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CLINICOPATHOLOGICAL AND HISTOPOTHOLOGICAL STUDIES ON THE USE OF ADIPOSE DERIVED STEM CELLS IN TREATMENT OF STREPTOZOTOCIN INDUCED DIABETIC NEPHROPATHY IN RATS

By

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ABSTRACT

Diabetic nephropathy (DN) is considered the major microvascular complication of diabetes mellitus and the main cause of chronic kidney disease. Stem cell therapy results are promising for treatment of renal tissue degeneration. Stromal vascular fraction (SVF) is easily and rapidly isolated from adipose tissue, subjected to enzymatic digestion. It is composed mainly of mesenchymal stem cells (MSCs) and other stem and regenerative cells. Forty Sprague Dawley male rats were randomly divided into four equal groups; (I) control group, (II) were diabetic untreated group, (III) and (IV) SVF single and multiple IP transplanted diabetic rats respectively. In the present study, DN was induced by single IP injection of streptozotocin (STZ) in a dose of 55 mg/ kg body weight. Adipose tissue was collected from abdominal subcutaneous region of 5 female rats, digested, centrifuged, and the SVF pellet was collected and activated using low intensity laser. Laser activated SVF was IP transplanted in group III and IV rats at the 7th day of STZ injection and injected again after two weeks in group IV. Blood and urine samples were collected three times every two weeks starting from STZ injection day. Rats were euthanized 6th weeks after STZ injection and kidney specimens were also collected. Hematological, biochemical, urine, and histopathological examinations were performed for all experimental groups. Diabetes mellitus resulted in abnormalities in the tested parameters and it were improved by transplantation of SVF. SVF multiple IP transplanted rats exhibited better improvement in all measured parameters that was confirmed by histopathological examination, thus repeated SVF transplantation is preferred for better regeneration of acute degenerated kidney.

Keywords:

Stromal Vascular Fraction,Adipose tissue,Stem cells,Low Intensity Laser Irradiation, Diabetic nephropathy.

INTRODUCTION

The predominant feature of both types 1and 2 diabetes mellitus (DM) is the regression of pancreatic islets β-cells **(Dong *et al.*, 2008)**. The major microvascular complication of DM is diabetic nephropathy (DN) which is the most prominent cause of chronic kidney disease that represents nearly 50% of all end-stage renal disease all over the world **(Thomas *et al.*, 2004; Wang *et al.*, 2013).**

Currently, medications for DN are concerned with enhancing blood pressure and regulating blood glucose levels. However, these treatments are not beneficial in blocking further renal tissue damage **(Burney*et al.*,2009).**Limited kidney tissue donors,concurrently with complications of long-term immune suppression, led to development of DN therapy based on renal tissue regeneration **(Ezquer *et al.*, 2008; Fang *et al.*, 2012).**

Mesenchymal stem cells (MSCs) are considered an attractive candidate for renal repair for their immunomodulatory,multipotentiality and self-renewal capabilities. In addition, nephrons are of mesodermal origin. Previous studies by **Wang *et al.* (2013) and Hamza *et al.* (2017)** stated that MSC attenuate albuminuria and preserve normal renal histology in diabetic mice. The last author stated that bone marrow derived mesenchymal stem cells (BMSCs) contribute to cell turnover and repair in many tissue types, including the kidneys, however, **Liu and Tang (2016)** reviewed that phenotype of BMSCs, was affected when subjected to in vitro culture conditions. Furthermore, aging and aging related disorders significantly impair the viability and potency of BMSCs. In the present study, adipose tissue was selected as a source for MSCs isolation its ease of access and repeatability. Stromal vascular fraction (SVF), resulted from adipose tissue enzymatic digestion, is an idyllic applicable stem cell alternative as it is easily isolated and have abundant viable stem cell number obviating extensive expansion in culture to obtain pure stem cells **(Zuk *et al.*, 2001; Rombouts and Ploemacher, 2003)**. Moreover, **Ishimura *et al.* (2008)** found that characterization of SVF surface markers proved that most of them are positive to MSCs markers. To further enhance the therapeutic effect of freshly isolated SVF, low intensity laser irradiation (LILI) was applied as it has been proved to improve viability, protein expression and homing of stem cells to degenerated tissue through the activation of mitochondrial respiratory chain, this method is of low cost,

safe and saving time than culture method **(Abrahamse, 2011; Abdallah *et al.*, 2016).**

The present experiment was conducted to evaluate the therapeutic effect of transplanted laser activated SVF using single and multiple IP injections. Also, through detection of alterations in hematological parameters,kidney serum biomarkers,serum electrolytes as well as microalbuminuria in STZ induced DN in rats at some intervals.

MATERIAL AND METHODS

1. Experimental animals:

The present study was accepted by Institutional Animal Care and Use Committee (Approval ID: CU/ II/ F/ 43/ 18). Forty apparently healthy male Sprague Dawley rats weighing 180-200 grams (12~14 weeks old) were housed in groups in separate cages. They were fed balanced diet and water was ad libitum.

2. Study Design:

The used rats in the present study were divided into four groups. DM was induced in all groups except group I as it was left as a control. Treatment with the SVF began at the 7th day of DM induction. Blood and urine sampling were carried out in all the experimental groups three times; every two weeks. The rats were euthanized at the 6th week of SVF treatment.

a) Group-I(Control group) (10 rats): rats injected IP (intraperitoneally) with phosphate buffered saline (PBS).

b) Group –II (Diabetic untreated group) (10 rats): STZ diabetic untreated rats.

c) Group -III (SVF single IP injected group) (10 rats): Diabetic rats received single IP injection of laser activated SVF.

d) Group –IV (SVF multiple IP injected group) (10 rats): diabetic rats received IP injection of laser activated SVF at the 7th day of DM induction, 2 weeks later a second transplantation was administered. Rats were euthanized at the 6th week of the second SVF transplantation.

3. Induction of Diabetes mellitus:

Diabetes mellitus was induced by single IP injection of 55 mg/kg body weight STZ (WXBC6558V, Sigma-Aldrich, USA) in 0.1 M citrate buffer, pH 4.5 into 16-18^{hr} fasted rats as previously stated by **Chen *et al.*, 2016.** STZ-treated rats were kept for the next 24 hours on 5% glucose solution in their cages to prevent initial drug induced hypoglycemic mortality **(Oche *et al.*, 2014).** After seven days, rats that exhibited blood glucose more than 250 mg/dL were considered diabetic and selected for the experiment.

4. SVF Isolation, processing and activation.

4.1. Adipose tissue collection: Five female Sprague Dawley rats were euthanized and adipose tissue (30-40g**)** was collected under aseptic conditions from sub-cutaneous region of the abdomen into a 50 ml sterile cup.

4.2. Tissue Processing, Isolation, and activation of Stem and Regenerative Cells: Adipose tissue underwent enzymatic digestion and centrifugation to obtain the SVF. According to ***Zuk et al.*** **(2001)** the tissue was minced and washed several times till obtain clear infranatent. A volume of 0.1%collagenase typeI(234153, Sigma-Aldrich,USA)solution was supplemented per volume of adipose tissue and placed in a 37°C shaking water bath for 30 minutes. SVF pellet containing the stem and regenerative cells was re-suspended into PBS and activated using low level laser for 20 minutes (LED Technology Adilight2®), after which it was ready for animal injection.

5. SVF Transplantation in Diabetic Animals.

Under aseptic precautions, laser activated SVF was injected IP at a dose of $1.5x10^6$ nucleated cells/ rat **(El-domouky *et al.*, 2017)** of group III and IV.

6. Evaluation.

6.1. Clinicopathological evaluation: Blood sampling was performed at the 2nd, 4th and 6th week of SVF transplantation from retro-orbital venous plexus, simultaneously; spot urine samples were collected by manual compression of the bladder. Glucometer (GlucoDr supersensor, AGM-2200, Germany) was used for estimation of blood glucose level in all experimental groups. Blood samples were collected on EDTA, for complete blood count (CBC) utilizing animal cell counter (ABC Vet, France). Prepared serum samples were used to determine the levels of serum urea (Spectrum, Egypt), creatinine (Spectrum, Egypt), sodium (Spectrum, Egypt), and potassium (Spectrum, Egypt). Urine samples were collected for estimation of microalbuminuria (MAU) (Spectrum, Egypt).

6.2.Histopathological examinations:

Kidney specimens collected from rats of all experimental groups were fixed in 10 % neutral buffered formalin, routinely processed and embedded in paraffin then sectioned and stained by hematoxyline and eosin (H&E) and Periodic acid Schiff (PAS) according to **Bancroft and Gamble (2008).**

7.Statistical Analysis.

Data were presented as Mean ± SD. Variables were statistically analyzed by One-way

ANOVA using software COSTAT (version 6.400, CoHort software, USA) **(Cardinali and Nason, 2013)**.

RESULTS

Rats having blood glucose level more than 250 mg/dL were considered diabetic so were included in the diabetic untreated group and the SVF treated groups.

Effect of SVF transplantation on erythrogram of diabetic nephropathy rats:

Mean values of erythrogram are illustrated in (Table 1). Statistical analysis of data revealeda significant decrease in the values of Hb concentration, PCV percent and RBCs count of group II (diabetic untreated rats) all over the experimental period compared to control group. Meanwhile, MCV was significantly increased with significant decrease in MCHC value of group II at different times of the experiment compared to control group. Group III and IV rats showed rather normalized pattern of the erythrocytes especially at the 6th week compared to diabetic untreated rats.

Table (1): Erythrogram of different experimental groups (means ± SD).

Time	Animal groups	PCV (%)	Hb (g/dL)	RBCs (x 10^6 /µL)	MCV (fL)	MCHC (%)
2nd Week	I	39.60 ± 0.89^a	14.37 ± 0.71^a	8.18 ± 0.31^a	48.45 ± 1.67^c	36.28 ± 1.53^a
	II	37.00 ± 1.58^b	12.66 ± 0.15^b	6.61 ± 0.19^c	55.99 ± 2.27^a	34.22 ± 1.39^{ab}
	III	40.80 ± 0.76^a	14.39 ± 0.68^a	7.70 ± 0.47^b	53.07 ± 2.39^b	35.27 ± 1.61^{ab}
	IV	34.80 ± 0.67^c	11.58 ± 0.70^c	6.32 ± 0.27^c	55.11 ± 1.27^{ab}	33.29 ± 2.16^b
4th Week	I	38.82 ± 0.97^a	13.78 ± 0.39^a	7.34 ± 0.32^a	52.93 ± 1.32^b	35.51 ± 1.25^a
	II	37.20 ± 0.84^b	11.78 ± 0.49^c	7.01 ± 0.28^{ab}	53.11 ± 1.72^b	31.68 ± 1.55^c
	III	38.40 ± 1.14^a	13.04 ± 0.22^b	7.36 ± 0.17^a	52.19 ± 1.56^b	33.97 ± 1.07^{ab}
	IV	38.00 ± 0.35^{ab}	12.82 ± 0.28^b	6.72 ± 0.31^b	56.64 ± 2.61^a	33.73 ± 0.47^b
6th Week	I	40.80 ± 0.84^a	14.16 ± 0.48^a	8.25 ± 0.56^a	49.60 ± 2.79^b	34.71 ± 1.19^a
	II	35.42 ± 0.80^c	11.46 ± 0.48^b	6.46 ± 0.39^b	56.78 ± 0.76^a	32.35 ± 0.72^b
	III	40.30 ± 0.57^a	14.02 ± 1.11^a	8.31 ± 0.19^a	48.52 ± 0.84^b	34.78 ± 2.73^a
	IV	38.67 ± 0.54^b	13.51 ± 0.43^a	7.80 ± 0.59^a	49.79 ± 3.74^b	34.95 ± 1.59^a

Note: **Mean values with different superscript letters in the same column are significantly different at ($p \leq 0.05$).**

Effect of SVF transplantation on leukogram of diabetic nephropathy rats:

Leukogram results illustrated in (Table 2) revealed a significant leukopenia associated with significant lymphopenia, eosinopenia and monocytopenia and increase in absolute count of neutrophils in group II (Diabetic untreated rats) compared to control group. Meanwhile, group III exhibited significant leukocytosis due to significant neutrophilia that was observed throughout the experimental period. Treatment of diabetic rats with single or multiple IP injections of SVF improved total and absolute differential leukocytic counts compared to group II but counts were still different than that of control rats.

Table (2): Leukogram of different experimental groups (means ± SD).

Time	Animal groups	TLC (x 10^3 /µl)	Neutrophil (x 10^3 /µl)	Lymphocyte (x 10^3 /µl)	Eosinophil (x 10^3 /µl)	Monocyte (x 10^3 /µl)
2nd Week	I	17.66 ± 1.01^{b}	2.94 ± 0.39^{c}	13.67 ± 0.98^{a}	0.41 ± 0.06^{b}	0.64 ± 0.11^{b}
	II	16.76 ± 0.79^{b}	4.10 ± 0.20^{a}	12.20 ± 0.74^{b}	0.18 ± 0.02^{d}	0.27 ± 0.04^{d}
	III	20.46 ± 0.50^{a}	4.28 ± 0.38^{a}	14.28 ± 0.47^{a}	0.34 ± 0.04^{c}	1.56 ± 0.19^{a}
	IV	15.17 ± 0.21^{c}	3.53 ± 0.25^{b}	10.29 ± 0.10^{c}	0.87 ± 0.03^{a}	0.45 ± 0.03^{c}
4th Week	I	18.42 ± 0.87^{a}	2.71 ± 0.15^{c}	14.59 ± 0.76^{a}	0.40 ± 0.06^{a}	0.71 ± 0.05^{a}
	II	12.78 ± 0.67^{c}	3.53 ± 0.33^{ab}	8.79 ± 0.96^{d}	0.28 ± 0.05^{b}	0.17 ± 0.02^{c}
	III	16.96 ± 0.66^{b}	3.68 ± 0.73^{a}	12.58 ± 0.54^{b}	0.31 ± 0.04^{ab}	0.36 ± 0.06^{b}
	IV	13.30 ± 0.20^{c}	2.97 ± 0.03^{bc}	10.17 ± 0.25^{c}	0.17 ± 0.02^{c}	0.00 ± 0.00^{d}
6th Week	I	18.88 ± 0.86^{a}	2.69 ± 0.32^{b}	14.68 ± 0.77^{a}	0.51 ± 0.07^{b}	1.01 ± 0.12^{a}
	II	11.42 ± 0.41^{c}	2.70 ± 0.19^{b}	8.44 ± 0.27^{c}	0.19 ± 0.03^{d}	0.21 ± 0.04^{c}
	III	18.52 ± 1.13^{a}	5.14 ± 0.43^{a}	12.78 ± 0.91^{b}	0.30 ± 0.04^{c}	0.31 ±0.05^{bc}
	IV	15.99 ± 2.15^{b}	1.92 ± 0.26^{c}	13.11 ± 1.76^{b}	0.64 ± 0.09^{a}	0.32 ± 0.04^{b}

Note: **Mean values with different superscript letters in the same column are significantly different at ($p \leq 0.05$).**

Effect of SVF transplantation on kidney biomarkers of diabetic nephropathy rats:

Serum urea and creatinine levels (Table 3) showed a significant increase in group II all over the experimental period compared to control group.Group IV (SVF multiple IP injected rats) didn't show an improvement in serum urea level compared to group II (Diabetic untreated rats), in contrast, group III (SVF single IP injected rats) showed significant improvement from the 2nd week till the end of the experiment.Group III and IV showed a significant reduction in serum creatinine levels at the 4th and 6th week compared to group II but failed to normalize. Microalbuminuria concentration was elevated in group II(diabetic untreated rats)at the 2nd and 4th week of the experiment compared to control rats.Concentrations were lowered in rats of group III and IV throughout the experiment compared to control rats and diabetic untreated ones.

Table (3): Renal biomarkers of different experimental groups (means ± SD)**.**

Time	Animal groups	Urea (mg/dL)	Creatinine (mg/dL)	Microalbuminuria (mg/L)
2nd Week	I	33.34 ± 3.65^{c}	0.64 ± 0.05^{b}	6.54 ± 0.35^{b}
	II	88.80 ± 6.41^{a}	1.22 ± 0.14^{a}	8.65 ± 0.41^{a}
	III	67.58 ± 5.83^{b}	0.86 ± 0.15^{b}	1.31 ± 0.03^{d}
	IV	69.90 ± 2.95^{b}	1.19 ± 0.30^{a}	3.74 ± 0.49^{c}
4th Week	I	39.08 ± 0.94^{c}	0.69 ± 0.07^{d}	4.74 ± 0.22^{b}
	II	92.83 ± 5.31^{a}	1.07 ± 0.08^{a}	6.30 ± 0.62^{a}
	III	79.37 ± 3.55^{b}	0.95 ± 0.06^{b}	1.30 ± 0.28^{c}
	IV	86.46 ± 8.05^{a}	0.81 ± 0.06^{c}	1.44 ± 0.23^{c}
6th Week	I	29.82 ± 3.11^{c}	0.81 ± 0.10^{c}	6.82 ± 0.36^{a}
	II	87.31 ± 8.76^{a}	1.10 ± 0.09^{a}	8.24 ± 1.53^{a}
	III	73.90 ± 2.97^{b}	0.87 ± 0.04^{bc}	1.52 ± 0.05^{c}
	IV	86.69 ± 8.00^{a}	0.91 ± 0.03^{b}	4.08 ± 0.11^{b}

Note: **Mean values with different superscript letters in the same column are significantly different at (p≤0.05).**

Effect of SVF transplantation on electrolytes of diabetic nephropathy rats:

Electrolytes concentrations as illustrated in (Table 4) revealed a significant increase in potassium and sodium levels in group II (Diabetic untreated rats) all over the experimental period compared to control group. Meanwhile, group III and IV (SVF treated rats) exhibited significant decrease in potassium level at the 4th and 6th week compared to group II but failed to normalize. Whereas, rats of group III and IV exhibited normalized pattern of sodium level at the 2nd week till the end of the experiment.

Table (4): Electrolytes concentration in different experimental groups (means ± SD).

Time	Animal Groups	Potassium (mmol/L)	Sodium (mEq/ L)
2nd Week	I	4.94 ± 0.78^{b}	107.34 ± 8.51^{b}
	II	6.92 ± 0.90^{a}	152.56 ± 5.46^{a}
	III	6.20 ± 0.46^{a}	107.76 ± 11.42^{b}
	IV	6.35 ± 0.54^{a}	100.40 ± 9.42^{b}
4th Week	I	5.30 ± 0.42^{c}	106.73 ± 3.99^{c}
	II	7.17 ± 0.90^{a}	161.40 ± 5.07^{a}
	III	6.15 ± 0.52^{b}	120.83 ± 7.95^{b}
	IV	6.30 ± 0.59^{b}	107.00 ± 11.51^{c}
6th Week	I	5.04 ± 0.99^{c}	115.72 ± 5.07^{b}
	II	7.75 ± 0.57^{a}	157.16 ± 11.23^{a}
	III	6.66 ± 0.45^{b}	120.90 ± 8.66^{b}
	IV	6.86 ± 0.75^{ab}	115.80 ± 11.97^{b}

Note: **Mean values with different superscript letters in the same column are significantly different at ($p \leq 0.05$).**

Histopathological findings:

Microscopic examination of renal interstitium of diabetic untreated rats showed mononuclear cells infiltration mainly lymphocytes and macrophages in periglomerular and perivascular areas, Fig.(1a),with appearance of regenerative renal tubules, thickened basement membrane, basophilic cytoplasm, and vesicular nuclei, Fig.(1b). Small aggregation of lymphocytes was observed in SVF single IP injected rats, Fig. (1c) with presence of small clusters of regenerative tubules without thickened basement membrane or karyomegally as observed in diabetic rats. The lymphocytic aggregation in renal interstitium was extremely reduced in SVF multiple IP injected group with appearance of similarly described clusters of regenerative tubules, Fig. (1d), in addition to hyperemia of peritubular and glomerular blood capillaries. Renal tissue of diabetic rats showed increased number of clear cell tubules (CCT) or the so called Armanni-Ebstein lesions, Fig. (2a), which predominantly affect distal tubules. Those distal tubules lined by vacuolated tubular epithelium and stained red by PAS indicating cellular accumulation of glycogen Fig. (2d). the proximal tubules showed low staining density of proximal tubular epithelial apical brush borders Fig. (2d). There was reduction in the number of CCT in distal tubules Fig. (2b) as well as improvement and restoration of staining density (PAS) of proximal tubule apical brush borders in SVF single IP injected group Fig. (2e). Marked improvement was achieved in both distal Fig. (2c), and proximal (stained by PAS) Fig. (2f) tubules in SVF multiple IP injected group.

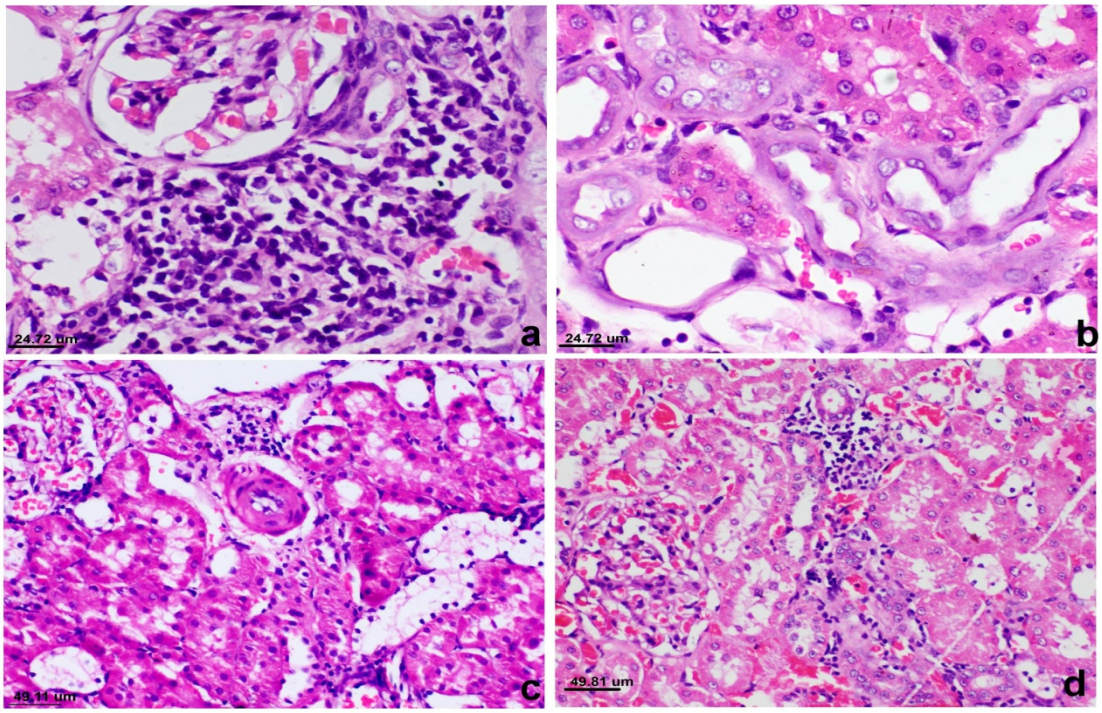

Fig. (1): Histopathological sections of kidney tissue stained by H&E.

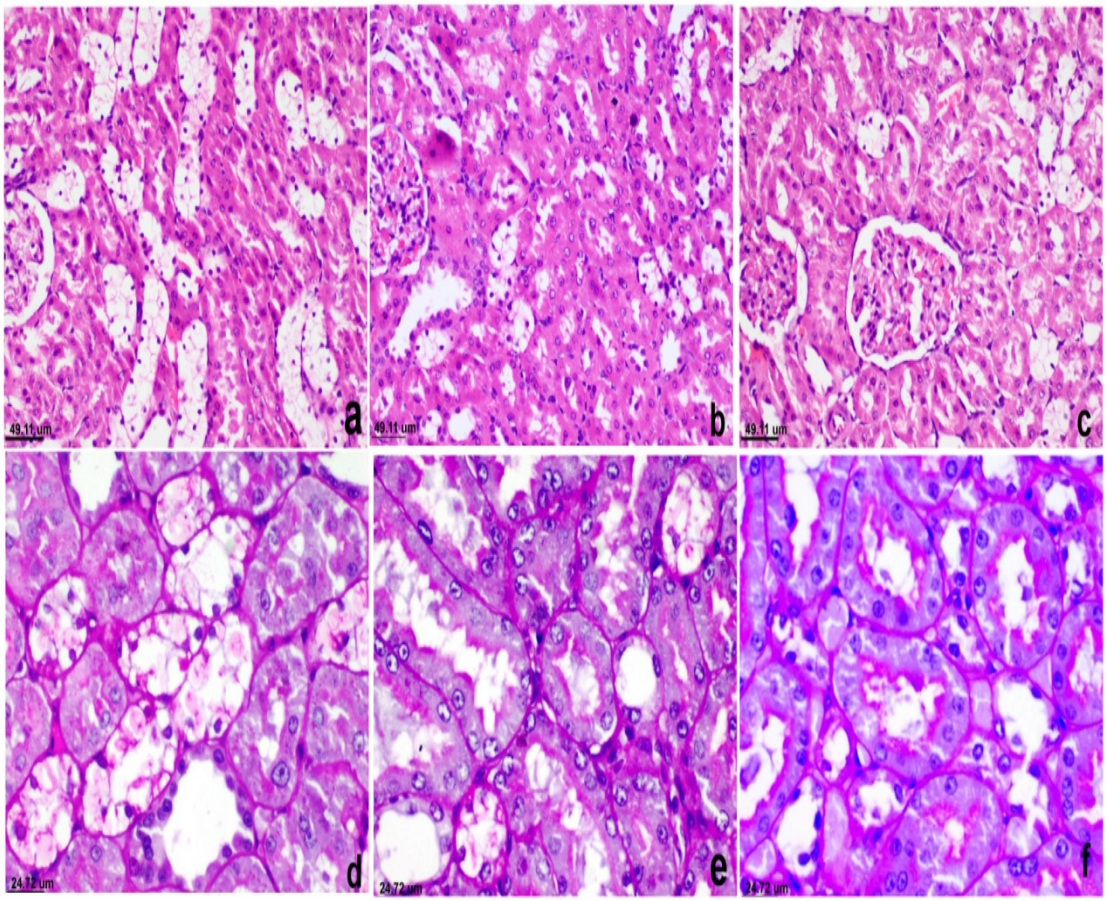

Fig. (2): Histopathological sections of kidney tissue from different experimental groups.

DISCUSSION

Diabetes mellitus is the most common cause of end stage renal disease and is, therefore, a common cause of anemia. **Thomas *et al.* (2004)** reported that in DM patients, anemia is a common complication and develops earlier than renal impairment. MSCs therapy exhibited promising outcomes in kidney disease, including experimental DN **(Liu and Tang, 2016)**. Current studies have proved the homing properties of systemic transplanted adipose derived stem cells (ASCs) to cell degenerated areas in DN rats, and a high percentage of adipose derived stem cells (ASCs) were found in injured tissues **(Hu *et al.,* 2015)**.

Hematological evaluations performed in the present experiment revealed the presence of anemia in group II (diabetic untreated rats). These observations agree with existing literature that anemia is a common pathophysiology associated with diabetes mellitus **(Kothari and Bokariya, 2012; Al-Mahmood *et al.,* 2016)**. Persistent hyperglycemia during diabetes causes lipid peroxidation and non-enzymatic glycosylation of body proteins, such as hemoglobin and RBCs membrane proteins, leading to RBCs hemolysis and anemia **(Tahmasebpour *et al.,* 2013)**. Red cell indices denoted the existence of macrocytic hypochromic anemia similar to that found by **Al-Mahmood *et al.* (2016)** which is a sign of regeneration following anemia. Both SVF transplanted groups of rats exhibited normalized pattern of erythrogram at the 6th week of SVF transplantation. **Li *et al.* (2010)** stated that MSCs provide the microenvironment for hematopoietic stem cells (HSCs) and communicate with hematopoietic cells in different junctions and secrete cytokines to support hematopoiesis. The niche microenvironments keep HSCs in a dynamic balance between self-renewal and differentiation.

The significant leukopenia associated with significant lymphopenia, eosinopenia and monocytopenia as well as neutrophilia observed in STZ diabetic rats at 4th and 6th week was similar to the findings of **Al-Mahmood *et al.* (2016)** who mentioned that leukopenia due to lymphopenia in diabetic rats indicate suppression of the immune system. This is also identical to the findings of **Warley *et al.* (1987)** who suggested that, the regression of lymphocytes in the peripheral blood indicates that there is loss of the thymus cells.

Significant neutrophilia observed in group III throughout the experiment may be attributed to systemic inflammation as reported by **Scridon *et al.* (2015)**. In our study treatment of diabetic rats with single or multiple IP transplantation of SVF improved total and differential leukocytic counts compared to diabetic untreated rats.

Renal biomarkers were evaluated in our experiment. Urea and creatinine are metabolic waste

products that are excreted from the kidneys, the blood level of both increases in renal diseases, where urea is an indicator of protein catabolism and creatinine is an indicator of muscle breakdown. Both creatinine and urea levels were significantly increased in group II (diabetic untreated rats) that was similar to the findings of **El-Barky *et al.* (2018)**. It has been proved that, the metabolic defects observed in uncontrolled diabetes lead to gluconeogenesis, breakdown of protein and consequently urea production **(Al-Mahmood *et al.*, 2016).**

The first indicator of diabetic kidney disease can be detected by the presence of microalbuminuria, a state known as incipient DN, where small amounts of albumin are present in the urine. Microalbuminuria is considered as the initial marker and predictive of the development of proteinuria or overt nephropathy, particularly in DM **(Davey *et al.*, 2014; Hu *et al*., 2016)**. MAU was significantly increased in group II (diabetic untreated rats) identical to that found by **Abdel Aziz *et al.* (2014)**. Values were lowered, however, in SVF treated rats, and similarly reported by **Ezquer *et al.* (2008).**

Serum creatinine and urea were significantly improved in group III (Single SVF treated rats) which could be related to the role of ASCs treatment in restoring kidney architecture.

Fang *et al*. (2012) reported that transplantation of MSCs can be used therapeutically to improve metabolic disorder, relieve and hinder further impairment of renal damage induced by DM. **Wang *et al*. (2013)** stated that MSCs treatment in diabetic rats could keep serum levels of urea and creatinine close to the control values suggesting the beneficial role of MSCs either directly or indirectly in providing protection against diabetic nephropathy. Moreover, the authors proved that MSCs decrease albuminuria and preserve normal renal histology in diabetic treated mice. **Rookmaaker *et al.* (2007)** declared that BMSCs home to injured glomerular endothelium, differentiate into endothelial cells, and participate in regeneration of the glomerular microvasculature.

Hyperglycemia in diabetic rats has induced tubular morphological and metabolic changes as result of glycogen deposition with appearance of Armanni- Ebstein lesion **(Dombrowski *et al.*,2007; Ribback *et al.*,2015)**. Basophilic tubules with thickened basement membrane and karyomegally observed in diabetic untreated rats was previously reported by **Pourghasem *et al.* (2015)** to develop in diabetic nephropathy. **Frazier *et al.* (2012)** added that tubular basophilia with thickened membrane developed in chronic progressive nephropathy and was not reparative process.

The regenerative tubules detected in stem cells injected rats (group III and IV) without

thickened basement membrane or karyomegally were considered regenerative reparative process of renal tubules. **Little and Kairath (2016)** demonstrated that MSCs had an ameliorative potential against acute renal injury induced in animal models and they thought to be a result of immunomodullatory effect of stem cells rather than integration into the necrosed tubular epithelium or transdifferentiation into an epithelial cell type.

SVF transplantation has reduced the number of altered distal tubules, the loss of proximal tubular brush borders, and the interstitial inflammation. This amelioration of kidney lesion was enhanced by SVF multiple transplantations, as discussed previously. It is assumed that MSCs have anti-inflammatory action and have role in reduction of clear cell renal tubules as described by **Pourghasem *et al.* (2015)**.

In conclusion, the current experiment results pointed out to attenuation of diabetic nephropathy as indicated by lowering of serum urea and creatinine, decrease of microalbuminuria, improvement of serum electrolytes and histopathological alterations in diabetic rats treated with SVF. Adipose derived stem cells were safe, feasible technique offering an alternative stem cell therapy avoiding further delays in culturing cells in vitro and offers the advantage of early therapeutic interference in such a progressive disease.

Potential conflict of interest:

The authors have no conflicting financial interest.

REFERENCES

Abdallah, A., Shamaa, A.,El-Tookhy, O. and Abd El-Mottaleb, E. (2016): 'Evaluation of low level laser-activated stromal vascular fraction as a single procedure for treatment of experimental chondral defects', *Asian Journal of Animal Sciences*. Science Alert, 10 (1), pp. 15-28.

Abdel Aziz, M., Wassef, M., Ahmedm, H., Rashed, L., Mahfouz, S., Aly, M., Hussein, R. and Abdelaziz, M.(2014):'The role of bone marrow derived-mesenchymal stem cells in attenuation of kidney function in rats with diabetic nephropathy', *Diabetology and Metabolic Syndrome*, 6 (34), pp. 1-10.

Abrahamse, H. (2011): 'Inducing stem cell differentiation using low intensity laser irradiation: a possible novel therapeutic intervention',*Central European Journal of Biology*,6 (5), pp.695-698.

Al-Mahmood, S., Razak, T., Abdullah, S., Ahmad, N., Mohamed, A. and Al-ani, I. (2016): 'A comprehensive study of chronic diabetes complications in streptozotocin-induced diabetic rat', *Makara Journal of Health Research*, 20 (2), pp. 48-56.

Bancroft, J. and Gamble, M. (2008): "Theory and Practice of Histological Techniques". 6th Ed., Churchill Livingstone, Elsevier, China.

Burney, B., Kalaitzidis, R. and Bakris, G. (2009): 'Novel therapies of diabetic nephropathy', *Current Opinion in Nephrology and Hypertension*, 18, pp. 107-111.

Cardinali, A. and Nason, G. (2013): 'Costationarity of locally stationary time series using costat', *Journal of Statistical Software*, 55 (1), pp. 1-22.

Chen, Y., Shibu, M., Fan, M., Chen, M., Viswanadha, V.,Lin, Y., Lai, C.,Lin, K., Ho, T., Kuo, W. and Huang, C. (2016): 'Purple rice anthocyanin extract protects cardiac function in STZ-induced diabetes rat hearts by inhibiting cardiac hypertrophy and fibrosis', *Journal* of *Nutritional Biochemistry*, 31, pp. 98 -105.

Davey, G.,Patil,S.,O'Loughlin, A. and O'Brien, T.(2014): 'Mesenchymal stem cell-based treatment for microvascular and secondary complications of diabetes mellitus',*Frontiers in Endocrinology*, 5 (86), pp. 1-16.

Dombrowski, F., Klotz, L., Bannasch, P. and Evert, M. (2007): 'Renal carcinogenesis in models of diabetes in rats- metabolic changes are closely related to neoplastic development', *Diabetologia*, 50, pp. 2580 -2590.

Dong, Q., Chen, L., Gao, G., Wang, L., Song, J., Chen, B., Xu, Y. and Sun, L. (2008): 'Allogeneic diabetic mesenchymal stem cells transplantation in streptozotocin-induced diabetic rat', *Clinical and Investigative Medicine*, 31 (6), pp. 328 -337.

El Barky, A., Ezz, A., Alm-Eldeen, A., Hussein, S., Hafez, Y. and Mohamed, T. (2018): 'Can stem cells ameliorate the pancreatic damage induced by streptozotocin in rats', *Canadian Journal of Diabetes*, 42 (1), pp. 61-70.

El-domouky, A., Hegab, A., Al-shahat, A. and Raafat, N. (2017): 'Mesenchymal stem cells and differentiated insulin producing cells are new horizons for pancreatic regeneration in type I diabetes mellitus', *International Journal of Biochemistry and Cell Biology*, 87, pp. 77-85.

Ezquer, F., Ezquer, M., Parrau, D., Carpio, D., Yañez, A. and Conget, P. (2008): 'Systemic administration of multipotent mesenchymal stromal cells reverts hyperglycemia and prevents nephropathy in type 1 diabetic mice', *Biology of Blood and Marrow Transplantation*, 14, pp. 631- 640.

Fang, Y.,Tian, X., Bai, S., Fan, J.,Hou, W.,Tong, H. and Li, D. (2012): 'Autologous transplantation of adipose-derived mesenchymal stem cells ameliorates streptozotocin-induced diabetic nephropathy in rats by inhibiting oxidative stress , pro-inflammatory cytokines and the p38 MAPK signaling pathway', *International Journal of Molecular Medicine*, 30, pp. 85 - 92.

Frazier, K., Seely, J., Hard, G., Betton, G., Burnett, R., Nakatsuji, S., Nishikawa, A., Durchfeld-Meyer, B. and Bube, A. (2012): *Proliferative and nonproliferative lesions of the rat and mouse urinary system*', *Toxicologic Pathology*, 40 (4), pp. 14 - 86.

Hamza, A., Al-bishri, W., Damiati, L. and Ahmed, H. (2017): 'Mesenchymal stem cells : A future experimental exploration for recession of diabetic nephropathy', *Renal Failure*, 39(1), pp. 67-76.

Hu, J., Fu, Z., Chen, Y., Tang, N., Wang, L., Wang, F., Sun, R. and Yan, S. (2015): 'Effects of autologous adipose-derived stem cell infusion on type 2 diabetic rats', *Endocrine Journal*, 62 (4), pp. 339-352.

Hu, J., Wang, Y., Gong, H., Yu, C., Guo, C., Wang, F., Yan, S. and Xu, H. (2016): 'Long term effect and safety of Wharton's jelly-derived mesenchymal stem cells on type 2 diabetes', *Experimental and Therapeutic Medicine*, 12, pp. 1857-1866.

Ishimura, D., Yamamoto, N., Tajima, K., Ohno, A., Yamamoto, Y., Washimi, O. and Yamada, H. (2008): 'Differentiation of adipose-derived stromal vascular fraction culture cells into chondrocytes using the method of cell sorting with a mesenchymal stem cell marker', *The Tohoku Journal of Experimental Medicine*, 216 (2), pp. 149 -156.

Kothari, R. and Bokariya, P. (2012): 'A comparative study of haematological parameters in type I mellitus patients and healthy young adolescents. *International Journal of Biological and Medical Research*, 3 (4), pp. 2429 -2432.

Li, J., Zheng, C. and Han, Z. (2010): 'Abnormal immunity and stem / progenitor cells in acquired aplastic anemia.', Critical Reviews in Oncology / Hematology, 75 (2), pp. 79 -93.

Little, M. and Kairath, P. (2016): 'Regenerative medicine in kidney disease', *Kidney International*, 90 (2), pp. 289-299.

Liu, Y. and Tang, S. (2016): 'Recent progress in stem cell therapy for diabetic nephropathy', *Kidney Disease*, 2, pp. 20 - 27.

Oche, O., Sani, I., Chilaka, N., Samuel, N. and Samuel, A. (2014):'Pancreatic islet regeneration and some liver biochemical parameters of leaf extracts of Vitex doniana in normal and streptozotocin-induced diabetic albino rats', *Asian Pacific Journal of Tropical Biomedicine*, 4 (2), pp. 124 -130.

Pourghasem, M., Shafi, H. and Babazadeh, Z. (2015): 'Histological changes of kidney in diabetic nephropathy', *Caspian Journal of Internal Medicine*, 6 (3), pp. 120 -127.

Ribback,S.,Cigliano, A.,Kroeger, N., Pilo, M., Terracciano, L., Burchardt, M., Bannasch, P., Calvisi, D.and Dombrowski,F.(2015):'PI3K/AKT/mTOR pathway plays a major pathogenetic role in glycogen accumulation and tumor development in renal distal tubules of rats and men', *Oncotarget*, 6 (15), pp. 13036 -13048.

Rombouts, W. and Ploemacher, R. (2003): 'Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture',*Leukemia*,17 (1),pp. 160 -170.

Rookmaaker, M., Verhaar, M., De Boer, H., Goldschmeding, R., Joles, J., Koomans, H., Grone, H. and Rabelink, T. (2007): 'Met-RANTES reduces endothelial progenitor cell homing to activated (glomerular) endothelium in vitro and in vivo', *American Journal of Physiology-Renal Physiology*, 293, pp. 624 - 630.

Scridon, A., Perian, M., Marginean, A., Fisca, C., Vantu, A., Ghertescu, D., Chevalier, P. and Serban, R. (2015): 'Wistar rats with long-term streptozotocin-induced type 1 diabetes mellitus replicate the most relevant clinical , biochemical , and hematologic features of human diabetes', *Revista Română de Medicină de Laborator*, 23 (3), pp. 263-274.

Tahmasebpour, N., Dehghan, G., Feizi, M. and Esmaeili, H. (2013): 'Variation in body weight and some hematological parameters in streptozotocin-induced diabetic rats, treated with teucrium orientale', *Pharmacology Online*, 3, pp. 32-36.

Thomas, M., MacIsaac, R., Tsalamandris, C., Molyneaux, L., Goubina, I., Fulcher, G., Yue, D. and Jerums, G. (2004): 'The burden of anaemia in type 2 diabetes and role of nephropathy: A cross-sectional audit', *Nephrology Dialysis Transplantation*, 19 (7), pp. 1792 -1797.

Wang, S., Li, Y., Zhao, J., Zhang, J. and Huang, Y. (2013): 'Mesenchymal stem cells ameliorate podocyte injury and proteinuria in a type 1 diabetic nephropathy rat model', *Biology of Blood and Marrow Transplantation*, 19, pp. 538-546.

Warley, A., Daniel, P. and Guthriet, D. (1987): 'Diabetes mellitus in the rat: Diminution in the number of cells in the thymus and of lymphocytes in the blood', *Journal of Experimental Physiology*, 72, pp. 609 - 615.

Zuk, P., Zhu, M., Mizuno, H., Huang, J., Futrell, J., Katz, A., Benhaim, P., Lorenz, H. and Hedrick,M.(2001): 'Multilineage cells from human adipose tissue : Implications for cell-based therapies', *Tissue Engineering*, 7 (2), pp. 211-228.