values (renal function tests) at days 4 & 7 (Fig. 2). Increased level of GSH and SOD in GIII, GIV, GV, GVI and a decrease of MDA. The improved was markedly detected in GIII & G V where cisplatin-intoxicated groups received AD-MSCs cultured in media containing normal renal homogenate or serum compared to less regenerated groups GIV and GVI which received AD-MSCs cultured in media containing pathogenic serum or renal homogenate (Fig. 3).

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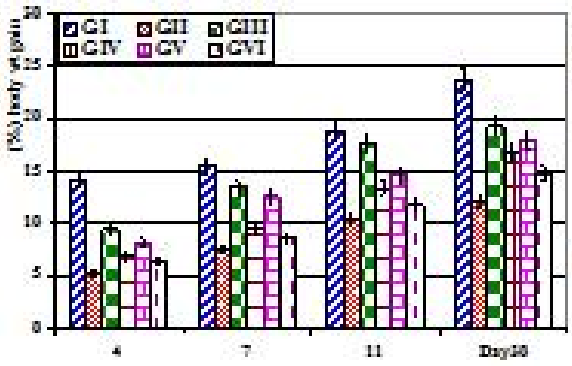

Fig. (A). Body weight

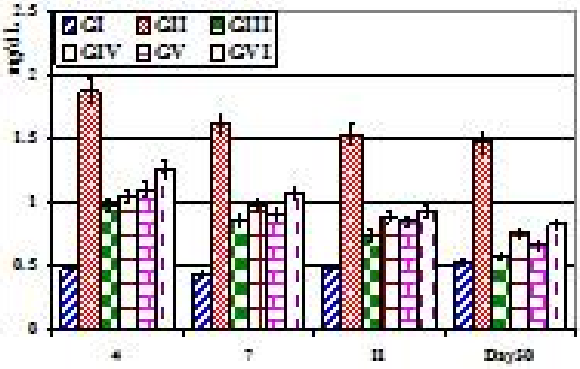

Fig. (B). Serum creatinine

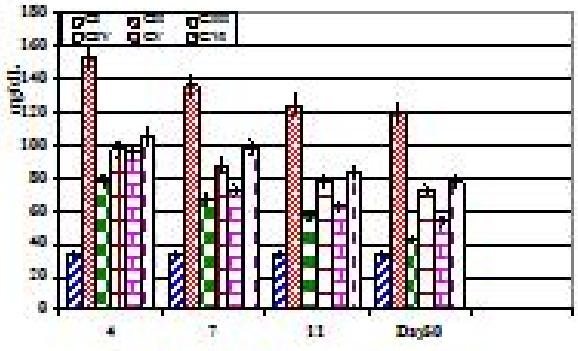

Fig. (C). Blood urea nitrogen

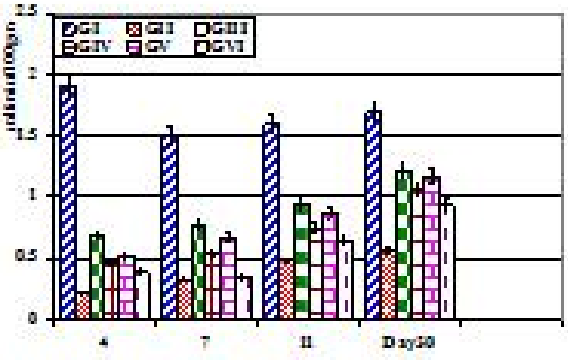

Fig. (D). Urine creatinine clearance

Figure (2). Body weight (g.) (A), serum creatinine (mg/dL) (B), blood urea nitrogen (BUN) (mg/dL) (C) and urine creatinine clearance (mg/dL) (D) of cisplatin-intoxicated rat received AD-mesenchymal stem cell with cultured in media containing either normal or pathogenic serum or rat renal homogenate. Each result represent the mean±SE (n=5) at intervals of 4, 7, 11 and 30 days. Cisplatin-intoxicated groups highly significant at $p < 0.001$ and other treated groups significant at $p < 0.05$.

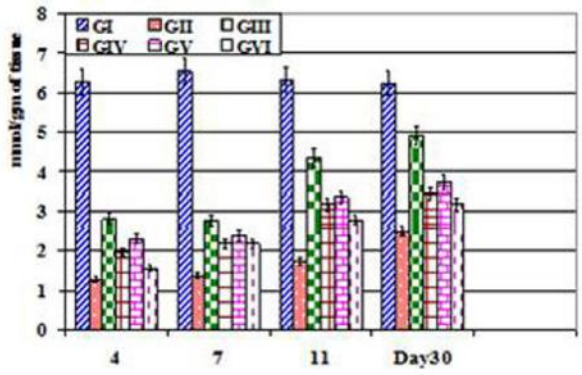

Fig. (A). Glutathione-S-reductase

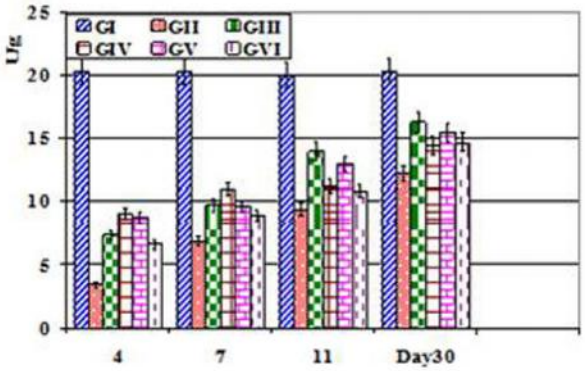

Fig. (B). Superoxide dismutase

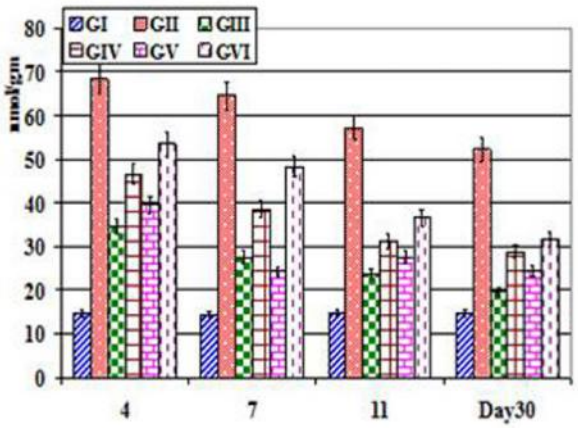

Fig. (C). Malondialdhyde

Figure (3). Glutathione-S-reductase (nmol/g of tissue) (A), superoxide-dismutase (U/g of tissue) (B) and malondialdhyde (mmol/gm of tissue) (C) of cisplatin-intoxicated rat received AD-mesenchymal stem cell with cultured in media containing either normal or pathogenic serum or rat renal homogenate. Each result represent the mean±SE (n=5) at intervals of 4, 7, 11 and 30 days. Cisplatin-intoxicated groups highly significant at $p< 0.001$ and other treated groups significant at $p< 0.05$.

3. Histological Observations:

From table (2), the histopathological scores were markedly increased in cisplatin-treated rats and improved in cisplatin-intoxicated group and received AD-MSCs cultured in media containing either normal serum or normal homogenate kidney compared to that cultured in media containing either pathogenic serum or kidney homogenate**.**

Histological observations of the control (G1), revealed normal pattern structure of renal medullary tissue including distal convoluted tubules and their lining epithelium and tubular lumina (Fig. 4A).

Experimental rats received cisplatin-intoxication exhibited marked degenerative changes in distal tubules of medullary region after 4 days-treatment. It varied from tubular cell vacuolar degeneration, up to complete tubular necrosis with apoptosis and shedding of tubular cells in about 90% of the tubules. No solid sheets were detected. Regenerative changes were also detected and varied from tubular cell enlargement with regenerative atypia, mitosis and interstitial solid sheet formation (5/10 HPF). The degenerative renal tubules proceed in the next period of treatment 7, 11 & 30 days (Fig.4B, 5A, 6A).

Table (2). Score of histopathological lesions of cisplatin-intoxicated rats recieved AD-mesenchymal stem cells cultured in media containing either with normal or pathogenic serum or rat renal homogenate.

Time (days)		Necrotic Tubules	Atrophic tubules	Mitotic Figures	R. interstitial solid sheets	R. Tubules	Inflamm. cells	Interst. fibrosis
4	G II	12.6±2.1	13.3±2.3	0.0±0.0	0.0±0.0	0.0±0.0	8.8±1.3	0.0±0.0
	GIII	4.2±0.4	7.3±1.5	0.0±0.0	4.6±0.3	1.5±0.4	1.6±0.4	0.0±0.0
	GIV	3.9±0.3	0.0±0.0	2.2±0.8	1.8±0.8	1.2±0.2	0.0±0.0	0.0±0.0
	GV	8.4±1.7	12.5±1.4	0.0±0.0	0.0±0.0	0.0±0.0	8.4±1.6	0.0±0.0
	G VI	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
7	G II	8.6±1.3	12.7±1.2	0.0±0.0	0.0±0.0	0.0±0.0	8.4±1.6	23.7±0.919.
	GIII	4.5±0.3	6.7±1.8	1.4±0.5	4.3±0.6	4.4±0.5	2.8±0.1	1.60.0±0.0
	GIV	1.6±0.4	0.0±0.0	2.7±0.2	1.8±0.6	2.7±0.2	0.0±0.0	23.1±1.4
	GV	8.3±1.5	12.5±1.3	0.0±0.0	0.0±0.0	0.0±0.0	8.3±1.9	0.0±0.0
	G VI	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	
11	G II	7.4±1.8	12.4±0.8	1.7±0.2	0.0±0.0	0.0±0.0	8.5±1.1	44.2±1.3
	GIII	4.6±0.3	5.4±1.7	4.3±0.4	4.5±0.3	7.6±1.5	0.0±0.0	17.6±1.5
	GV	7.5±2.2	12.3±0.6	1.5±0.3	0.0±0.0	0.0±0.0	8.1±1.5	42.8±2.3
	G VI	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
30	G II	4.7±0.1	4.4±0.2	1.7±0.2	1.3±0.2	1.4±0.2	4.7±0.2	37.6±2.6
	GIII	0.0±0.0	2.2±0.5	4.8±0.1	6.4±2.6	8.4±2.3	0.0±0.0	11.8±1.4
	GV	2.4±0.3	4.6±0.2	1.5±0.2	1.4±0.4	1.6±0.3	4.4±0.3	33.7±2.7
	G VI	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Experimental group intoxicated with cisplatin and received treatment of AD-MSCs in media containing serum or normal renal homogenate showed mild regenerative changes after 4 days-treatment. These were localized mainly in the inner strip outer medulla (ISOM) in the form of many regenerating tubules (70-80%) lined by large cells with large hyperchromatic active nuclei with few solid sheets (less than 1/20 HPF). The interstitium was the seat of mild inflammatory cellular infiltrate (Fig.4 C&D). At 7 days, the regenerative changes were also detected in about 20% of all fields examined in the experimental group, varied from tubular cell enlargement, mitosis and interstitial solid sheet formation. Regenerative changes were detected in the form of solid sheets with regeneration and mitosis. The interstitium was the seat of solid sheets and mild round cell infiltration and mild fibrosis (Fig.5 D &F). At 11 days, tubular dilatation with focal tubular necrosis (2-4 tubules /HPFs) was detected. Regenerative changes were increased mainly in the form of tubular regenerative atypia & stratification. The interstitium was the seat of solid sheets, mild round cells and some fibrosis (Fig.6 B&C). At 30 days, intact tubular architecture with focal degenerative changes (5% of all studied fields in the group), mainly in the form of tubular atrophy and dilatation. Regenerative changes were detected mainly in the form of tubular regenerative atypia. The interstitium was the seat of mild round cells and mild fibrosis (Fig.6 D&F).

Experimental group intoxicated with cisplatin and received AD-MSCs cultured media containing either pathogenic serum or renal homogenate for 4 and seven days showed less marked degenerative changes observed mainly in the outer medullary region**.** The degenerative changes varied from tubular cell vacuolar degeneration, up to complete tubular necrosis with apoptosis and shedding of tubular cells in about 60-70% of the tubules. Regenerative changes were also detected in both outer and inner medulla in the form of many interstitial solid sheets (2/10 HPF) and tubules lined by large cells with prominent nucleoli and with occasional mitosis (Fig. 4 E&F and 5 B&C).

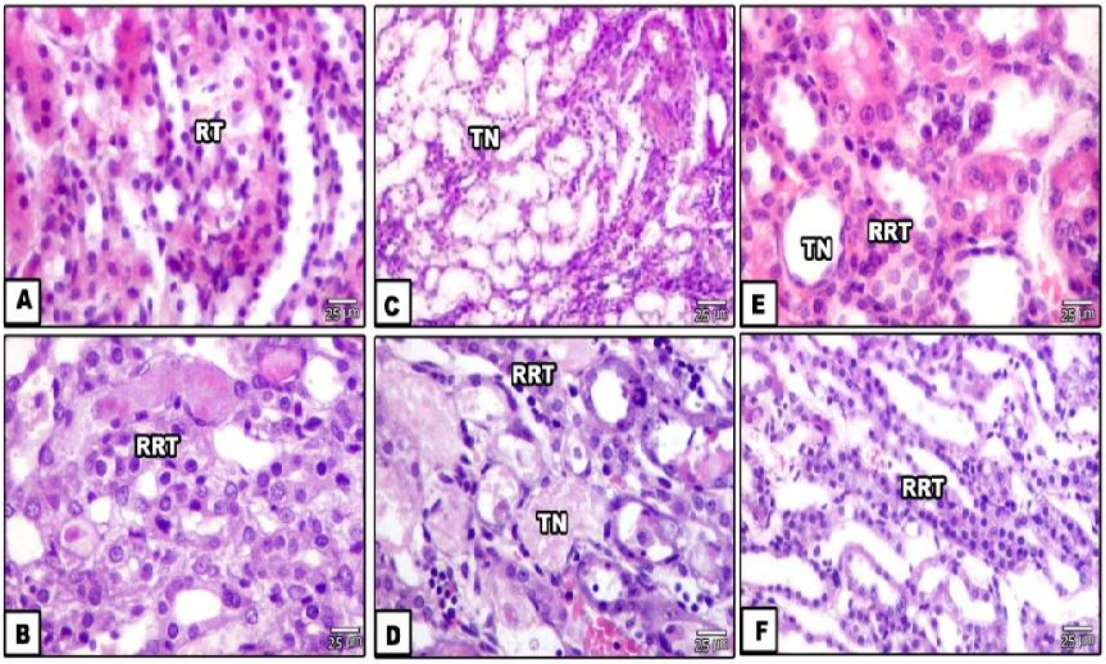

Figure 4(A-F). Photomicrograph of histological structure of rat kidny. A. Control showing intact medullary renal tubules (RT) at day 0. B. Cisplatin-treatment (G1) at 4 day showing necrotic dilated tubules. C&D. Cisplatin-intoxicated received AD-MSCs cultured in media containing normal serum or renal homogenate at 4 day. Note varying degrees of tubular necrosis (TN) infiltrated by regenerative changes in the inner strip outer medulla. E &F. Group IV showing regenerated (RRT) few solid sheets of tubular lining cells infiltrated tubulo-necrosis. HX&E

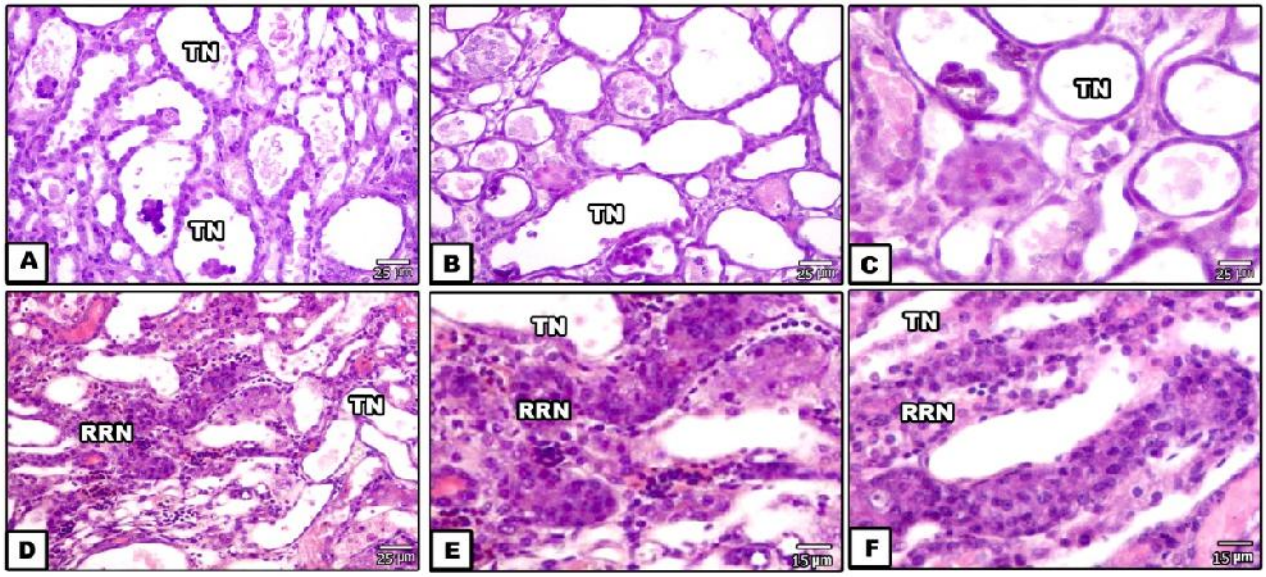

Figure 5 (A-F). Photomicrographs of histological section of rat kidney at 7 days. A. Cisplatin -intoxication for 7 days showing massive degenerative renal tubules infiltrated by few regenerating tubules (RRN) with lining cells having prominent hobnail festooning cells with bulging nuclei. B&C. Cisplatin-intoxication received AD-MSCs in incubating media containing either pathogenic serum or renal homogenate showing necrotic & dilated tubules (B) and regenerated tubules with stratification solid sheets in the interstium (C). D-F. Cisplatin-intoxication received AD-MSCs in media containing either normal serum or kidney homogenate showing tubular necrosis (TN) infiltrated with many regenerated interstitial solid sheets in inner and outer medulla. H&E.

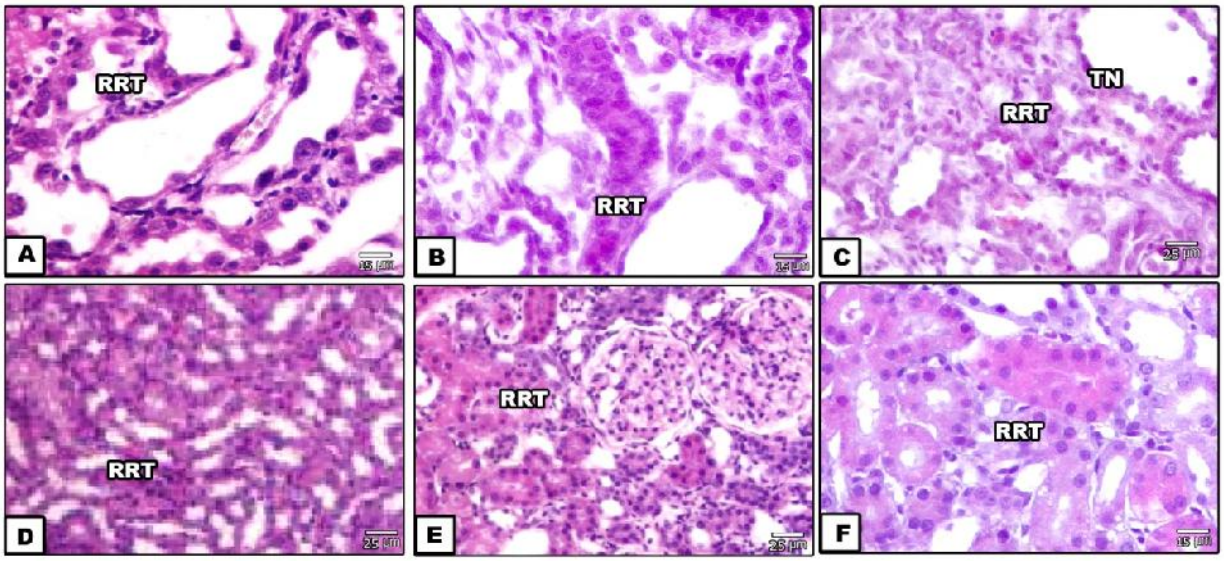

Figure 6 (A-F). Photomicrographs of histological section of rat kidney. A. Cisplatin -intoxication for 11 days showing massive tubular necrosis (TN) at OSMO with regenerating tubular cell arrangement. B&C. Cisplatin-intoxication received AD-MSCs in media containing normal serum or renal homogenate showing few intact renal tubules (B) and regenerated renal tubules (RRN) with luminal bulging cells having a hobnail appearance (C). D-F. Cisplatin-intoxication and received normal AD-MSCs therapy at 30 days showing regeneration of tubules. H&E.

Discussion

Adipose tissue derived mesenchymal stem cells are one of the source of adult stem cells [31] with high ability of trans-differentiation and self-renewal ability and possess potential role in regenerative medicine [32]. Recently, mesenchymal stem cell therapy suppressed inflammation and reduces renal damage of some systemic diseases such as systemic lupus erythematosus (SLE) and Crohn's disease patients [33,34].

According to Lin [35], AD-MSCs are known to have anti-inflammatory properties and immune modulation. AD-MSCs exhibit long-term proliferation, self-renewal and multipotent differentiation. The obtained AD-MSCs possessed a specific surface protein expression positive CD13, CD29, CD105 and CD90 and negative for the endothelial and hematopoietic-specific markers CD34 and CD 14.

Acute kidney injury (AKI) is resulted from the accumulation of waste products such as urea [36]. Cisplatin a chemotherapeutic drug that used for the treatment of different cancers [37, 38] and contributed to different pattern of nephrotoxcity and renal failure [39, 40]. These led many authors to select it as a model for animal studies [41].

The present findings revealed that cisplatin-intoxication resulted in the development of different pattern of degenerated tubular epithelium associated with increased interstitial inflammatory cell infiltration, internal haemorrhage and accumulation of hyaline casts.

Miller et al reported similar damage of renal tubules. [42] post-cisplatin-intoxication.

The present findings supported the work of Jouan-Lanhouet et al. [43] and Su et al., [44] whom reported that cisplatin-treatment resulted in various forms of cell deaths such as apoptosis, necrosis, and autophagic cell death in epithelial cells of proximal tubules.

Also, the observed tubular necrosis was assessed by impairment of renal functions were confirmed by increase of blood urea nitrogen nitrogen and creatinine levels and urine creatinine clearance in cisplatin-intoxicated rats.

Similar findings were reported by Arunkumar et al. [45] in patients treated with cisplatin.

Peres and Cuncha Junior [13] attributed cisplatin nephrotoxicity to its high binding affinity with amino acids containing thiol group like cysteine. Also, it is dissociated in blood with subsequent release of chloride ligands and formation of positively charged platinium ions which bind to DNA, impairing its replication and transcription, leading to cell damage [46].

Also, high retention of cisplatin in renal tissues led to apparent tubular necrosis, glomerular atrophy and reduction of glomerular filtration rates [8,46].

Following administration of AD-MSCs cultured in media containing normal serum or renal homogenate decreased the incidence of renal damage in cisplatin-intoxicated rats. The regenerated sheet of renal tubules firstly appeared as solid sheets, and then proliferated during the periods of treatment. The score of histopathological lesions were markedly decreased.

Similar findings were reported by Villanueva et al. [47,48] who reported that AD-MSCs revealed significant reduction in plasma creatinine levels in rat model of chronic renal failure.

Human umbilical cord derived MSCs detected predominantly in peritubular areas and acted to reduce renal cell death in mice subjected to cisplatin induced renal damage [49].

Also, AD-MSCs-treatment led to marked improved of renal function tests including serum creatinine, blood urea nitrogen, urine creatinine clearance and increased antioxidant defense of glutathion-s-reductase and superoxide dismutase parallel with a decreased level of malondialdhyde.

Mesenchymal stem cell therapy led to manage antioxidative defense and decreased liberation of reactive oxygen species [50].

Mesenchymal stem cells have a great therapeutic potential. Kunter et al.[18] injected intra-arterial BM-MSCs in a rat model of anti-Thy1.1mesangioproliferative glomerulonephritis and detected the presence of adipocytes in 20% of glomeruli which facilitated of preserving glomerular structure and reducing proteinuria.

On the other hand, treatment with AD-MSCs cultured in media containing pathogenic serum or renal homogenate exhibited mild regenerative capacity compared to that of normal components.

Morigi et al. [19] and Kim et al. [51] attributed the therapeutic potential of AD-MSCs against cisplatin induced nephrotoxicity to the liberation of certain types of factors otherwise their contribution directly to the cells of the damaged tubules.

AD-MSCs was found to up-regulated growth factors facilitated ameliorated adenine induced renal damage [52].

Finally the authors concluded that cisplatin associated nephrotoxicity explained by direct depletion of the antioxidant defense of GSH and SOD initiating tubular epithelium damage, improved by AD-MSCS cultured in media containing either serum and renal homogenate which modulate antioxidantive-defense. These led to scavenge of free radical and improve renal function tests and decrease apoptosis by reducing the level of malondialdhydes.

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