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ORIGINAL RESEARCH PAPER

The effect of exosomes derived from mesenchymal stem cells in the treatment of induced type 1 diabetes mellitus in rats

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Abstract
Aim The aim of the current study was to evaluate the therapeutic and regenerative effects of MSCs derived exosomes in the treatment of type 1 DM and to compare its effects with MSCs themselves. The experiment was done on forty albino rats grouped as follows, group (1): Ten healthy rats, group (2): Ten induced type 1 DM rats, group (3): Ten induced type 1 DM rats received exosomes intraperitoneally, and group (4): Ten induced type 1 DM rats received MSCs intraperitoneally. Serum glucose and plasma insulin levels were assessed weekly. QRT-PCR was done to assess regeneration of pancreatic beta cells by measuring *insulin, Pdx1, Smad2, Smad3 and TGFβ* genes. Additionally, histopathological and immune-histochemical examinations were done to confirm pancreatic tissue regeneration.
Results Regarding the assessed genes (insulin, *Pdx1, Smad2, Smad3 and Tgfβ*) gene expression in MSCs treated group showed significant increase compared to diabetic group (*p value* < 0.001) and gene expression in exosomes treated group was increased significantly compared to diabetic and MSCs treated groups (*p value* < 0.001). Histopathological and immune-histochemical examination revealed regeneration of pancreatic islets in both treated groups.
Conclusion MSCs Derived exosomes showed superior therapeutic and regenerative results than MSCs themselves

Keyword Type 1 DM · MSCs · Exosomes · Insulin · *Pdx1* · *Smad2* · *Smad3* · *Tgfβ*

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Introduction

Type 1 diabetes mellitus is caused by chronic insulin deficiency resulting from destruction of pancreatic islets beta cells by the immune system. The long term macrovascular and microvascular complications can be destructive. The long term survival for patients with the disease has been improved since insulin discovery about 100 years ago. Each year trials to discover a cure are carried out but much work is still required to eliminate the disease [1]. Mesenchymal stem cells (MSCs) are multipotent cells capable of differentiating into mature cells of several mesenchymal tissues. MSCs are one of the most commonly researched stem cells due to their ability to differentiate into mesoderm and non-mesoderm derived tissues, their immunomodulatory effects, their availability and their role in maintaining and replenishing endogenous stem cell niches [2]. The minor MSCs engraftment rate

observed in damaged tissues strengthens the postulation that MSCs achieve their curative role by replacing damaged cells with newly differentiated cells. Thus, it was postulated that the roles achieved after MSCs use in different animal models of experimental tissue injury may be achieved by the soluble factors secretion acting in a paracrine manner [3]. Over the past decades, the theory that extracellular vesicles, microvesicles and exosomes work as cellular housekeepers and as factors for communication among and between cells and tissues, had been one of the most hopeful areas in the biomedical field. Many researches are performed to test the use of extracellular vesicles especially exosomes as therapeutic agents. However, their exact way of biogenesis and function in cellular and tissue homeostasis is still undiscovered [4]. The extracellular vesicles are of different size and can be grouped into two main sub types: microvesicles (100–1000 nm) and exosomes (20–200 nm), that are present in most body fluids including blood, breast milk, semen and saliva [5].

The current study aimed to evaluate and compare the therapeutic effects of MSCs derived exosomes in the treatment of type 1 diabetes mellitus with the effects of MSCs themselves.

Materials and methods

Diabetic rat model preparation

The present study included forty albino female rats inbred strain (Cux1: HEL1) of matched age and weight (4–6 months and 120–150 gm respectively). Rats were inbred in the Experimental Animal Unit, Faculty of Medicine, Cairo University.

Rats were kept as stated by the standard guidelines of the Institutional Animal Care and Use Committee (IACUC) and all animal experiments were given approval from the Institutional Review Board (approval number: CU III F 6418).

Animals were provided a semi-purified diet containing (gm/kg): 200 gm/kg casein, 100 gm/kg cellulose, 555sucrose gm/kg, 100 gm/kg fat blends, 35 gm/kg mineral mix and 35 gm/kg vitamin mix.

Experimental design

Forty rats were divided into four groups as illustrated in the flow chart (Fig. 1).

- *Group* (1) Ten healthy white albino rats as negative control rats received standard diet i.e. normal control.
- *Group* (2) Ten white albino rats that received streptozotocin (STZ) in a dose of 50 mg/Kg once intraperitoneally to induce type1 diabetes mellitus as positive control [6].
- *Group* (3) Ten white albino experimentally induced type 1 diabetic rats received MSCs intraperitoneally once in a dose of 10^7 cells per rat [7].
- *Group* (4) Ten white albino experimentally induced type 1 diabetic rats received exosomes derived from MSCs injected with four prepared doses (once per week) intraperitoneally at a dose of 200 μg/mL in a fresh or a frozen pellet [8, 9].

Preparation of bone marrow derived mesenchymal stem cells (BM-MSCs)

Rat bone marrow was used for separation of mononuclear cells (MNCs). Short, low speed centrifugation of a two-phase system (Ficoll solution and blood) (Gibco-Invitrogen, Grand Island, NY) was used [10]. We flushed the BM cells from tibia of white albino rat bones with Phosphate-Buffered Saline (PBS). 15 mL of the flushed bone marrow cells was layered on 15 mL Ficoll-Paque (Gibco-Invitrogen, Grand Island, NY), then centrifugation was done for them for 35 min. at $400\times g$ rpm. Aspirated of the upper layer was done leaving the MNC layer undisturbed at the interface. The interface layer (MNC layer) was carefully aspirated and then washing was done twice in PBS which contains 2 mM ethylene diamine tetra acetic acid (EDTA) and then centrifugation for 10 min was done at $200\times g$ rpm at 5 °C. The cell pellet was resuspended in a final volume of 300 μL of PBS. Isolated BM-MSCs were cultured and propagated on 25 mL culture flasks in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 10% Fetal Bovine Serum (FBS), 0.5% penicillin, streptomycin and incubated at 37 °C and 5% CO_2 for 12–14 days. Changing the media was done every 2–3 days. Cultured MSCs were characterized by

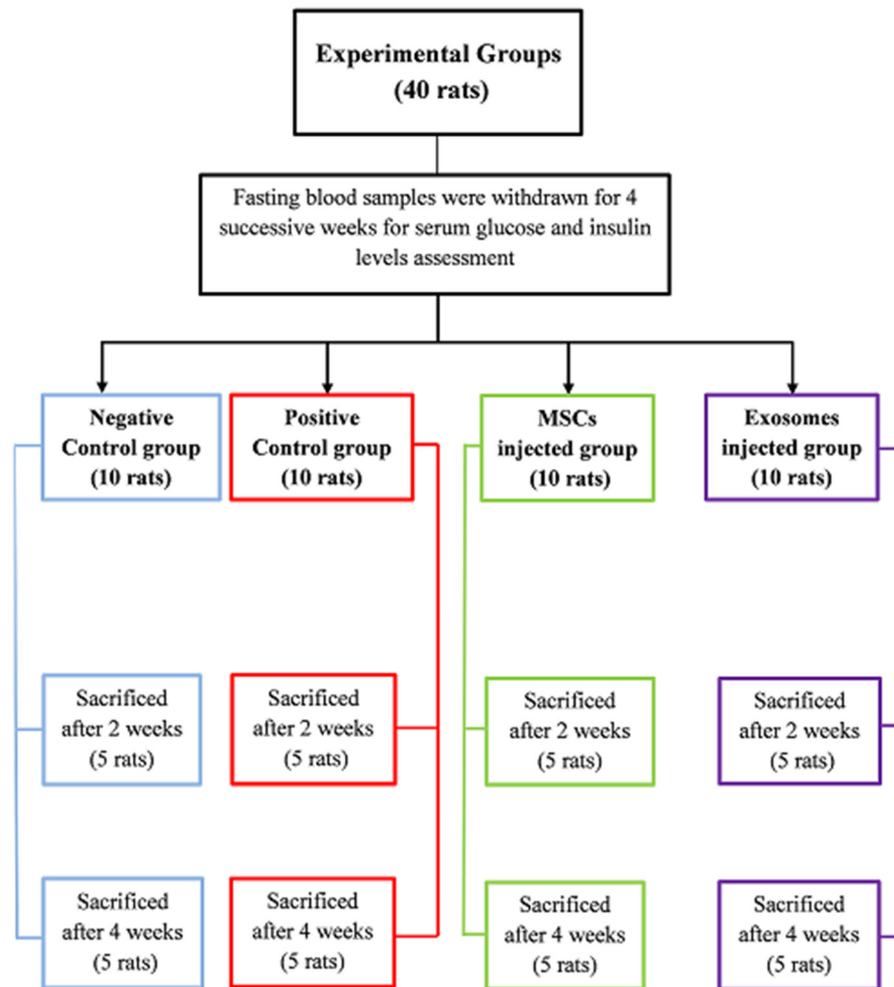

Fig. 1 Flow chart showing the sacrificing schedule of all groups

morphology, adherence and FACS (fluorescence activated cell sorting) by assessment positivity of CD29 and CD 105 and negativity of CD34 specific to MSCs. When large colonies developed, cultures were washed twice with PBS and cells were trypsinized with 0.25% trypsin in 1 mM EDTA to obtain a second passage.

Preparation of exosomes derived from BM-MSCs

Exosomes were obtained from supernatants of MSCs cultured overnight in RPMI without FBS. To obtain exosomes, after differential centrifugation at 3000×*g* to remove cells then 10,000×*g* for 20 min to eliminate the debris, centrifugation was done to cell-free supernatants at 100,000×*g* (Beckman Coulter Optima L-90 K ultracentrifuge) for one hour at 4 °C, washed in 25 mM of serum-free medium 199 containing N-2-Hydroxy Ethyl Piperazine-N'-2-Ethane Sulfonic acid (HEPES) (Sigma) and then a second ultracentrifugation in the same conditions was preformed [11]. Exosomes were characterized by transmission electron microscopy (TEM). Exosomes were fixed with 2.5% glutaraldehyde for 2 h, after being washed; exosomes were ultra-centrifuged and suspended in 100 µL human serum albumin (HSA). A total of 20 µL of exosomes was loaded onto a formvar/carbon-coated grid, negatively stained with 3% aqueous phosphor-tungstic acid for one minute and observed by TEM. Exosomes pellet was re-suspended with 100 µL distilled water and diluted pellets were stored at (− 80 °C) till the intended time of their injection. The protein content of exosomes

was quantified by Bradford method (BioRad, Hercules, CA, USA).

Estimation of serum glucose level

We measured plasma glucose by glucose Liquizyme GOD—PAP (Single Reagent) kit (spectrum Cat no # 250 001).

Assessment of plasma insulin level by ELISA

The kit used was supplied by Chongqing biospes co., Ltd., for quantitative detection of Insulin in Rat serum, plasma, body fluids and tissue lysates. Catalog No.: BEK1243.

Quantitative reverse transcriptase—polymerase chain reaction (qRT-PCR)

Total RNA extraction was performed using the kit supplied by Thermo Fisher Scientific Inc. Germany, (GeneJET, Kit, #K0731).

Quantitative real time—PCR kit was provided by Vivantis, ViPrimePLUS One Step Taq RT-qPCR (reverse transcription-quantitative polymerase chain reaction) Green Master Mix I with ROX (SYBR Green Dye) (cat no #QLMM14-R). The prepared reaction mix samples were applied in real time PCR (StepOne Applied Biosystem, Foster city, USA). ViPrimePLUS One Step Taq RT-qPCR Green Master Mix I with ROX Kit was compatible with three-step cycling, where reverse transcription occurs at 55 °C for 10 min as one cycle, enzyme activation at 95 °C for 8 min as one cycle, denaturation occurs at 95 °C for 10 s and annealing and extension at 60 °C for 60 s for forty cycles. After the RT-PCR run the data were expressed in Cycle threshold (Ct). The RQ of each target gene is quantified by normalization against house-keeping gene according to the calculation of delta-delta Ct (ΔΔCt). The primer sequence of all studied genes was presented in (Table 1).

Western blot

Tissues were lysed in RIPA buffer supplied by Bio BASIC INC. (Marhham Ontario L3R 8T4 Canada). The lysates were boiled for 5 min with lamellae buffer, and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate the proteins and transferred to an Immobilot membrane (Millipore). Antigen affinity-purified monoclonal mouse insulin Antibody was used (2D11-H5: sc-8033, Santa Cruz Biotechnology, Inc.). After incubation in 5% non-fat dry milk, Tris–HCL, 0.1% Tween 20 for one-hour, monoclonal antibody was added to one of the membranes containing specimen samples and then incubation was done at 4 °C overnight. Secondary antibodies were incubated for 2 h at room temperature. Washing was done twice in 1 × TBS-T, then densitometric analysis of the immunoblots was performed to quantify the amounts of insulin against β-actin by normalization using Image analysis software on the ChemiDoc MP imaging system (version 3) produced by Bio-Rad (Hercules, CA).

Table 1 Primers sequence of all the studied genes

Gene	Primer sequence (5′-3′) (F: Forward primer), (R: Reverse primer)
Insulin	F: GGGAGCGTGGATTCTTCTAC
	R: CAGTGCCAAGGTCTGAAGGT
	(#Accession number: AH002190.2)
Pdx1	F: GGATGAAATCCACCAAAGCTC
	R: TTCCACTTCATGCGACGGT
	(#Accession number: NM_022852.3)
Smad 2	F: GCCCCAACTGTAACCAGAGA
	R: GCCAGAAGAGCAGCAAATTC
	(#Accession number: XM_006254945.3)
Smad 3	F: GGCTTTGAGGCTGTCTACCA
	R: GGTGCTGGTCACTGTCTGTC
	(#Accession number: XM_008766216.2)
Tgf β	F: TGGGGCAGCTGTACATTGAC
	R: GGCAGAAGTTGGCGTGGTA
	(#Accession number: NM_021578.2)
Gapdh	F: ATGGTGAAGGTCGGTGTGAACG
	R: TGGTGAAGACGCCAGTAGACTC
	(#Accession number: XM_017592435.10)

Histopathological examination of the pancreas

Pancreatic tissue was collected into PBS and fixed overnight in 40 g/L paraformaldehyde in PBS at 4 °C. Hematoxylin and Eosin (H&E) was used to stain serial sections of the pancreas and then histopathological examination was done for them and unstained sections

were examined by immunohistochemistry by anti-insulin antibody marker.

Statistical analysis

Data was summarized using mean and standard deviation. Comparisons between groups were performed using analysis of variance (ANOVA) with multiple comparisons post hoc test when comparing more than two groups. Comparisons between the two durations were done using paired *t* test [12]. p values less than 0.05 were considered as statistically significant.

Results

MSCs and exosomes characterization

MSCs were identified by morphology where MSCs were characterized by their fibroblast spindle shape and colony forming unit (Fig. 2a–e). Phenotypic FACS analysis MSCs were positive for surface marker CD29 (99.9%) and CD105 (90.38%), but negative for CD34 (0.2%) (Fig. 2f). Exosomes were identified by TEM according to their cup shaped spheroidal morphology and by their size (100 nm) (Fig. 3).

Glucose and insulin level

Concerning serum glucose levels there was significant increase in serum glucose levels in diabetic compared to normal group throughout the 4 weeks (p $value < 0.001$). There was significant decrease in serum glucose levels in MSCs treated group compared to diabetic group at three and 4 weeks (p $value < 0.001$). There was significant decrease in serum glucose levels in exosomes treated group compared to diabetic and MSCs groups at 2 weeks and significant decrease compared to diabetic group only at 3 and 4 weeks (p $value < 0.001$) (Fig. 4a). Concerning plasma insulin levels there was significant decrease in plasma insulin levels in diabetic group throughout the 4 weeks (p $value < 0.001$). There was significant decrease in plasma insulin levels in MSCs treated group compared to normal group throughout the 4 weeks with significant increase compared to diabetic group at 3 and 4 weeks (p $value < 0.001$). There was significant decrease in plasma insulin levels in exosomes treated groups compared to normal group (p $value < 0.001$) and significant increase in plasma insulin levels in exosomes treated group compared to diabetic and MSCs groups (p $value < 0.001$) at 2 weeks with significant increase in exosomes treated group compared to diabetic group (p $value < 0.001$) but no significant difference compared to normal group at 3 and 4 weeks (Fig. 4b).

Genes expression

Concerning the genes that indicate cell proliferation and regeneration after their destruction by STZ, there was significant decrease in *Tgfß, Smad2 and Smad3* genes expression (Fig. 5a–c respectively) in diabetic groups compared to normal group at 2 and 4 weeks *(*p $value < 0.001$*)*. There was significant decrease in *Tgfß, Smad2 and Smad3* genes expression in MSCs treated groups compared to normal group at 2 weeks *(*p $value < 0.001$*)* but no significant difference between MSCs treated groups and normal group at 4 weeks *(*p $value > 0.05$*)*.There was significant increase in *Tgfß, Smad2 and Smad3* genes expression in MSCs treated group compared to diabetic group at 4 weeks *(*p $value < 0.001$*)*. There was no significant difference between exosomes treated group and normal group at 2 weeks *(*p $value = 0.9, 0.72, 0.9$ *respectively)* or 4 weeks *(*p $value > 0.05, = 1, = 1$ *respectively)*. But there was significant increase in exosomes treated group compared to diabetic and MSCs treated groups at 2 weeks and 4 weeks for *Tgfß (*p $value < 0.001, 0.04$ *respectively), Smad2 (*p $value < 0.001, 0.012$ *respectively) and Smad3 (*p $value < 0.001, 0.04$ *respectively)* genes expression.

Concerning genes indicating insulin synthesis and secretion by beta cells (differentiation of the regenerated cells into insulin producing cells), there was significant decrease in insulin and *Pdx1* genes expression in diabetic group compared to normal group (p $value < 0.001$) at 2 and 4 weeks. There was significant decrease in insulin and *Pdx1* genes expression in MSCs treated group compared to normal group at 2 weeks (p $value < 0.001$) and 4 weeks (p $value < 0.017$). There was significant increase in insulin and *Pdx1* genes expression in MSCs treated group compared to diabetic group (p $value = 0.004, < 0.001$ *respectively*) at 4 weeks. There was no significant difference between exosomes treated group and normal group at 2 weeks (p $value = 0.71, = 0.94$

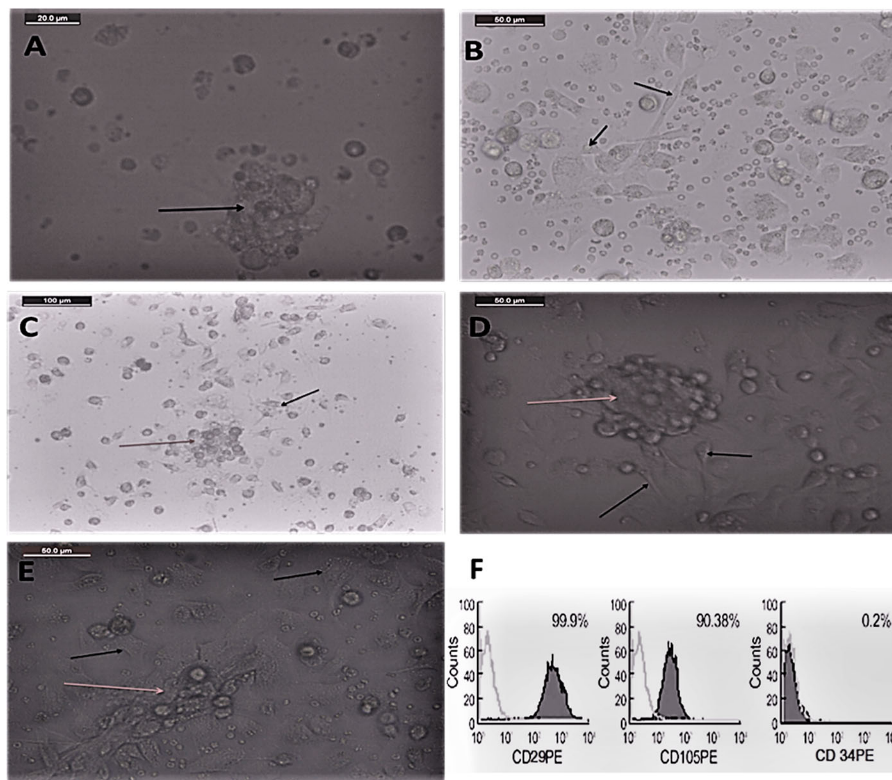

Fig. 2 MSCs in culture. **a** MSCs at 0 day of isolation have round shape (black arrow). **b** MSCs at day 2 they started taking the spindle shape (black arrows) with about 20% confluence of cells. **c** MSCs after 1 week showed colonies forming unit (CFU) (red arrow) and spindle shaped cells (black arrow) with about 50% confluence. **d** MSCs after 10 days represented CFU (red arrow) and spindle shaped cells (black arrows) with about 60% confluence. **e** MSCs after 14 days formed CFU (red arrow) and spindle shaped cells (black arrows) with about 70–80% confluence. **f** Flow cytometric analysis of cultured MSCs (They were positive for CD29 and CD105 but negative for CD34)

respectively) or 4 weeks (*p value = 0.1, = 1 respectively*). In exosomes treated group compared to diabetic and MSCs treated groups there was significant increase in insulin (*p value* < 0.001, *0.016 respectively*) and *Pdx1* (*p value* < 0.001, *0.02 respectively*) at 2 and 4 weeks (Fig. 5d, e).

Histological assessment

Compared to the control and STZ induced diabetic rats, H&E stained pancreatic tissues of the rats treated with exosomes showed marked improvement while those treated with MSCs showed moderate improvement as showed at Table 2 and Fig. 6.

Western blot

Concerning western blotting results of insulin protein, in diabetic group there was significant decrease in insulin levels compared to normal group at 2 and 4 weeks (*p value* < 0.001). There was significant decrease in insulin protein expression in MSCs treated groups compared to normal group at 2 and 4 weeks (*p value* < 0.001, *0.019 respectively*) with significant increase in insulin protein expression in MSCs treated group compared to diabetic group at 4 weeks (*p value = 0.008*). There was significant decrease in insulin protein expression in exosomes treated group compared to normal group at 2 weeks (*p value* < 0.005) but no significant difference at 4 weeks (*p value = 0.1*) with significant increase in insulin protein expression in exosomes treated group compared to diabetic group at 2 and 4 weeks (*p value = 0.036,*

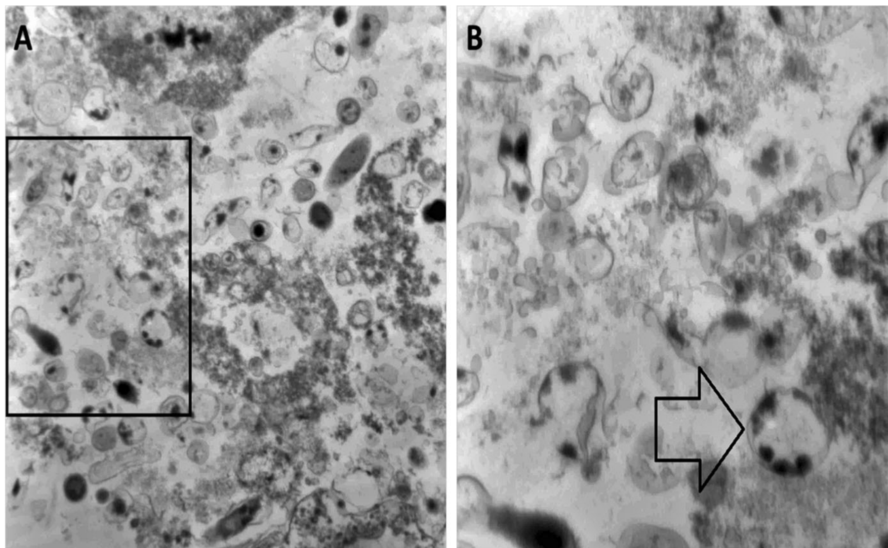

Fig. 3 **a** Exosomes showed their cup shaped morphology and their size (100 nm). **b** Black arrow is the magnification of the marked rectangular area in picture A)

< 0.001 *respectively*) with no significant difference between MSCs treated group at 4 weeks (*p value* = *0.95 respectively*) (Fig. 7).

Immunohistochemistry

Concerning insulin immunohistochemistry staining of pancreatic tissues there was significant decrease in insulin protein in diabetic group compared to normal group at 2 and 4 weeks (*p value* < 0.001). There was no significant increase in insulin in MSCs treated group compared to diabetic group at 2 weeks (*p value* = *0.99*) but with significant increase at 4 weeks (*p value* < 0.001). There was significant increase in insulin exosomes treated groups compared to diabetic group and MSCs treated groups at 2 and 4 weeks (*p value* < 0.001) (Fig. 8).

Discussion

Our research concluded that both MSCs derived exosomes and MSCs themselves have therapeutic effects when injected in STZ induced diabetic rats with superior and more rapid effect regarding exosomes. The treated groups showed lowering in blood glucose levels together with elevation of plasma insulin levels indicating regeneration of pancreatic beta cells with re-production of insulin hormone. Type 1 DM is a type of diabetes in which beta cells of the pancreas are destroyed by the immune system. This leads to noticeable decrease in insulin level accompanied by marked hyperglycemia [13]. MSCs have been thought of as a hopeful tool for the cure of type 1 DM in the last few years, because they have the capability to differentiate into glucose responsive insulin producing cells. The MSCs regenerative ability can be achieved by the release of certain paracrine factors, which includes extracellular vesicles (EVs), formed mostly of microvesicles and exosomes. MSCs derived exosomes have genetic and protein material that when transferred to recipient cells can initiate the activation of various repair mechanisms [14]. Exosomes have huge potentials in tissue regeneration and injury repair as MSCs [15]. To our own knowledge, this is considered the first research applied to assess and compare the efficacy of two types of cell based therapy "MSCs derived exosomes and MSCs themselves" in the treatment of type 1 DM. In the current study, cultured MSCs derived from bone marrow were characterized by being positive for surface marker CD29 (99.9%) and CD105 (90.38%) and negative for CD34 (0.2%). This agreed with Kobolak et al. [16] results who reported in their study that MSCs were characterized by their morphology as well as by being positive for CD29 and CD105 but negative for CD34.

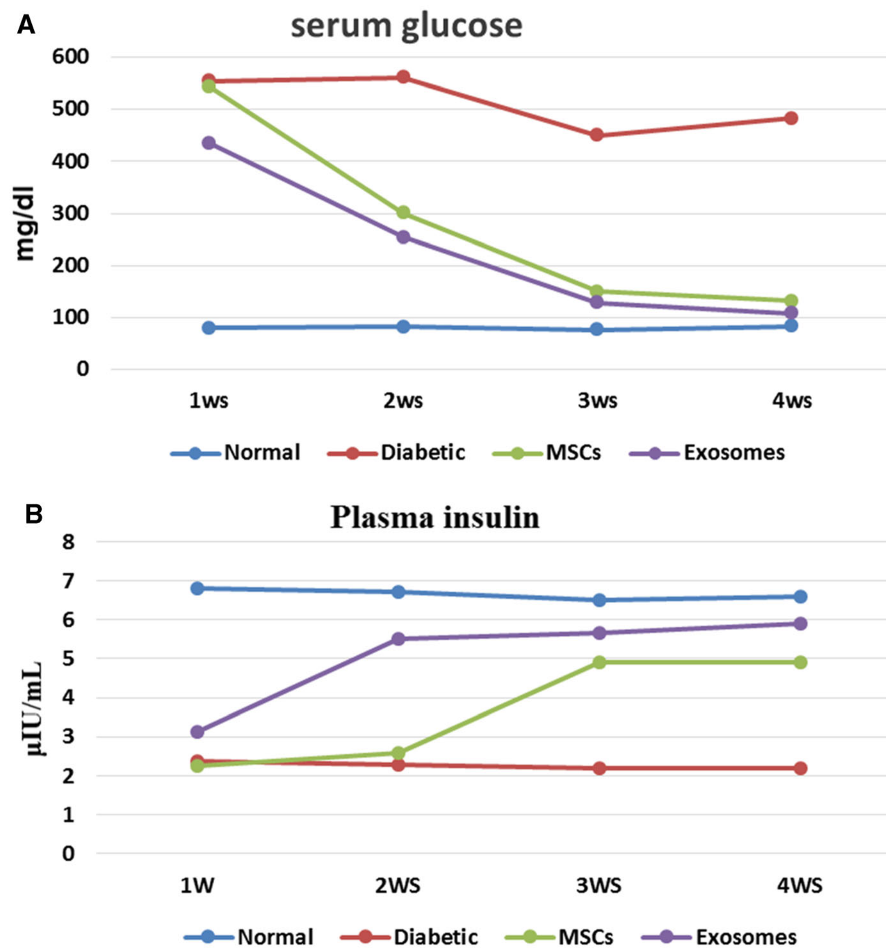

Fig. 4 Serum glucose (**a**) and plasma insulin (**b**) mean levels among different studied groups all through the experiment. **a** Normal group showed no significant difference between all durations (*p value* > *0.05*). Diabetic group showed no significant difference between all durations (*p value* > *0.05*). MSCs group showed significant decrease in serum glucose levels till 3 weeks (*p value* < *0.001*) but no significant difference between 3 and 4 weeks durations (*p value* > *0.05*). Exosomes group showed significant decrease in serum glucose levels till 3 weeks (*p value* < *0.001*) but no significant difference between 3 and 4 weeks durations (*p value* > *0.05*). **b** Normal group showed no significant difference between all durations (*p value* > *0.05*). Diabetic group showed no significant difference between all durations (*p value* > *0.05*). MSCs group showed significant increase in plasma insulin levels after 3 and 4 weeks compared to their levels after 1 and 2 weeks (*p value* < *0.001*). Exosomes group showed significant increase in plasma insulin levels after 2, 3 and 4 weeks compared to their levels after 1 week (*p value* < *0.001*), but no significant difference between their levels after 2, 3- or 4-weeks durations (*p value* > *0.05*)

Also, in the present study, exosomes derived from MSCs were identified by transmission electron microscopy (TEM) confirming their size being 100 nm and their spheroidal cup shaped morphology. In consistence with these results a study carried out by Torre Gomez et al. [17] reported that exosomes were characterized by their size (50-200 nm) and spheroidal cup shaped morphology by TEM examination. In a study carried out by Barky et al. [18] type 1 DM was proved by obtaining fasting blood glucose levels above 200 mg/dL, as well as insulin levels below 3 µIU/mL. Moreover DM was established by the presence of both fibrosis as well as marked decrease in size and number of islets of Langerhans by histopathological examination. That was matching with our work where experimentally induced type 1 DM rats having blood glucose levels above 200 mg/dL with insulin levels below 3 µIU/mL were considered diabetic. Histopathological examination showed results similar to the previous study results. Concerning serum

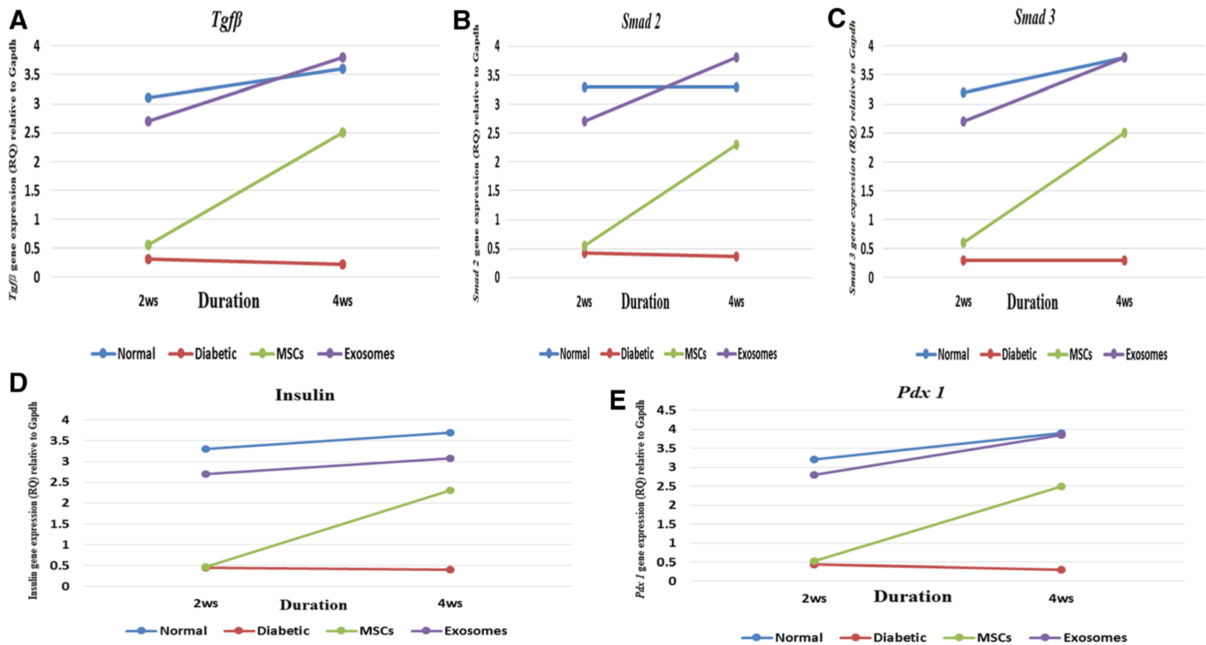

Fig. 5 quantitative expression means levels of all five studied genes *(A: Tgfβ, B: Smad2, C: Smad3, D: Insulin and E: Pdx1)* among different studied groups all through the experiment. For all the studied groups; normal group showed no significant difference between the two durations, diabetic group showed no significant difference between the two durations, MSCs group represented significant increase in genes expression in 4 weeks duration compared to the 2 weeks duration and exosomes group represented no significant difference between the two sacrifice durations

Table 2 H and E histopathological findings in pancreatic tissues of rats

Islets of Langerhans	Signs of inflammation	Vacuolar degeneration	Congestion	Fibrosis	Size of islets	Number of islets/field (X40)
Normal pancreas	–	–	–	–	Normal	10–14
STZ diabetic rats	+ +	+ +	+ +	+ + +	Marked decrease	2–3
MSCs-treated rats (sacrificed after 2 weeks)	+	+ +	+ +	+ +	Marked decrease	2–3
MSCs-treated rats (sacrificed after 4 weeks)	–	–	–	+	Variable	5–9
Exosomes-treated rats (sacrificed after 2 weeks)	–	–	–	–	Variable	6–8
Exosomes-treated rats (sacrificed after 4 weeks)	–	–	–	–	Increase	9–14

glucose and plasma insulin levels together with the histopathological and immunohistochemistry analysis in MSCs and exosomes treated groups, the present study showed a significant decrease in blood glucose levels with significant increase in plasma insulin levels throughout the 4 weeks. Histopathological examination also revealed islet cells regeneration with increase in the number and size of islets of Langerhans with decrease in both fibrosis and inflammation denoting their improvement. Yet the results obtained by exosomes were superior to those obtained by MSCs. These results agreed with Carlsson et al. [19] who studied the effect of MSCs in patients who were recently diagnosed to be type 1 DM and the study

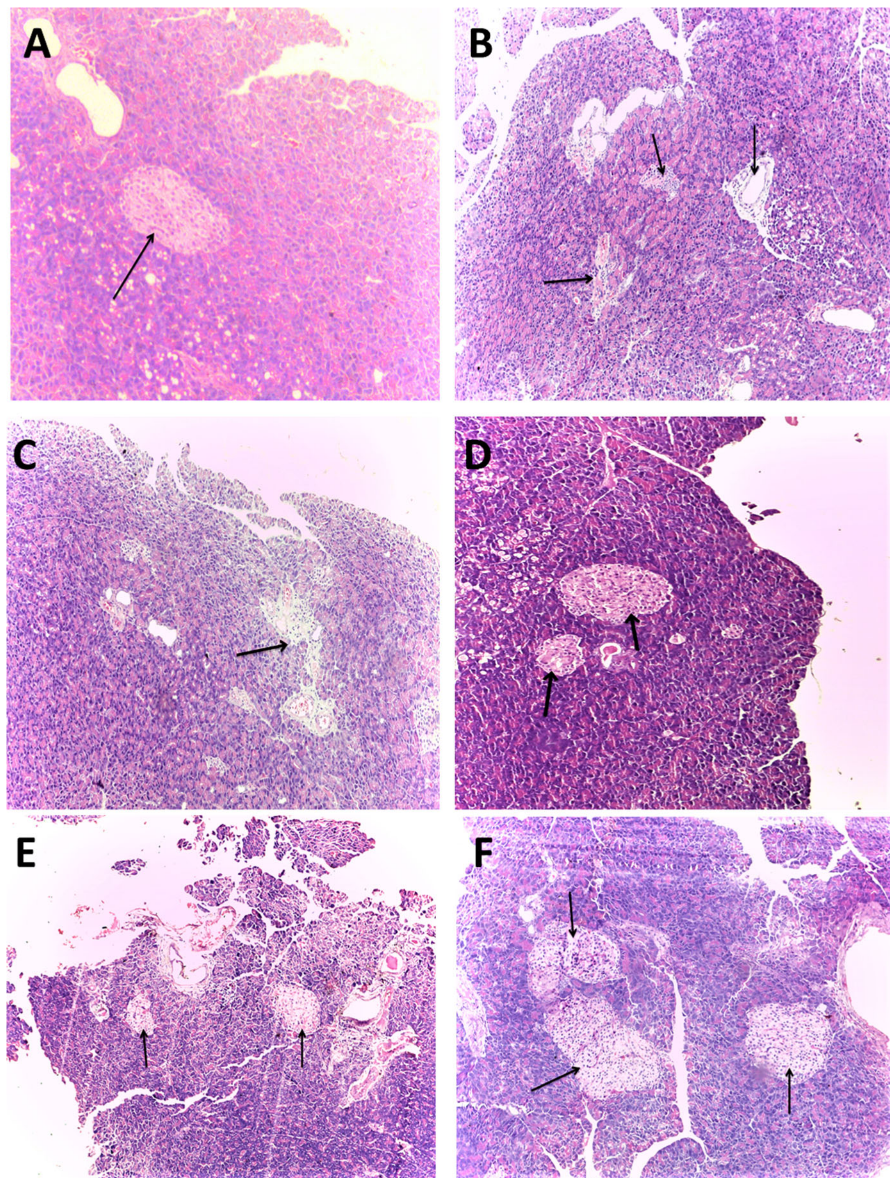

Fig. 6 Hematoxylin and Eosin staining of pancreatic islets with black arrows pointing to the islets of Langerhans. **a** Normal pancreatic islet (X100). **b** Fibrosed pancreatic islet of STZ diabetic rat (X100). **c** Fibrosed pancreatic islet showing minimal improvement of rat receiving MSCs and sacrificed after 2 weeks (X100). **d** Improved pancreatic islet showing moderate improvement of rat receiving MSCs and sacrificed after 4 weeks (X100). **e** Improved pancreatic islet showing moderate improvement of rat receiving exosomes and sacrificed after 2 weeks (X100). **f** Improved pancreatic islet showing marked improvement of rat receiving exosomes and sacrificed after 4 weeks (X100)

revealed that groups which received MSCs as a treatment had a decrease in plasma glucose levels together with rise in both insulin and C-peptide levels. Agreeing with the current study results, Sun et al. [20] found that exosomes derived from human umbilical cord MSCs intravenous injection into STZ-induced DM rat model, lead to the reduction of blood glucose level as a chief paracrine approach of MSCs. Also, an increase in the glycogen storage in the liver was detected to maintain glucose homeostasis. Exosomes derived from human umbilical cord MSCs can inhibit STZ induced βcell apoptosis to re-establish the insulin

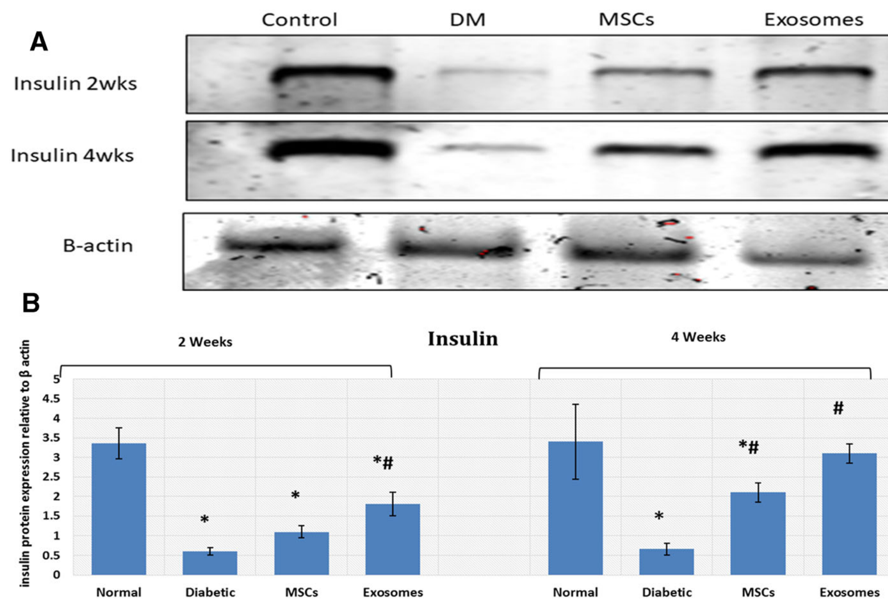

Fig. 7 Western blot of insulin protein versus β-actin in pancreatic tissues. **a** Scanning blot of insulin in different studied groups versus the two durations (2 and 4 weeks). **b** Significant quantitative analysis of insulin between different studied groups in two durations. (*)Significant difference versus normal group. (#)Significant difference versus diabetic group

secreting function of pancreatic beta cells. Rani et al. [21] stated that exosomes can be used as a new alternative to whole cell therapies. In comparison with their parent cells, exosomes may possess a better safety profile. Moreover, exosomes can be stored in a safe manner without affecting its function. The current study also assessed the degree of pancreatic islets of Langerhans regeneration, after injection of MSCs or exosomes, by qRT-PCR for insulin, *Pdx1, Smad2, Smad3* and *Tgf-β* genes in pancreatic tissue. The results showed significant up regulation of the expression of all five assessed genes. This up regulation was more in exosome treated group than MSCs treated group. On the other hand, the untreated group showed significant down regulation of the expression of all five genes. MSCs acted through the activation of *Tgf-β* as well as *Smad* family genes [22].The superfamily members of *Tgf-β* are responsible for the regulation of the establishment of tissue differentiation by affecting cell proliferation, differentiation, development, growth and migration. The action of the *Tgf-β* family is obtained through a combinatory set of ligands and receptors such as the SMAD transcription factors; the most crucial are SMAD2 and SMAD3. They function as transcription factors mediating the signal transduction pathway of the *Tgf-β* superfamily, this leads to the regulation of cell growth, differentiation and development. As a result of this new beta cells are formed with expression of insulin as well as *Pdx1* genes [23, 24, 25]. Nojehdehi et al. [26] performed an experimental study on STZ induced type 1 DM mouse model with injections of exosomes derived from adipose tissue derived MSCs. Blood glucose, plasma insulin and *Tgfβ* expression levels were measured. The treated group showed a significant increase in the *Tgfβ* gene expression levels, a significant increase in plasma insulin levels and a significant decrease in blood glucose levels compared to untreated group. There was also significant regeneration of pancreatic islets proved by H&E staining and immunohistochemistry analyses. *Pdx1* is considered one of the transcription factors implicated in pancreatic organogenesis and has a pivotal role in early lineage marker of pancreatic specification in addition to differentiation of β cells. In mature pancreas, *Pdx1* controls plenty of genes contributing in the maintenance β cell identity and function. PDX1 attaches firmly to the insulin gene promoter causing up regulation of its expression. *Pdx1* expression obviously supports the production of insulin by beta cells. Moreover, it plays an important role in beta-cell differentiation as well as survival [27]. Regarding the expression of insulin and *Pdx1* genes by

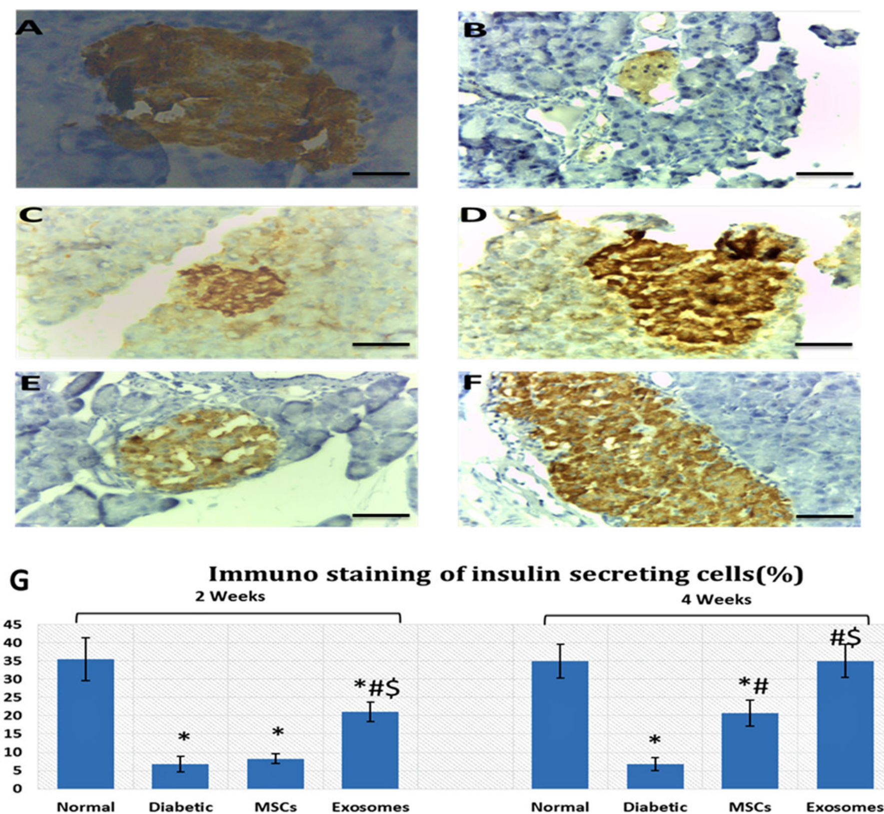

Fig. 8 Immunohistochemistry staining of pancreatic islets by anti-insulin antibody. **a** It showed normal pancreatic islet (X400). **b** It showed STZ diabetic rat with low staining intensity and small sized islet (X400). **c** It showed pancreatic islet of rat treated by MSCs and sacrificed after 2 weeks represented small intermediate stained islet (X400). **d** It showed pancreatic islet of rat treated by MSCs and sacrificed after 4 weeks represented with increased number and sized islet and intense cytoplasmic staining (X400). **e** It showed pancreatic islet of rat treated by exosomes and sacrificed after 2 weeks showed moderate cytoplasmic staining with increase in the number of the islet (X400). **f** It showed pancreatic islet of rat treated by exosomes and sacrificed after 4 weeks represented strong intensity of cytoplasmic staining with marked improvement in size of the islet (X400). (Scale bare was denoted by). Significant quantitative analysis of insulin between different studied groups in two durations. (*)Significant difference versus normal group. (#) Significant difference versus diabetic group. ($)Significant difference versus MSCs group

quantitative RT-PCR, the current study showed significant down regulation of the expression of both genes in diabetic untreated group, and significant up regulation of the expression of the same genes in the two treated groups, confirming beta cell regeneration, which was more evident in the group treated by exosomes proving more islet cell regeneration and differentiation than the MSCs treated group. These results agreed with Fujimoto and Polonsky [28] results who stated that the expression levels of both *Pdx1* as well as insulin were decreased in many animal models with induced diabetes and explained the decrease in *Pdx1* and insulin expression as a result of diabetes. They also reported that the significance of *Pdx1* in the pancreas is emphasized by the development of pancreatic agenesis in *Pdx1* null mice. Their study concluded that *Pdx1* has a vital role in β cell survival and glucose homeostasis, not only for being the main regulator of insulin gene expression, but it is also needed for the normal development of the pancreas most likely by influencing the differentiation and maturation of common pancreatic precursor cells in the developing gut. Rhee et al. [29] stated that the pre-adipocyte factor 1 contributes in not only the proliferation but also in the differentiation of numerous precursor cells. The pre- adipocyte factor 1 leads to the activation of signaling pathway, which induces the transcription of *Pdx1* gene and leads to the

differentiation of human pancreatic ductal cells into β like cells and an increase in insulin synthesis with increase in insulin gene expression, which demonstrates that *Pdx1* gene expression is necessary for beta cell differentiation, maturation and insulin hormone production. The present study provided promising results for the use of exosomes-cell free therapy instead of MSCs; being more safe, rapid and easier to inject with more efficient results than the cells themselves.

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Compliance with ethical standards

Conflict of interest All authors declare that no conflict of interest.

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