Role of stem cells in decreasing renal failure in rats

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ABSTRACT

Stem cells are homogenous cells able-to-proliferate and differentiate producing almost all types of cells. They can yield a great number of differentiated functional cells creating the tissue after hurt. Stem cell therapy is a recent type of intervention strategy that is introduced-to-treat-a-specific-illness. Renal failure is one of the greatest common reasons of morbidity-and-mortality-all-over-the-world. Treatment options of chronic renal failure (CRF) mainly involve renal transplantation and blood dialysis. Kidney transplantation is limited by shortage of donors while dialysis is-associated---with--several-socioeconomic-problems-for-the-patients.-In this study we tried to estimate the use of stem cells for antagonizing induced renal failure via apoptotic parameters. The animals were classified into five groups which received chemotherapeutic drug (cisplatin) to induce kidney damage, tissue and blood samples were collected. The oxidative content measured by glutathione. The serum urea, creatinine and IL18 biomarkers levels were decreased significantly. This confirms that under the conditions of this study, stem cells can heal the renal damage made by cisplatin in rats.

INTRODUCTION

Cisplatin is a chemical anti-cancer drug; it is one of the most important agents in chemotherapy with applications of almost50% of human cancers among 700 FDA-approved drugs *(Boulikas, 2007)*. It is one of the most economic and effective chemotherapeutic drugs used in treatment of several types of solid tumors such as testicular, head, ovarian, neck and lung carcinoma *(Barabas et al., 2008 and Jia et al., 2011)*.

Nevertheless, the use of thisremedy is limited in human treatment due to its major side effects. Thus, prevention of these side effects is one of the maindifficulties in treating cancer. Although, many trials have been tested to prevent the side effects of cisplatin (such as the concurrent use of anti-oxidants), scientists did not approve its clinical use *(Chang et al., 2002; Vickers et al., 2004).*

El-Sayed et al. (2008), Noori and Mahboob (2008) and Abdel Gawad and Mohamed (2010) stated that administration of cisplatin resulted in a major increase in the levels of

creatinine in comparison to standard parameters. Furthermore, cisplatin precipitated noticeable decrease in glutathione (GSH) content of kidney tissue compared to control group*. (Miller et al., 2010).*

Davis et al. (2001) and Ganesan and Brian(2004)said-that-the-nephrotoxic-effect-of-this chemotherapeutic drug is mediated by decreased protein synthesis, DNA damage, mitochondrial dysfunction and membrane peroxidation.

In last decade, many researches were published regarding the medical use of bone marrow-derived stromal cells (BMSCs) in treatment of kidney diseases. Some results declared that BMSCs contributed significantly to healing of renal tissue and to a high degree during recovery from tissue injury*(Morigi et al., 2004)*.

Stem cell therapy is a modern technique of intervention strategy in which cells are transported to damaged tissue in order to treat a specific disorder or injury. Many scientists believe that stem cells have the ability to alter the future of human disease and terminate pain. The potential of stem cells to multiply and give rise to subsequent generations with different degrees of differentiation dimensions offers marked potential for the generation of tissues that promote functional and structural repair *(Fa-Ming and Xiaohua 2016)*

Bi et al. (2007) declared that injection of BMSCs reduced the severity of cisplatin-induced acute renal failure, decreased tubular cell apoptosis and improved tubular cell proliferation next to injury. These studies were agreed by ***Wan et al. (2010)*** who stated that BMSCs could differentiate into the tubular epithelial cells directly, improving the functions of kidney and curing acute renal failure in mice after cisplatin administration.

MATERIALS AND METHODS

Aim of the work and plan

We aim to evaluate the protective effect of stem cells in rats with induced kidney failure. Fifty mature male albino rats were used in the study. The rats were held in metal cages. All the ethical rules for animal treatment were followed and directed by the animal house, Faculty of science, Al- Azhar University. The rats were divided into five equal groups, ten rats in each cage:

Group A (plain control): ten rats did not receive any drug.**Group B (cisplatin):** ten rats received a single dose of intraperitoneal injection of **5mg/kg** body weight cisplatin and killed after 5 days to confirm renal damage *(Chang et al., 2002)*.**Group C: (Cisplatin and saline)**: ten rats were intraperitoneally injected by a single dose of **cisplatin, 5mg/kg** body weight followed by a single dose of 1 ml saline (IV) after 5 days *(Chang et al., 2002)*.**Group D (Cisplatin and Mesenchymal stem cells treated-group)-**each-rat-was injected intraperitoneally with a single dose of **cisplatin, 5mg/kg**

body weight followed after 5 days by venous injection of **mesenchymal stem cells** in the tail vein of the subject, in a dose of (10^6) **cells / animal** *(Tögel et al.,2005).*

Group E (Recovery group): ten rats received-a-single-dose-of intraperitoneal injection-of-**5mg/kg**-body-weight-cisplatin for each and sacrificed after 30 days to evaluate recovery.

Standard-deviation-(SD), Statistical significance and difference from control and test values were evaluated by analysis of variance (ANOVA) using SPSS. P values <0.05 was considered statistically significant, while P < 0.01 was considered statistically highly significant.

Isolation of BM-derived MSCs from rats:

Bone marrow was harvested by flushing the tibia and femur of six weeks old male albino rats with Dulbecco's modified Eagle's serum (DMEM,GIBCO/BRL)-supplemented-by-ten%-fetal-bovine-medium   (GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll/Paque-(Pharmacia)].

The cells were incubated at 37° C in 5% humidified CO_2 for 12-15-days-as-primary-culture-upon-formation-of-large-colonies.When-large colonies developed-(80-90%-confluence),-cultures-were-washed twice with phosphate buffer saline (PBS) and cells were trypsinized with 0.25% trypsin in 1mm EDTA (GIBCO/BRL) for 5-minutes-at-37∘-C.-After-centrifugation (at 2400 rpm for 20-minutes)-cells-were-re-suspended-in-serum-supplemented medium and incubated in 50 cm^2 culture flask (Falcon). The produced cultures were referred to as first passage cultures **(Abdel Aziz et al., 2007)**.

Biochemical results

After 30 days, serum urea, creatine and IL18 biomarkers levels in addition to tissue glutathione content were measured using colorimetric method. The results were subjected to statistical analysis.

Histo-pathological study

At the end of trial, rats were killed and kidneys are prepared for pathological examination by light microscope.

A- Preparation for paraffin blocks and sections

Each kidney was fixed and processed for paraffin blocks. Histological sections (5-6μ thick) were stained with:

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

B- Stains

1- Haematoxylin and Eosin stain (Bancroft and Gamble, 2008).

Method

1. The deparaffinization of the sections were done in xylol . Hydration was done in descending grades of alcohol.

2. The sections were stained in haematoxylin for ten minutes .

3.Washing of excess blue colour under running tap water for five minutes.

4. Dryness was done on hot plate at 40°C until tissue appeared dryed and stretched.

5.Counterstain in 1% solution of eosin for ten minutes was done .

6. Washing excess colour under running tap water was carried out

7. Dehydration in ascending grades of alcohol, clearing in xylol and mounting were performed.

Statistical analysis

The results of biochemical studies were expressed as mean± standard

Results and Discussion

Control groups (Group A)

In control group, no changes were observed between morphological findings of this group and known normal rat kidney appearance.

Histological examination of the animal kidney specimens showed renal cortex containing Malpighian corpuscles and convoluted tubules. Each renal Malpighian corpuscle seemed as a rounded structure and formed of a glomerulus surrounded by Bowman's capsule. The glomerulus consisted of a capillary tuft which lined by endothelial cells with numerous nuclei. Bowman's capsule had visceral layerattached to the glomerulus and thin parietal layer which surrounded by a basement membrane and lined with simple squamous epithelium. Bowman's space (urinary or capsular space) occurred between the visceral and parietal layer (fig.1). The cells of macula densa were seen close to vascular the renal corpuscle. Their nuclei appear to be much closer to each other (fig.1).

The proximal convoluted tubules had narrow lumens and lined by a few number of cuboidal cells with rounded nuclei in addition to apical brush border (fig.1). The distal

convoluted tubules had wide lumens and were lined by cuboidal cells with rounded nuclei and rested on basement membrane with no brush border (fig. 1).
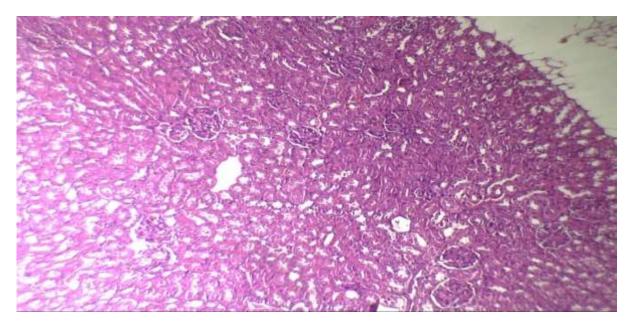

Fig. (1): Section from the kidney showing normal-sized glomeruli, uniform tubules lined by cuboidal cells with no evidence of cystic dilation or cast formation (H&E, 100X)

Cisplatin administration group (Group B)

As compared to the control groups, light microscopic analysis of kidney specimens of the animals exposed to cisplatin injection revealed degenerative changes in the form of shrunken glomeruli, with wide Bowman`s space as well as thickening of parietal layer of Bowman`scapsule, ill-defined outline of some renal glomeruli (fig.2)
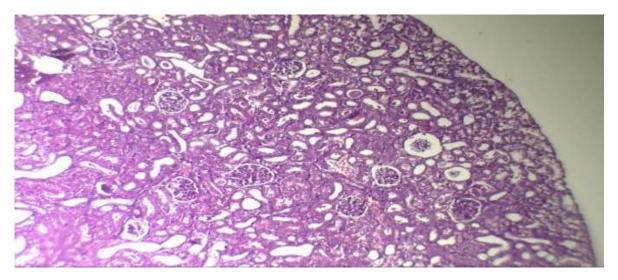

Fig. (02): Section from the kidney showing acute tubular necrosis with dilatation of tubules, epithelial flattening, brush border loss in proximal tubules, and shedding of

Mesenchymal stem cells treated group after cisplatin administration (Group C)

As compared to the control groups administration of bone marrow derived stem cells one day next to cisplatin injection caused reduction of the pathological changes as regard to the extent and degree-produced-by-cisplatin-administration-alone.

Light-microscopic-examination of the rat kidney specimensshown almost normal presence of most of the renal tubules and glomeruli. Each renal glomerulus appeared rounded with preserved normal histological construction. The glomerulus consisted of a capillary tuft with numerous nuclei (fig.3). The parietal layer-of-Bowman´s capsule was seen as single layer similar to the control group as well as normal appearance of the capsular space. However, minor shrinkage of some renal glomeruli and dilatation of Bowman`s (capsular) space were seen (fig.3) denoting the variable degree of regeneration triggered by BMSCs.
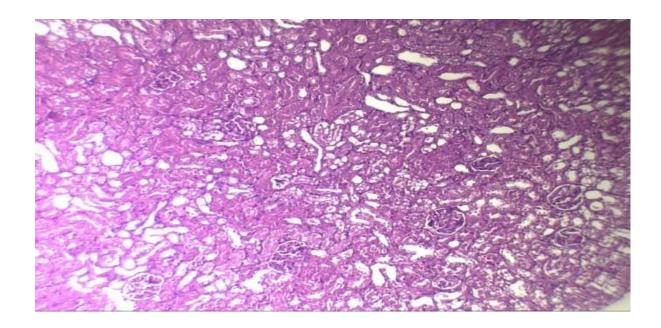
Group injected with saline (group D)

Figure3: Section from the kidney showing moderate dilatation of tubules, moderate atrophic glomeruli (H&E, 100X)
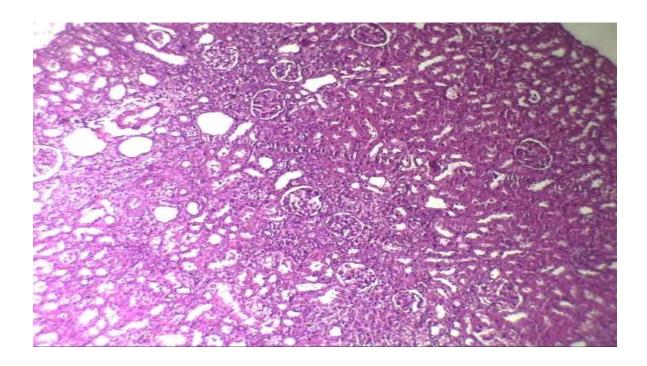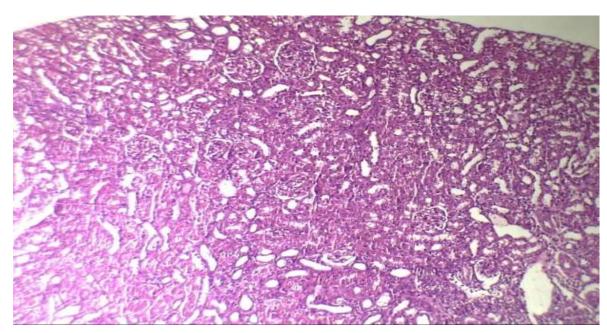
Fig. (4): Section from the kidney showing chronic pyelonephritis. Where, interstitial and periglomerular fibrous areas (H&E, 100X)

Recovery group (E)

Fig 5: Section from the kidney showing few atrophic glomeruli and glomerulonephritis, moderate tubular necrosis with dilatation of tubules (H&E, 100X)

Table 1: concentrations of glutathione, urea, creatinine and IL18 in normal, cisplatin, mesenchymal, saline and recovery groups.

		Control (10)	Cisplatin (8)	Mesenchymal (8)	Saline (7)	Recovery (7)
Glutathione in-tissues	(mmol/g)	**24.85**	**17.65**	**22.23**	**20.4**	**19.84**
Serum urea	(mg-dl)	**22.5**	**22.5**	**22.5**	**22.5**	**22.5**
Serum creatinine	(mg-dl)	**0.464**	**0.464**	**0.464**	**0.464**	**0.464**
Serum IL18	(pg/ml)	**32.4**	**32.4**	**32.4**	**32.4**	**32.4**

Table 2: The change in concentration of glutathione, urea, creatinine and IL18 in cisplatin, mesenchymal, saline and recovery groups compared to control.

group / Parameter	Creatinine (mg-dl)	Urea (mg-dl)	IL18 (pg/ml)	Glutathione (mmol/g)
Cisplatin	+ (431) **%**	+ (209) %	+ (106) **%**	- (28) **%**
Mesenchymal	+ (89) **%**	+ (3.3) %	+ (10.7) **%**	- (10.5) **%**
Saline	+ (238) **%**	+ (20.6) %	+ (24.3) **%**	- (26.7) **%**
Recovery	+ (183) **%**	+ (15.6) **%**	+ (20.4) **%**	- (20) **%**

Nephrotoxicity is a well-known side effect of cisplatin which limits its use as anti-cancer remedy. Acute kidney injury is one of the most serious and common complication made by cisplatin administration which occurs in 20–30% of patients treated by this drug *(Miller et al., 2010 and Al-Kharusi et al., 2013)*

In the ongoing work, administration of cisplatin **(5 mg/kg i.p.)** resulted in an overt nephrotoxicity as evidenced by the detection of histological and morphological changes by light microscope in addition to bio-chemical manifestations of diminished renal functions.

Morphologically, rats intoxicated by a single injection of cisplatin showed no noticeable changes in body weight as compared to control group. In similar study, *Gautier et al. (2010)* informed that rats injected by cisplatin showed no variations in their body weights.

In the current work, cisplatin injection caused structural alterations in the renal glomeruli which seemed shrunken with consequent widening of Bowman`s space as well as ill-defined outline of some renal glomeruli and obliteration of Bowman`s space. These results were similar to that of *Shirwaikar et al. (2003)*and *Abdel Meguid et al. (2010).*The previous results of the present study clarified by *Giuseppa et al.(2009)* who stated that the reduction in the glomerular size and glomerular collapse were a sequel of glomerular damage process.

Moreover, the collective effect of cisplatin and its by product culminate their cytotoxic effects through interaction with DNA leading to cell death. In an aqueous environment, the chloride ligands of cisplatin are substituted by water molecules generating a positively charged electrophile which reacts with nuclear DNA. The produced cisplatin-DNA intra-strand cross-links resulted in cytotoxicity and were thought to be the reason for cellular death *(Galea and Murray, 2002 and Boulikas andVougiouka, 2003).*Another suggestion was that ROS in cisplatin induced nephrotoxicity directly proceededto DNA and destroyed it *(Ajith et al., 2007).* These reports could explain the observed lesions in the present work.

Biochemically, this study verified that treatment with cisplatin administration impaired renal oxidation load which manifested by decreasedglutathione as compared to control. Similar results obtained by *Somani et al. (2000)and Aydogan et al. (2008)*, who proposed that tubular injury which include obstruction and back leak of glomerular filtrate could be the cause of the decrease in glomerular functions in cisplatin-treated rats. The alterations in glomerular functions may also be secondary to reactive free radicles which induced mesangial cells contraction, altering the filtration surface area and altering the ultrafiltration coefficient factors that reduced the glomerular filtration rate

Significant exhaustion of anti-oxidant content was observed in our study in cisplatin group compared to normal control; this is in matching with *Helen H. and MacusTienKuo.(2010)* who noticed that this may be because cisplatin inhibits glutamylcysteine synthesis (γ-GCS), the rate-limiting enzyme for GSH biosynthesis.

*Morigi et al. (2004)*clarified that injury to a target organ can be detected by the injected bone marrow stem cells that travel to the site of the injury, undergo differentiation and promote functional and structural repair. MSCs homed in the damaged renal tissue and differentiated into tubular epithelial cells, thereby restoring renal structure and function indicating that the exogenous administration of MSCs could promote both functional and structural renal healingthrough the trans-differentiation of MSCs into tubular epithelium.

Rookmaaker et al. (2007) declared that bone-marrow-derived stem cells may home to damaged glomerular endothelium, differentiate into endothelial cells and participate in restoration of the highly specialized glomerular microvasculature.

We published a paper titled (THE MODERN USE OF STEM CELLS IN TREATMENT OF RENAL FAILURE IN RAT MODEL) and tested other parameters : cystatn c,super oxide dismutase and malondaldehde.

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