

EFFECTS OF ADIPOSE TISSUE MESENCHYMAL STEM CELL THERAPY ON DIABETIC RATS

By

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ABSTRACT

Background: Globally, diabetes mellitus has become one of the most common serious diseases to public health. Mesenchymal stem cells provide an alternative option for treatment of diabetes. **Objective:** Investigation of adipose tissue-derived stem cells (AT-MSCs) potential as an available less invasive source to produce insulin producing cells (IPCs). **Material and methods:** Forty female and ten male adult rats of Sprague Dawley strain between 8-9 weeks-old weighing 150-200 g were used. The male rats were used as donors for mesenchymal stem cells, while female rats were assigned to four groups: Group I (control group), Group II (diabetic group), Group III (diabetic group injected by 2 doses of phosphate buffer saline) and Group IV (diabetic group treated with MSCs isolated from adipose tissue). At the end of the experiment, serum levels of glucose, HbA1c, C-peptide and lipid profile, as well as the genes expressing the pancreatic regeneration (Pdx1 and Neuro D) were determined for all groups. The study was confirmed by histopathological and immunohistochemical examination of pancreatic tissue specimens. **Results:** STZ injection significantly increases the blood glucose, HbA1c, cholesterol, TG and LDL levels; while C-peptide and HDL levels significantly decreased. Furthermore, marked pancreatic destruction was also detected by significant decrease of pancreatic genes (Pdx1 and Neuro D) expression. All these parameters showed improvement with adipose tissue stem cells therapy. This improvement could not reach the normal levels when compared to the normal control group. These results were supported by the results of histological examination of pancreatic tissues. **Conclusion:** AT-MSCs therapy possessed anti-diabetogenic actions that might be mediated by their reducing effect on the blood glucose, HbA1c, lipid profile and expression of Pdx1 and Neuro D genes. The study demonstrated the potentiality of AT-MSCs as a safer, more promising source of IPCs providing a possible future therapeutic option for diabetes.

Key words: Diabetes mellitus, AT-MSCs, C-peptide, HbA1c.

INTRODUCTION

Irrespective of the type of diabetes, the current focus is to target β -cell dysfunction and/or mass to achieve the cure of diabetes. Exogenous administration of insulin is routinely used to control both types of diabetes, but it does not sufficiently replace β cells and the adverse short and long- term effects of the disease remain (*Cantarelli et al., 2015*). Therefore, the cure for diabetes lies in the possibility to replace the lost β cell mass

with a new endocrine component capable of assessing blood sugar level, and secreting appropriate level of insulin in the vascular bed.

Mesenchymal stem cells (MSCs) provide an alternative option for treatment of diabetes (*Vanikar et al., 2011*). Their ability to differentiate into many cell types and high expansion potential *ex vivo*, make them an attractive therapeutic tool for cell transplantation and tissue engineering (*Aali et al., 2014*). It can

differentiate into insulin producing cells and improve pancreatic regeneration which generated hope that this therapeutic notion could become a reality (*Jiang et al., 2011*). MSCs were chosen for treatment of diabetes to avoid the ethical considerations as well as practical problems and malignant proliferation caused by embryonic stem cells (*Wei et al., 2013*).

MSCs are originally derived from bone marrow, but they have been isolated from other tissues such as adipose tissue, periosteum, synovial membrane, synovial fluid, muscle, dermis, deciduous teeth, pericytes, trabecular bone, infrapatellar fat pad and articular cartilage (*Chen & Tuan, 2008 and Mimeault & Batra, 2008*). Cultured MSCs secrete various bioactive molecules which have got anti-apoptotic, immunomodulatory, angiogenic, antiscarring and chemo-attractant properties, providing basis for their use as tools to create local regenerative environment *in vivo* (*Dubei et al., 2014*).

Adipose tissues isolated from human lipoaspirates are also called AT-MSCs with several priorities over other sources in the way that they can be accessed easily in large amounts, and possess differentiation capacities similar to BM-MSCs (*Ouyang et al., 2014*). Moreover, MSCs from bone marrow and adipose tissue share similar cell populations and it has also been reported to contain significantly greater number of MSCs than bone marrow per unit weight (*Nakao et al., 2010*).

The present study aimed to investigate the potentiality of adipose tissue MSCs to produce functional insulin producing cells

(IPCs) on streptozotocin induced diabetic rat.

MATERIAL AND METHODS

Animals

Sprague Dawley rats (forty females and ten males, 8-9 weeks old, with an average weight of 150-200 g) were obtained from Nile center for experimental studies and researches (Al-Mansoura, Egypt). All rats were housed (one rat /25 x 30 x 30 cm cage) under specific pathogen-free conditions in facilities maintained at 27-32°C with a 40-60% relative humidity and normal light/dark cycle. Rats were handled regularly to accustom them to the procedure, and to minimize the effect of stress during the experiment. All rats had *ad libitum* access to standard rodent chow and filtered water, and were acclimated for 2 weeks prior to initiation of the experiment. All procedures were approved by the Animal Care Committee of Al-Azhar University. The principles of laboratory animal care were followed, as well as specific national laws where applicable.

For the experiment, male rats were used as donors for MSCs, while female rats were randomly allocated into four equal groups.

Group I: Normal control group.

Group II: Diabetic control group.

Group I II: Diabetic rats supplied by 2 doses of phosphate buffer saline (PBS 1 ml/dose) by intravenous injection in the rat tail at the time of MSCs administration (1st dose on the 3rd day after induction of diabetes, and the 2nd dose 1 week apart after the 1st dose).

Group IV: Diabetic rats treated with two doses of MSCs isolated from adipose tissues in the form of 2 doses (2.5×10^6 cells/rat suspended in PBS) (*Hu et al., 2015*).

Induction of diabetes: Streptozotocin was purchased from Sigma Company (St. Louis, MO, USA) in the form of powder. It was solubilized in sodium citrate buffer. The solution was prepared at pH 4.5 and injected I.P. within 15 minutes of its preparation (*Dong et al., 2008*). Diabetes was identified by measuring the non-fasting serum glucose concentration after the injection of STZ. Rats with a blood glucose level above 300 mg/dL were considered to be diabetic and were used in the experiment.

The blood glucose level of all groups was monitored under non fasting conditions every 3 days. The samples obtained from the tail vein using Accutrend strip (Roche Diagnostic, Indianapolis, IN).

At the end of the experiment, after 12 hours over night fasting, morning blood samples were collected from the heart by intracardiac blood collection method (*Parasuraman et al., 2010*) under deep general anesthesia by halothane. The spurting blood was collected into two aliquots: one was anti-coagulated for HbA1c assessment, the second aliquot was allowed to clot at room temperature and then centrifuged at 3000 rpm for 15 minutes. Resultant sera were stored at -20°C until assayed for estimation of:

1. Blood glucose (*Burtis and Ashwood, 2011*).

2. Glycosylated hemoglobin (HbA1c) (*Nathan et al., 2008*).

3. C- Peptide by quantitative immuno-enzymatic colorimetric method using NOVATEC IMMUNDIAGNOSTICA GMBH, Germany (Cat number DNOV112) (*Jones and Hattersley, 2013*).

4. Total serum cholesterol and HDL by quantitative enzymatic colorimetric determination of total and HDL cholesterol in serum using biomed diagnostic assay kits (*Moghadasian et al., 2002*).

5. Serum triglycerides by quantitative enzymatic colorimetric determination of triglycerides in serum using Cayman colorimetric assay kit (*Cole et al., 1997*)

6. Serum LDL cholesterol from the values of total cholesterol (TC), HDL and triglycerides using Friedewald equation: $\text{LDL (mg/dl)} = \text{TC} - \text{HDL} - (\text{TG}/5.0)^2$ (*Ahmadi et al., 2008*).

7. AT-MSCs of animals were isolated and cultured (*Zuk, 2002*). Transplantation was carried out 3 days after injection of STZ (after the animals confirmed to be diabetic). The 2nd dose was administered 1 week after the 1st dose. Animals underwent 12-h fasting. Following successful general anesthesia, the animals were fixed to the operating table in the supine position. 2.5×10^6 cells/rat/dose was injected intravenously through tail vein (*Bhansali et al., 2015*).

The animals were sacrificed under general anesthesia, a midline incision ≈ 4

cm in length was made in the abdomen, and the pancreas was dissected out and used for:

- Polymerase chain reaction technique to detect (Homing of male derived MSCs in the pancreatic tissue, Pdx1 and Neuro D gene expression)
- Histopathological and immunohistochemistry examination: Half of the pancreatic tissue (including tail part) was fixed in 10% paraformaldehyde and subsequently embedded in paraffin. Tissue sections of 5 μ m were then prepared and stained by hematoxylin and eosin (H&E). Immunohistochemical detection of insulin was performed on 4 μ m section using a standard chain polymer-conjugated technique (*Mehrazma et al., 2012*).

Morphometric assessment was done on H&E stained slides using the image analyzer optical micrometer (TS view), objective lens of magnification 10 and eye piece of magnification of 40 binuclear microscope. The number of islets per

fixed square area of 11703.6 μ m² was counted. Average area of the islets was determined by measuring the area of 4 islets in each section and totally 20 islets in each group. All measurements for each group were averaged and these results were subjected to statistical analysis.

Statistical analysis was processed by SPSS 17.0 software. Data with normal distribution were expressed as mean \pm SEM. One-way analysis of variance (ANOVA) followed by a Bonferroni post hoc tukey multiple comparison tests. $P < 0.001$ was considered to indicate statistical significance.

RESULTS

Spindle-shaped forms characteristic of MSCs began to appear in culture within the first week. 80% confluence for adipose tissue stem cells was reached within 10-15 days, where it showed uniform fibroblast-like cells (Figure 1).

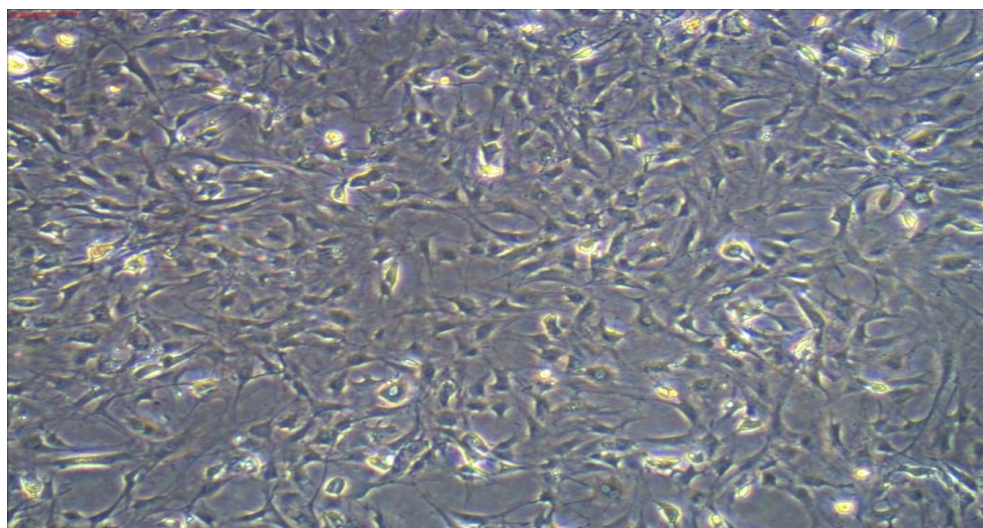


Figure (1): Morphology of MSCs of adipose tissue origin. Undifferentiated spindle-shaped of adipose tissue origin after the third passage.

Cell surface antigen phenotyping was performed on AT-MSCs by flowcytometry (figure 2). AT-MSCs revealed expression of surface markers. Receptor molecule protein CD 44 was positive

(97.85%), while hematopoietic markers CD34 and CD45 were negative (0.43%, 0.22 %). Such results correlate well with the criteria of MSCs (*Dominici et al., 2006*).

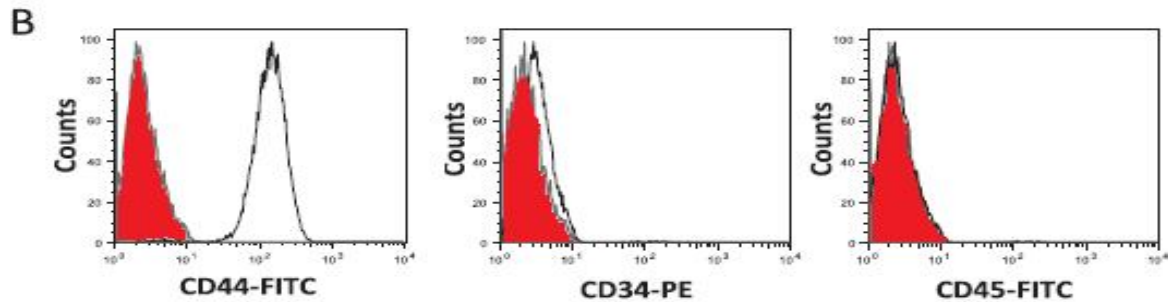


Figure (2): Characterization of rat MSCs for CD44. These cells were negative for CD34 and CD45 on primary cultures of AT-MSCs.

There was insignificant change ($p > 0.001$) of the blood glucose level in all groups in day 0. STZ treatment induced significant elevation ($p < 0.001$) of blood glucose level in treated groups when compared to the control group. This increase was continued till the end of the experimental period in a significant manner ($p < 0.001$). However, insignificant change ($p > 0.001$) was observed in blood glucose level between II and III (table 1).

MSCs injection from adipose tissue origin improved the hyperglycemia induced by STZ injection. There was a significant decrease ($p < 0.001$) of the blood glucose level in group IV when compared to group II and III respectively. This decrease was noticed at day 8 after the 1st dose of MSCs injection and continued till the end of the experimental period. However, there was still a significant increase ($p < 0.001$) of blood glucose in these groups when compared to the control group.

Induction of type I DM by STZ injection resulted in a significant increase ($p < 0.001$) of HbA1c level in diabetic groups (II and III) compared to the control one. Group II and III was insignificantly different ($p > 0.001$). Rats received AT-MSCs showed a significant decrease ($p < 0.001$) of HbA1c level when compared to diabetic groups (table 2).

Induction of type I DM by STZ injection induced a significant decrease ($p < 0.001$) of C peptide level in diabetic groups (II and III) when compared to control group. There was insignificant change ($p > 0.001$) of C- peptide level between diabetic groups. A significant elevation ($p < 0.001$) of C- peptide level diabetic rats treated with AT-MSCs (Group IV) when compared to diabetic groups, but this decrease failed to return its level to the normal (Table 2).

Table (1): Changes in blood glucose level (mg/dl) after induction of diabetes and MSCs therapy at various durations.

Groups		<i>Group I</i>	<i>Group II</i>	<i>Group III</i>	<i>Group IV</i>
Day 0	Mean	104.1	110.5	106.5	110.00
	SEM	2.83	3.69	4.15	2.29
Day 4	Mean	101.9	405.2 ^a	444.5 ^a	434 ^a
	SEM	4.1	10.97	10.76	11.85
Day 8	Mean	107.2	410.5 ^a	438.5 ^a	367 ^{abc}
	SEM	3.68	11.04	7.71	8.95
Day 12	Mean	100.8	455.2 ^a	469.5 ^a	323.5 ^{abc}
	SEM	2.57	7.51	6.97	8.53
Day 16	Mean	114.1	472.5 ^a	483 ^a	288.5 ^{abc}
	SEM	2.69	6.11	4.16	6.46
Day 20	Mean	108.8	493.50 ^a	502 ^a	265.5 ^{abc}
	SEM	3.1	4.22	3.59	5.19
Day 24	Mean	110.3	508 ^a	504 ^a	244.5 ^{abc}
	SEM	3.05	9.4	4	3.29

SEM: Standard error of mean

^a: significance relative to Group I

^b: significance relative to Group II

^c: significance relative to Group III

There were significant differences on comparing all groups with each other in serum concentration of total cholesterol and triglycerides respectively. STZ injection significantly increased the serum total cholesterol level ($P < 0.001$) in diabetic groups vs control rats. In comparison to diabetic groups, AT-MSCs treatment showed significantly lower total cholesterol and triglycerides levels.

There were significant differences on comparing all groups with each other in

serum concentration of LDL and HDL respectively. Similar to the above results, STZ injection increased the serum LDL in diabetic rats compared to control rats and MSCs adipose tissue derived significantly lowered LDL level. On the other hand, a significant reduction in serum HDL was observed diabetic rats when compared to control rats. AT-MSCs significantly elevated HDL level when compared to diabetic rats, this elevation reached to the normal levels.

Table (2): Changes of serum HbA1c (%), C peptide (ng/ml), Cholesterol (mg/dl), Triglycerides (mg/dl), LDL (mg//dl) and HDL (mg/dl) in different groups.

Groups Parameters	Group I (control)	Group II (diabetic)	Group III (Diabetic received PBS)	Group IV (diabetic-AT MSCs)	P value
Serum Hb A1c (%)	5.02±0.01	14.12 ^a ±0.10	15.01 ^a ±0.04	6.57 ^{abc} ±0.56	P < 0.001
C peptide (ng/ml)	1.37±0.04	0.48 ^a ±0.04	0.46 ^a ±0.05	1.08 ^{abc} ±0.07	P < 0.001
Serum cholesterol (mg/dl)	91.3±5.47	186.7 ^a ±7.87	188.3 ^a ±10.63	108.6 ^{bc} ±8.72	P < 0.001
Serum triglycerides (mg/dl)	78.2±5.31	167.1 ^a ±12.21	169.1 ^a ±10.85	103.008 ^{bc} ±6.83	P < 0.001
Seum LDL (mg//dl)	38.6±3.668	116.57 ^a ±11.38	117.23 ^a ±10.69	51.6963 ^{bc} ±3.98	P < 0.001
Seum HDL (mg/dl)	37.6±2.34	23.40 ^a ±2.15	22.6 ^a ±1.99	34.7 ^{bc} ±1.83	P < 0.001

^aValue significantly different vs. control group , ^b from diabetic groups, ^c: vs. group III at P<0.00

Induction of type I DM by STZ injection led to a significant decrease (p <0.001) in pancreatic Pdx1 and Neuro D gene expressions in diabetic groups vs control

group. Stem cell injection (AT-MSCs) revealed a significant increase (P<0.001) in Pdx1 and Neuro D genes expression in diabetic rats (Table 3).

Table (3): Changes Pdx1 (%) and Neuro D gene (%) expression in all groups.

Groups Parameters	Group I (control)	Group II (diabetic)	Group III (Diabetic received PBS)	Group IV (diabetic-AT MSCs)	P Value
Pdx1 gene (%)	0.689±0.008457	6.8x10 ^{-5 a} ± 6.49x10 ⁻⁶	8.5 x10 ^{-5 a} ±8.36 x10 ⁻⁶	2.99 ^{abc} ±0.1759	P<0.001
Neuro D gene (%)	1.06±0.07	0.11 ^a ±0.01	0.09 ^a ±0.006	3.82 ^{abc} ±0.27	P<0.001

^aValue significantly different vs. control group or ^bfrom diabetic groups, ^c vs, group III at P<0.001.

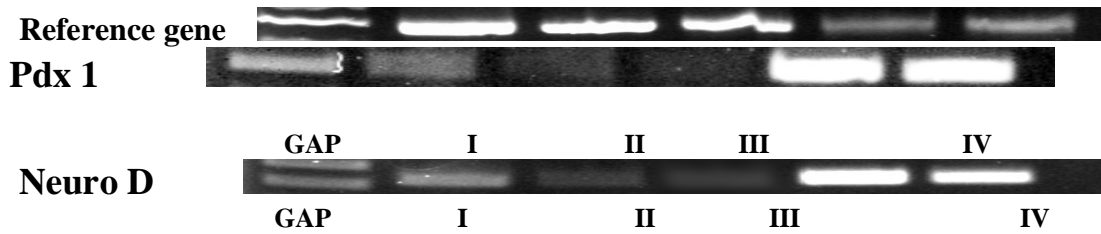


Figure (3): Expression of both Pdx1 and Neuro D genes as regards the reference gene (GAP gene) in all groups.

GAP: Reference gene

Lane I: Group I (control group)

Lane II: Group II (diabetic group)

Lane III: Group III (Diabetic group treated with PBS)

Lane IV: Group IV (Diabetic group treated with MSCs of adipose tissue origin).

Histological changes by Hematoxylin & Eosin (H&E) stain and Immunohistochemistry

Normal morphological pattern of pancreatic tissues, consisting of many pancreatic islets of Langerhan's scattered in pancreatic tissue. The pancreatic islets were regular with well-defined boundaries and contained small endocrine cells, predominantly made up of beta cells with marked immunoreactivity seen in positive cells (Figures 4&5 and table 4).

Microscopic examination of STZ induced diabetic rats showed variable sized pancreatic islets with reduction of its

cellular components than normal. The exocrine pancreatic tissue showed perivascular cellular infiltration, focal necrosis, dilated ducts. The number of pancreatic islets decreases and the lobular distribution of islets were greatly reduced in number. Insulin positive cells immune staining showed no proliferate activity. There was a significant reduction ($p < 0.001$) in the insulin positive cells in the pancreas of diabetic rats (6.91 ± 0.23 to $0.18 \pm 0.02\%$) compared to the control group (Figures 6, 7 and table 4).

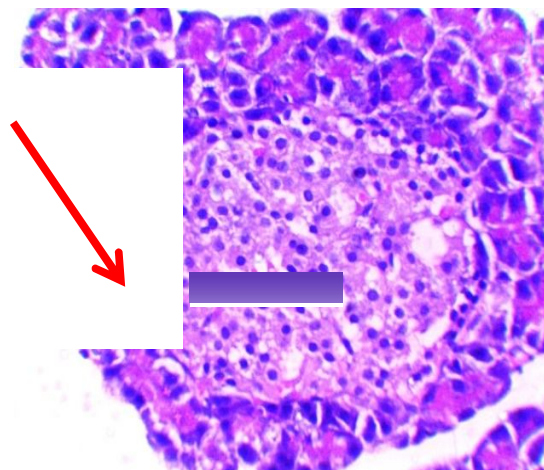


Figure (4): Pancreatic tissue of the control showing the normal pancreatic structure where the nucleus of the acinar cells is located at the base of cell (thin arrow).The islets of Langerhans (thick arrow) were also evident (H&Ex400).

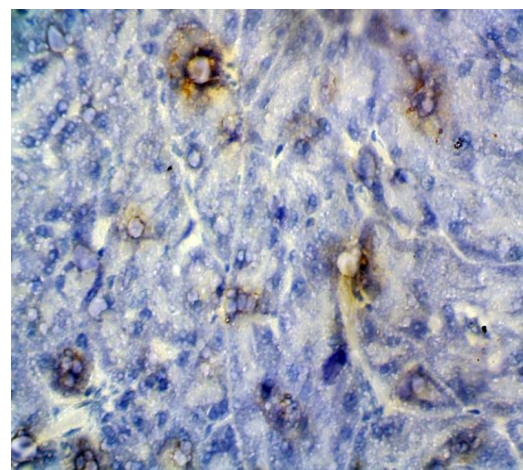


Figure (5): Pancreatic tissue of normal rat showed marked immunoreactivity. Stained immunoreactive cells seen between acini (brown color)(anti-insulin immunostaining x400).

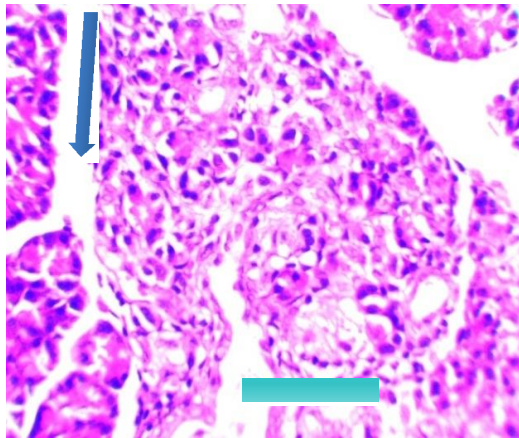


Figure (6): Pancreas of diabetic rat showed indistinct boundary between the endocrine and exocrine part (blue arrow). Notice inflammatory cells (red arrow) infiltrating through CT septae (H&E) x400

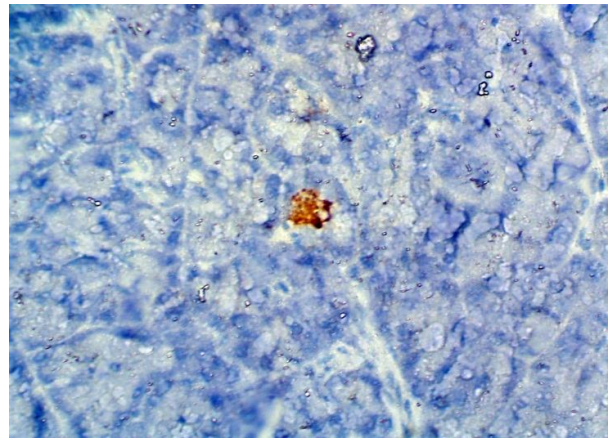


Figure (7): Pancreatic tissue of diabetic rat showed marked decreased immunoreactivity with mild immunostained cells in the central part of islet (brown color)(antiinsulin immune-staining x400)

Microscopic examination of group III, showed nearly similar morphological patterns like that of the diabetic group. The pancreatic islets were very small, cord like and difficult to be distinguished from the surrounding exocrine cells with which they merged. The pancreatic islet cells showed variable degrees of

degeneration extending from vacuolation to dropout necrosis. Insulin positive cells immune staining revealed a significant reduction ($p < 0.001$) of the percentage area from $(6.91 \pm 0.23$ to $0.59 \pm 0.06\%$) when compared to the control group (Figures 8, 9 and table (4)).

Table (4): Effect of induction of diabetes and mesenchymal stem cell therapy on insulin positive cells in all groups.

Groups	Group I	Group II	Group III	Group IV	P Value
Parameters					
Insulin positive cells	6.91 ± 0.23	$0.18^{a} \pm 0.02$	$0.59^{a} \pm 0.06$	$3.52^{abc} \pm 0.14$	$P < 0.001$

^aValue significantly different vs. control group, ^bfrom diabetic groups, ^c vs. group III, $P < 0.001$.

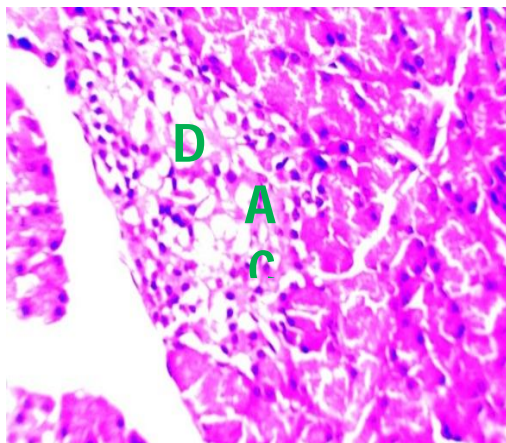


Figure (8): Pancreatic tissue of the diabetic and Saline group showing abnormal architecture of acinar cells (AC) with degenerated many cells (D)(Hx &E x400)

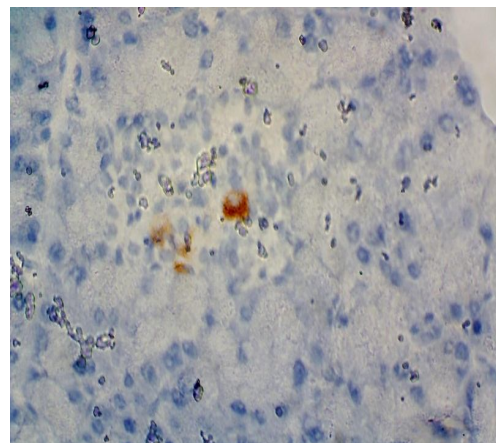


Figure (9): pancreatic tissue showed marked decreased immunoreactivity in central part of an islet of Langerhans (anti-insulin immune-staining)

Microscopic examination of AT-MSCs treated rats, showed increased number of pancreatic islets due to pancreatic regeneration. There was great variability in size of the pancreatic islets as compared to diabetic and normal groups. Some islets showed compensatory hypertrophy. Insulin positive immune staining showed reorganization of islet and partial restoration of β -cell as indicated by high insulin reactivity compared with diabetic

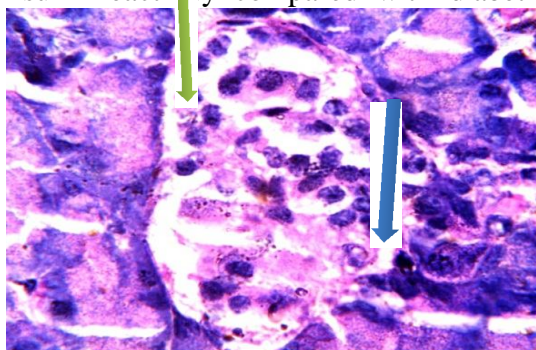


Figure (10):pancreatic tissue of adipose group showing moderate restoration of some β cells (green arrow) with hydropic degeneration (blue Arrow) (Hx&Ex1000).

DISCUSSION

Bone marrow-derived stem cells are considered the well-established source for stem cells (*Barlow et al., 2008*), and showed the potentiality to differentiate into functional insulin-producing cells (IPCs) (*Xin et al., 2016*). However the combined invasive procedure and reported low yields stem cells (*Lee et al., 2006*), provoked further research for other sources of MSCs. Adipose tissue derived stem cells are available, non invasive and non expensive. So, the present study aimed to investigate the potential of adipose tissue MSCs to produce functional IPCs. This was assessed through observing the changes of blood

groups. There was a significant increase ($p < 0.001$) in number of insulin positive cells in this group from (0.18 ± 0.02 to $3.06 \pm 0.19\%$) when compared to group II and from (0.59 ± 0.06 to $3.06 \pm 0.19\%$) when compared to group III. However, this increase did not reach to that of the control group (Figure 10 & 11 and table 4).

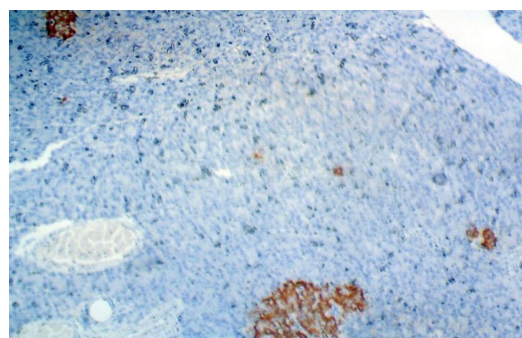


Figure (11): Pancreas of AT-MSCs treated rats showing dense immunoreactivity (anti-insulin immunostaining x400) (anti-insulin immunostaining)

glucose, HbA1c, C peptide, lipid profile levels, pancreatic gene expressions (Pdx1 and Neuro D gene) and histomorphological changes.

The current study showed significant increases in blood glucose levels on the 3rd day of streptozotocin (STZ) injection to rats. The hyperglycemia was supported by the studies of *El Said et al. (2013)*, *Ismail et al. (2013)* and *Gabr et al. (2015)*.

Eleazu et al. (2013) attributed this hyperglycemia to that STZ induces cell death through altered NF- κ B based cell signaling. It selectively inhibits the activity of the glycoside hydrolase O-GlcNAc case in the β cell, which is

responsible for removing O-GlcNA from proteins. This causes irreversible O-glycosylation of intracellular protein resulting in β cell apoptosis. Another biochemical mechanism for the STZ cytotoxicity was reported by *Daisy et al. (2012)* that β cell death occurs as a result of DNA fragmentation induced by selective pancreatic beta cell toxicity after STZ injection. Oxidative stress also was shown to be responsible at least in part for pancreatic β -cell dysfunction caused by glucose toxicity in hyperglycemia. STZ treatment causes significant increase in malondialdehyde but decreases antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase activities (*Sobrevilla et al., 2011*).

During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin (*Marcovecchio et al., 2009*). The present study revealed that there was a significant increase of HbA1c level in rats treated with STZ injection. This increase was in accordance with the studies of *Haller et al. (2013)*, *Hu et al. (2013)* and *Mesples et al. (2013)*.

Haque and Siddiqui (2013) reported that the rate of formation of HbA1c is directly proportional to the blood glucose concentration. A rise of 1% in HbA1c corresponds to an approximate average increase of 2 mmol/L (36 mg/dl) in blood glucose.

Measures of serum C-peptide has been widely used as a marker of insulin secretion (*Akinlade et al., 2014*). The peripheral insulin levels may not accurately reflect portal insulin secretion as about 50% of insulin produced by pancreas is first pass metabolized by the

liver, and its peripheral clearance is variable. Meanwhile, C-peptide has negligible extraction by the liver and constant peripheral clearance. Therefore, it circulates at concentrations approximately five times higher than insulin in the systemic circulation (*Ghorbani and Shafiee-Nick, 2015*). The present study revealed that induction of diabetes led to significant decrease in the levels of C peptide when compared to control group. These results were consistent with those of *Abu-Abeeleh et al. (2010)*, *Kanchana et al. (2011)* and *Gabr et al. (2015)*. *Daisy et al. (2012)* explained this reduction to the destruction of the β cells of pancreas by inhibiting insulin release.

Streptozotocin injection induced disturbances in the blood lipid levels in diabetes induced rats. There was a significant increase in the cholesterol, TG and LDL cholesterol levels, whereas the HDL level showed significant decrease when compared to control group. *Ahmed et al. (2013)* attributed the hypercholesterolemia occurred in diabetic rats to metabolic abnormalities as HMG Co-A reductase enzyme is accountable for synthesis of cholesterol. Insulin has an inhibitory effect on HMG-Co-A reductase and deficiency of insulin will enhance the generation of cholesterol. Another mechanism was reported by *Almeida et al. (2012)* that diabetes increases the activity of hormone-sensitive lipase which catalyzes the mobilization of fatty acids from TAG stored in adipocytes. Thus, the greater quantities of fatty acids returning to the liver are reassembled into TAG and secreted in VLDL. Moreover, *Verges (2015)* reported that insulin promotes the clearance of LDL by increasing receptor expression and activity. So plasma LDL

increase in DM due to absence of insulin action.

STZ injection also induced marked pancreatic destruction as shown by significant reduction in the levels of pancreatic genes (Pdx1 and Neuro D) expression in diabetic rats. Pdx1 is not only a key regulator of insulin gene expression, but it is also essential for normal development of the pancreas (*Ardestani et al., 2014*). *Migliorini et al. (2014)* reported that the activating transcription factor 2 (ATF2) during morphogenesis plays an important role in the regulation of insulin gene expression in mature islets. ATF2 interacts with key β cell transcription factors such as Pdx1 and Neuro D. altogether, besides classical signaling pathway and growth factors known to control β cell homeostasis, islet architecture and regulating β cell function.

β cell damage was confirmed by the histopathological changes of pancreatic tissue in the present study which revealed degenerated islet's cells with decrease in number of β cell, nuclear pyknosis and fragmentation with abnormal architecture of acinar cells. Immunohistochemistry also showed significant reduction of insulin positive cells. These results were supported by the study of *Bhansali et al. (2015)* who reported that diabetic rat pancreatic section revealed swelling, vacuolation and degranulation in the cytoplasm of some islet cells denoting necrotic changes. Moreover, some nuclei showed fragmented chromatin and dissolution denoting apoptosis due to STZ injection.

The dose of MSCs is an important determinant of glucose-insulin homeostasis outcome. The dose varying from 2-

10 million cells either as single injection (*Azab et al., 2011*), or as multiple injections (*Aali et al., 2014*) have been used in experimental animals. In the present study, injection of two doses of 2.5×10^6 cells of AT-MSCs led to improvement of diabetic picture. There was a significant decrease in blood glucose and HbA1c levels associated with an increase in C-peptide level when compared to diabetic rats.

These results were in accordance with the studies of *Yang et al. (2010)*, *Si et al. (2011)*, *Li et al. (2012)* and *Silva et al. (2014)* who reported that incomplete reversion of diabetes promoted by MSCs therapy may be a result of an incomplete pancreatic islet regeneration. The dose of MSCs would represent a good strategy to improve therapy efficiency.

Hu et al. (2015) explained this improvement after AT-MSCs infusion, these cells not only reduce glucose toxicity, but also inhibit inflammation; cell apoptosis and promoting angiogenesis evidenced by decreased the activity of caspase-3 in islets of diabetic rats after AT-MSCs infusion. Another mechanism was reported by *Yoo et al. (2013)* that administration of AT-MSCs modulates the levels of TGF- β in the pancreatic tissue which is a regulatory cytokine that plays pleiotropic role in immune system, and promotes protection against autoimmune diabetes. They found that there were increased levels of TGF- β in STZ-induced experimental diabetes that could decrease the inflammatory process in the pancreatic islets. *Calafiore and Basta (2015)* reported that adipose-derived MSCs represent a very promising approach to diabetes since they are enriched with a

large number of bioactive mediators such as leptin, adiponectin and visfastin which are known to regulate glucose homeostasis and lipid metabolism.

As regards the effect of AT-MSCs therapy on lipid profile level, there was an improvement in a significant manner compared to diabetic group. These results were in consistent with *Fang et al. (2012)* and *Cao et al. (2015)* who found that there were fewer fat cell infiltrations in livers of diabetic animals received single dose of AT-MSCs accompanied by restoration of lipid profile (reduced serum TG and increased HDL), with significant decrease of plasma glucose, urea nitrogen and creatinine associated with increased serum insulin level to moderate extents.

Regarding to Pdx1 and Neuro D gene expression, the present study revealed upregulation of their levels after AT-MSCs injection to diabetic rats which indicate pancreatic regeneration. These results were supported by the studies of *Chandra et al. (2009)*, *Zhang et al. (2011)* and *Dang et al. (2015)*.

This improvement also was confirmed by histopathological results of the present work which revealed moderate restoration of some β cells and small preserved islets as well as partial beta cells regeneration which was evidenced by increase in the number of insulin positive cells as shown by immunohistochemistry technique.

Cui et al. (2007) reported that adipose tissue derived stem cells had an inherent regenerative angiogenic potential, and anti-apoptotic capability through their secretion of trophic factors. Also, they have anti-inflammatory and immunomodulatory properties, including suppression of T-cell proliferation. Therefore, they can

potentially allow improved engraftment of transplanted islets with enhanced vascularization and suppression of inflammation (*Peak et al., 2014*).

In conclusion, AT-MSCs possessed significant anti-diabetogenic properties. MSCs may contribute to reduce the hyperglycemia by improving β - cells proliferation and insulin secretion. However, further studies are needed to evaluate the potential value of AT-MSCs for the management of diabetes, the most effective duration for treatment to return the all parameters to the normal levels.

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تأثير العلاج بالخلايا الجذعية المستخلصة من الأنسجة الدهنية علي الجردان المصابة بمرض البوال السكري

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خلفية البحث: يعد مرض السكري من أهم التهديدات التي تواجه الصحة العامة. وقد أصبح العلاج بالخلايا الجذعية علاجاً بديلاً لمرض البوال السكري.

الهدف من البحث: دراسة الدور المحتمل للخلايا الجذعية المستخلصة من الأنسجة الدهنية كمصدر جديد قادر علي تجديد خلايا بيتا البنكرياسية.

مواد وطرق البحث: تم تنفيذ الدراسة الحالية على ٤٠ من إناث و ١٠ من ذكور الجردان البيضاء البالغة، وتم عزل الخلايا الجذعية من ذكور الجردان البالغين بينما قسمت الإناث الي أربع مجموعات متساوية على النحو التالي :

المجموعة الأولى : المجموعة الضابطة.

المجموعة الثانية المجموعة المصابة بالبوال السكري.

المجموعة الثالثة: المجموعة المصابة بالبوال السكري يتم حقنها بمحلول PBS ١ مجم لكل جرعة من خلال الحقن في الوريد الذليل للجرذ في نفس وقت العلاج بالخلايا الجذعية.

المجموعة الرابعة المجموعة المصابة بالبوال السكري معالجة بالخلايا الجذعية المستخلصة من الانسجة الدهنية بجرعة ٥ مليون خلية مقسمة الي جرعتين متساويتين.

وفي نهاية التجربة تم تقييم تأثير العلاج بالخلايا الجذعية علي الجردان المصابة بالبوال السكري من خلال قياس نسب كل من جلوكوز الدم والهيموجلوبين السكري و السي بيبتايد ونسب الدهون المختلفة (الكوليستيرول، الدهون الثلاثية، الدهون منخفضة الكثافة والدهون عالية الكثافة) هذا الي جانب اخذ عينات من البنكرياس للبحث الجيني عن كل من Pdx1 و Neuro D حيث أنهما دلالات هامة تثبت حدوث تجديد لخلايا البنكرياس التالفة بسبب البوال السكري ، علاوة علي ذلك فقد تم اخذ عينات اخري من البنكرياس للفحص المجهرى والفحص الكيمياءى الهستولوجي المناعي.

النتائج: الاستخدام العلاجي للخلايا الجذعية المستخلصة من الأنسجة الدهنية في الجردان المصابة بمرض البوال السكري أدى إلي تحسن ذو دلالة احصائية في مرض البوال السكري المستحدث في صورة انخفاض مستويات الجلوكوز بالدم و الهيموجلوبين السكري والكوليستيرول و الدهون الثلاثية بالإضافة الي الدهون منخفضة الكثافة. بينما أحدثت ارتفاع ذو دلالة احصائية في نسب السي بيبتايد والدهون عالية الكثافة مقارنة بالمجموعتين المصابتان بالسكري الا ان هذا التحسن لم يصل للمستوي الطبيعي مقارنة بالمجموعة الضابطة. إضافة الي ذلك فان نسب التعبير الجيني لكلا من Pdx1 و Neuro D قد اعطي ارتفاعا ذا دلالة احصائية مقارنة بالمجموعات المصابة بالبوال السكري. وقد أوضح الفحص النسيجي تحسناً جزئياً للتأثير المدمر الناجم عن حقن الاستريبتوزوتوسين بظهور جزر جديدة والذي اكده الفحص الكيمياءى الهستولوجي المناعي بحدوث ارتفاع ذو دلالة إحصائية في نسب الخلايا الكاشفة للانسولين مقارنة بتلك الموجودة في المجموعتين المصابتين بالبوال السكري.

الإستنتاج: إستخدام الخلايا الجذعية المستخلصة من الأنسجة الدهنية له دور علاجي في ضبط مرض البوال السكري.