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Clinicopathological Studies on the Use of Bone Marrow Mesenchymal Stem Cells in the Treatment of Streptozotocin-Induced Diabetes mellitus in Sprague Dawley Rats

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Diabetes mellitus (DM) is a systemic disease affecting all body systems. Bone marrow mesenchymal stem cells (BM-MSCs) therapy has a promising value in the management of kidney, liver and pancreatic degeneration. Fifty Sprague Dawley male rats were used in this experiment. Five rats were used as a source of BM-MSCs and Five (STZ-diabetic) rats were used in the cell tracking. The remaining 40 rats were randomly divided into 4 equal groups; (I) control group, (II) stem cell group, (III) untreated diabetic group and (IV) treated diabetic (BM-MSCs) group. group.DM was induced by single intraperitoneal injection of streptozotocin (STZ) in a dose of 55 mg/ kg body weight. The BM-MSCs were collected from the bone marrow of 10 male rats, cultured, characterized by flowcytometry analysis and labeled with Qtracker 655 cell labeling. BM-MSCs were transplanted in group (IV) at the 7th day of STZ injection. Rats were euthanized 4 weeks after STZ injection. Blood samples were collected at the end of experiment (4th week of BM-MSCs transplantation). The pancreas, kidney and liver tissues were collected. Hematological, biochemical, insulin gene expression and histopathological examination were performed for all experimental groups. Untreated diabetic group showed significant disturbances in liver, kidney and pancreatic functions with excessive production of inflammatory cytokines in kidney tissues and increased oxidative stress in liver tissues. The BM-MSCs treated diabetic group showed significant improvement in organs functions, insulin gene expression as well as hematological and histopathological examinations. BM-MSCs therapy in diabetic rats triggered improvement of the diabetic state and ameliorated some of its complications.

Keywords: BM-MSCs, Diabetes mellitus, Sprague Dawley male rats, Flow cytometry, Labeled cells, Insulin gene expression, Oxidative stress, TNF-α.

INTRODUCTION

Beta (β)-cells in the pancreatic islets of Langerhans are responsible for the production of insulin hormone. Therefore, the majority of the pathologic disorders of diabetes mellitus can be attributed to the loss of β-cell number and function (Ferrannini et al. 2010). Eventually, restoration of a functional β-cell mass constitutes the central goal of recent diabetes therapy (Malmgren et al. 2013).

The main aim of the cell-based therapy that has emerged as a strategic treatment for many diseases is to replace, repair and/ or enhance the biological function of damaged tissues in an organ. The main biological materials for this purpose are “stem cells” which are obtained either

from embryonic or adult tissue-specific stem cells (Karaoz et al. 2011). Several studies suggested that the insulin secreting cells can be generated in-vitro from mouse bone marrow stromal cells (Hoesli et al. 2012) and mature pancreatic cells including mouse and human pancreatic duct cells (Loomans et al. 2018). In this regard, the epithelium of pancreatic duct contains progenitor cells which are involved in pancreas growth and renewal (Movassat and Portha, 2007). Furthermore, there are other cell types within the pancreas that are able to proliferate and differentiate to beta cells (Milanesi et al. 2011).

Since MSCs could easily be isolated from bone marrow and differentiated into a variety of cell types, MSCs are known as the most used stem cells in tissue engineering and regenerative medicine (Wang et al. 2013). The ability of mesenchymal stem cells particularly the bone marrow derived (BM-MSCs) to differentiate into many cell types, as well as their high ex-vivo expansion potentiality, makes them an attractive therapeutic tool for cell transplantation and tissue engineering (Phinney and Sensebé, 2013).

Several evidences suggested that administration of MSCs have highly potentiality to recover endogenous β cells in diabetes mellitus due to their ability to differentiate into many cells and tissues under specific conditions (Tang et al. 2012). These studies indicated that MSCs are promising tools for the treatment of different types of tissue damages, because they secrete a multitude of bioactive molecules that ultimately lead to reformation and regeneration of injured tissues (Miranda et al. 2015). Therefore, it has been shown that MSCs migrate and dock preferentially into the injured or damaged tissue sites promoting the survival of surrounding cells (Zhang et al. 2017). However, the precise function of BM-MSCs in β cell regeneration is controversial.

Based on the minimal criteria of International Society of Cellular Therapy (ISCT), MSCs are adult stem cells which identified by adherence to plastic, expression of some cell surface markers as CD29, CD44, CD90, CD49a-f, CD51, CD73, CD105, CD106, CD166 and Stro-1 with lack of the expression of the CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules. Moreover, the MSCs have no immunogenic effect and could replace the damaged tissues. These criteria are directed to the development of progressive methods of isolation and characterization of MSCs from various sources for therapeutic uses in regenerative medicine. (Maleki et al. 2014).

Flowcytometry is the method of choice for immunophenotyping MSCs using a recommended specific cell surface signature for MSCs of the International Society for Cell Therapy (ISCT).

Diabetic patients may have a significant defect in antioxidant defence mechanism; however, oxidative stress and free radicals may be responsible for the diabetes mellitus itself, and its complications (Oliveira et al. 2014).

Tumour necrosis factor- alpha (TNFα) is one the most important pro-inflammatory mediator that is critically involved in the autoimmune process leading to beta cell damage (Akash et al. 2018).

The present experiment was conducted to evaluate the therapeutic effects of bone marrow MSCs transplantation in STZ induced diabetes mellitus in Sprague Dawley rats through confirming that MSCs are homing into pancreas, detecting the changes in β- cells functions by RT-PCR for insulin gene expressions and histopathological studies as well as observing the alterations in hematologic and biochemical parameters, total antioxidant capacity and TNFα level in all experimental groups.

MATERIALS AND METHODS

Animals

Fifty Sprague Dawley male rats, 12-14 weeks old and weighing 180-200 g were used in this study. Rats were housed in standard cages and kept at environmentally controlled room. The rats were fed standard rat chow diet and had *ad-libitum* access to food and water. The procedures of this study were carried out after a week of acclimatization and all experimental protocols and techniques were approved (CU/II/F/126/18) by the Institutional Animal Care and Use Committee of Cairo University (IACUC).

Experimental design

The 50 rats were divided into: Five rats were used as a source of BM-MSCs. Five (STZ-diabetic) rats were used in the cell tracking group. The remaining 40 rats were randomly allocated into four groups each contains 10 rats, as the following:

Group I (Control group):

The rats were treated with single intravenous (i.v.) injection of 0.2 ml Dulbecco's Modified Eagles Medium (DMEM) via penile vein.

Group II (Stem cell group):

The rats were treated with single i.v. injection of BM-MSCs (1×10^6 cells/ rat) in 0.2 ml DMEM via penile vein.

Group III (Untreated diabetic group):

Diabetes mellitus was induced in the rats of this group. After 7 days, these diabetic rats were treated with single i.v. injection of 0.2 ml DMEM via penile vein.

Group IV (Treated diabetic group):

Diabetes mellitus was induced in the rats of this group. After 7 days, these diabetic rats were treated with single i.v. injection of BM-MSCs (1×10^6 cells/ rat) in 0.2 ml DMEM via penile vein (Abdel Aziz et al. 2011).

Induction of diabetes mellitus

Diabetes mellitus was induced in the fasted rats by a single intraperitoneal (i.p.) injection of Streptozotocin (STZ; Sigma-Aldrich, USA, 55 mg/kg b.w.) in 0.1 M citrate buffer, pH 4.5. The tail vein blood was drawn from all animals, and the blood glucose of each animal was daily monitored using a glucometer (AGM-2200, Germany). After 7 days, the STZ-injected rats that exhibited blood glucose level more than 250 mg/dL were considered diabetic and allocated for groups (III) and (IV) as well as the tracking group (Chen et al. 2016).

Isolation and culture of rat BM-MSCs

Five rats were euthanized by i.p. anesthetic overdose (Na thiopental, 120 mg/kg b.w.), then, the bone marrow cells were collected (femurs and tibias) under aseptic conditions, resuspended in phosphate buffer saline (PBS, pH7.4), and passed through a 70 µm cell strainer to remove the bone debris and blood aggregates. Then, the cells were cultured in 20 ml complete DMEM and incubated at 37 ℃ in humidified CO_2 (5%) incubator for 7-10 days as primary culture or upon formation of large colonies (Abdel Aziz et al. 2014).

Characterization of BM-MSCs by Flow cytometry analysis

The BM-MSCs were analyzed by a fluorescence activated cell sorting (FACS) Caliber flow cytometer (BD Bioscience, USA) for CD105, CD73, CD45 and CD34 (Draz et al. 2015).

Tracking the BM-MSCs homing in pancreatic tissues

The stem cell homing in the injured pancreas was tracked by injecting the rats of tracking group with Qtracker 655 labeled BM-MSCs at the 7th day of STZ injection. The BM-MSCs were labeled using a Qtracker 655 Red Fluorescent Linker Kit (Sigma, USA), according to the manufacturer's protocol. Seven days later, the rats were euthanized by i.p. anesthetic overdose (Na thiopental, 120 mg/kg b.w.), and their pancreases were examined using a fluorescent microscope (Leica, Germany) (Dong et al. 2008).

Clinicopathological evaluation

Collection of blood samples and tissue harvesting

Blood samples were drawn by tail prick for the intraperitoneal glucose tolerance test, at the 10th day of BM-MSCs transplantation from the rats of group (1), (3) and (4). Moreover, at the end of experiment (4th week of BM-MSCs transplantation), the rats of all experimental groups were anesthetized by isoflurane, and the maximum amount of blood was collected via cardiac puncture. A portion of blood samples was collected on EDTA for complete blood count (CBC), while the remaining portion was used for serum separation.

Following the collection of blood samples, the rats were euthanized by opening the chest and abdomen for the harvest of liver, kidney and pancreas. A part of the collected organs was stored in formalin (10%) for histopathological examination and the remaining parts were stored at liquid nitrogen for biochemical analysis and RT-PCR.

Intraperitoneal glucose tolerance test

At the 10th day of BM-MSCs transplantation, the rats were challenged by i.p. administration of glucose solution (2 g/kg b.w.). Blood samples were drawn by tail prick for determination of blood glucose by using a glucometer (AGM-2200, Germany) at baseline (0 minute), 30, 60, 90 and 120 minutes after glucose load (Andrikopoulos et al. 2008).

Hematological investigations

Complete blood count (CBC) was evaluated utilizing the EDTA blood by the animal cell counter (ABC Vet, France). CBC includes the erythrogram (RBCs count, Hb concentration, PCV, MCV, MCH and MCHC), Leukogram (TLC) and platelet count.

DLC was evaluated by microscopic examination of Leishman-stained blood smears.

Assessment of serum chemical biomarkers

Serum was utilized to assay the biomarkers by using the commercially available biochemical diagnostic kits and according to manufacturer's instructions. The kit of serum insulin (rat ELISA kits) was supplied by Millipore, St. Charles, MO (Dedania et al. 2011**)**. Serum total proteins, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine were assayed by kits provided by Biomérieux, France, while serum alkaline phosphate (ALP) and glucose were assayed using kits supplied by Stainbio Laboratory, Texas, USA. Moreover, serum triglycerides, cholesterol and high-density lipoproteins (HDL) were measured by kits supplied by RANDOX, England.

Measurement of TNF-α in kidney tissues

The kidney tissues stored at liquid nitrogen were homogenized in cold PBS, centrifuged and the supernatant was collected to measure kidney TNF-α using rat TNF-α Sandwich-ELISA kit supplied by Biodiagnostics, Egypt, according to (Majid 2011).

Assessment of total antioxidant capacity in liver tissues

The liver tissues stored in liquid nitrogen were homogenized in PBS, centrifuged, and the supernatant was collected to measure the hepatic total antioxidant capacity (TAC) using a kit provided by Biodiagnostics, Egypt, according to Erel (2004).

Insulin gene expression analysis in pancreatic tissues

The pancreatic tissues stored in liquid nitrogen was used for total RNA extraction using the TriFast ™ reagent RNA extraction kit (PeqLab Biotechnologie GmbH, Germany) according to the manufacturer's instructions. The purity and concentration of obtained RNA were determined spectrophotometrically (Nanodrop 2000, Thermo Scientific, USA) at A260/A280 nm. The obtained purity of RNA was 1.8–1.9. About 100 ng cDNA per reaction, using the SensiFAST ™ cDNA Synthesis Kit (BIO-65053) for reverse transcription of the purified RNA. For high yield and purity of RNA, use the Bioline ISOLATE II RNA Mini Kit (BIO-52072).

Insulin gene specific primers were designed and purchased from Bioline, UK. The forward primer sequence was GGGGATCTTCAGACCTTGGC, while the reverse primer sequence was GGGATGCAGGGATGATGTTCT. Thermal cycling reaction was performed using thermal cycler (CFX96, BioRad, USA) with the following program: initial denaturation; 94°C for 5 minutes, 35 cycles of 94°C for 0.5 minute denaturation, 60°C for 1 minute (StAR protein), 58°C for 30 sec (Cytochrome P450scc), and 60°C for 1 minute (RPS 16) as primer annealing, 72°C for 1 minute extension and Final extension at 72°C for 7 min. Gene expression levels were determined by calculating the ratio between the square pixel value of the target gene in relation to the control GAPDH gene (QIAGEN GmbH, Germany).

Histopathological examination

Liver, kidney and pancreas samples were stored in formalin (10%), processed and stained with Hematoxylin and Eosin (Kiernan 2001). Pancreatic tissues were examined for swelling, vacuolation and cytoplasmic degranulation of islet cells (Ismail et al. 2013). Kidney tissues were examined for tubular epithelial cell vacuolization, degeneration, necrosis, glomerular sclerosis and inflammatory cell infiltration. Liver tissues were examined for periportal fibrosis, vacuolated cytoplasm and cellular infiltration (Mostafa et al. 2013).

Statistical Analysis

Data were expressed as mean ± SEM. Significant differences were determined by using ANOVA and post-hoc tests for multiple comparisons using COSTAT (version 6.400, CoHort software, USA). Results were considered significant at $p < 0.05$ (Cardinali and Nason, 2013).

RESULTS

Characterization of MSCs by Flow cytometry analysis

The isolated MSCs showed homogenous fibroblastic like morphology in vitro that tightly attached to the culture flask (Figure 1). Results of flow cytometric analysis on expression of cell surface antigens revealed that MSCs expressed high levels of CD73 and CD105 with low levels of CD34 and CD45 (Figure 2).

Tracking the BM-MSCs homing in pancreatic tissues

The pancreatic tissues of tracking group that were transplanted by Qtracker 655 labeled BM-

MSCs showed fire red auto-fluorescence when examined by fluorescent microscope on the 7th day of transplantation, confirming that these cells were actually seeded into the pancreatic tissue (Figure 3).

Intraperitoneal glucose tolerance test

Compared to control group, the intraperitoneal glucose tolerance test showed impaired glucose tolerance in group III (untreated diabetic rats) with significant improvement in that of group IV (treated diabetic rats) (Figure 3).

Effect of BM-MSCs therapy on hematological parameters

Compared to group I (control group), RBCs count, Hb concentration, PCV MCH and MCHC showed significant decrease with significant increase in MCV in group III (untreated diabetic rats). Meanwhile, Group IV (treated diabetic rats) showed significant improvement in these parameters compared to group III. The leukogram of group III (untreated diabetic rats) showed a significant leukopenia with neutropenia, lymphopenia, eosinopenia and monocytopenia compared to control group. On the other hand, group IV (treated diabetic rats) revealed significant leukocytosis with neutrophilia, lymphocytosis, eosinophilia and monocytosis compared to group III. The group III (untreated diabetic rats) revealed significant thrombocytopenia compared to control group, while there was a significant improvement in platelets count in group IV (treated diabetic rats) compared to group III (Table 1: **Effect of BM-MSCs therapy on hematological parameters of different experimental groups**.

Effect of BM-MSCs therapy on the glucose profile

A significant hyperglycemia with decreased insulin was recorded in group III (untreated diabetic rats) compared to control group. On the other hand, the values of blood glucose and serum insulin levels in group IV (treated diabetic rats) showed no significant differences (Effect of BM-MSCs therapy on hematological parameters

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Effect of BM-MSCs therapy on the lipid profile

The serum triglycerides, cholesterol and LDL showed significant increase, while HDL showed significant decrease in group III (untreated diabetic rats) compared to control group. However, group IV (treated diabetic rats) showed improved results with no significant difference in comparison to control group (Effect of BM-MSCs therapy on hematological parameters

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Effect of BM-MSCs therapy on liver and kidney functions

The group III (untreated diabetic rats) showed a significant reduction in total proteins, serum albumin and serum globulins compared to the control group. Moreover, the liver enzymes (ALT,

AST and ALP) activities were significantly higher in the same group. However, the group IV (treated diabetic rats) showed no significant change compared to group I. The A/G ratio showed non-significant difference in all groups compared to control group (Effect of BM-MSCs therapy on the glucose profile

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Markers of kidney functions (serum creatinine and BUN) were significantly higher in group III (untreated diabetic rats) compared to control group. However, these significant changes disappeared in group IV (treated diabetic rats) when compared to the control group (Effect of BM-MSCs therapy on the glucose profile

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Effect of BM-MSCs therapy on the TNF-α in kidney tissues and TAC in liver tissues

In comparison to control group, group III (untreated diabetic rats) showed a significantly increased level of TNF-α in kidney tissues with a significantly decreased TAC in liver tissues. Interestingly, the group IV (treated diabetic rats) showed no significant change in the same parameters when compared to the control group (**Error! Reference source not found.**).

Effect of BM-MSCs therapy on the insulin gene expression in pancreatic tissues

The expression of insulin gene (in pancreatic tissues) showed a significant decrease level in group III (untreated diabetic rats) and a significant increase level in group IV (treated diabetic rats) when compared to control group (Figure 3).

Histopathological findings

Pancreas

Microscopic examination of pancreatic tissues revealed normal islet of Langerhans and pancreatic granular acini in control group (Figure 6a). Meanwhile, the pancreatic tissues of group III (untreated diabetic rats) showed necrosis in β cell of islets of Langerhans and marked vacuolation of pancreatic glandular acini (Figure 6b, c). Moreover, the pancreatic tissues of group IV (treated diabetic rats) showed mild degeneration and vacuolation of both pancreatic acinar cells of the exocrine portion; both pancreatic acini and islets cell and mostly within normal limit (Figure 6d).

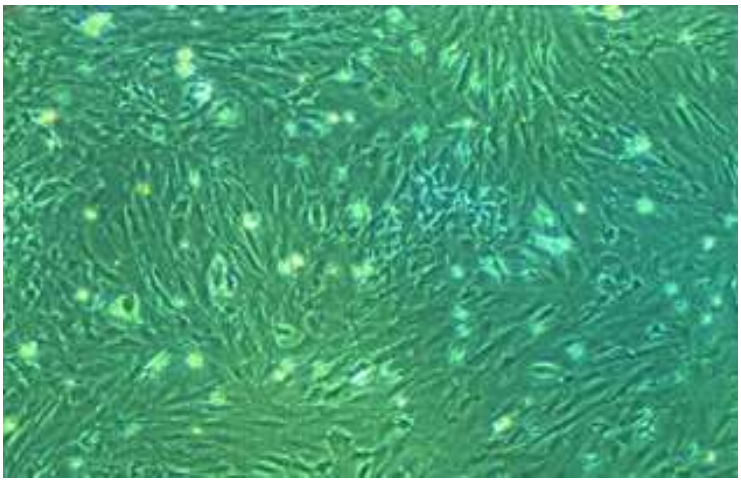

Figure 1: Mesenchymal stem cells with fibroblast- like morphology.

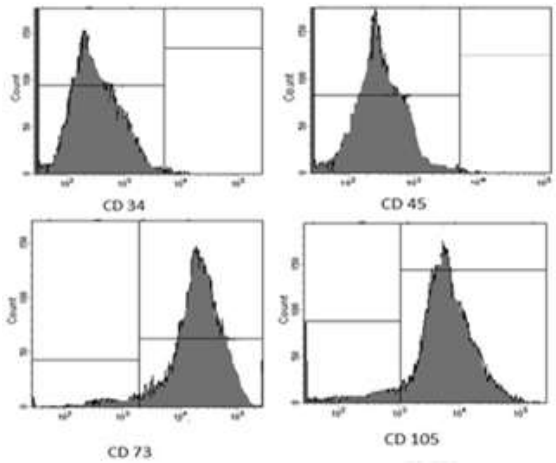

Figure 2: Flow cytometry analysis of cell surface markers (CD 34, CD45, CD 73 and CD 105).

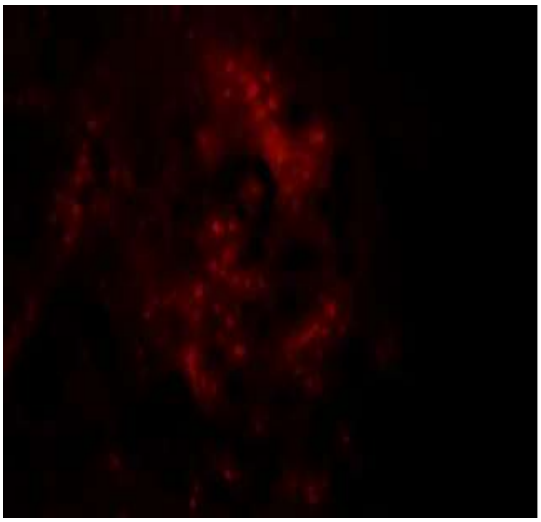

Figure 3: Pancreatic tissue with Qtracker 655 labeled stem cells showing red auto-fluorescence.

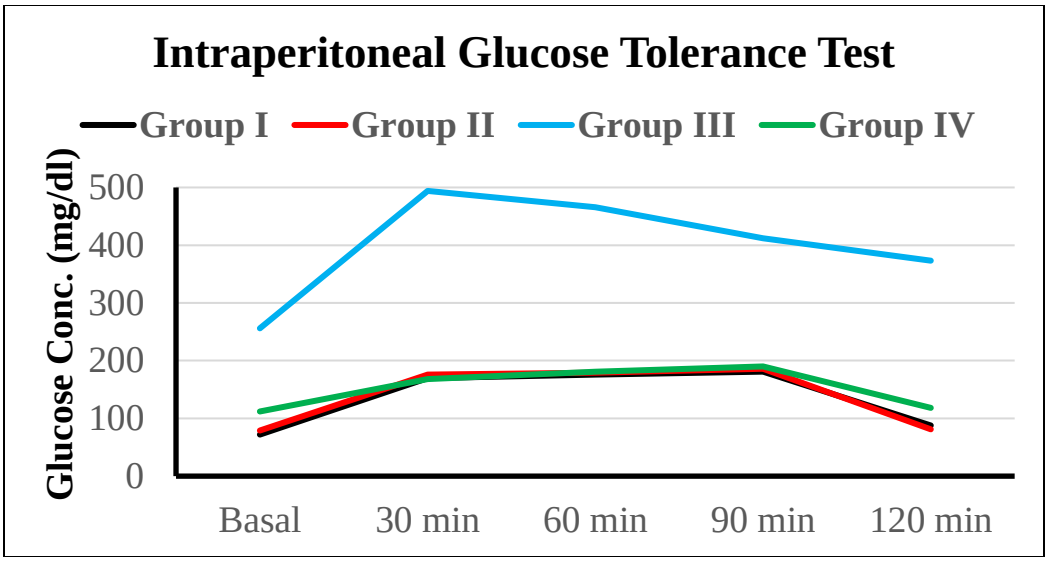

Figure 4: Glucose tolerance curve in different groups.

Table 1: Effect of BM-MSCs therapy on hematological parameters of different experimental groups.

Parameter	Unit	Group I	Group II	Group III	Group IV
RBCs	$\times10^6$/µl	6.92±0.11^a	6.85±0.03^a	6.40±0.07^b	6.85±0.09^a
Hb conc.	g/dl	14.49±0.05^a	14.14±0.09^a	12.19±0.09^b	14.11±0.09^a
PCV	%	54.50±0.19^a	53.50±0.57^a	52.00±0.53^b	54.25±0.56^a
MCV	fl	78.76±1.08^a	78.10±0.68^a	81.30±0.69^b	79.20±0.56^a
MCH	pg	20.93±0.32^a	20.64±0.12^a	19.06±0.15^b	20.60±0.20^a
MCHC	g/dl	26.85±0.05^a	26.43±0.11^a	23.44±0.32^b	26.01±0.07^a
TLC	$\times10^3$/µl	6.97±0.03^a	6.69±0.08^a	5.06±0.05^b	7.26±0.10^a
Neutrophil	$\times10^3$/µl	2.25±0.04^a	2.18±0.02^a	1.89±0.02^b	2.85±0.09^a
Lymphocyte	$\times10^3$/µl	3.67±0.03^a	3.49±0.06^a	2.57±0.05^b	3.44±0.01^a
Eosinophil	$\times10^3$/µl	0.411±0.06^a	0.432±0.03^a	0.267±0.05^b	0.392±0.04^a
Monocyte	$\times10^3$/µl	0.642±0.01^a	0.591±0.02^a	0.373±0.01^b	0.584±0.04^a
Platelets	$\times10^3$/µl	532.50± 11.53^a	502.50± 4.12^a	307.50± 4.09^b	492.50± 3.13^a

Data were expressed as mean ± SE. Values with different superscripts in the same raw are significantly different at (p≤0.05).

Table 2: Effect of BM-MSCs therapy on glucose and lipid profile of different experimental groups.

Parameter	Unit	Group I	Group II	Group III	Group IV
Bl. Glucose	mg/dL	120.33 ± 0.88^a	119.75 ± 1.11^a	280.00 ±0.82^b	130.50 ±0.65^a
S. Insulin	U/ml	20.47 ± 0.49^a	21.72 ± 0.29^a	6.79 ± 0.15^b	19.96 ±0.31^a
Cholesterol	mg/dl	85.00 ±0.27^a	82.00 ±0.53^a	121.50 ± 1.02 ^{b}	90.26 ± 0.27^a
Triglycerides	mg/dl	69.00 ± 0.27^a	65.50 ± 0.98^a	98.97 ± 0.43^b	71.00 ± 0.38^a
HDL	mg/dl	45.93 ± 0.14^a	42.60 ± 0.33^a	28.66 ± 0.87^b	41.35 ± 0.35^a
LDL	mg/dl	15.81 ± 0.15^a	16.30 ± 0.39^a	25.05 ± 0.70^b	18.70 ± 0.26^a

Data were expressed as mean ± SE. Values with different superscripts in the same raw are significantly different at (p≤0.05).

Table 3: Effect of BM-MSCs therapy on liver functions and kidney functions of different experimental groups.

Parameter	Unit	Group I	Group II	Group III	Group IV
T. Proteins	g/dl	6.93± 0.05^{a}	6.75± 0.05^{a}	4.49± 0.13^{b}	5.91± 0.06^{a}
Albumin	g/dl	3.37± 0.02^{a}	3.29± 0.04^{a}	2.24± 0.05^{b}	2.91± 0.08^{a}
Globulins	g/dl	3.53±0.02^{a}	3.41± 0.03^{a}	2.25± 0.02^{b}	3.00± 0.12^{a}
A/G ratio		0.95±0.01^{a}	0.96±0.01^{a}	0.97±0.01^{a}	0.97±0.01^{a}
ALT	U/L	41.33±0.56^{a}	41.75±0.62^{a}	64.75±2.27^{b}	41.32±0.40^{a}
AST	U/L	29.66±0.56^{a}	28.00±0.96^{a}	55.75±0.49^{b}	38.50±0.63^{a}
ALP	U/L	26.90 ±0.34^{a}	26.65±0.22^{a}	31.67±0.83^{b}	27.19 ±0.67^{a}
Creatinine	mg/dl	0.70 ± 0.00^{a}	0.68 ± 0.02^{a}	1.40 ± 0.12^{b}	0.71 ± 0.03^{a}
BUN	mg/dl	16.02 ± 0.22^{a}	15.65 ± 0.21^{a}	22.79 ± 0.19^{b}	16.36 ± 0.18^{a}

Data were expressed as mean ± SE. Values with different superscripts in the same raw are significantly different at (p≤0.05).

Table 4: Effect BM-MSCs therapy on kidney TNFα and liver TAC of different experimental groups.

Parameter	Unit	Group I	Group II	Group III	Group IV
TNFα	pg/ml	289.00±0.96^{a}	286.25± 2.04^{a}	2362.50± 4.33^{b}	305.00±4.43^{a}
TAC	mM/L	3.37± 0.04^{a}	3.44± 0.06^{a}	2.33±0.03^{b}	3.82± 0.06^{a}

Data were expressed as mean ± SE. Values with different superscripts in the same raw are significantly different at (p≤0.05).

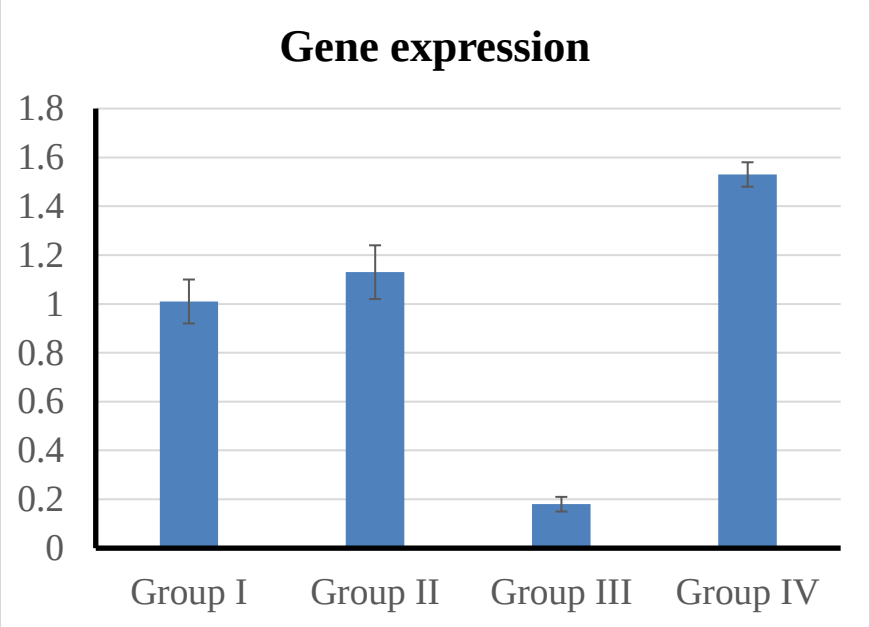

Figure 5: Relative expression of mRNA of insulin gene in different groups.

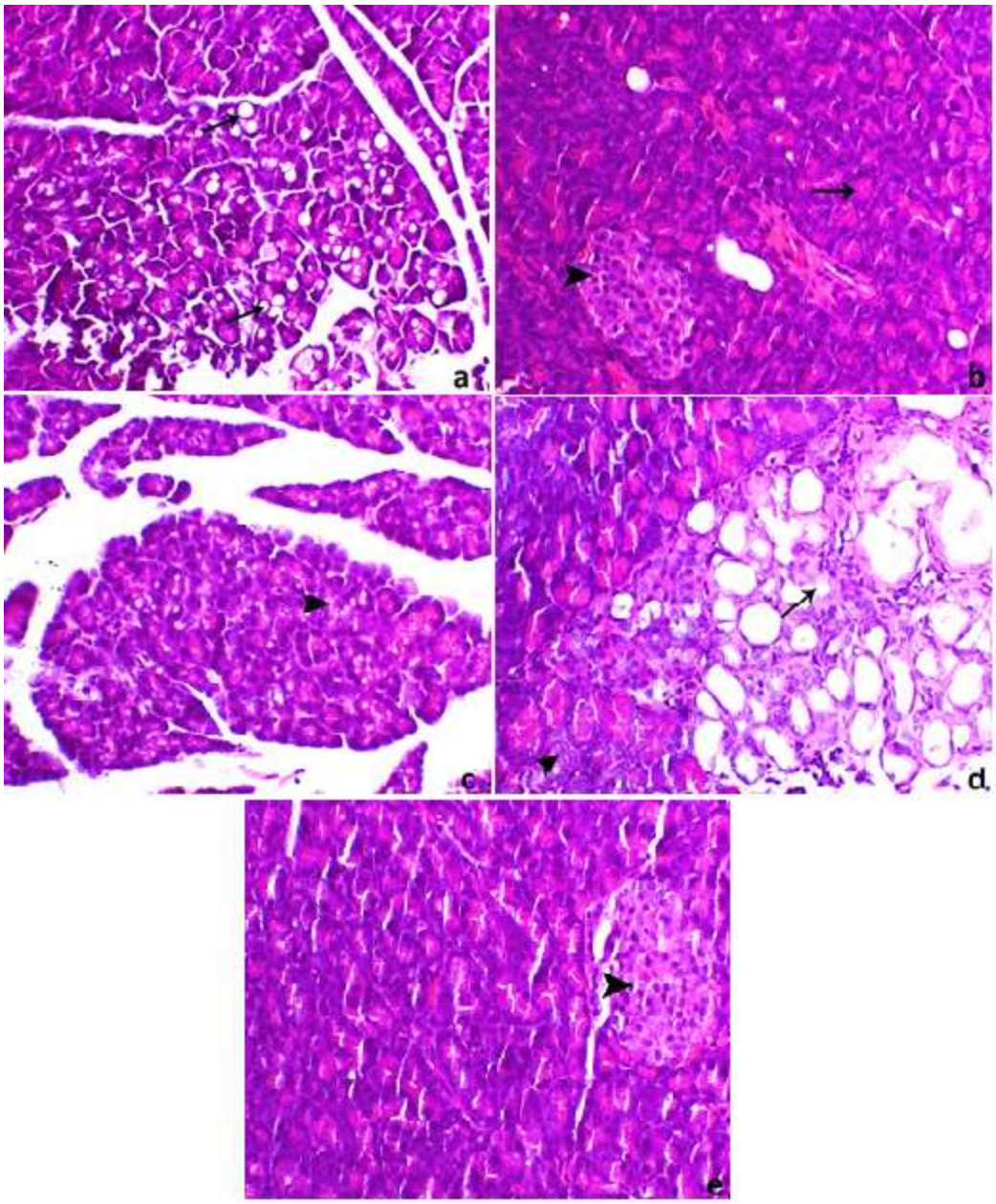

Figure 6: Pancreatic histopathological sections (H&E, X200).

a-Control rats showing normal pancreatic glandular acini and islets of Langerhans (arrow and arrowhead, respectively).

b-Untreated diabetic rats revealing marked vacuolation of pancreatic glandular acini (arrows).

c-Untreated diabetic rats showing degeneration of the exocrine pancreas (arrowhead) and necrosis of β cells associated with cystic hyperplasia within the endocrine pancreatic portion (arrow).

d-Treated diabetic rats showing mild degeneration and vacuolation of both pancreatic acinar cells of the exocrine portion (arrowhead).

e-Treated diabetic rats with mild degeneration of both pancreatic acini and islets cell and mostly within normal limits (arrowhead).

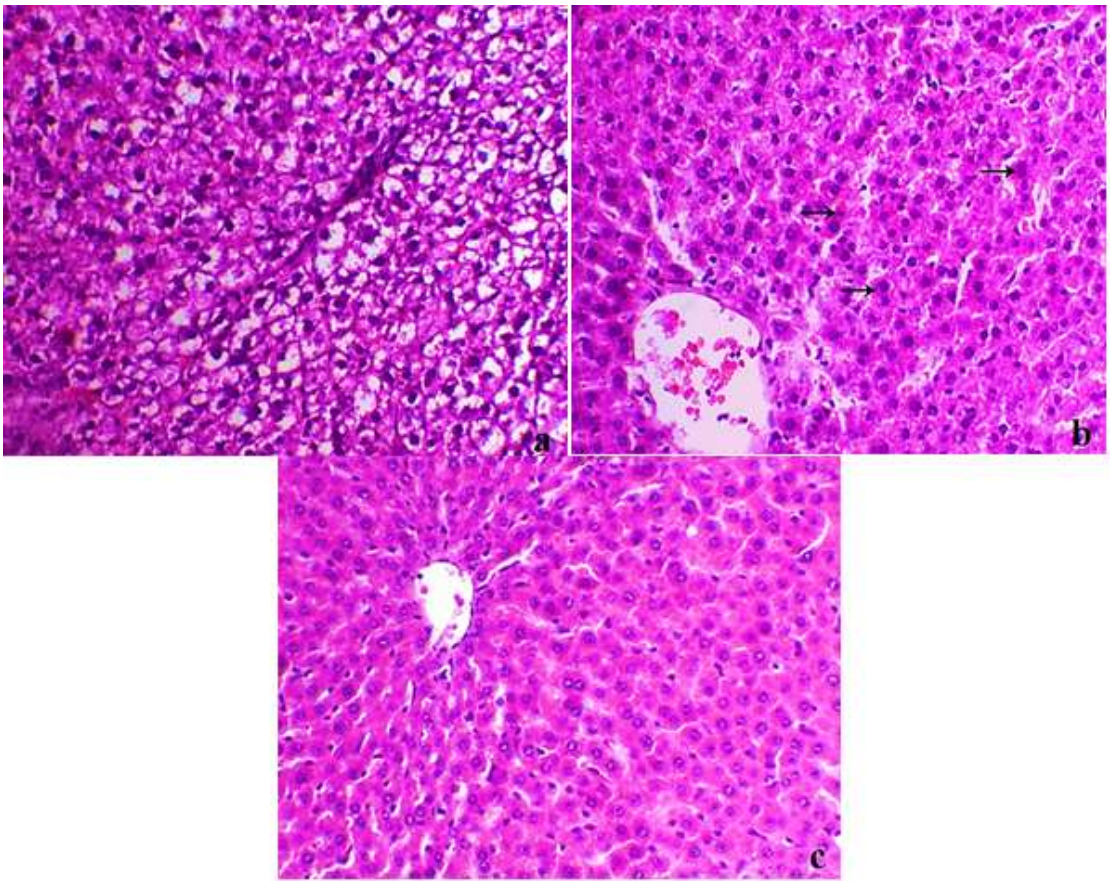

Figure 7: Liver histopathological sections (H&E, X200).
a-Liver of control rats showing vacuolation of hepatocytes.
b-Liver of untreated diabetic group showing increase apoptotic hepatocytes (arrows).
c-Marked reduction in number of apoptotic bodies in Treated diabetic rats.

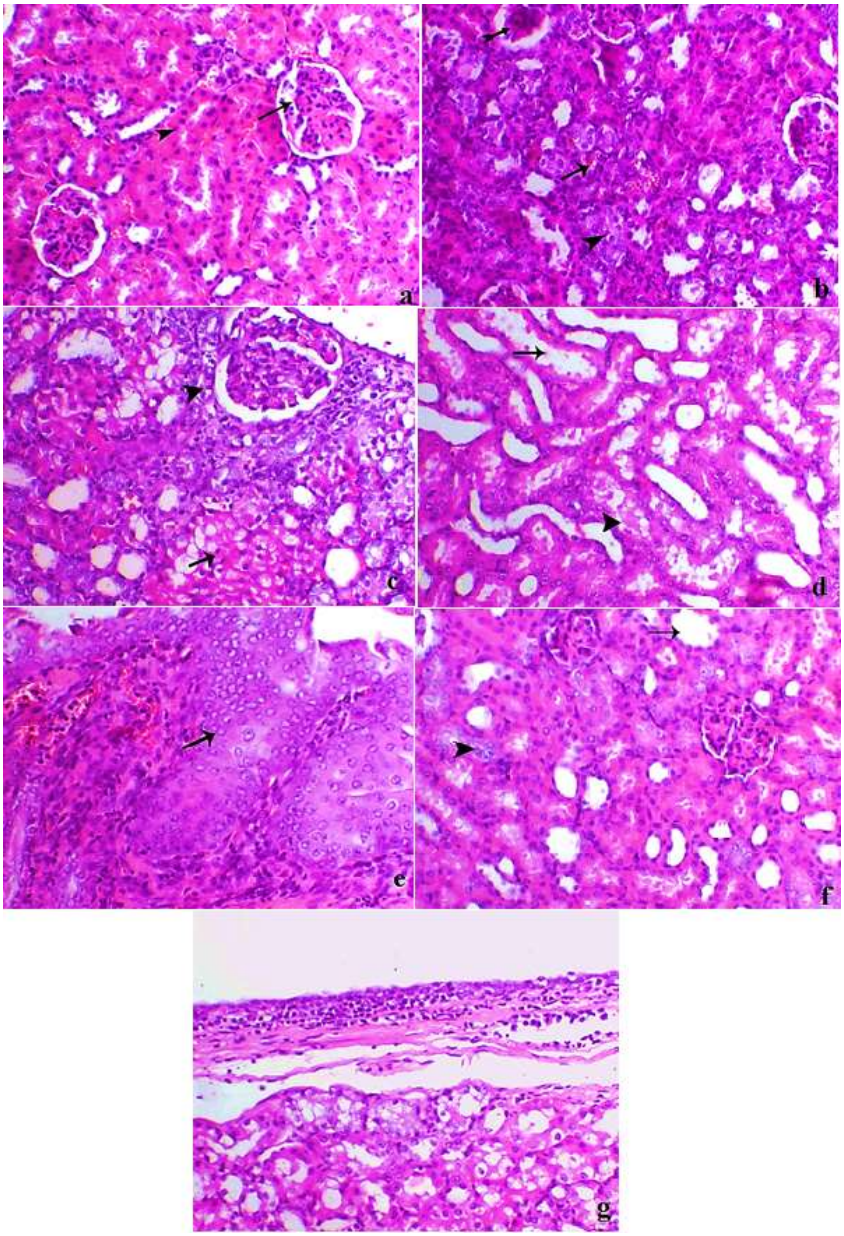

Figure 8: Renal histopathological sections (H&E, X200.).

a-Kidney of control rats showing normal renal glomerular and tubular structures (arrow and arrowhead respectively).

b-Kidney of untreated diabetic rats showing marked glomerular sclerosis (tailed arrow), tubular degeneration associated with tubular basophilia (arrowhead) and hemosiderosis (arrow).

c-Kidney of untreated diabetic rats showing tubular necrosis (arrow) and periglomerular fibrosis and infiltration of inflammatory cells (arrowhead).

d-Kidney of untreated diabetic rats showing marked renal hydronephrosis (arrow) associated with degeneration of renal tubular epithelium (arrowhead).

e-Kidney of untreated diabetic rats showing squamous metaplasia of renal pelvis epithelium (arrow).

f-Kidney of Treated diabetic rats showing mild degeneration of renal tubular epithelial lining associated with mild degree of renal tubular basophilia (arrowhead) and hydronephrosis (arrow).

g-Kidney of group IV showing normal renal pelvis lining epithelium.

Liver

Microscopic examination of liver tissues revealed normal vacuolation of hepatocytes in control group (

Figure 7a). On other hand, the liver tissues of group III (untreated diabetic rats) showed increase apoptotic hepatocytes, bi-nucleated cells surrounded by hollow zone representing apoptosis and sinusoidal cells activation (

Figure 7b). Moreover, the liver tissues of group IV (treated diabetic rats) showed marked amelioration of hepatotoxic effect of STZ represented by decrease number of apoptotic cells (

Figure 7c).

Kidney

Microscopic examination of kidney tissues revealed normal renal glomerular and tubular structures in control group (Figure 8 a:c). On other hand, the kidney tissues of group III (untreated diabetic group) showed marked glomerular sclerosis, tubular degeneration associated with tubular basophilia and hemosiderosis, tubular necrosis and periglomerular fibrosis and infiltration of inflammatory cells and squamous metaplasia of renal pelvis epithelium (Figure 8d:f). Moreover, the kidney tissues of group IV (treated diabetic rats) showed mild degeneration of renal tubular epithelial lining associated with mild degree of renal tubular basophilia and hydronephrosis and normal renal pelvis lining epithelium (Figure 8g).

DISCUSSION

Bone marrow-derived stem cells (BM-MSCs) represent new horizons for the development of cell-based therapeutic strategies in regenerative medicine and tissue repair. They are easily isolated and have multilineage potential and extensive propagation in cultures (Sudkamp 2007). In the current work, BM-MSCs were isolated from the femurs and tibias of Sprague Dawely rats, cultured and characterized by their spindle shape, adhesiveness and by expressing the MSCs surface markers (CD73 and CD105) but negative for endothelial and hematopoietic markers (CD34 and CD45) (Zhao et al. 2014; Draz et al. 2015). Moreover, seeding and homing of the BM-MSCs in pancreatic tissues (at the 7th day of transplantation) was proved in the tracking group via Qtracker 655 labeled BM-MSCs and fluorescent microscopy.

In the present study, a rat model of Diabetes Mellitus type-1 was adopted via administration of STZ (55 mg/kg b.w.) to Sprague Dawely male rats. The estrogen in females may interfere with the diabetogenic action of STZ, so, male rats were used in this study (Sayed et al. 2019). A significant and persistent hyperglycemia with hypoinsulinemia in the untreated diabetic rats were achieved. Moreover, significant impairment in glucose tolerance test in the untreated diabetic rats was found. The hyperglycemia and hypoinsulinemia in rats after STZ administration were attributed to the destruction of pancreatic beta cells (β-cells) by STZ. Via the glucose transporter 2 (GLUT2), The STZ enters the pancreatic β-cells, causes alkylation of the DNA of β-cells and reduces the cellular NAD via the nitrosourea moiety and carbonium ions (Zafar and Naqvi, 2010; Saumya and Basha, 2011). Moreover, STZ generates reactive oxygen species (ROS) and selectively lead to β-cells destruction (Goyal et al. 2016). These changes may adversely affect the enzymes of cellular respiration with alteration of mitochondrial function leading to the irreversible apoptosis β-cells resulting in suppression and disturbance of biosynthesis of insulin secretion with the resultant permanent hyperglycemia. The current work demonstrated a significant reduction in the insulin level in pancreatic tissues at the level of mRNA suggesting destruction of β-cells of islets of Langerhans.

Moreover, the results of the treated diabetic group demonstrated significant improvement in the glucose tolerance test (10 days post BM-MSCs therapy). Similar early results were reported by Lv et al. (2014). Interestingly, the improvement persisted and reflected on the blood glucose and insulin levels at the end of experiment (4 weeks post BM-MSCs therapy).

These findings are similar to the results of Dong et al. 2008 and Ezaquer et al.2008 who reported that a single infusion of BM-MSCs ameliorate the blood glucose in STZ diabetic rats. After systemic administration of BM-MSCs, the stem cells migrate to the site of injured pancreatic β-cells, proliferate and differentiate into insulin secreting cells (Dong et al. 2008). This hypothesis was confirmed by the detection of BM-MSCs in the pancreatic tissues of the tracking group. Moreover, a significant increase in insulin gene expression at the level of mRNA in the treated diabetic rats suggesting more increase in insulin expression and secretion.

Regarding, lipid profile, the current work showed significant increase in the serum levels of TC, TGs and LDL with significant decrease in HDL in diabetic rats. These findings are in line

with those reported by Komolafe et al. (2009) and Radhika et al. (2010). They reported hyperlipidemia in DM in the form of increased levels of TC, TGs and phospholipids with changes in lipoprotein composition. The disturbance in lipid metabolism might be due to deficiency of insulin or inadequate insulin efficiency which results in elevated free fatty acids (FFAs) mobilization from adipose tissue to the blood leading to excessive transformation of FFAs to ketone bodies or through their incorporation into liver triglyceride and VLDL, and through diminished lipogenesis. Also, the current work showed that stem cell therapy in diabetic rats caused significant reduction of serum levels of TC, TGs and LDL with significant increase level of HDL compared to diabetic rats. These are in line with those reported by El-Tantawy and Halem, (2014). They found that treatment of type 1 diabetic rats with MSCs normalized lipid profile pattern.

Regarding the hematological changes in DM, diabetic rats have significantly lower values of Hb, PCV, MCH, TLC and platelet count suggesting significant reduction in hematopoiesis. These findings are in line with the findings of previous studies which demonstrated 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. Also, the significant leukopenia associated with significant lymphopenia, eosinopenia and monocytopenia as well as neutrophilia observed in STZ diabetic rats 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.

On the other hand, treatment with stem cell caused significant improvement in these parameters suggesting that BM-MSCs enhance hematopoiesis which might be due to migration of the stem cell to the bone marrow and generation of blood cells or release of growth factors that stimulate blood cell formation. 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 next point investigated in the current work was the liver injury in diabetic rats. Liver damage was confirmed in the present study by the presence of elevated serum ALT, AST and ALP enzymes levels with morphological changes in liver histopathological examination. STZ prevents DNA synthesis in mammalian cells and prevents cellular reproduction by inhibiting the substrate binding to the DNA or inhibiting many of the enzymes involved in DNA synthesis (Holemans et al. 1997) leading to impairment of liver function, and leakage of these enzymes in the blood. Liver injury in DM is multifactorial and is not controllable only via inhibition of hyperglycemia (Waer and Helmy, 2012).

Hyperglycemia in DM causes excessive formation of advanced glycation end products (AGEs) which facilities free radicals production via disturbance in ROS production. Recent studies demonstrated the effect of oxidative stress in liver during DM, in both humans and rodents. The current study demonstrated significant reduction in total antioxidant capacity (TAC) in liver tissues of diabetic rats suggesting the development of oxidative stress in liver of diabetic rats. Increased ROS generation in the liver of diabetic individuals has been involved in the progression of nonalcoholic fatty liver disease (NAFLD). As increased ROS levels are related to morphological changes in hepatocytes and liver DNA damage (Cubero and Trautwein, 2011; Dechandt et al. 2014). On the other hand, stem cell therapy caused significant improvement in liver enzymes activity and liver morphology with significant increase in total antioxidant capacity suggesting hepatoprotective and antioxidant effect for BM-MSCs therapy.

One of the major diabetic nephropathies is the end stage renal disease (ESRD) which is manifested by elevated serum levels of creatinine, urea and uric acid with reduced creatinine clearance (Yan et al. 2007; Davey et al. 2014). In the current work, diabetic rats showed elevated serum creatinine and BUN with significant deteriorations of the kidney morphology suggesting development of renal impairment. In line with these findings, Mir et al. (2008) postulated that the elevation in serum levels of urea and creatinine in diabetic animals might be due to the functional and/or morphological changes in the kidneys. The mechanisms underlying development of DN involve oxidative stress, considerable injury in the glomeruli, a

marked increase in the protein glycosylation, disorders in matrix protein synthesis and increase in transforming growth factors-beta (TGF- β) (Patel et al. 2009; Erejuwa et al. 2011). Also, we found in the current study significant increase in TNF-α inflammatory cytokine in kidney tissues; one the most important pro-inflammatory mediator that is judgmentally involved in the autoimmune process leading to beta cell damage (Akash et al. 2018).

On the other hand, BM-MSCs therapy caused significant improvement in kidney functions and morphology suggesting its renoprotective and anti-inflammatory activity of stem cells. In line with these findings, Abdel Aziz et al. (2014) demonstrated significant improvement in kidney function in diabetic rats with single dose of 1×10^6 MSCs per rat. They attributed this improvement in kidney function in MSCs treated group to their paracrine action via different growth factors such as VEGF, TGF-β and TNF- α and antiapoptotic effects via Bax and Bcl2 genes.

CONCLUSION

In conclusion, the current work demonstrated that diabetic rats treated with BM-MSCs showed hopeful results in stimulating insulin secretion from beta cells of islets of Langerhans. Also, they were able to improve the dyslipidemia, liver and kidney dysfunctions in diabetic rats. This may be helpful in the prevention of some diabetic complications such as diabetic nephropathy and liver dysfunctions.

CONFLICT OF INTEREST

The authors declared that there is no conflict-of-interest present in the study.

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AUTHOR CONTRIBUTIONS

SYS and SIS designed and supervised the experiments. SYY wrote the first draft. KMAM, SIS and SYY analyzed the data, conducted the research, managed the literature search and edited the manuscript. All authors read and approved the final version.

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