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Infusion of bone marrow mesenchymal stem cells alleviates insulin resistance and initiates pancreatic regeneration in experimental type 2 diabetic-rats

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ABSTRACT

Insulin resistance and defect in insulin-producing pancreatic beta cells are the key features of type 2 diabetes (T2DM). Mesenchymal stem cells isolated from bone marrow (BM-MSCs) have differentiation, anti-inflammatory and immunosuppressive characteristics, making them suitable for treating T2DM. Therefore, the present study aimed at investigating the possible therapeutic mechanisms of BM-MSCs infusion in high fat diet/streptozotocin (HFD/STZ)-induced T2DM rats. Thirty male Wistar rats were divided into 3 groups: Normal control, T2DM, and T2DM treated with BM-MSCs. The anti-diabetic effect of BM-MSCs was evidenced by ameliorating the state of hyperglycemia, hyperinsulinemia, and hyperlipidemia. BM-MSCs enhanced significantly insulin sensitivity in diabetic rats *via* decreasing leptin levels in serum and consequently, increasing adiponectin/leptin ratio, compared to T2DM group. Furthermore, BM-MSCs improved glucose homeostasis by up-regulating gene expressions of hepatic glucokinase and glycogen synthase in the liver and skeletal muscles as well as increasing their levels. Treatment of T2DM with BM-MSCs stimulated pancreatic regeneration in diabetic rats by up-regulating the expression of insulin-like growth factor-1 receptor gene, compared to diabetic and normal control groups. Histopathological results show that BM-MSCs therapy ameliorated HFD/STZ-induced atrophy in islands of Langerhans cells with the presence of mild cystic dilatation in the duct system. In conclusion, BM-MSCs might be an ideal therapeutic option for T2DM since they ameliorated peripheral insulin resistance and promoted beta cell regeneration.

Introduction

The global prevalence of diabetes in adults has been increasing over recent decades, with 425 million diabetes (aged 20 to 79 years) in 2017 and an estimate of 629 million new diabetes (20 - 79 years old) in 2045, accounting for 9.9 % of the world population [1]. The development of type 2 diabetes mellitus (T2DM), accounting for 90 % of all diabetes cases, has been determined by both genetic and environmental factors [2]. The prevalence of T2DM is increasing rapidly in Egypt and considered one of the major health problems in the Eastern Mediterranean region [3]. T2DM is characterized by disturbances in the metabolism of lipids and carbohydrates, as well as insulin resistance and dysfunction in pancreatic β-cells [4]. T2DM can be developed experimentally by feeding animals with a high fat diet (HFD) followed by a single streptozotocin (STZ) injection resulting in β-cells necrosis and insulin resistance [5]. HFD elevates body weight and visceral fat depots and alters the metabolism of carbohydrate and lipid metabolism as well as the level of adipokines, which regulate energy and glucose metabolism [6,7]. Leptin controls food intake, energy expenditure and whole-body energy balance in rodents and humans [8]. Adiponectin enhances insulin sensitivity and protects against obesity-associated metabolic syndrome [9].

The liver and skeletal muscles regulate glucose homeostasis [10] *via* controlling several glucose metabolic pathways including glycolysis and glycogenesis [11]. Glucokinase (GK) has important role in glucose utilization and glycogen synthesis. Its level decreases in diabetic human and rodents [12]. Hence, restoration of hepatic GK activity may provide a possible therapeutic strategy of diabetes treatment. Glycogen synthesis is

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impaired in type 2 diabetic-patients [13].

Insulin-like growth factor-1 acts mainly via insulin-like growth factor-1 receptor (IGF-1R). Upon activation of IGF-1R, many transduction pathways are activated leading to cell growth and mitogenesis. In tissues, IGF-1 is capable of increasing cell numbers by inhibiting apoptosis [14]. Therefore, IGF-1R can induce transcriptional activity to promote survival, self-renewal and differentiation of mesenchymal stem cells (MSCs) [15]. Targeted ablation of IGF-1R function in pancreatic cells impairs glucose-induced insulin secretion, without changes in cell proliferation [16].

Currently, many anti-diabetic medications are available, however they have known existing adverse side effects and increasing costs [17]. Hence, new therapeutic strategies against T2DM should be developed. Stem cells are immature tissue precursor cells with self-renewal and differentiation into multiple cell lineages [18]. The capability of stem cells therapy to regenerate damaged tissues received a great attention. Bone marrow is an important source of easily obtained adult mesenchymal stem cells (MSCs) [19], which can produce numerous trophic cytokines to improve the microenvironment of the pancreas and promote the expansion of endogenous pancreatic stem cells [20,21]. However, these findings were still not adequate to explain the therapeutic contribution of MSCs to T2DM, which is prominently characterized by peripheral insulin resistance. Accordingly, the present study was undertaken to investigate the therapeutic potential of BM-MSCs infusion against HFD/STZ-induced type 2 diabetic rat model *via* studying BM-MSCs effects on some diabetic biomarkers, lipid profile, insulin sensitivity, pancreatic regeneration and some glucose-regulating enzymes.

Subjects and Methods

Isolation, culture and identification of BM-MSCs

Young (6 - 8 weeks old) male Wistar rats weighing 60 - 75g were provided from the animal house of the Medical Ain Shams Research Institute (Faculty of Medicine, Ain Shams University) to establish BM-MSCs cultures according to the methods of Porter *et al.* [22] & de Hemptinne *et al.* [23]. Briefly, rats were killed by cervical dislocation and femurs and tibias were removed from the back limbs, placed in 70 % ethanol for a few secs and washed with 1× phosphate buffered saline (PBS). In the laminar flow (Nuaire 425-300, USA), the end of femurs and tibias were cut open with a scissor in a 10 cm Petri dish containing 5 ml 1× PBS. The marrow was flushed from bones into a 50 ml Falcon tube by inserting a needle filled with 5 ml Dulbeco Modified Eagle Medium (DMEM) provided from Sigma (USA). Bone marrow samples were pipetted to get rid of clumping of cells to obtain single cell suspension, and then cells were added to 5 ml DMEM solution and centrifuged at 2000× g for 5 min. The isolated BM-MSCs were washed twice with 1× PBS. The BM-MSCs were re-suspended in 5ml working DMEM solution and centrifuged. The cell pellet was collected and cultured in a 25-cm^2 tissue culture flask for a total of 10 flasks in 10 ml working DMEM solution (2 million cells/ flask). The culture flasks were incubated in a 5 % CO_2 incubator at 37°C (Nuaire 4950, USA) for about 1 week. The media were changed every 2 - 3 days when the color of phenol red indicator was converted from red to pale red.

BM-MSCs were counted and trypsinized according to the method of Zhang and Chan [24]. Briefly, BM-MSCs were counted through the inverted fluorescence microscope (Axiovert 100, Germany). For each suspended two million cells, the medium was aspirated and 2 ml trypsin-EDTA were added to each flask. The flasks were incubated for 5 mins to allow cell detachment. Once cells were detached, equal amount of culture medium was added to inactivate trypsin. Cell suspension was collected into a 15 ml tube and cells were spanned down at 200× g at 4°C for 5 mins. The supernatant was discarded from each tube and the cell pellet was dissolved in 0.5 ml 1× PBS to be immediately injected in the tail vein of each diabetic rat.

Before intravenous injection, BM-MSCs were identified by flow cytometry *via* detecting positive BM-MSC markers (CD44) as well as hematopoietic markers (CD19) using fluorescein isothiocyanate-conjugated mouse monoclonal antibody specific for rat CD44 and phycoerythrin (PE)-conjugated mouse monoclonal antibody specific for rat CD19, respectively (Dako Agilent Technologies, Inc., USA). The Beckman Coulter (Navios Flow Cytometer, Ireland) was used and the data were analyzed using Beckman Coulter Navios software 1.2.

Animals

For acclimatization, male Wistar rats (3 - 4 months old) weighing 100 - 150 g were housed in steel mesh cages (5 rats/ cage) under standard laboratory conditions of 12/12 h light/dark cycles and temperature (28°C) for 1 week before the start of the experiment and kept under these conditions throughout the experimental period. The animals were fed standard pellet diet with a total caloric value of 25 kJ/kg (Meladco, Cairo, Egypt) and water *ad libitum*. The experimental protocol was carried out according to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, 1985).

Induction of T2DM

T2DM was induced in 20 out of 30 rats by feeding them a high-fat diet (22 % fat, 48 % carbohydrate and 20 % protein, with a total caloric value of 44.3 kJ/kg) for 3 consecutive months [25]. After HFD administration, rats were injected intraperitoneally (i.p.) once with streptozotocin (STZ) (III Kirch, France) (35 mg/ kg b.w.) prepared in 0.1M citrate buffer (pH 4) [26] and kept on drinking water containing sucrose (3 %) for 3 days. After 3 days from STZ injection, fasting blood sample was collected from the tail vein to measure the glucose level to ensure the successful induction of hyperglycemia by using a digital glucometer (Mannheim, Germany). Rats having blood glucose above 250 mg/dl were considered

diabetic and were further included for the experiments.

Study Design

Thirty rats were divided equally into 3 groups as follows: Group I (Normal Control): normal rats were i.p. injected with sterile citrate buffer (1 ml kg^{-1}); Group II (T2DM): normal rats were fed HFD followed by a single i.p. injection of STZ; Group III (T2DM+BM-MSCs): diabetic rats were treated 3 days after STZ injection by a single i.v. injection of 2×10^6 BM-MSCs prepared in sterile saline. Rats were observed for a further 5 weeks period and the fasting blood glucose levels from the tail vein were monitored regularly using a digital glucometer (Mannheim, Germany).

Blood sampling and collection of body organs

At the end of the experimental period (17 weeks), the animals were fasted for 8 h, weighed, and the blood samples were taken from the retro-orbital venous plexus under light ether anesthesia with or without potassium oxalate/sodium fluoride mixture. Serum samples were collected, aliquoted, and stored at -80°C until analyses. After blood collection, the rats were then sacrificed by cervical dislocation. Pancreas, liver, and skeletal muscles were excised, rinsed thoroughly in isotonic sterile saline containing heparin, blotted dry with a filter paper. The pancreas was divided into 2 parts; one part was stored at -80°C until analyses. The other part of pancreas was kept in 10 % formalin for histological examination. In addition, liver and skeletal muscles were stored at -80°C until analyses.

Diabetic biomarkers and lipid profile

Fasting blood glucose was determined using a commercial colorimetric kit (Spectrum, Egypt) [27]. Fasting serum insulin was measured using rat insulin ELISA kit provided from MyBiosource (USA). Indices of both homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA-β) were calculated according to the empirical formulae: HOMA-IR = fasting insulin (mU/L) × fasting glucose (mmol/l) / 22.5; and HOMA-β = [(fasting insulin "mU/L" × 20) / (fasting glucose "mmol/L"] – 3.5 [28]. Serum total cholesterol (TC), triacylglycerol (TAG), and high-density lipoprotein cholesterol (HDL-C) were determined using commercial colorimetric kits (Spectrum, Egypt) [29,30,31]. Low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were calculated according to the equations: LDL-C = TC – [HDL-C + TAG/5] [32] and VLDL-C = TAG/5 [33], respectively. Atherogenic index-1 was calculated from the ratio = TC/HDL–C and atherogenic index-2 was calculated from the ratio = LDL-C/ HDL-C [34].

Assessment of insulin sensitivity markers

Adiponectin and leptin levels were quantified in sera samples using rat specific ELISA research kits provided by Assaypro and CUSABIO (USA), respectively. Adiponectin/Leptin (A/L) ratio was then calculated according to Jung *et al*. [35].

Expressions of glucokinase, glycogen synthase and IGF-1R genes

Total RNA was extracted and purified from pancreas, liver, and skeletal muscles using RNeasy Mini Kit according to manufacturer's instructions (Qiagen, Germany). The purity of the extracted RNA was assessed spectrophotometrically at 260/280 nm using a UV-spectrophotometer (PhotoBiometer, Eppendorf, Germany). A total of 1μg of RNA was reverse transcribed into single-stranded complementary DNA (cDNA) using QuantiTect reverse transcription kit (Qiagen, Germany). cDNA synthesis was performed using Gene Amp PCR System 9700 (Applied Biosystems, USA).

Quantitative real time PCR (qPCR) was performed using SYBR green master mix (Qiagen, Germany) to determine the relative expressions of the glucokinase in liver, glycogen synthase in liver and skeletal muscles, and IGF-1R in pancreas. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene for normalizing mRNA expression for genes of interest. qPCR was performed in an optical 96-well plate with an ABI PRISM 7500 fast sequence detection system (Applied Biosystems, Carlsbad, California) and universal cycling conditions (10 mins at 95°C, followed by 50 cycles at 95°C for 15 sec, 60°C for 60 sec, and 72°C for 15 sec). The used primers (Biosearch Technologies, USA) for qPCR are listed in **Table 1**. Gene expression was expressed in relative units ($RQ = 2^{-\Delta\Delta CT}$) according to the method of Livak and Schmittgen [36].

Preparation of liver and skeletal muscles homogenates

Ten percent of liver and muscle tissue homogenates were prepared by homogenization in ice-cold phosphate-buffered saline (pH 7.4) using an electric homogenizer (Universal Laboratory Aid MPW-309, Poland). The whole homogenates were centrifuged at 18000× g for 20 mins at 4ºC (Cooler Microfuge Laborzentrifugen, Sigma, Germany) to obtain the cytosolic fraction. The cytosolic fractions were then collected, aliquoted and stored at -80^{0}C until analyses.

Total protein measurement

The total protein concentration was determined in the cytosolic fractions of both liver and skeletal muscle homogenates according to the method of Bradford [37].

Assay of glucokinase and glycogen synthase contents

Liver glucokinase, as well as liver and skeletal muscles glycogen synthase contents were quantified by rat specific ELISA research kits (Immunoconcept, India) in their respective homogenates. The contents of the enzymes were expressed as ng/mg protein.

Histological examination

Pancreatic specimens of the different groups were fixed in 10 % formalin solution for at least 3 days at 4ºC for subsequent staining with hematoxylin and eosin. Examination of the slides was performed under the electric light microscope (Olympus, Japan).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science version 20 for Windows (SPSS software package, Chicago, USA).

Individual data in experimental groups were analyzed using one-way Analysis of Variance (ANOVA) followed by Duncan test as posthoc testing for multiple comparisons between the different treated groups. *P* value was considered significant at $p < 0.05$. The percentage of change was calculated in comparison with the normal control. The results were represented as mean ± SD of 10 values.

Results

Characteristics of the isolated BM-MSCs

The isolated BM-MSCs showed the fibroblastic morphology that is characteristic of MSCs **Fig. 1A**. In addition, analysis by flow cytometry demonstrated that BM-MSCs were 91.8 % pure for CD44. The percentage of contaminated populations of hematopoietic stem cells positive for CD19 was 8.9 % **Fig. 1B.**

Table 1: Primers list for qPCR

Gene	Sequence (5´- 3´)	Tm	GC%	Self 3´ Complementary
Pancreatic IGF-1R NM_052807.2	**Forward primer** GACCTGCTGATCCTCGACCC	62.02	65	0
	Reverse primer TCCACTGAGATACAGGAGGCT	59.71	52.38	3
Glucokinase AH002177.2	**Forward primer** CACCCGAGGTCAGCTTCCAC	62.79	65	2
	Reverse primer TGATTTCGCAGTTGGGTGTCA	60.48	47.62	3
Glycogen synthase NM_001109615.1	**Forward primer** GATTCAGTGTCGCCGCTCAC	61.69	60	3
	Reverse primer TAACGGGTGTCCTACGGGAA	60.25	55	0
GAPDH NM_017008.4	**Forward primer** TGTGAACGGATTTGGCCGTA	59.96	50	2
	Reverse primer ACCAGCTTCCCATTCTCAGC	60.03	55	2

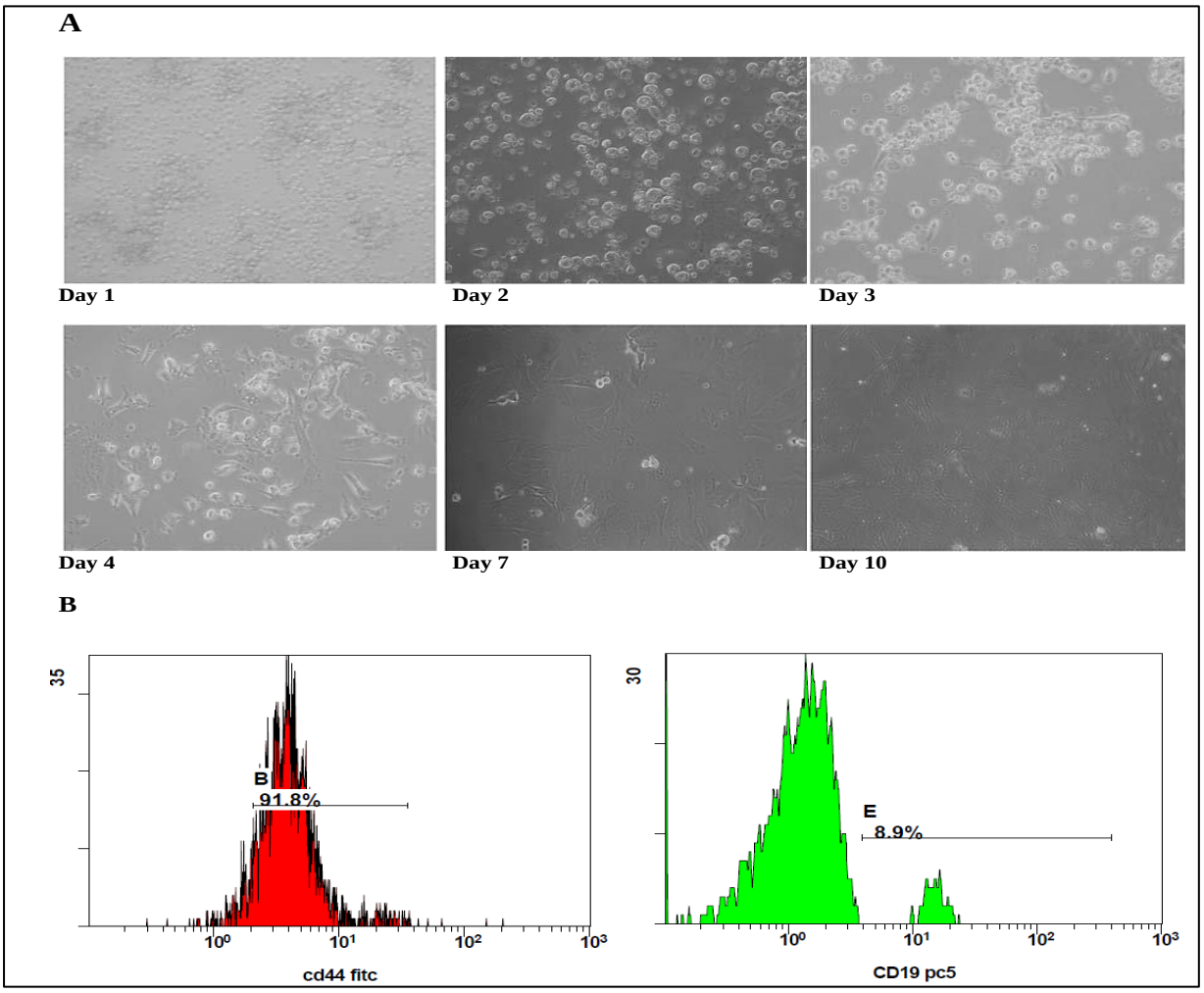

Fig. 1: Morphology and immunophenotyping of rat BM-MSCs. **(A)** Rat BM-MSCs showed homogenous, fibroblast-like morphology at the 10th day of the first passage. **(B)** Fluorescence-activated cell sorting analysis of immune markers in rat BM-MSCs demonstrating that rat BM-MSCs were positive for CD44 but negative for CD19.

Diabetic biomarkers and lipid profile

Table 2 shows that induction of T2DM in rats by HFD followed by a single i.p administration of STZ elevated significantly ($p < 0.0001$) fasting blood glucose and serum insulin levels (430.14 and 127.74 %, respectively), compared to the normal control rats. On the other hand, treatment of diabetic rats with BM-MSCs reduced significantly ($p < 0.0001$) fasting blood glucose and serum insulin levels, compared to type 2 diabetic-rats, however, they were still significantly higher ($p < 0.0001$) than the normal levels (46.25 and 31.75 %, respectively). Calculating HOMA-IR & HOMA-β indices of type 2 diabetic-rats showed a significant increase in HOMA-IR (1108.89 %, $p < 0.0001$) with a significant reduction ($p < 0.0001$) in HOMA-β index (57.2 %). Meanwhile, BM-MSCs therapy normalized the values HOMA-IR & HOMA-β.

Furthermore, data shown in **Table 2** demonstrate that type 2 diabetic-rats had significant elevations ($p < 0.0001$) in the serum levels of TC (103.49 %), TAG (64.12 %), VLDL-C (64.15 %), and LDL-C (284.82 %), as well as atherogenic indices 1 & 2 (202.16 and 476.19 %, respectively), in association with a significant reduction ($p < 0.0001$) in serum HDL-C level (31.3 %), compared to the normal control rats. On the other hand, treatment of type 2 diabetic-rats with BM-MSCs normalized TC, TAG, VLDL-C, LDL-C, atherogenic indices 1 & 2. In addition, BM-MSCs therapy improved serum HDL-C level, compared to type 2 diabetic-rats, but was still significantly ($p < 0.0001$) lower than the normal level (13.41 %).

Adipokines and glucose metabolizing enzymes

Table 3 reveals a slight significant reduction ($p < 0.05$) in the serum adiponectin level in HFD/STZ and T2DM+BM-MSCs groups (16.2 and 13.64 %, respectively), compared to the normal control rats. Furthermore, serum leptin levels were increased significantly ($p < 0.0001$) in type 2 diabetic-rats (97.74 %), compared to the normal control rats. Meanwhile, treatment of type 2 diabetic-rats with BM-MSCs normalized serum leptin level. Adiponectin/ leptin (A/L) ratio showed a marked significant reduction $p < 0.0001$ (57.32 %) in HFD/STZ-induced type 2 diabetes; and this reduction was significantly improved ($p < 0.0001$) after BM-MSCs therapy (23.98 %), compared to the normal control group.

Data shown in **Fig. 2A&B** indicate that there were significant downregulations in the relative gene expression of liver GK as well as liver and skeletal muscles GS in HFD/STZ-induced T2DM rats, compared to the normal control rats. After BM-MSCs therapy, liver GK and muscle GS genes were significantly up-regulated, compared to the type 2 diabetic group, however, their expressions were still significantly lower than the normal expression levels. Furthermore, liver GS was up regulated, compared to both type 2 diabetic rats and normal control rats. In addition, data shown in **Fig. 2C** report a significant down regulation in relative expression of IGF-1R in pancreas of HFD/STZ-diabetic rats, compared to the normal expression level while; treating type 2 diabetic-rats with BM-MSCs upregulated its expression, compared to the normal expression level.

Table 4 shows that there were significant reductions ($p < 0.0001$) in the contents of liver GK (28.75 %) and GS (25.15 %), as well as muscle GS (43.46 %) in HFD/STZ group, compared to the normal control rats. Treatment of type 2 diabetic-rats with BM-MSCs increased significantly ($p < 0.0001$) the contents of liver GK (23.32 %), liver GS (25.95 %), compared to both HFD/STZ and normal control groups, while BM-MSCs normalized muscle GS content.

Table 2: Diabetic biomarkers and lipid profiles of the experimentally studied groups

	NC	T2DM	T2DM+BM-MSCs
Fasting blood glucose (mg/dl)	81.30 ± 6.67^a	431.00 ± 57.71^b	118.90 ± 24.06^c
Fasting serum insulin (ng/ml)	2.74 ± 0.3^a	6.24 ± 0.39^b	3.61 ± 0.27^c
HOMA-IR	15.86 ± 2.31^a	191.73 ± 31.12^b	30.68 ± 7.04^a
HOMA-β	348.09 ± 45.23^a	148.98 ± 21.92^b	319.58 ± 46.8^a
Triacylglycerol (mg/dl)	109.76 ± 10.35^a	180.14 ± 28.86^b	109.06 ± 15.51^a
Total Cholesterol (mg/dl)	107.31 ± 11.47^a	218.37 ± 44.55^b	116.30 ± 15.72^a
LDL-C (mg/dl)	39.13 ± 11.19^a	150.58 ± 45.68^b	54.46 ± 20.09^a
HDL-C (mg/dl)	46.23 ± 3.03^a	31.76 ± 4.6^b	40.03 ± 6.16^c
VLDL-C (mg/dl)	21.95 ± 2.07^a	36.03 ± 5.77^b	21.81 ± 3.1^a
Atherogenic Index-1	2.32 ± 0.2^a	7.01 ± 1.83^b	2.98 ± 0.71^a
Atherogenic Index-2	0.84 ± 0.23^a	4.84 ± 1.73^b	1.43 ± 0.69^a

[Results are mean ±SD of 10 values. NC = normal control, T2DM = type 2 diabetes, BM-MSCs = Bone marrow-mesenchymal stem cells]. Similar characters denote insignificance between groups.

Table 3: Serum adiponectin and leptin levels in experimentally studied groups

	NC	T2DM	T2DM+BM-MSCs
Adiponectin (µg/ml)	17.16 ± 2.41[a]	14.38 ± 3.1[b]	14.82 ± 1.49[b]
Leptin (ng/ml)	7.09 ± 0.82[a]	14.02 ± 3.55[b]	8.09 ± 1.42[a]
A/L Ratio	2.46 ± 0.53[a]	1.05 ± 0.19[b]	1.87 ± 0.3[c]

[Results are mean ± SD of 10 values. NC = normal control, T2DM = type 2 diabetes, BM-MSCs = Bone marrow-mesenchymal stem cells, A/L = adiponectin/ leptin]. Similar characters denote insignificance between groups.

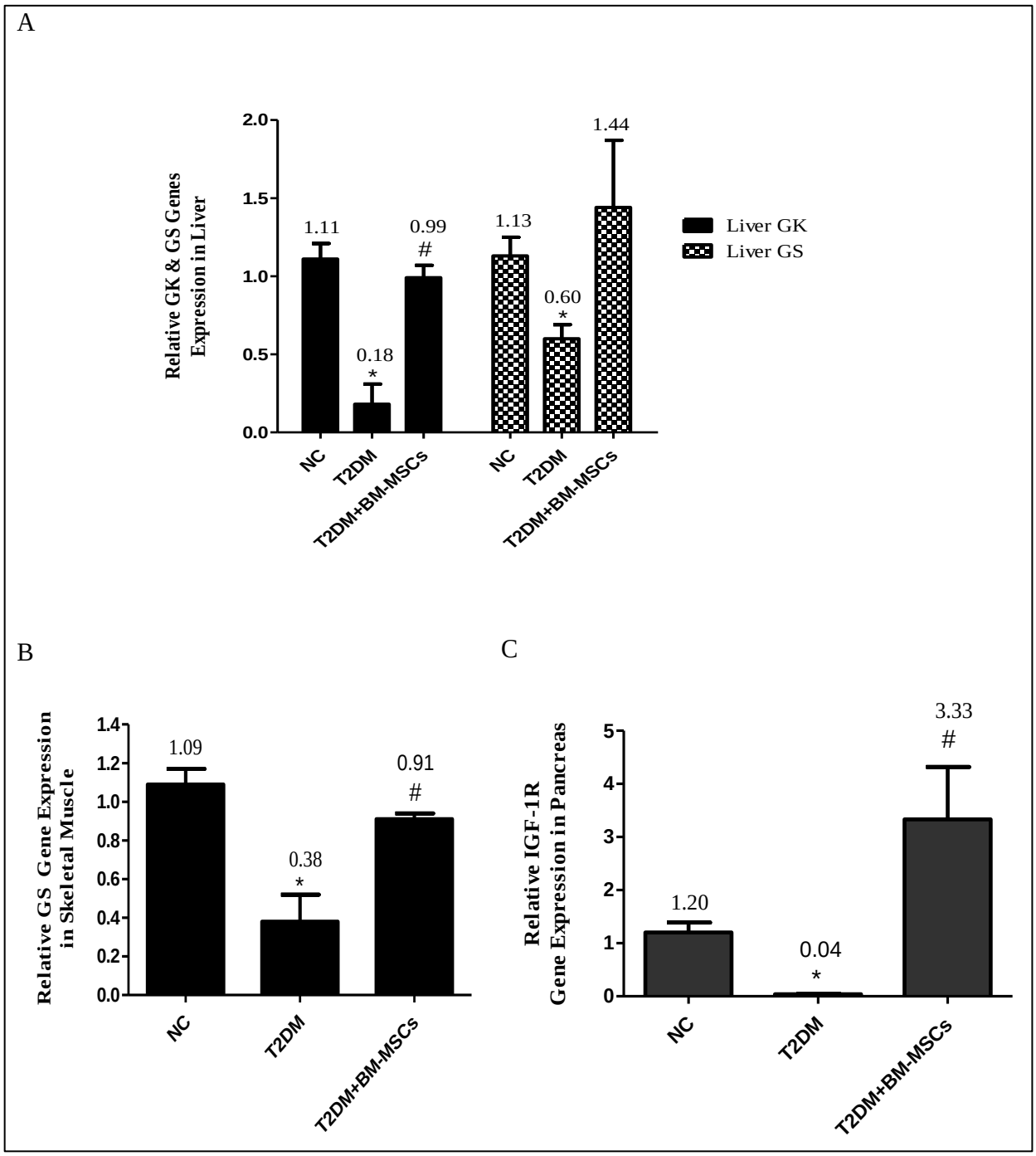

Fig. 2: Relative gene expression of glucokinase (GK) and glycogen synthase (GS) in liver **(A),** glycogen synthase in skeletal muscles **(B),** and IGF-1R in pancreas **(C)** of all the experimental groups. The data of the qPCR were normalized by GAPDH as a housekeeping gene. Data are presented as mean ± SD of 6 values/group. [$^{*,\#}$ denote significance at $p < 0.0001$]

Table 4: Glucokinase and glycogen synthase contents in liver and/or muscles of the experimentally studied groups

	NC	T2DM	T2DM+BM-MSCs
Liver GK Conc. (ng/mg protein)	453.79 ± 63.25[a]	323.32 ± 23.78[b]	559.60 ± 98.9[c]
Liver GS Conc. (ng/mg protein)	5.01 ± 0.68[a]	3.75 ± 0.34[b]	6.31 ± 1.22[c]
Muscle GS Conc. (ng/mg protein)	4.51 ± 0.61[a]	2.55 ± 0.56[b]	5.12 ± 0.18[a]

[Results are mean ±SD of 10 values. NC = normal control, T2DM = type 2 diabetes, BM-MSCs = Bone marrow-mesenchymal stem cells, GK = glucokinase, GS = glycogen synthase]. Similar characters denote insignificance between groups.

Histological examination

The histological examination of the H&E-stained pancreas sections of the normal control group (Gr.1) under the light microscope revealed normal histological structure of the islands of Langerhans as endocrine portion as well as the acini with the duct system as exocrine were recorded in **Fig. 3A**. On the other hand, pancreas sections from HFD/STZ-induced T2D (Gr.2) showed the presence of atrophy in a diffuse manner all over the islands of Langerhans cells **Fig. 3B**, associated with severe congestion in the stromal interlobular blood vessels **Fig. 3C**. The interstitial stroma showed fat vacuoles as well as inflammatory cells infiltration **Fig. 3D**. Pancreas sections from T2DM rats treated with BM-MSCs (Gr.3) showed that the islands of Langerhans cells were nearly intact but smaller in size than the normal control one **Fig. 3E**. In addition, cystic dilatation was detected in the duct system **Fig. 3F**.

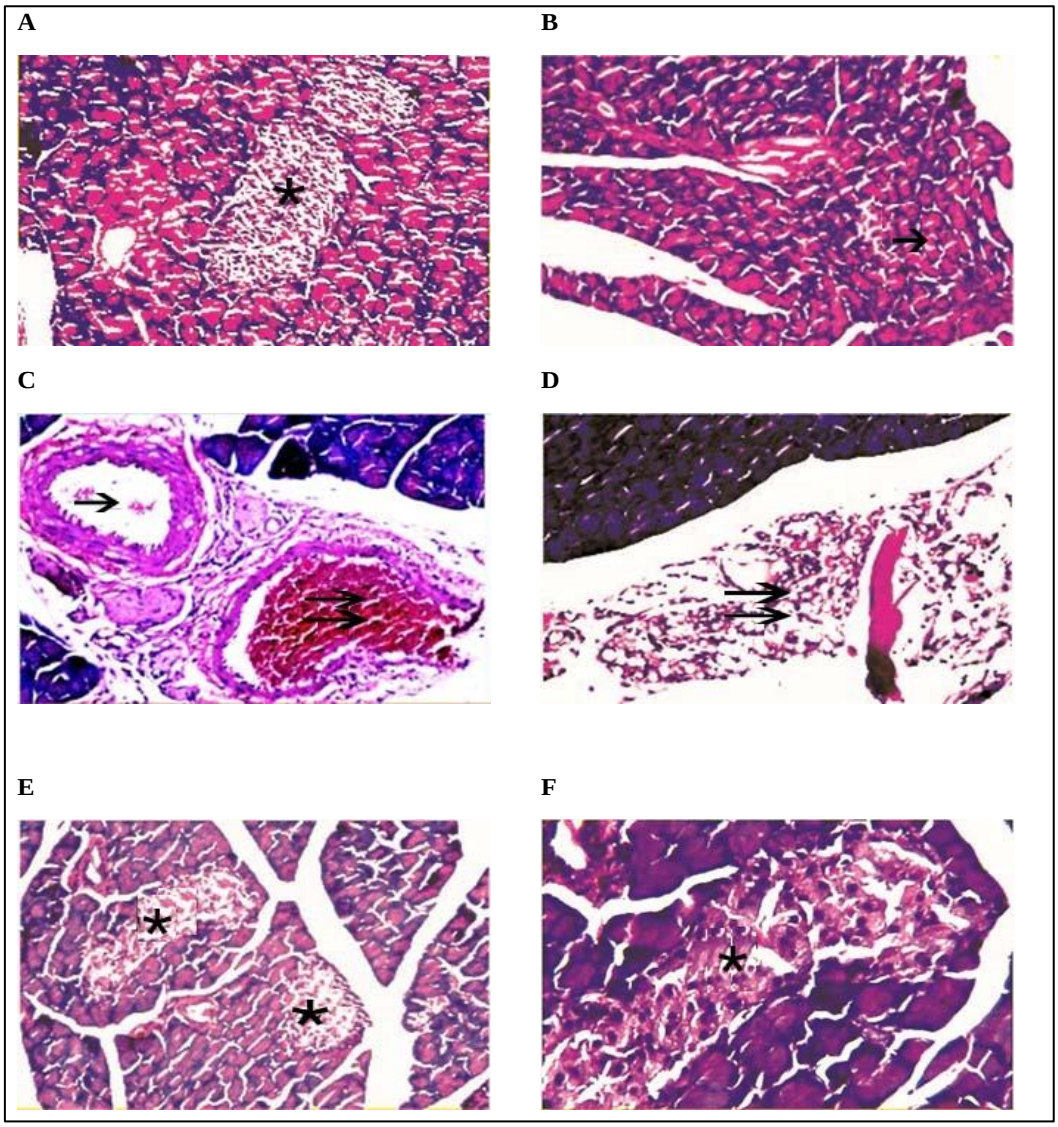

Fig. 3: Photomicrographs of pancreatic sections of rats in normal control (A), T2DM (B-D), and diabetic rats treated with BM-MSCs (E-F). Normal rats showed normal histological structure of the islands of Langerhans (*), while diabetic rats showed atrophy (→) as well as inflammatory cells infiltration (⇉). Diabetic rats treated with BM-MSCs showed nearly intact islands of Langerhans cells (*) but smaller in size than the normal control one. In addition, cystic dilatation was seen in the duct system. [Hematoxylin and Eosin, x400].

Discussion

Strategies to attenuate the peripheral insulin resistance and enhance the regeneration of pancreatic beta cells may be the ideal therapeutic options for T2DM [38]. The reports of Ho *et al.* [39], Hao *et al.* [40] and Hughey *et al.* [41] have illustrated beneficial effects of stem cells transplantation on the metabolic status in both type 1 and 2 diabetic patients and in rodent models of diabetes.

The experimental induction of type 2 diabetes in rats using HFD/STZ model was chosen in the present study. This model was successfully generated and characterized by persistent hyperglycemia, hyperinsulinemia, and insulin resistance which was evidenced from the elevated HOMA-IR index as well as the declined HOMA-β index. These results agree with Duan *et al.* [42] and Tan *et al.* [43]. Experimentally induced Type 2 DM was confirmed in the current research also from the observed histological alterations in the pancreas, including atrophy in the cells of islands of Langerhans, severe congestion in the stromal interlobular blood vessels, and presence of fat vacuoles as well as inflammatory cells infiltration in the interstitial stroma. These findings are in line with Song *et al.* [44]. Single i.v. infusion of BM-MSCs to type 2 diabetic-rats ameliorated hyperglycemia, hyperinsulinemia, and HOMA-IR & HOMA-β indices, which indicates an enhancement in insulin sensitivity and the function of pancreatic β-cells. This is also evidenced from the observed amelioration in the histological alterations induced in pancreas due to STZ administration. These findings are in line with the reports of Hussien *et al.* [45] and Mansor *et al.* [46]. In addition, Mansor *et al.* [46] explained the ameliorated efficacy of MSCs by their ability to possess tissue repair as well as cytoprotective effects possibly *via* their preferential homing property to injured pancreatic tissues with significant islet reconstruction.

The present research reports that feeding rats with HFD produced hyperlipidemia manifested by increasing serum levels of TC, LDL-C, TAG, and VLDL-C as well as decreasing HDL-C level. These results are in line with the previous reports (Soliman [47] and Naidu *et al.* [48]). The most common risk factor of T2DM is obesity [49], which elevates serum triacylglycerol in association with insulin resistance [50]. The observed hyperlipidemia was improved after treatment with BM-MSCs. These results are in agreement with those of Ahmed *et al.* [51], who attributed the improvement of lipid profile by MSCs due to improving β-cell function and decreasing insulin resistance.

Previously it was reported that disturbances in the serum levels of adiponectin and leptin are associated with the etiology of obesity and type 2 diabetes [52,53]. In the current study, along with the significant reduction in serum adiponectin level, a significant elevation in serum leptin level was observed and consequently lowered A/L ratio in the untreated type 2 diabetic-rats. These results are in line with the previous reports of Diwan *et al.* [54] and Sakai *et al.* [55]. Decreased levels of serum adiponectin in type 2 diabetic rats was explained by a down regulation of adiponectin gene expression in adipose tissue due to obesity and insulin resistance [56]. The observed elevation in serum leptin level may be due to leptin resistance since obesity is known to decrease the sensitivity to leptin resulting in an inability to detect satiety despite high energy stores and high levels of leptin [57]. Fortunately, treatment with BM-MSCs improved the serum level of adiponectin and restored a normal serum leptin level causing an improvement in A/L ratio and consequently an increase in insulin sensitivity. These results suggest that BM-MSCs therapy can reverse the leptin resistance. According to previous studies, MSCs are considered the major source of adipocyte generation and are implicated in adipogenesis [58-61] through a range of transcription factors that regulate adipogenesis such as peroxisome proliferator-activated receptor-gamma (PPAR-gamma) and several members of the CCAAT/enhancer-binding proteins (C/EBPs) [62].

It is well known that T2DM can disturb basal and insulin-stimulated glucose metabolism in insulin target tissues, including skeletal muscle and liver [63]. Hepatic glucokinase is a rate-limiting enzyme in glycolysis [11] *via* the phosphorylation of glucose. Glycogen synthase (GS), a crucial and rate-limiting enzyme in glycogen synthesis, catalyzes the conversion of glucose 6 phosphate to uridine diphosphate (UDP)-glucose to elongate glycogen chain through α-1,4-glycosidic bonds [64]. The current study revealed down expressions of GK gene in liver as well as GS gene in both liver and muscles in HFD/STZ-induced T2DM, accompanied by a reduction in their contents in the examined tissues. These results suggest an inhibition in the rate of glycolysis in the hepatic tissues with decreased glucose removal and a defect in glycogen storage capacity, which may be related to the decrease in insulin sensitivity in those rats. The present results are in line with that of Wang *et al.* [65]. The study of Sandu *et al.* [66] reported that HFD has pro-oxidant and pro-inflammatory compounds that have been linked to impaired insulin sensitivity, and therefore decreases insulin-stimulated glycolysis and glycogenesis [67], diminishes GLUT-4 expression [68] and impairs insulin function on glucose transport in skeletal muscle [69]. Treatment of HFD/STZ-induced type 2 diabetic-rats with BM-MSCs has beneficial effects on both enzymes since it upregulated the expression of these genes in the examined tissues accompanied by elevations in their contents compared to the diabetic group suggesting an enhancement in glucose uptake and metabolism. The improvement in the overall glucose homeostasis can be due to BM-MSCs induced increase in the insulin-responsive effects. The regulation of GK expression at the transcription level was reported in the control of hepatic GK activity [70]. Insulin up-regulates the expression of GK through the AKT-signaling pathway [71] and enhances glycogen synthesis by stimulating GS activity [72] *via* dephosphorylation [73].

In diabetes research, signaling by receptor tyrosine kinases of the insulin/IGF family affects pancreatic cell function received a great attention [74]. IGF-1R is a receptor tyrosine kinase which triggers the phosphatidylinositol-3 kinase (PI3k)/AKT signaling pathway [75] to exert several physiological processes including cell growth, proliferation, and survival [76]. The present study demonstrates a significant reduction in the pancreatic IGF-1R gene expression after STZ administration. This result is in line with that of Lai *et al*. [77] and Juárez-Vázquez *et al*. [78] who reported that STZ-induced T2DM reduces significantly the relative IGF-1R gene expression in pancreas, heart, liver, and colon. Meanwhile, treatment of HFD/STZ-induced T2DM with BM-MSCs overexpressed IGF-1R gene in the pancreas. Similarly, the role of MSCs and IGFs has been documented in treating diabetes [79]. The study of Kato *et al.* [80] reported the therapeutic role of BM-MSCs against experimentally induced diabetic foot ulceration *via* improving wound-healing and elevating the expression of IGF-1. Consequently, activation of IGF-1 activates IGF-1R which signals mitogenic, anti-apoptotic, and transforming activities. Therefore, MSCs expressing IGF system components are needed for stimulating tissue repair of the damaged organs and attenuating diabetes [81].

Conclusion

In conclusion, the therapeutic effect of BM-MSCs infusion in HFD/STZ-induced T2DM was verified. Multiple roles and mechanisms were involved including a) improving glucose homeostasis *via* enhancement of glucose kinase and glycogen synthase gene expressions and protein contents, b) initiating pancreatic regeneration *via* insulin-like growth factor-1 receptor, c) improving adiponectin/leptin ratio. These findings established an important foundation for exploring BM-MSCs infusion to enhance insulin sensitivity in T2DM therapy.

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Declaration and conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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