ORIGINAL ARTICLE

Role of Mesenchymal Stem Cell Therapy in Cisplatin Induced Nephrotoxicity in Adult Albino Rats: Ultrastructural & Biochemical Study

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ABSTRACT

Objective: Mesenchymal stem cells (MSCs) have generated a great deal of excitement and promise as a potential source of cells for cell-based therapeutic strategies. These data provide the clue of using MSCs in the current work in correcting cisplatin-induced nephrotoxicity, the severest adverse effect of the well-known anticancer drug; cisplatin. **Methods:** MSCs of bone marrow origin of femora and tibiae of adult albino rats were separated, grown, propagated in culture then identified by both morphology and CD29 surface marker detection. MSCs were injected into the rats" tail veins one day after a single dose (5 mg/kg body weight) of intraperitoneal injection of cisplatin. Four weeks later kidney tissue was examined histopathologically and ultra-structurally. Renal functions s(urea, creatinine) as well as serum electrolytes levels (Na, K) were estimated **Results:** Cisplatin group demonstrated atrophied glomeruli, thickened glomerular basement membrane, dilated urinary space, loss of proximal convoluted tubules brush borders, loss of podocyte pedicels and collagen deposition. Tubular cells showed vacuolization and nuclear membrane degeneration. Serum levels of urea, creatinine, Na and K were significantly elevated. MSCs ameliorated cisplatin-induced nephrotoxicity to a great extent as evidenced histologically, ultra-structurally and biochemically. **Conclusion:** MSCs have a potential therapeutic effect against cisplatin induced nephrotoxicity.

Keywords: Mesenchymal stem cells, Cisplatin, Kidney, Ultra-structures

INTRODUCTION

Cisplatin is widely used as an anticancer drug of considerable value for chemotherapy of several human neoplasms. However, this agent often causes renal toxicity.[1] On other hand, acute renal failure is known as a public health problem worldwide. Acute renal failure complicates approximately 5% of all hospitalized patients with a higher prevalence in critical care unites.[2] Also chronic kidney disease has become a worldwide public health problem. Renal transplantation is the treatment of choice for end-stage renal disease, but is limited by a small number of organ donors and the immune barrier.[3]

Cetin[4] suggested that cisplatin treatment caused significant oxidant load to kidneys through both xanthine oxidase activation and impaired antioxidant defense system. Cisplatin administration caused a significant increase in blood urea nitrogen (BUN) and creatinine levels with marked elevation in lipid peroxides accompanied by a significant decrease in reduced glutathione (GSH) content of kidney tissue as compared to control group.[5]

Histopathological findings also revealed significant damage in the kidney tissues from cisplatin-treated rats. Renal interstitial fibrosis a major complication of cisplatin treatment, occurred due to the increased accumulation of extracellular matrix (ECM) proteins whose remodeling is important for the development of normal tissues.[6] With cisplatin administration, the kidney revealed acute tubular necrosis along with dilatation and slogging of epithelium.[7]

Nabila et al[8] stated that cisplatin administration produced cellular and sub-cellular changes in kidney in the form of increased kidney weight, atrophic glomeruli, dilated urinary space, loss of proximal convoluted tubule brush borders, hypertrophied podocyte pedicles, thickened glomerular basement membrane as well as tubular cell vacuolization.

After cisplatin administration, the kidney showed glomeruli congestion, marked ATN in proximal and distal tubule, decrease height of epithelial cells, shedding of atypical cytoplasm and loss of brush border and marked congestion in vessels. Marked increased in levels plasma urea and creatinine are noticed as compared to control.[9]

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Stem cell treatment is a type of intervention strategy that introduces new cells into damaged tissue in order to treat disease or injury. Many medical researchers believe that stem cell treatment has the potential to change the face of human disease and alleviate suffering. The ability of stem cells to self-renew and give rise to subsequent generations with variable degree of differentiation capacities, offers significant potential for generation of tissues that can replace diseased and damaged areas in the body, with minimal risk of rejection and side effects.[10]

There are contradictory reports regarding the role of stem cells in treatment of renal failure, also only few studies have been published on the effects of stem cells in treatment of cisplatin-induced kidney failure. Experimental evidence suggested that administrating exogenous mesenchymal stem cells during acute and chronic kidney injury may improve functional and structural recovery of the tubular, glomerular and interstitial kidney compartments.[11]

Marina et al.[12] mentioned that injury to a target organ can be sensed by bone marrow stem cells that migrated to site of damage, undergone differentiation and promoted structural and functional repair. They stated that injection of mesenchymal stem cells protected cisplatin-treated mice from renal function impairment and severe tubular injury.

Bi B et al.[13] confirmed that injection of bone marrow-derived stromal cells (BMSC) reduced the severity of cisplatin-induced acute renal failure, enhanced tubular cell proliferation after injury and decreased tubular cell apoptosis.

In contrast to the previous studies, Yousof et al.[14] confirmed that stem cells have no role in protecting the kidney against kidney failure induced by cisplatin administration.

MATERIAL AND METHODS

A- Animals

Forty adult male albino rats (Sprague-Dawley strain) will be used in the current study, weighing 180-200 gm. The rats will be housed in cages, four rats in each cage, under standardized laboratory conditions with controlled light-dark cycle, temperature 22±2 and moisture 60-70%, animals had food and water supply ad libitium. The rats will be divided into four equal groups:

Group I: (plain control): Consists of ten rats which will received no treatments

Group II**: (sham control):** Consists of ten rats which will receive single dose of intraperitoneal injection of normal saline.

Group III: Consists of ten rats. Each rat will be intraperitonealy injected by a single dose of cisplatin, 5 mg/kg body weight.

Group IV: Consists of ten rats, Each rat will be intraperitonealy injected by a single dose of cisplatin, 5 mg/kg body weight by followed by intravenous injection of mesenchymal stem cells after one day. All rats will be sacrificed after 4 weeks.

B-Chemicals

1- **Cisplatin (Platinol):** Obtained in form of yellowish crystalline powder from Bristol-Myers Squib Company. Each vial containing 10 mg diluted in 10 ml normal saline to get final concentration of 1 mg/ml.

2- **Isolation and cultivation of rat bone marrow derived stem cells:** Bone marrow will be harvested by flushing the tibiae and femurs of 6-week old rats with Dul-becco's modified Eagle's medium (Gibco/BRL, Carlsabad, CA, USA) supplemented with 10% fetal bovine serum (Gibco/BRL). Nucleated cells will be isolated with density gradient (Ficoll/paque, Pharmacia Biotech AB, Uppsala, Sweden) suspended in complete culture medium supplemented with 1% penicillin-streptomycin (Gibco/BRL). The cells will be incubated at 37° C in 5% humidified CO2 for 12-14 days as primary culture or up on formation of large colonies. When large colonies will develop (80%-90% confluence), the cultures will be washed twice with phosphate buffered saline and the cells will be treated with 0.25% trypsin in 1 mmol ethylene daimine tetra acetate (Gibco/BRL) for 5 min at 37 °C. After centrifugation, the cells will be resuspended with serum-supplemented medium and will be incubated in 50 cm^2 cultues flask. Theses cultures were referred to as first-passage cultures.[15]

C-Morphological Study

The morphometric parameters of the rat kidney will be investigated in order to shed light on any anatomical features. The rats will be weighted alive and the animals the sacrificed. A mid-ventral abdominal incision will be made on each animal, the peritoneum will be reflected and intestine displaced to gain access to the renal system. The kidney will be removed and weighted. The data obtained will be subjected to statistical analysis.

D-Histological and Ultrastructural Study

Light microscopic study

At the end of the duration of the experiment, rats will be sacrificed. In each rat, both kidneys will be dissected and removed. They will be fixed in 10 % neutral buffered formalin. After fixation, specimens will be processed for paraffin embedding. The kidneys will be sectioned at the thickness of 5-7 microns to be stained with:

1- **Haematoxylin and Eosin:** For the histological examination of the general architecture of the studied organ.

2- **Masson's trichrome:** For detection of collagenous fibers.

3- **Periodic acid Schiff (PAS) reaction:** For detection of mucopolysaccharide and glycogen.

Electron microscopic study

Procedure for conventional EM studies[16]

Kidneys tissues from all experimental animals will be immediately prepared after kidney extraction for electron microscopic study. The tissue will be fixed in 3% glutaraldehyde in phosphate buffer then 1% osmium tetraoxide in phosphate buffer. Dehydration will be accomplished in graded ethanol. The specimens will be left in equal volumes of epon andacetone or propylene oxide for one hour, to facilitate the infiltration of the resin. The hardness of the final block will be controlled by adjusting the ratio of the two mixtures.[17] The fixed tissue specimens will be disposed in the beam capsule. Polymerization will be done at 60°C for 24 hours. Ultrathin sections (10-100 nm thickness) with a sliver or golden color,[18] will be cut by diamond knife to make a ribbon of sections. The section will be stained and prepared for electron microscopic examination using Ziess EM transmission electron microscope.

C-Biochemical Study

Serum will be collected and serum creatinine, urea, K and Na measured in order to assess renal functions.

RESULTS:

Effect of BMSCs on Biochemical Parameters:

Cisplatin group demonstrated high significant elevation ($P \leq 0.01$) of serum levels of urea and creatinine as compared to control group. On the other hand, BMSCs/cisplatin treated animals revealed high significant reduction of the elevated levels of serum urea and creatinine ($P \leq 0.01$) as compared to cisplatin group. However serum urea and creatinine were not completely ameliorated and were still significantly elevated as compared to control ($P \leq 0.05$) (Table 1).

Effect of BMSCs Treatment on Serum Electrolytes:

Cisplatin produced high significant increase in serum sodium (Na) and potassium (K) levels ($P \leq 0.01$) as compared to control group, while BMSCs/cisplatin group revealed highly significant reduction in serum levels of Na and K ($P \leq 0.01$) as compared to cisplatin group. BMSCs completely ameliorated the elevated serum K level with no significant difference compared to control, while Na serum level was still significantly elevated ($P \leq 0.05$) (Table 2).

Effect of BMSCs Treatment on Body Weight and Kidney Weight:

No significant change in rats' body weight was observed in all groups, while the mean value of rat's kidney weigh of cisplatin treated group was markedly elevated with significant difference ($P \leq 0.05$) as compared to control. The increase kidney weight was completely corrected in BMSCs/cisplatin group (Table 3).

Control and Sham Control Groups:

Both groups were indistinguishable from each other showing normal architecture.

Cisplatin-induced nephrotoxicity:

In comparison with control group (Fig. 1a), Hx.&E. stained sections revealed distinct glomerular degenerative changes including shrunken and lobulated glomeruli, numerous cytoplasmic vacuolizations, wide Bowman`s space and thickened parietal layer of the Bowman`s capsule (Fig. 1b).

Table 1: Effect of BMSCs on biochemical parameters

	Control N=10	Sham Control N=10	Cisplatin N=10	Cisplatin/ BMSCs N=10
Serum Urea (mg/dl)	46.5±3.98	47.93±3.41	81.9±14.1	50.9±3.33
P1		0.4	0.0001**	0.015*
P2				0.0001**
Serum Creatinine (mg/dl)	0.21±0.046	0.22±0.036	0.54±0.198	0.25±0.038
P1		0.9	0.0001**	0.039*
P2				0.0003**

P1: Comparison with control group P≤0.05: Significant*
P2: Comparison with cisplatin group P≤0.01: Highly significant**

Table 2: Effect of BMSCs on serum electrolytes

	Control N=10	Sham Control N=10	Cisplatin N=10	Cisplatin/ BMSCs N=10
Serum Sodium (meq/L)	136±1.21	136.97±2.056	163.1±163.1	140±4.02
P1		0.65	0.0001**	0.014*
P2				0.0001**
Serum Potassium (meq/L)	3.56±0.299	3.51±0.292	4.86±0.744	3.88±0.267
P1		0.92	0.0001**	0.02*
P2				0.001**

P1: Comparison with control group P≤0.05: Significant*
P2: Comparison with cisplatin group P≤0.01: Highly significant**

Table 3: Effect of BMSCs on body weight and kidney weight

	Control N=10	Sham Control N=10	Cisplatin N=10	Cisplatin/ BMSCs N=10
Body weight (g)	250±7.62	251±7.42	244±4.59	248±7.89
P1		0.53	0.06	0.67
P2				0.18
Kidney weight (g)	0.59±0.024	0.60±0.0337	0.84±0.112	0.61±0.405
P1		0.49	0.0001**	0.16
P2				0.0001**

P1: Comparison with control group P≤0.05: Significant*
P2: Comparison with cisplatin group P≤0.01: Highly significant**

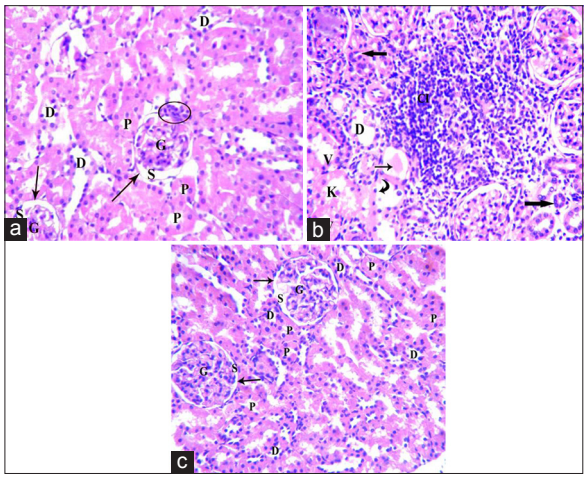

Figure 1: Micrographs of normal control (1a), cisplatin group (1b) and cisplatin/BMSCs group (1c). In comparison with control group (1a) renal sections of animals exposed to cisplatin injection revealed shrunken and lobulated glomeruli (G), numerous cytoplasmic vacuolization (arrowhead), wide Bowman`s space (S) and thickened parietal layer of the Bowman`s capsule (thin arrow). Proximal convoluted tubules were collapsed, had thick wall and lost brush border (P). Distal convoluted tubules showed variable degrees of dilatation (D). Their lining epithelial cells demonstrated cytoplasmic vacuolization (V) and variable signs of nuclear degeneration as karyorhexis (thick arrow), karyolysis (K) as well as pyknotic like changes (curved arrow). Intraluminal shedding of degenerated epithelial cells (L) and hyaline casts were also observed inside the renal tubules (1b). Mononuclear cellular infiltration is obvious (CI). With BMSCs treatment Renal glomeruli preserved the normal histological architecture reappearance of the macula densa (encircled). Proximal (P) and distal (D) convoluted tubules are comparable to the control (1c). (Hx. & E. x400)

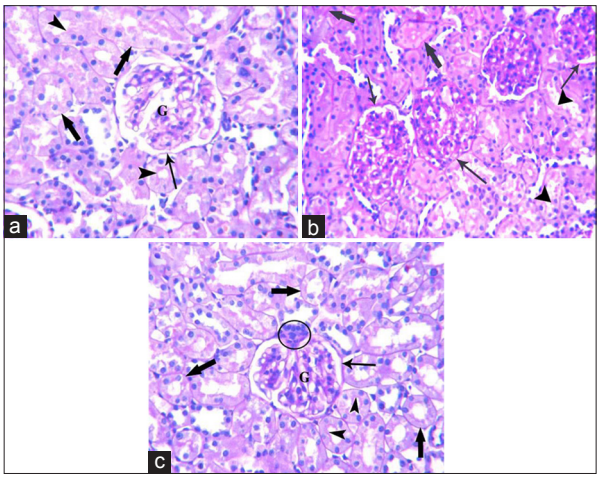

Figure 2: Micrographs of normal control (2a), cisplatin group (2b) and cisplatin/BMSCs group (2c). In comparison with control group (2a) PAS stained sections of cisplatin group showed marked thickening of the parietal layer of Bowman`s capsule (thin arrow) and hyalinosis and obliteration of Bowman`s space. The basement membrane of proximal and distal convoluted tubules was also thickened (thick arrow) and the brush border of the proximal convoluted tubules was lost with deposition of intraluminal hyaline casts were (arrowheads) (1b). BMSCs treated group displayed restoration of normal histological architecture of the renal glomeruli and tubules. The brush border of the proximal convoluted tubules (arrowheads) and macula densa (encircled) were restored (1c). (PAS x400)

Histopathological changes of cisplatin-induced nephrotoxicity were reflected mainly in the proximal and distal convoluted tubules. Proximal convoluted tubules were collapsed, had thick wall and lost brush border. Distal convoluted tubules showed variable degrees of dilatation and sometimes they were ballooned (Fig. 3b). Their lining epithelial cells demonstrated cytoplasmic vacuolization and variable signs of nuclear degeneration as karyorhexis, karyolysis as well as pyknotic like changes. Intraluminal shedding of degenerated epithelial cells and hyaline casts were also observed inside the renal tubules (Fig. 1b).

Signs of inflammation were expressed by mononuclear cellular infiltration among renal tubules (Fig. 1b).

As compared to the control group (Fig. 2b), PAS stained sections showed thickening of the parietal layer of Bowman`s capsule and hyalinosis and obliteration of Bowman`s space. The basement membrane of proximal and distal convoluted tubules was also thickened and the brush border of the proximal convoluted tubules was lost (Fig. 2b).

Masson's trichrome stained sections exhibited increased collagen deposition around the parietal layer of Bowman`s capsule and the basement membrane of proximal and distal convoluted tubules. Interstitial fibrous tissue was also increased (Fig. 3b) when compared to control (Fig. 3a).

Effect of BMSCs treatment in cisplatin-induced nephrotoxicity:

BMSCs induced impressive regenerative effect on cisplatin-induced renal pathologic changes. Injection of BMSCs almost completely abrogated tubular damage. Renal glomeruli appeared rounded with preserved normal histological architecture, normal thickness of the parietal layer of Bowman's capsule as well as normal appearance of the capsular space. The macula densa was also preserved (Fig. 2c). However, mild shrinkage of some renal glomeruli and dilatation of Bowman`s space was noticed denoting variable degrees of regeneration triggered by BMSCs (Fig. 1c).

Injection of BMSCs almost completely abrogated tubular damage. However, some renal tubules were collapsed other showed degenerative changes. Marked reduction of areas mononuclear cellular infiltration could be observed (Fig. 1c).

PAS stained sections displayed disappearance of glomerular hyalinosis and regaining of the normal thickness of the parietal layer of Bowman's capsule and basement membrane of renal tubules. The brush border of the proximal convoluted tubules was also retained. However, infrequent areas of increased thickness of the parietal layer of Bowman's capsule and tubular basement were seen (Fig. 2c).

BMSCs injection regressed collagen deposition around renal tubules and in the interstitial tissues which appeared

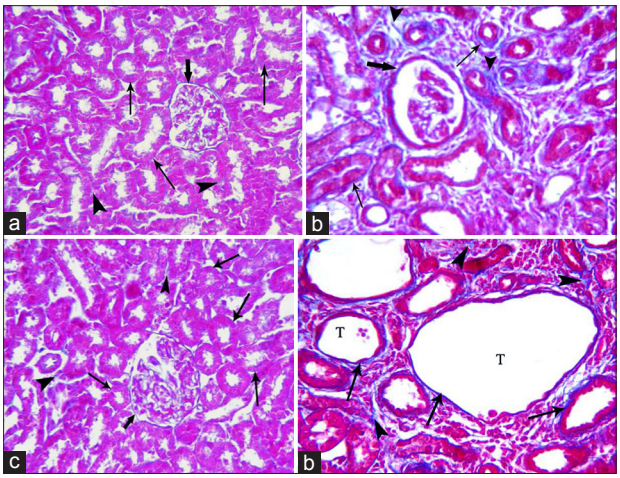

Figure 3: Micrographs of normal control (3a), cisplatin group (3b) and cisplatin/BMSCs group (3c). In comparison with control group (1a) Masson's trichrome stained sections renal sections of cisplatin group exhibited increased collagen deposition around the parietal layer of Bowman`s capsule (thick arrow) and the basement membrane of proximal and distal convoluted tubules (thin arrow). Interstitial fibrous tissue was also increased (arrowheads) (3b). BMSCs injection regressed collagen deposition around renal glomeruli (thick arrow) and tubules (thin arrow) and in the interstitial tissues which appeared similar to the control group (3c). (Masson's Trichrome x400)

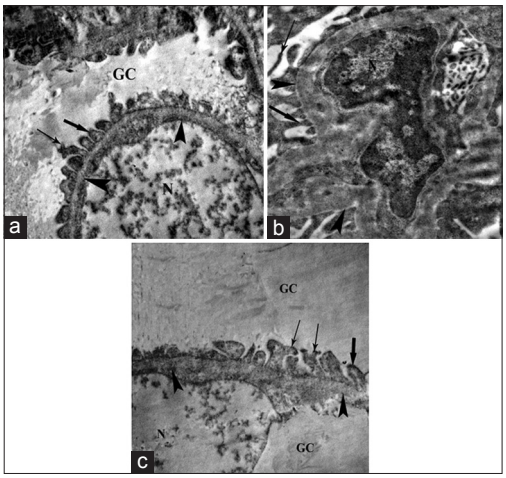

Figure 4: Ultramicrographs of normal control (4a), cisplatin group (4b) and cisplatin/BMSCs group (4c) revealing podocytes ultrastructural changes in the experimental groups as compared to the control. In cisplatin group the nucleus is irregular and has electron dense, clumped and marginated chromatin (N). The foot processes appeared either coalesced with disfigured appearance (thick arrow), detached in the glomerular capillaries (thin arrow) or totally lost. The basal lamina is thick, homogenous, irregular and lacked its trilaminar appearance (arrowhead) (4b). Specimens of BMSCs/cisplatin group regained the normal structural appearance. The podocytes have a well developed primary (thick arrow) and secondary pedicles (thin arrow). The nucleus has electron lucent peripherally distributed euchromatin (N). The basal lamina has trilaminar appearance (arrowhead) (4c). (E.M. x 15000)

similar to the control group, except of small infrequent areas (Fig. 3c). Electron microscopy:

Cisplatin-induced nephrotoxicity:

Ultrastructural surveillance of kidney specimens of animals exposed to cisplatin injection revealed glomerular changes; the podocytes' nuclei were irregular and possessed electron dense, clumped and marginated chromatin. The foot processes appeared either coalesced with disfigured appearance or totally lost. Some of them were detached in the glomerular capillaries. The basal lamina became thick, homogenous, irregular and lacked its trilaminar appearance (Fig. 4b).

Lining epithelial cells of proximal tubules suffered from marked degenerative changes, they had complete loss of the brush border of their luminal surface, thick basal lamina, multiple cytoplasmic vacuolization and extensive cytoplasmic rarefaction. Their nuclei displayed electron dense, irregular, clumped and marginated chromatin together with degenerated segments of the nuclear envelope. Mitochondria were variable in size and exhibited vacuolization, loss of their cristae, rupture of their outer membrane and early ballooning (Fig. 5a).

However, the distal convoluted tubules of the same group showed fewer degenerative changes as compared to that of proximal convoluted tubules. There was partial degeneration of the apical mitochondria with a partial destruction of the luminal part of the cells. Also, there was a partial clumping of nuclear chromatin together with thickness and irregularity of the basal lamina as well as appearance of cytoplasmic rarefaction (Fig. 6a).

Effect of BMSCs treatment in cisplatin-induced nephrotoxicity:

As compared to the control group (Fig. 4a), rats of this group almost regained the normal ultrastructural appearance. The podocytes appeared to have well developed primary and secondary pedicles. The nucleus showed electron lucent peripherally distributed euchromatin. The basal lamina retained its trilaminar appearance (Fig. 4c).

The proximal convoluted tubules retained normal appearance of their nuclei which were seen oval, basal and euchromatic. The basement membrane was thin and regular and most of mitochondria appeared similar to the normal control. The luminal surface the renal tubular cells carried well developed microvilli. However, partial loss of microvilli and infrequent appearance of small-sized intracytoplasmic vacuoles were also detected (Fig. 5c).

With the exception of appearance of very few intracytoplasmic vacuoles, the distal convoluted tubules appeared cuboidal, lacking brush border and contained oval, central and euchromatic nuclei with distributed chromatin. The cytoplasm contained numerous rounded and oval mitochondria of normal appearance (Fig. 6c).

DISCUSSION

As predicted, administration of cisplatin (5 mg/kg i.p.) in the present work resulted in an overt nephrotoxicity as evidenced biochemically, morphologically and

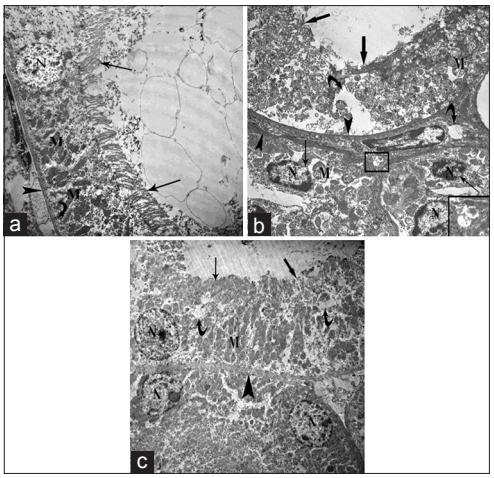

Figure 5: Ultramicrographs of normal control (5a), cisplatin group (5b) and cisplatin/BMSCs group (5c) revealing proximal convoluted tubules ultrastructural changes in the experimental groups as compared to the control. In cisplatin group, lining epithelial cells of proximal tubules show complete loss of brush border of the luminal surface (thick arrow) and thick basement lamina (arrowhead), multiple cytoplasmic vacuolization (curved arrow) and extensive cytoplasmic rarefaction. The nucleus has electron dense, clumped and marginated chromatin together (N) with degenerated segments of the nuclear envelope (thin arrow). Mitochondria are variable in size and exhibit vacuolization (M), loss of their cristae or rupture of outer membrane and early ballooning (boxed) (5b). BMSCs treated rats regained intact nuclei (N), microvilli (thick arrow), thin regular basement membrane (arrow head) and numerous mitochondria (M) of the proximal convoluted tubules. Scattered areas of vacuolization (curved arrow) and partial loss of microvilli (thick arrow) are seen (5c). (E.M. x 3000)

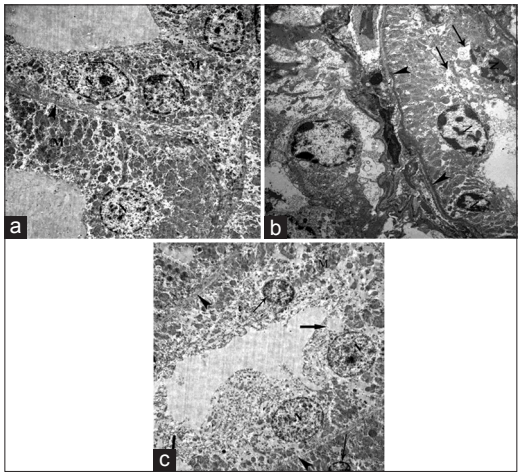

Figure 6: Ultramicrographs of normal control (6a), cisplatin group (6b) and cisplatin/BMSCs group (6c) revealing distal convoluted tubules ultrastructural changes in the experimental groups as compared to the control. In cisplatin group, their lining epithelial cells exhibit thick irregular basement membrane (arrow heads) and multiple cytoplasmic vacuolization (arrows). The nuclei (N) have clumped marginated chromatin. Partial degeneration of the apical mitochondria and partial destruction of the luminal part of the cells are noticed (6c). BMSCs treated rats show intact nuclei (N), thin regular basement membrane (arrow head) and numerous mitochondria (M). Scattered areas of vacuolization (thick arrow) are still seen (6c). (E.M. x 3000).

histologically which were detected by both light and electron microscope. The above mention results reflected manifestations of impaired renal functions.[19]

Biochemically, measurement of serum urea and creatinine levels thirty days after cisplatin injection revealed a high significant elevation of their levels as compared with control. These cisplatin-induced changes could be attributed to reduction in glomerular filtration rate. Moreover, tubular injury induces back leak obstructing glomerular filtrate in cisplatin-treated rats.[20] Another postulation demonstrated that alterations in glomerular function was secondary to the elevated reactive oxygen species. The later reactions lead to mesangial cells contraction and reduces the filtration surface area thus modifying the ultrafiltration coefficient factors with decreased glomerular filtration.[21]

Moreover serum electrolytes disturbance was recorded in the present work as indicated by high significant elevation of serum sodium and potassium levels as compared with control. These findings were analogous to the findings of similar studies.[22] However, serum sodium and potassium diminished instead of being elevated after cisplatin administration.[23] The later authors attributed their observation to tubular damage and dysfunction.

On the other hand, morphologically, there were no significant changes noticed in the rats' body weight as compared with control group. Identical findings regarding the body weight were reported.[24] Contradicting studies, reported a significant decrease in the rats' body weight after cisplatin injection which might be attributed to gastrointestinal disorders caused by cisplatin.[25]

However, there was observable increase in kidney weight in the current work. Similar results were described and suggested to be an indication of renal damage due to the cellular degenerative changes, including cytoplasmic vacuolization of the proximal tubular cells, tubular dilation and mononuclear cellular infiltration.[26] On contrary to the later authors and the current findings, a significant decrease in the kidney weight was monitored after cisplatin treatment.[27]

In the current study, cisplatin injection altered the histological architecture of the renal glomeruli which appeared shrunken and lobulated with consequent widening of Bowman`s space. These histological changes were similar to that observed.[28] On the other hand, few researcher observed no glomerular changes but still the latest in conclusion.[29]

Ultrastructurally, the basal lamina of glomerular podocytes lacked its trilaminar appearance[25] and were suggested to be the sequel of glomerular sclerosing process after cisplatin treatment.[30]

The results of the present study indicated that cisplatin brought significant histopathological changes in both proximal and distal convoluted tubules. The PCT appeared

collapsed with thick wall, while DCT showed variable degrees of dilatation with flattening of their epithelial lining cells. Additionally the tubular system were disfigured or ballooned in many cases. Similar findings were also observed.[31]

Furthermore, renal tubular lining epithelial cells exhibited cytoplasmic vacuolization and variable signs of nuclear degeneration as karyorhexis, karyolysis and ghost cells which were signs of necrosis. In some previous works, regarding cellular changes, Pyknotic like changes were also seen indicating apoptosis and necrosis.[32]

An extensive damage of the proximal convoluted tubules were recorded in the form of loss of the brush border, cytoplasmic vacuolization and desquamated epithelial cells. These histological findings were in accordance to other cisplatin studies.[33]

A characteristic hyaline casts were observed inside the renal tubules. A considered explanation to the presence of such finding stated that necrotic epithelial cells provides the matrix for tubular cast formation with subsequent tubular obstruction.[16]

Moreover, ultrastrucural surveillance of the lining epithelial cells of PCT showed irregular basal lamina, multiple cytoplasmic vacuolization and rarefaction. Mitochondria were variable in size and exhibited vacuolization, lost cristae, rupture of their outer membrane and early ballooning of the inner membrane. However, DCT showed fewer degenerative changes as compared to that of the PCT. The degeneration was limited to the apical mitochondria. Irregular basal lamina and cytoplasmic rarefaction were also seen. Both tubular data were comparable to similar study.[25] Additionally, PCT histological findings were analogous to previous reported data.[34]

An explanation of the cisplatin injurious effect was based on its selective nephrotoxicity especially high exposure of kidney to cisplatin. The major route of cisplatin elimination is filtration at the glomeruli and secretion in the PCT. It had been established that cisplatin concentration in the PCT is about five times the serum concentration. Cisplatin enters the cell through passive diffusion and transporter-mediated process (YAO).[23] Copper transporter receptor1 (Ctr1) and organic cation receptor2 (Oct2) are highly expressed in the PCT and actively transport cisplatin. This disproportionate accumulation of cisplatin in the PCT contributes to cisplatin-induced nephrotoxicity and extensive tubular injury. [35]

There were several interconnected mechanisms responsible for cisplatin-induced nephrotoxicity and cell death. Cisplatin undergoes metabolic activation in the kidney to more potent toxic compounds. The cumulative effect of cisplatin and its product mediate their cytotoxic effects through their interaction with DNA. Cisplatin binds to DNA leading to the formation of inter- and intrastrand cross-links, thereby arresting DNA synthesis and replication. So that, the induction of cell cycle arrest and DNA damage-inducible genes correlate with enhanced apoptosis as a key pathway for cisplatin induced growth arrest. However, cells can differ in their response to cisplatin according to the extent of damage of proteins or molecules, as high doses of cisplatin induce necrosis of tubular epithelial cells, while lower doses generate apoptosis.[32]

Unfortunately, beside inhibition of protein synthesis of tubular epithelial cells, cisplatin directly binds to the antioxidant cellular molecules such as glutathione (GSH) and disrupts the cellular oxidant defense system. Moreover, cisplatin enhances the formation of reactive oxygen species (e.g. superoxide anion and hydrogen peroxide) as well as reactive nitrogen species (peroxynitrite and nitric oxide). They directly act on cell components, including DNA, lipids, proteins, and destroy their structure.[23] Furthermore, these free radicals consequently decrease the renal anti- oxidant enzymatic activity with enhanced lipid peroxidation and more DNA damage and cell death.[36]

Several lines of evidence suggested that mitochondrial DNA or other mitochondrial targets were more important than nuclear DNA damage in mediating cisplatin-induced cell death. Thus, the sensitivity of cells to cisplatin appeared to correlate with both the density of mitochondria and the mitochondrial membrane potential. This observation may explain the particular sensitivity of PCT to cisplatin toxicity, as this segment exhibits one of the highest densities of mitochondria in the kidney.[37]

In the current work, signs of inflammation were expressed by variable degrees of mononuclear cellular infiltration among renal tubules. This histological apperance was in compliance with other similar works.[29,36]

There was a growing recognition of the importance of inflammatory mechanisms, in addition to direct cellular toxicity, the pathogenesis of cisplatin nephrotoxicity through recruitment of inflammatory cells, such as macrophages and leukocyte that contribute to cisplatin-induced damage. Furthermore, cisplatin increased renal expression of a variety of inflammatory chemokines and cytokines, such as tumor necrosis factorα-(TNFα) and interleukin (IL)-1β.[38]

In the current study, PAS reaction displayed thickened tubular and glomerular basement membranes as well

as hyalinosis of some renal glomeruli. Accumulation of glycoproteins due to the induced renal damage could support and explain the results of the current work.[39]

Histological study using Masson's trichrome stained sections exhibited increased collagen deposition around renal glomeruli and tubular basement membrane together with increased interstitial fibrous tissue. Similar observation were reported.[40]

Conflicting postulations concerning the underlying mechanism of the development of irreversible tubulointerstitial fibrosis were encountered. It was suggested that fibrosis develops as a result of the excessive production of matrix proteins by the activated and phenotypically altered resident cells tauighty.[41] However, other studies elucidated that macrophages may play an important role in renal interstitial fibrosis via the production of certain fibrogenic factors as tumor necrosis factor α (TNF-α) and transforming growth factor β (TGF-β). Such tumor factors mediate induction of myofibroblastic cells capable of producing extracellular matrices[42] On the other hand, ROS were claimed to evoke the development of renal interstitial fibrosis which, in turn, lead to renal function impairment.[43]

The co-administration of BMSCs and cisplatin in the current work induced outstanding morphological and histologically recovery, approaching the normal parameters. However, biochemical parameters reveled marked improvement but still elevated as compared with the control one.

BMSCs were highly significantly reduced the elevated levels of serum urea and creatinine as well as sodium and potassium levels as compared to cisplatin group. However, these parameters were still significantly higher than normal levels. In partial agreement with the present work, other reports documented that, MSCs completely recovered the cisplatin-disturbed kidney functions.[44] This improvement could be related to the role of BMSCs treatment in restoring the kidney architecture, thereby preserving GFR which detected biochemically.[45]

On the other hand, morphologically, BMSCs administration revealed kidney enlargement with highly significant reduction in cisplatin-induced elevated mean kidney weight which return to its normal value. Identical findings were reported by in MSCs treated animals with gentamycin-induced nephrotoxicity.[46]

Histologically, light microscopic examination of the rat kidney specimens revealed the restoration of the normal architecture of most of the glomeruli in BMSCs treated group. Comparable findings were observed in BMSCs treated rats with anti-Thy1.1 induced –nephrotoxicity.[47]

Ultrastructurally, glomerular podocytes also restorted its normal structure with reappearance of well developed primary and secondary pedicles, trilaminar basal lamina and euchromatic nucleus. Comparable results were obtained in MSCs treated rats with adriamycin-induced nephropathy.[48] The authors emphasized that MSCs increased glomerular vascular endothelial growth factor (VEGF) expression and limited microvascular rarefaction which may explain the noticeable pro survival effect by stem cell therapy.

In the current work, BMSCs remarkably regained the normal architecture of the proximal and distal convoluted tubules with disappearance of most of cisplatin-induced histopathological changes. However, some renal tubules showed degenerative changes and collapsing.

Additionally, ultrastructural surveillance of the proximal and distal convoluted tubules confirmed the protective effect of BMSCs, as they retained their normal appearance, most of mitochondria appeared intact and similar to the normal control. The luminal surface of proximal renal tubular cells carry well developed microvilli and the basement lamina reattained its thin trilaminar appearance. A partial loss of microvilli and infrequent appearance of small-sized intracytoplasmic vacuoles were noticed in limited cases. In this respect, administration of conditioned medium derived by murine BM-MSC to mice with cisplatin induced AKI, enhanced tubular cell proliferation, and limited renal cell apoptosis.[49]

Moreover, human BM-MSC infusion preserved the integrity of the tubular epithelium and prolongs survival in AKI mice.[28]

In the present work, BMSCs markedly reduced mononuclear cellular infiltration stimulated by cisplatin administration. Similar observations were reported.[28] In fact, the anti inflammatory action of MSCs is exerted by suppressing the release of TNF-α and IL-1β from renal tubular cells; the inflammatory cytokine mediating cisplatin-induced renal toxicity.[28,50] Hyaline cast formation was decreased and only few casts could be detected in BMSCs/cisplatin treated group. these finding was concurring with comparable study.[44]

BMSCs injection after cisplatin administration produced marked regression of the increased interstitial fibrous tissue and collagen deposition around renal tubules. Confirming to these results, MSCs treatment reduced levels of fibrosis in rats with chronic renal failure.[51] The authors clarified the immunosuppressant, anti-inflammatory and apoptic effects of MSCs through the reduction of renal interleukin (IL)-6, tumor necrosis factor (TNF)-α, and all serum cytokine which were decreased in MSC-treated animals. Meanwhile, it was reported that, tumor necrosis factor α (TNF-α)

was one of the important fibrogenic factors in cisplatin-induced nephrotoxicity, which mediates the induction of myofibroblastic cells capable of producing extracellular matric.[42]

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