ROLE OF ADIPOSE DERIVED MESENCHYMAL STEM CELLS IN IMPROVING STREPTOZOTOCIN-INDUCED DIABETES MELLITUS IN RATS

By

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ABSTRACT

Background: Diabetes mellitus (DM) is one of the most recognizable endocrine metabolic disorders characterized by chronic hyperglycemia. Mesenchymal stem cells (MSCs) are promising tool to treat DM type I (DM 1). They can be isolated from many sources including adipose tissue because of its easy availability. **Objective:** Evaluation of the effect of adipose derived MSCs (ADMSCs) on streptozotocin (STZ)-induced DM in rats. **Materials and Methods:** Forty eight adult male albino rats were included and divided into 4 equal groups. Group I: control untreated, group II: diabetic group, group III: insulin-treated group, and group IV: ADMSCs-treated group. After 21 days from treatment, animals were sacrificed and pancreas was obtained for histological assessments. Stem cell homing was detected by polymerase chain reaction for human specific Alu gene. **Results:** ADMSCs caused lowering of blood glucose levels starting at the 14th day of treatment and increased blood insulin levels accompanied by reversal of the histopathological changes that were found in the diabetic group and increase insulin secreting cells. **Conclusion:** ADMSCs improved blood glucose levels and reversed the histopathological changes of the pancreas in experimentally induced DM I.

Key words: Adipose derived stem cells, mesenchymal stem cells, type I diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease, affects over 347 million subjects all over the world (**Mathers & Loncar, 2006 and Danaei et al., 2011**). It was reported in Egyptian manuscript about 3000 years ago (**Ahmed, 2002**). There was about 382 million people had DM in 2013 and it is expected to be 592 million by 2035 (**International Diabetes Federation, 2013**).

Treatment options for type 1 diabetes (DM 1) include pancreas or pancreatic islet transplantation (**Ryan et al., 2005 and Vergani et al., 2010)** which are relatively effective in normalizing blood glucose and hemoglobin A1c levels, as well as restoring insulin and C-peptide production (**Ramiya and Schatz, 2004**).

Only less than 0.5% of DM 1 benefit from this therapy (**Miszta-Lane et al., 2006**) due to shortage of donors. Life-long requirement of immunosuppression by its adverse effects often leads to non-compliant patient (**Lechner, 2004 and Miszta-Lane et al., 2006**). Recurrence of autoimmunity against β-cells continues to be a major challenge with transplantation therapies (**Ramiya and Schatz, 2004**).

Mesenchymal stem cells (MSCs) have shown a promising option for DM 1 cell therapy due to their potential for differentiation into insulin secreting cells (**Vija et al., 2009**). Adipose tissue is an attractive source of MSCs due to its stem cell abundance and ease of tissue procurement through a minimally invasive and relatively inexpensive procedure (**Chandra et al., 2009, Gimble et al., 2010 and Doi et al., 2013**). **Paek et al. (2014)** revealed that, the development of ADSC based treatments for diabetes are still in its early steps, and numerous challenges and opportunities still lie ahead.

The aim of this study was to evaluate the effect of adipose derived MSCs (ADMSCs) on streptozotocin (STZ)-induced DM in rats.

MATERIALS AND METHODS

Animals: Forty eight adult male albino rats weighing 130-160 grams of local strain were included in the study. They were brought from The Ophthalmology Research Institute (Giza, Egypt) and were acclimatized for one week before starting the experiment. They were housed four per cage (60 cm length × 40 cm width × 35 cm height), on normal dark - light cycle, with free access to food and water.

Animals were anesthetized before scarification. Rats were divided into four equal groups: Group I (control) received no treatment; group II (diabetic untreated): DM was induced by a single intraperitoneal injection of streptozotocin (STZ) supplied in powder form (Sigma-Aldrich, St. Louis, MO, USA) dissolved in citrate buffer (10 ml distilled water + 100 mg citric acid + 180 mg sodium citrate) in a dose of 65 mg/kg, and after 72 hours, blood glucose was measured to ensure the occurrence of DM. Animals with blood glucose level more than or equal 300 mg/dl were considered diabetic (**Aali et al., 2014**) and received IV injection of phosphate buffer saline (PBS). Group III (insulin-treated): DM was induced as group II and received subcutaneous injection of NPH insulin (Novopharma) in a dose of 4 U/day (**Pinheiro et al., 2011**). Group IV (ADMSCs-treated group): 72 hrs after DM induction, animals were treated by IV injection of 2×10^6 cells/rat (**Lin et al., 2009**) through tail vein without immunosuppression. After 21 days of treatment, animals were sacrificed and pancreas was fixed in formalin, paraffin processed and sectioned for histological assessment.

Body weight and blood glucose measurements: Body weight of all rats was measured weekly throughout the study. Blood glucose was measured before DM induction, 72 hrs after induction, and then every 2 days until scarification using one Touch Glucometer (MDSS GmbH, Germany). The measurement was done through puncturing the tail vein to obtain a blood drop.

Blood serum preparation: Whole blood was collected in test tubes containing no anticoagulant. The tubes were incubated in an upright position at room temperature for 30 min to allow clotting, and then centrifuged for 15 min at 1500 rpm. The supernatant (serum) was carefully aspirated using Pasteur pipette. The samples were maintained at 2-8°C while handling. The serum was aliquoted into cryovials, and stored at –20°C until used.

Insulin level measurement: Serum insulin was measured before induction of diabetes, 72 hrs after DM induction, and at the time of scarification by ELISA (DRG Insulin (rat\mouse) EIA-4127 ELISA insulin kit, Sigma Aldrich) according to the manufacturer. Blood was obtained from retro-orbital venous plexus.

Glycated hemoglobin (Hb A1C) measurement: Hb A1C levels were measured in freshly obtained peripheral blood (tail vein puncture) before DM induction and at the time of scarification by using automation (Hitachi 912).

Collection of adipose tissue: Human adipose tissue was obtained from elective liposuction procedure for plastic purposes after a written consent from donors. About 400 ml of lipoaspirate was collected in a sterile container and stored at 4°C till processing within 24 hours.

Preparation of the culture media: The culture media used was modified eagle's medium (MEM) (Biowest company) supplemented with 10% Fetal Bovine Serum (FBS) (cat.no.F6178, Sigma-Aldrich) and 1% Penicillin-Streptomycin (cat. No. P4333, Sigma-Aldrich). The prepared complete media was 500 ml MEM + 50 ml FBS + 5 ml Penicillin-Streptomycin, then the prepared media

was divided into aliquots of 50 ml in different sterile falcon tubes and was stored at 8 °C **(Lin et al., 2008)**.

Processing of adipose tissue samples: Adipose tissue samples were diluted in phosphate buffer saline (PBS) (Lonza-belgium, cat. No. BE17-516F) in a proportion of 1:1, and then washed several times till obtaining clear fluid. The adipose tissue was enzymatically treated with collagenase type I (cat. no. C-0130, Sigma- Aldrich), then filtered through mesh filter paper and exposed to density gradient centrifugation at 1200 rpm for 5 minutes at room temperature in order to obtain the stromal vascular fraction containing the low density mono-nuclear cells (MNCs). The MNCs were transferred carefully to a new sterile tube and washed twice with PBS through centrifugation at 2000 rpm for 10 minutes **(Bunnell et al., 2008)**.

Culture of mesenchymal stem cells (MSCs) from mononuclear cells: Isolation of MSCs was possible due to its capacity of adhesion to the plastic flasks, which were eliminated from the culture during the procedures of media change by using immunomagnetic separation method using CD34⁻, CD45⁻ and CD105⁺ markers **(Dominici et al., 2006).**

Subculture of mesenchymal stem cells: Trypsin (Lonza,Walkersville, cat. No.D2287) was allowed to thaw in room temperature. When the cells reached 80% confluence, media was discarded from the flask. The adherent cells were washed twice with pre-warmed PBS, and the wash solution was discarded. Two milliliters of trypsin were added, and the flask was gently rocked to ensure dispersing the trypsin solution over the cells. The flask

was incubated at 37 °C for 5 minutes, monitored periodically for cell detachment by observing the cells under the inverted microscope. Once cells started to be round and detached, tapping the side of the flask was done to aid more cells detachment. Once > 90% of cells detached or 10 minutes passed, the trypsin was neutralized by equal amounts of pre-warmed prepared media. The cell suspension was centrifuged at 1200 rpm for 5 minutes at room temperature. The cell pellet was ready to be injected (**Bunnell et al., 2008**).

Histological assessments: The pancreas paraffin blocks were sectioned at 4 µm thickness for H&E staining. Histopathological changes in the form of cell degeneration and necrosis, shrinkage in the pancreatic islets diameter and number, infiltration of the islets with lymphocytes and congested dilated blood capillaries were captured with High Power Field (HPF) of 40X and intermediate power field of 10X using UIS optical system (Universal Infinity System, Olympus®, Japan). The number and diameter of pancreatic islets were counted using ImagJ software (**Lee et al., 2006).**

Polymerase chain reaction (PCR) analysis of human-specific Alu gene: To provide evidence that MSCs can home the pancreas of diabetic rats, human specific marker, Alu gene, was used to identify donor derived cells in rats using PCR technique (**Zhang et al., 2013**). A small piece of the pancreas was immediately frozen in liquid nitrogen and kept in -80℃. Genomic DNA was isolated from the pancreas of rats using InnuPREP DNA Mini Kit for DNA extraction (Life Science, Germany, Cat. No. 845-ks-

1040050) according to the manufacturer's protocol. DNA from normal rats (contol group) was used as negative controls. Human-specific marker, Alu gene primer sequences is used to amplify human specific regions. The DNA samples were transferred into thermal cycler PCR. The initial denaturation was at 95°C for 1 min, annealing was at 66°C for 1 min, extension at 72°C and final extension at 72°C for 3 min.

Statistical analysis: Statistical analysis was done using Statistical Package for Social Science SPSS for WINDOWS software (version 19.0; SPSS INC, Chicago, IL) for all statistical analysis. Data were presented as mean ± standard deviation. Paired t-test and Wilcoxon test were used to determine the difference between each two groups. One way analysis of variance ANOVA test was used to test the difference among several groups and followed by post Hoc Tukey test. P-value of < 0.05 was considered significant.

RESULTS

Effect of treatment with ADMSCs on blood glucose: At the end of the study the mean glucose level was 90.80±5.53, 534.29 ± 46.70, 359.22 ± 65.17 and 247.25±215.05 mg/dl in groups I, II, III and IV respectively. ANOVA test revealed significant difference between all groups (p<0.001) and post Hoc Tukey test revealed that both treated groups had a significantly lower blood glucose level than diabetic group (p<0.001) and higher levels than control group (p<0.001). Group IV had significantly lower blood glucose level than group III (p<0.000) denoting that ADMSCs improved blood glucose level better than insulin although

it could not be restored to normal level. However, they improved blood glucose levels on comparing them to group II (Fig. 1).
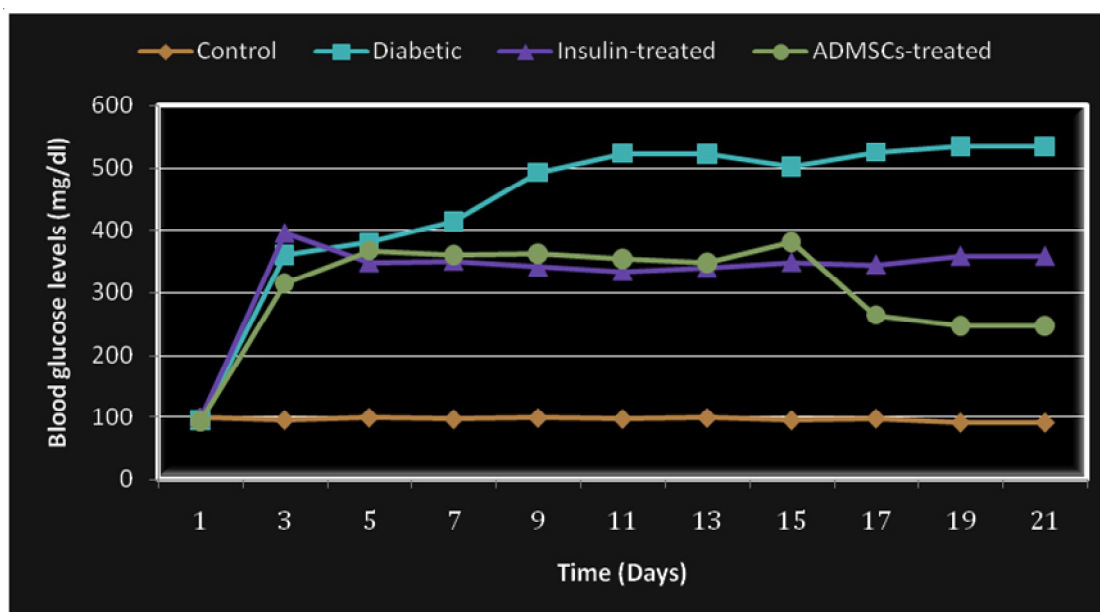

Figure (1): Blood glucose levels (mg/dl) through the study in all the studied groups.

Effect of treatment with ADMSCs on serum insulin level: At the end of the study, ANOVA test revealed significant difference between all groups (p=0.001). By using post Hoc Tukey, group I was significantly higher than groups II and III (p=0.001). ADMSCs-treated group showed a significantly higher insulin level than normal group (p=0.001) indicating that ADMSCs led to increased insulin level on comparing them to negative control group. The basal insulin level in group I did not differ from insulin level at the end of the study. However, in groups II and III, insulin level at the end of the study was significantly lower than basal insulin level, and in group IV, insulin level was significantly higher than basal level (Table 1).

Table (1): Insulin levels (μIU/mL) at the beginning (basal insulin) and at the end of the study (final insulin) in the different study groups.

Insulin level / Study groups	Basal Insulin	Final Insulin	p- value
Control group	1.48 ± 00.00	1.47 ± 00.00	1.000
Diabetic group	1.42 ± 00.00	0.21 ± 00.00	0.001*
Insulin-treated group	1.27 ± 00.00	0.82 ± 00.00	0.001*
ADMSCs-treated group	1.42 ± 0.19	2.86 ± 1.48	0.001*

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Effect of treatment with ADMSCs on body weight: At the end of the study, ANOVA test revealed significant difference between all groups (p=0.026), and post Hoc Tukey test revealed that body weight in groups II and III was significantly lower than group I (p=0.015 and 0.023 respectively). However, there was no significant difference between group IV and group I (p=0.290).

Wilcoxon test revealed that, at the end of the study, the body weight in group I was significantly higher than its basal level. In groups II and III, the body weight at the end of the study significantly decreased from their basal level. On the other hand, in group IV the body weight at the end of the study did not differ from its basal level (Table 2).

Table (2): Body weight (g) at the beginning (basal body weight) and at the end of the study (final body weight) in all the study groups.

Body weight / Study groups	Basal body weight	Final body weight	p- value
Control group	191.60 ± 35.00	215.80 ± 31.00	0.005*
Diabetic group	209.43 ± 10.34	168.29 ± 8.67	0.02*
Insulin-treated group	206.40 ± 13.00	193.67 ± 12.00	0.01*
ADMSCs-treated group	211.20 ± 15.19	215.60 ± 45.48	0.72

Effect of treatment with ADMSCs on Glycated haemoglobin (Hb A1C): At the end of the study, ANOVA test revealed significant difference between all groups (p=0.001). post Hoc Tukey test revealed that, group I was significantly lower than groups II, III and IV (p=0.001). Paired t-test revealed that, at the end of the study, Hb A1C level did not differ from its basal level in group I, but it was significantly higher than basal level in groups II, III and IV (Table 3).

Table (3): Glycated haemoglobin (Hb A1C) (%) at the beginning (basal Hb A1C) and at the end of the study (final Hb A1C) in all the study groups.

Hb A1C / Study Groups	Basal Hb A1C	Final Hb A1C	p- value
Control group	4.10 ± 00.00	4.10 ± 00.00	1.000
Diabetic group	4.10 ± 00.00	6.56 ± 00.00	0.001*
Insulin-treated group	4.40 ± 00.00	5.15 ± 00.00	0.001*
ADMSCs-treated group	4.15 ± 0.16	6.13 ± 0.48	0.001*

Histopathological results: There was significant difference between groups regarding pancreatic islets number and diameter. Pancreas from diabetic rats

contained smaller islets (Fig. 2), and decreased number of islets per section (Fig. 3). Post hoc Tukey test revealed that, there was significant difference between ADMSCs-treated group and the other 3 groups regarding the number and diameter of the pancreatic islets denoting that, it improved the pancreatic structure but it did not restore it to the normal. Fig. (4) showed the histopathological changes in the four studied groups. In the pancreas of ADMSCs-treated group, the islets appeared larger compared with islets from untreated diabetic rats (Fig. 4 D).

Polymerase chain reaction: The amplification plot for the presence of human ALU repeat sequence was negative in group I and strong positive in group IV (Fig. 5).
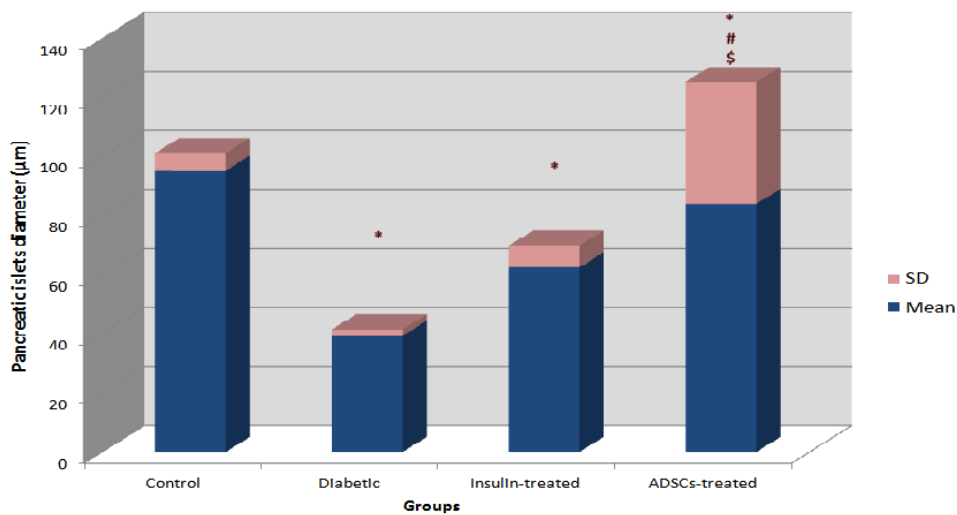
Figure (2): Pancreatic islets diameter (μm) in all the studied groups. (*) significantly different from control group. (#) significantly different from diabetic group. ($) significantly different from insulin-treated group.
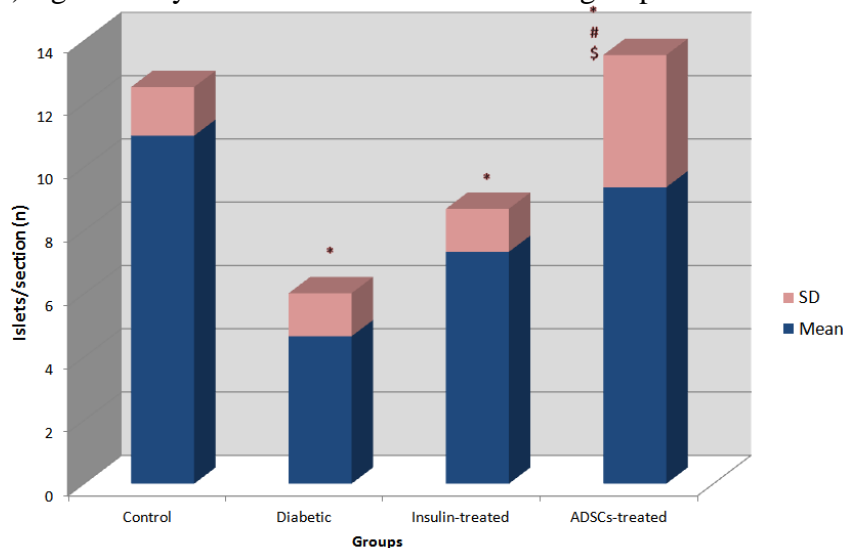
Figure (3): Pancreatic islets number/section (n) in all the studied groups. (*) significantly different from control group. (#) significantly different from diabetic group. ($) significantly different from insulin-treated group.
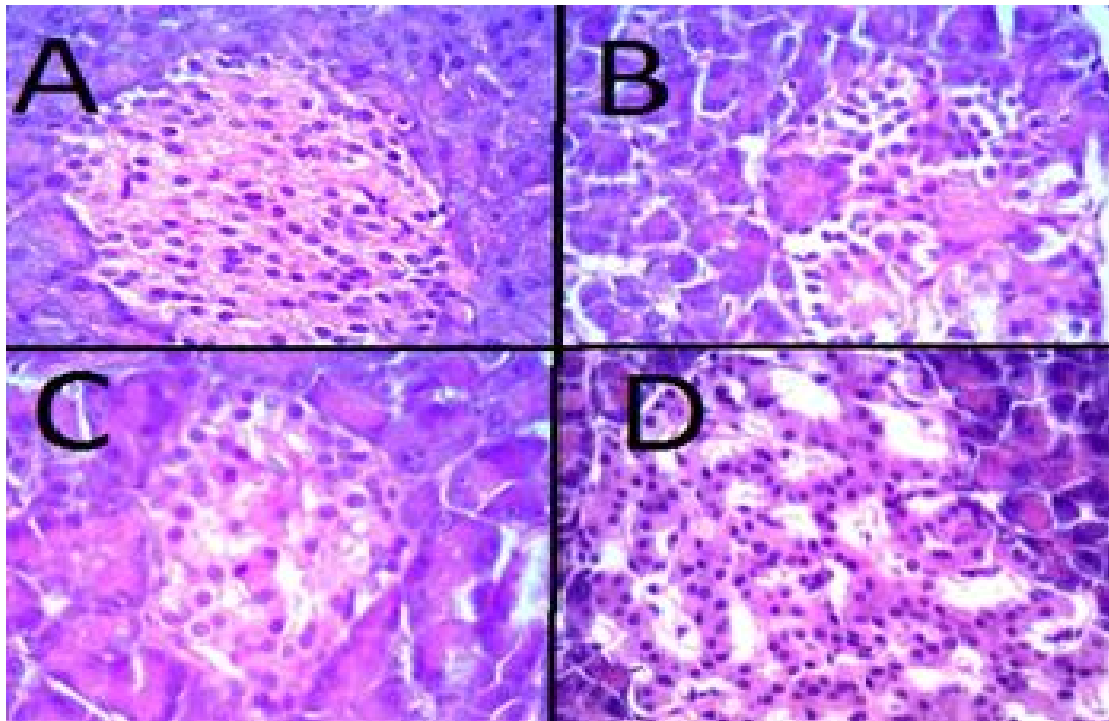
Figure (4): **A photomicrograph of a section in the pancreas. (A): negative control group (GI)** showing normal pancreatic architecture and arrangement of islet cells, having central nucleus and abundant pale eosinophilic cytoplasm arranged in groups within pancreatic tissue with scattered thin walled capillary size vessels (40 X). **(B): diabetic group (GII)** showing distorted islets cells with swelling, vacuolar degeneration and degranulation in the cytoplasm. Other cells showed small darkly stained nuclei with condensed chromatin and deep acidophilic cytoplasm. There were moderate lymphocytic infiltration in-between islet cells (40 X). **(C): insulin-treated group (GIII)** showing mild improvement in the architecture of islet cells in comparison with group II with still focal vacuolar degeneration and minimal lymphocytic infiltration (40 X). **(D): ADMSCs treated group (GIV)** showing marked improvement of islet cell morphology, no vacoulation or lymphocytic infiltration. There was hyperplasia of islet cells: increased number of islets, reaching 2-5 islets per 10X objective. The cells also showed the acinar architecture of cells (normal arrangement) and increased cell mass inside each islet.
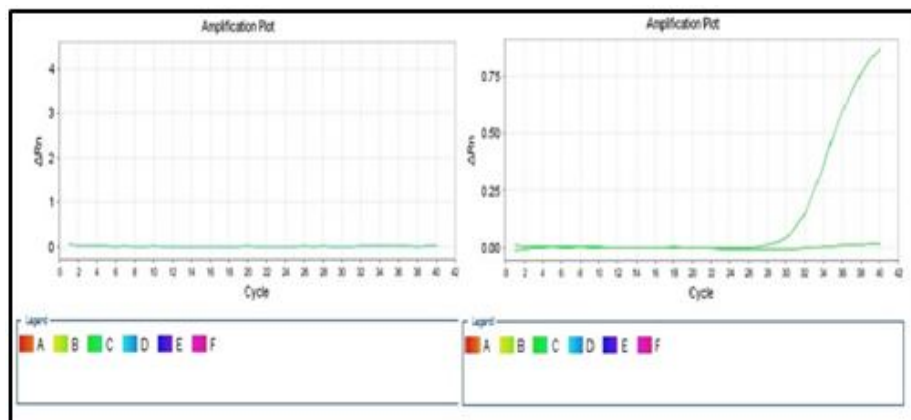

Figure (5): Amplification plot of samples for presence of ALU repeat sequence in groups I and IV.

DISCUSSION

DM1 is a T cell-mediated autoimmune disorder caused by decrease insulin production due to destruction of β-cells. Mesenchymal stem cells had immuno-modulatory properties and had the power to differentiate into insulin-secreting cells making it a promising therapeutic target for diabetes (**Vija et al., 2009**). Mesenchymal stem cells are multipotent cells reside mainly in the bone marrow showing tremendous potential for regenerative medicine due to their self-renewing ability, differentiation into different tissues and their availability in many sources (**Jiang et al., 2002, Porada et al., 2006 and Abdi et al., 2008**). They can be isolated from different tissues including umbilical cord blood (**Lazarus et al., 2005**) and adipose tissue (**Zuk et al., 2001**), and expand for several passages without losing their self-renewing capacity (**Raghunath et al., 2005 and Polak & Bishop, 2006**).

The present study addressed the anti-diabetic effect of mesenchymal stem cells derived from adipose tissue (ADMSCs) against streptozotocin (STZ)-induced DM in rats. ADMSCs are self renewing stem cells that can be isolated from human lipoaspirates. Moreover, ADMSCs compared to MSCs from other sources have the highest proliferation capacity and also retain their pluripotency after some passages (**Taléns-Visconti et al., 2007 and Taha & Hedayati, 2010**). ADMSCs were defined by adherence to plastic in culture, expression of cell surface markers such as CD105 and lack of expression of CD45 and CD34 (**Dominici et al., 2006**).

Diabetic rats were treated with ADMSCs without immunosuppression because ADMSCs have been shown to possess immunosuppressive properties (**Puissant et al., 2005 and Djouad et al., 2007**) and had the ability to survive in xenotransplantations (**Kang et al., 2003 and Rodriguez et al., 2005**).

Treatment with ADMSCs caused significant decrease in blood glucose level after two and three weeks when compared with groups II and III. This decrease might be related to secretion of insulin by the newly differentiated β. cells which was supported by the increase in serum insulin at the end of the study. The insulin increase was generally ascribed to the improved function of pancreatic islets mediated by MSCs. These results were consistent with previous studies using human bone marrow MSCs in immunodeficient STZ-induced diabetic mice (**Lee et al., 2006**), rat bone marrow MSCs in STZ-induced diabetic rats (**Dong et al., 2008 and Ezquer et al., 2008**), autologous bone marrow-derived stem cell transplantation (**Bhansali et al., 2009**) and human placenta derived MSCs in patients with type 2 diabetes (**Jiang et al., 2011**). **Li et al. (2012)** revealed that ADSCs could decrease fasting blood glucose in STZ-induced DM 1 animals. Although most of the studies reported that single transplantation of MSCs could reduce blood glucose in STZ-induced diabetic rats (**Dong et al., 2008 and Ezquer et al., 2008**) which resemble the present study, **Aali et al. (2014)** found that single transplantation of 2.5×10^6 MSCs could not reduce blood glucose levels of diabetic rats, but repeated injection of the same dose at week four could significantly reduce blood glucose levels compared to diabetic controls.

Diabetic control animals lost their body weight and became slim during the experiment, and the treatment with ADMSCs could prevent body weight loss significantly compared to diabetic rats. These results were similar to those of **Aali et al. (2014)** who revealed that administration of MSCs and their supernatant to diabetic animals not only could prevent loosing but also could increase their body weight significantly compared to normal diabetic rats and co-administration of MSCs with supernatant has synergistic effect on the body weight of animals improving their body weight significantly more than either MSCs or supernatant treated animals.

As the study lasted only three weeks, there was no improvement in glycated hemoglobin (HbA1c) in diabetic rats. **Nathan et al. (2007)** revealed that HbA1c is a glycemic indicator that reflects the average plasma glucose over the previous 8 to 12 weeks.

Histopathological assessment showed that STZ- injection caused β. cell damage of the diabetic and insulin treated groups. This was consistent with **Donath & Halban, (2004)** and **Akbarzadeh et al. (2007)** who reported that the area of islets of Langerhans decreased in diabetic groups because of decreased β. cell mass mostly due to necrosis and apoptosis which occurred with sustained hyper-glycemia. Also, **Homo Delarche et al. (2006)** concluded that development of fibrosis occurs secondary to hyper-glycemia which stimulates the secretion of fibronectin, collagen I and III by endothelial cells and vascular smooth muscle cells. On the other hand, ADMSCs treated group showed a normal structure

of the pancreas nearly similar to that of the control group indicating survival and differentiation of transplanted MSCs into islet cells in diabetic rats. This was contradicted by **Lechner et al. (2004)** who reported that MSCs could be feeder cells for islet differentiation, proliferation and vascularization, but do not differentiate into β. cells. **Ezquer et al. (2008)** found increased morphologically normal β. pancreatic islets in MSCs-treated rats. **Li et al. (2012)** reported that ADSCs ameliorated damage of pancreatic islets.

Pancreas from diabetic rats contained smaller islets and decreased islets number per section , however, treatment with ADMSCs increased the number and diameter of pancreatic islets. These results were consistent with those of **Lee et al. (2006)** who revealed that islets appeared larger in human MSCs-treated mice compared to untreated diabetic mice, and **Aali et al. (2014)** who reported an increase in number and size of islets per section in MSCs treated groups.

To improve homing of ADMSCs in rat pancreas, DNA from control negative and ADMSCs treated rat pancreas was extracted and amplified by PCR using primers specific for human Alu repeat sequences. Quantification of the human specific *Alu* sequences allows high sensitivity in detecting implanted cells without genetic modification or labeling of the cells. Amplification plot of samples for presence of Alu repeat sequence was positive in ADMSCs-treated rats and negative in control group which improved that implanted cells migrated and homed in the pancreas of transplanted animals. Human Alu gene expression was detected

in lung and spleen 4 weeks after ADSCs injection in diabetic nephropathy rat model **(Zhang et al., 2013)**, and locally up to 6 months after intra-articular injection **(Toupet et al., 2013)**. These findings were in accordance with **Sordi et al. (2005)** who found that intravenously infused MSCs are capable of migrating to pancreatic islets in experimental mice models. Other reports inform that MSCs migrate and dock preferentially into the injured or damaged tissues promoting survival of the surrounding cells **(Meirelles Lda et al., 2009 and Shi et al., 2010)**. **Aali et al. (2014)** demonstrated migration of MSCs to injured tissues by observation of labeled MSCs in pancreatic sections under fluorescent microscope.

So, ADMSCs transplantation improved DM physiologically and structurally indicated by the decreased glucose level, increased insulin level and improved pancreatic islets histologically. **Li et al. (2012)** revealed that ADMSCs may be able to differentiate into pancreatic β-cells, or support the survival of β-cell progenitors through secretion of growth factors. **Paek et al. (2014)** showed that ADMSCs exhibit unique characteristics well suited for trans-differentiation into a pancreatic endocrine lineage.

The limitation in this study was the short period of follow up of rats after stem cell therapy.

CONCLUSION

Systemic transplantation of ADMSCs in a dose of $2*10^6$ improved hyperglycemia, and the histopathological changes of pancreatic islets induced by STZ in rats after 15 days of treatment, and continued till the end of the experiment indicating that stem cell transplantation improved the pancreas structurally and functionally.

Conflict of Interest: There was no conflict of Interest.

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خلفية البحث: إن مرض البوال السكرى من أكثر أمراض الغدد الصماء وإضطرابات التمثيل الغذائي شيوعا ويتميز بفرط سكر الدم المزمن. الخلايا الجذعية المتعلقة باللحمة المتوسطة مبشرة لعلاج مرض البوال السكرى وهذه الخلايا يمكن فصلها من العديد من الأنسجة ومنها النسيج الدهني لسهولة الحصول عليه.

الهدف من البحث: تقييم تأثير زراعة الخلايا الجذعية المتعلقة باللحمة المتوسطة والمستمدة من النسيج الدهني على مرض البوال السكري المحدث بالإستربتوزوتوسين في الجرذان.

مواد وطرق البحث: أجريت هذه الدراسة على 48 جرذاً ذكراً تم تقسيمهم إلى أربع مجموعات متساوية. المجموعة الأولى: المجموعة الضابطة الغير معالجة، المجموعة الثانية: المصابة بمرض البوال السكري، المجموعة الثالثة: المعالجة بالإنسولين والمجموعة الرابعة: المعالجة بالخلايا الجذعية المتعلقة باللحمة المتوسطة المستمدة من النسيج الدهني. بعد 21 يوم من العلاج تم التضحية بالحيوانات والحصول على البنكرياس لتقييم التغيرات الهستوباثولوجية. ولاثبات وصول الخلايا الجذعية داخل البنكرياس تم تقصي وجود Alu جين البشري داخل نسيج البنكرياس باستخدام تقنية تفاعل البلمرة المتسلسل.

النتائج: الخلايا الجذعية المتعلقة باللحمة المتوسطة المستمدة من النسيج الدهني سببت انخفاض مستويات السكر في الدم بدءاً من اليوم الرابع عشر من العلاج وزيادة مستوى الإنسولين في الدم مصاحبا بعكس التغيرات التشريحية المرضية التي وجدت بالمجموعة المصابة بمرض البوال السكري وزيادة الخلايا الفارزة للإنسولين.

الإستنتاج: الخلايا الجذعية المتعلقة باللحمة المتوسطة المستمدة من النسيج الدهني البشري حسنت مستويات السكر في الدم و عكست التغيرات التشريحية المرضية في بنكرياس الجرذان المحدث لها مرض البوال السكري.