Antioxidant and Anti-Inflammatory Therapeutic Roles of BM-Mscs in Enhancing Pancreatic Auto-Immunity and Apoptotic Status in TIDM

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Abstract: Diabetes mellitus (DM), a global health crisis, affecting humanity regardless of the geographic location or socioeconomic profile of the population, as it considered the principle cause of great economic loss that can impede nation's development. In recent years; the transplantation of MSCs has achieved great therapeutic effects in animal models due to their multipotency along with their paracrine secretion of cytokines, angiogenic and neurotrophic factors, angiogenic factors, anti-inflammatory effects and immunomodulatory substances. Therefore, the current study was designed to assess the probable antioxidant, anti-inflammatory, immuno-suppressive and anti-apoptotic protective capacities of bone marrow derived mesenchymal stem cells (BM-MSCs) in comparison with insulin treatment in diabetic rats. Animals were divided into four groups; control group, diabetic group (D) which received a single intraperitoneal STZ dose (45 mg/kg b.w), D + insulin (0.75 IU/ 100 gm b.w., daily) group and D + BM-MSCs (single intravenous dose of 10 6 cell/rat); and the study continued for four consecutive weeks. Obtained results showed that either insulin or BM-MSCs administration remarkably improve the oxidative stress status resulting from diabetes induction; as reflected by lowered pancreatic MDA, ROS and XO levels; and enhanced the antioxidant defense system capability via increasing pancreatic contents of GSH, SOD, CAT, GST and TAC, compared to the diabetic group. Also, treatment of diabetic rats with insulin or BM-MSCs significantly ameliorated the inflammatory disorders as indicated by markedly decreased serum inflammatory markers; such as CRP, TNF-α, TGF-β and CD 95; compared to the untreated diabetic rats. In addition, insulin or BM-MSCs therapy was found to suppress pancreatic auto-immunity resulting in an obvious pancreatic cells apoptosis arrest in diabetic rats; which was confirmed by declined pancreatic CD4+, CD8+, annexin, P53 and caspase-3 levels accompanied by BCl-2 level elevation; compared to the diabetic group. Current findings provided additional evidence that BM-MSCs therapy has antioxidant, anti-inflammatory, immunomodulatory and anti-apoptotic characteristics which may greatly ameliorate diabetic patients' health through minimizing various DM side effects.

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Key words: Antioxidant –Apoptosis – BM-MSCs – Diabetes – Inflammation –Oxidative stress– Streptozotocin.

1. Introduction

Diabetes mellitus (DM) is considered as a chronic metabolic disorder due to irregularities in glucose metabolism, as a result of insulin dysregulation that characterized by elevated blood glucose levels with carbohydrate, protein, and fat metabolism abnormalities **(Jayakrishnapillai *et al.*, 2017)**. Type 1 DM (T1DM) is a chronic autoimmune disorder in which the immune system attacks endogenous pancreatic β cells resulting in insulin deficiency, chronic hyperglycemia, and long-term complications (**Chen *et al.*, 2017).** This form of diabetes is caused by a complex combination of genetic and environmental factors. When the susceptible population is exposed to environmental factors, T cells alter their function and secrete large amounts of interleukin-2 (IL-2). Cytokines such as γ-interferon (IFN-γ) trigger an inflammatory response in the pancreatic islets, which damages β cells, resulting in dysfunction and insulin secretion deficiency, thus triggering T1DM **(Liu *et al.*, 2013).**

T1DM are insulin-dependent, and it is generally lethal unless treated with daily exogenous insulin injections; which still the gold standard and the primary treatment of for these diabetic patients to replaces missing hormone in order to alleviate the symptoms, and nor diet neither exercise can prevent or reverse this type **(Xv *et al.*, 2017).** However, insulin treatment neither precisely controls the blood sugar levels, nor prevents the diabetes complications. It is impossible to maintain blood glucose levels within a range similarly with exogenous insulin similar to endogenous insulin secreted by β-cells of pancreas **(Jaen *et al.*, 2017).** Therefore, it is crucial to find new strategies to reverse hyperglycemia and alleviate the many debilitating complications of diabetes.

Stem cell-based strategies are therapeutically

potent for treating T1DM owing to their intrinsic regenerative capacity and immunomodulatory properties to arrest autoimmune β-cell destruction, preserve residual β-cell mass, facilitate endogenous regeneration, ameliorate innate/alloimmune graft rejection, restore β-cell specific unresponsiveness in absence of chronic immunosuppression and to reverse hyperglycemia **(Thakkar *et al*., 2017).** Stem cell-derived islet cell can avoid allograft rejection, thereby reducing the necessity for immunosuppressive therapy. They are also capable of secreting a variety of cytokines and improving the local microenvironment of pancreatic lesions, thereby improving the prognosis.

Addressing the inflammatory response may provide an opportunity for T1DM therapy, with the aim of controlling or arresting the progression of β-cell destruction and restoring glycemic control and immune hemostasis **(Davies *et al*., 2016).** Hence, in this review, we discuss the potential antioxidant and anti-inflammatory capacities of BM-MSCs in alleviation and suppression of the pancreatic experimentally-induced diabetic oxidative stress and apoptotic status; owing to theirimmunomodulating potency; which may encourage the further future study of using stem cell therapy in treatment of other different tissue diabetic complications.

2. Materials and Methods

Chemicals:

STZ was purchased from MP Biomedicals Company. (Bp 50067, Lllkrich, France). While, Insulinagypt containing insulin (100 IU/ml) was purchased from local pharmacy in El-Mansoura city. The drug was produced and supplied by Medical Union Pharmaceuticals Company, Egypt.

BM-MSCs:

A. Preparation of BM-MSCs:

Isolated samples were obtained from the tibias and femurs of 6–8-week-old albino rats. From these samples, (BM-MSCs) were obtained. These were then suspended in DMEM media, which contained 10% fetal bovine serum and penicillin/streptomycin as an antibiotic, respectively. The atmospheric state was adjusted at 5% carbon dioxide. Afterwards, an inverted microscope was used to conduct morphological characterization in order to confirm the identity of BM-MSC **(Hamza *et al*., 2016)**.

B. Characterization of BM-MSCs via flow cytometry:

PE-conjugated CD44 and CD49 antibodies and FITC conjugated CD45 antibodies were used to perform flowcytometry analysis on the BM-MSCs. This was done to verify that the phenotype of the stem cells was retained after expanding in the cell culture. As the BM-MSCs were incubated, each of the antibodies was placed against the surface markers: for 4 min for the CD44 and CD90 antibodies and for 30 min for the CD45 antibody, all at -4 ºC. Afterwards, flow cytometry analysis was performed on the samples **(Hamza *et al*., 2016)**.

All isolated MSCs from T1D donors and HCs were confirmed by flow cytometry to express the MSC marker profile according to the International Society for Cellular Therapy guidelines (positive for CD73, CD90, CD105, and HLA-I and negative for CD14, CD34, CD45, and HLA-II) **(Davies *et al*., 2016)**.

Animals

Adult male albino rats (*Rattusrattus*), weighing 100-120 g. Animals were obtained from the laboratory animal facility of the National Research Center, Dokki, Giza, Egypt. They were housed in plastic cages and were maintained at 22°C under a photoperiod of 12 h light / 12 h darkness, where food and water were provided *ad libitum*. After a week of acclimatization before dietary manipulation, the rats were divided into four groups each of six animals. All experimental procedures were conducted in accordance with internationally accepted principles for laboratory animal use and care; and were approved by the Ethics Committee in the Faculty of Science, Mansoura University, Egypt.

Induction of diabetes

Overnight fasting rats were injected intraperitoneally with a single dose of freshly prepared STZ solution (45 mg/kg body weight) dissolved in citrate buffer, pH 4.6. Seventy-two hours after induction, diabetes was confirmed by examining blood glucose level using Glukotest of diagnosis glucose level by ACCU–CHEKGo apparatus obtained from Roche Company, Germany **(Abo-Youssef and Messiha, 2013).** Rats with fasting blood glucose level over 200 mg/dl were considered as diabetic rats **(Koroglu *et al*., 2015).**

Experimental design

1. Control group: Animals received intraperitoneal single dose of citrate buffer, pH 4.6.

2. Diabetic untreated group: Animals received intraperitoneal single dose of STZ (45 mg/kg b.w.), dissolved in citrate buffer, pH 4.6.

3. Diabetic insulin-treated groups: Animals received intraperitoneal single dose of STZ (45 mg/kg b.w.), dissolved in citrate buffer, pH 4.6, then these diabetic rats received subcutaneous insulin injection dose (0.75 IU/100 g b.w dissolved in 0.75 ml normal saline), once daily **(Abdel-Razek, 2010).**

4. Diabetic BM-MSCs-treated groups: Animals received intraperitoneal single dose of STZ (45 mg/kg b.w.), dissolved in citrate buffer, pH 4.6, then these diabetic rats received intravenous single dose of BM-MSCs ($1x10^6$ cell/rat).

✓ All treatments were carried out for continuous 4 weeks.

Samples collection

At the end of the experimentation period, overnight fasted rats were anesthetized using diethyl ether before being dissected. Pancreas specimens were quickly separated and an appropriate part was labeled and kept at -20 °C for subsequent flowcytometric analysis.

Biochemical determinations

Pancreatic levels of MDA, GSH and TAC as well as the activities of SOD, CAT and GST were estimated by the methods of **Prins and Loose (1969), Nishikimi *et al*. (1972), Habig *et al*. (1974), Bock *et al*. (1980), Ohkawa *et al*. (1982), and Koracevic *et al*. (2001),** respectively, using kits from Bio Diagnostic Company (Egypt) according to the instructions of the supplier. According to the method of **Young, (2001)**, tissue ROS content was measured by kit purchased from AMSBIO, UK.; while XO activity measurement occurred by using XO–kit from Bio Vision Company, USA.

Using kits from SPINREACT diagnostics Company, Spain; pancreatic CRP level was estimated by the method of **Vaishnavi, (1996).** Meanwhile, flowcytometric analysis of pancreatic TNF-α, TGF-β, CD 95, CD 4, CD 8, annexin, P53, caspase-3 and BcL-2were determined according to the method of **Tribukait *et al*. (1975),** using FACS caliber flowcytometer (Becton Dickinson, Sunnyvale, CA, USA), equipped with a compact air cooked low power 15 m watt argon iron laser beam (488nm). Average evaluated nuclei per specimen are 20.000 (120 nuclei/second). Dean and Jett computer program for mathematical analysis was used to obtain the DNA histograms **(Dean and Jett, 1974).**

Statistical analysis

Obtained data were statistically evaluated with SPSS 17.5 software. *P* values equal or less than 0.05 were considered the minimal level of significance. All the results were expressed as the mean ± SE for six animals in each group. Percentage of change in the treated groups was calculated.

3. Results

Table (1): Pancreatic MDA, ROS and XOlevels.

Group Parameter		Control	Diabetic (D)	D + Insulin	D + BM-MSCs
MDA (n mol/g)	Mean ± SEM	10.46 ± 0.52	29.48 ± 1.47 [a]	20.13 ± 1.01 [ab]	14.63 ± 0.73 [abc]
	*		+ 181.83	+ 92.44	+ 39.86
	**			- 31.71	- 50.37
	***				- 27.32
ROS (n mol/g)	Mean ± SEM	0.78 ± 0.04	2.04 ± 0.10 [a]	1.12 ± 0.06 [ab]	0.88 ± 0.04 [b]
	*		+ 161.53	+ 43.58	+ 12.82
	**			- 45.09	- 56.86
	***				- 21.42
XO (nmol/min/ml)	Mean ± SEM	21.40 ± 1.07	49.40 ± 2.47 [a]	27.80 ± 1.39 [b]	26.40 ± 1.32 [b]
	*		+ 130.84	+ 29.90	+ 23.36
	**			- 43.72	- 46.55
	***				- 5.03
GSH (mg/gm)	Mean ± SEM	53.20 ± 2.66	20.56 ± 1.03 [a]	50.28 ± 2.51 [b]	50.26 ± 2.51 [b]
	*		- 61.35	- 5.48	- 5.52
	**			+ 144.55	+ 144.45
	***				- 0.03
SOD (u/gm)	Mean ± SEM	10.84 ± 0.54	4.56 ± 0.23 [a]	9.78 ± 0.49 [b]	9.68 ± 0.48 [b]
	*		- 57.93	- 9.77	- 10.70
	**			+ 114.47	+ 112.28
	***				- 1.02
CAT (u/gm)	Mean ± SEM	0.58 ± 0.03	0.34 ± 0.02 [a]	0.49 ± 0.02 [b]	0.52 ± 0.03 [b]
	*		- 41.37	- 15.51	- 10.34
	**			+ 44.11	+ 52.94
	***				+ 6.12
GST (Mmol/gm)	Mean ± SEM	6.24 ± 0.31	2.80 ± 0.14 [a]	5.18 ± 0.26 [ab]	5.42 ± 0.27 [b]
	*		- 55.12	- 16.98	- 13.14
	**			+ 85.00	+ 93.57
	***				+ 4.63
TAC (mg/gm)	Mean ± SEM	1.52 ± 0.08	0.58 ± 0.03 [a]	1.28 ± 0.06 [b]	1.50 ± 0.08 [b]
	*		- 61.84	- 15.78	- 1.31
	**			+ 120.68	+ 158.62
	***				+ 17.18

Values expressed as mean ± SEM (n = 6). **a, b** and **c** are Significant differences ($P \leq 0.05$) comparing to control, diabetic and diabetic insulin-treated groups respectively.*. ** and *** are % of change comparing to control, diabetic and diabetic insulin-treated groups respectively.

The data presented in (**table 1)** showed pancreatic MDA, ROS, XO, GSH, SOD, CAT, GST and TAC concentrations. The diabetic group showed a significant increase in pancreatic MDA, ROS and XO while showed a marked decline in GSH, SOD, CAT, GST and TAC levels when compared to normal control values. The results revealed that treatment of diabetic rats with either insulin or BM-MSCs showed significant decrease in MDA, ROS and XO, while a significant increase in GSH, SOD, CAT, GST and TAC levels; when compared to the diabetic group; while a non-significant changes compared to control group were observed; except for MDA in both diabetic treated groups and ROS in case of insulin-treated diabetic rats which were still significantly higher while GST level in insulin-treated diabetic rats was still markedly lower, compared to the control group. However, there were no remarkable changes between the results of insulin and BM-MSCs treated diabetic rats except for MDA which showed a marked enhancement in treatment with BM-MSCs than insulin treatment.

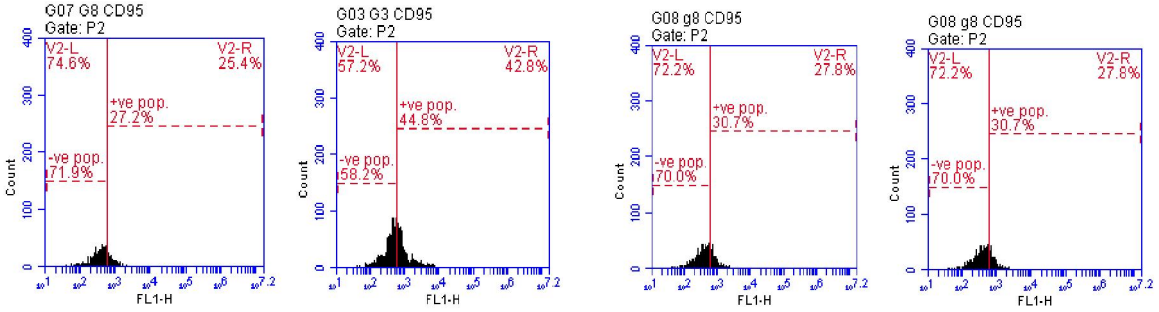
Table 2 illustrated the pancreatic CRP, TNF-α, TGF-β and CD95 levels. The present study showed significant increases in all inflammatory markers as seen in diabetic group when compared to control one. Concerning treatment, both treated groups showed significant decreases in all inflammatory markers compared to the diabetic group, while markedly declined values; for CRP in both groups in addition to TGF-β and CD95 in case in insulin treated group; still significantly higher when compared to the control group. No detectable changes were recorded in CRP, TNF-α and CD95 levels between the two diabetic treated groups, while TGF-β level exhibited a marked enhancement in BM-MSCs treated rats, compared to insulin group.

Table (2): Pancreatic CRP, TNF-α, TGF-β and CD95levels.

Group Parameter		Control	Diabetic (D)	D + Insulin	D + BM-MSCs
Serum CRP (g/dl)	**Mean ± SEM**	3.60 ±0.18	18.20 ± 0.91 [a]	7.75 ±0.39 [ab]	7.33 ± 0.37[ab]
	*		+ 405.55	+ 115.27	+ 103.61
	**			- 57.41	- 59.72
	***				- 5.41
Pancreatic TNF-α (%)	**Mean ± SEM**	27.80 ±1.39	44.02 ±2.20 [a]	33.70 ±1.69 [b]	30.00 ±1.50 [b]
	*		+ 58.34	+ 21.22	+ 7.91
	**			- 23.44	- 31.84
	***				- 10.97
Pancreatic TGF-β (%)	**Mean ± SEM**	17.40 ±0.87	46.94 ±2.35 [a]	32.25 ±1.61 [ab]	20.88 ±1.04 [bc]
	*		+ 169.77	+ 85.34	+ 20.00
	**			- 31.29	- 55.51
	***				- 35.25
Pancreatic CD95 (%)	**Mean ± SEM**	27.17 ± 1.36	48.20 ± 2.41 [a]	36.00 ± 1.80 [ab]	32.17 ± 1.61 [b]
	*		+ 77.40	+ 32.49	+ 18.40
	**			- 25.31	- 33.25
	***				- 10.63

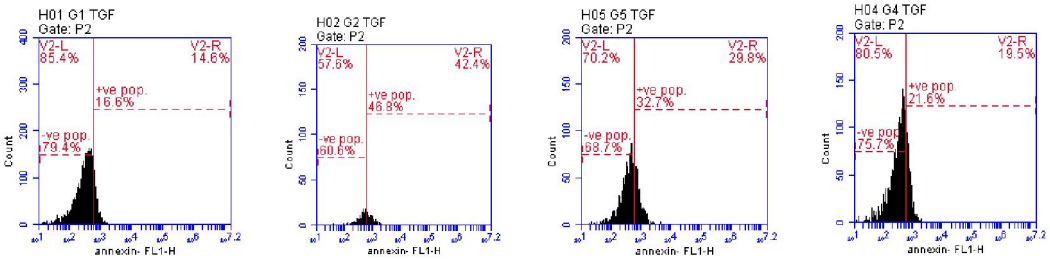
Values expressed as mean ± SEM (n = 6). **a, b** and **c** are Significant differences ($P \leq 0.05$) comparing to control, diabetic and diabetic insulin-treated groups respectively. ***.** ** and *** are % of change comparing to control, diabetic and diabetic insulin-treated groups respectively.

TNF-α %



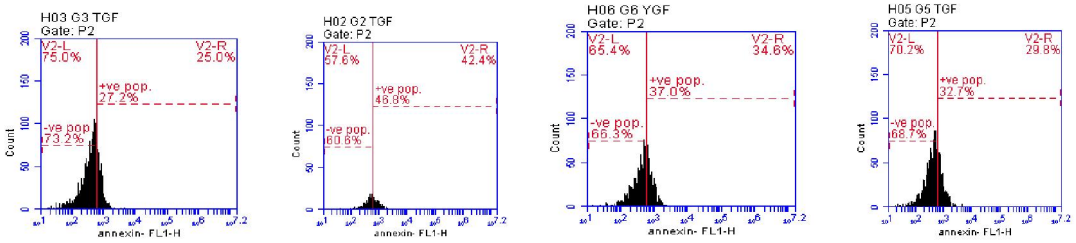
Control group Diabetic group D + Insulin group D + BM-MSCs group

TGF-β %



Control group Diabetic group D + Insulin group D + BM-MSCs group

CD95 %



Control group Diabetic group D + Insulin group D + BM-MSCs group

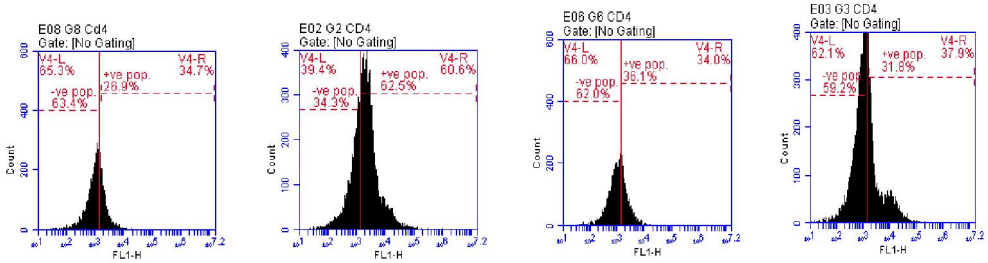
Table 3 represented pancreatic CD4 and CD8 %. The obtained results showed that significant increases were seen in diabetic group when compared to normal control one. On the other hand, both diabetic treated groups showed significant decreases in both parameters compared to the diabetic group while a non-significant elevation when compared to the control group. The results reveal non-significant changes between insulin and BM-MSCs treated diabetic rats.

Table (3): Pancreatic CD4 and CD8 %in different animal groups.

Group Parameter		Control	Diabetic (D)	D + Insulin	D + BM-MSCs
Pancreatic CD4 (%)	**Mean ± SEM**	27.97 ±1.40	60.17 ±3.01 [a]	36.03 ±1.80 [b]	32.17 ±1.61 [b]
	*		+ 115.12	+ 28.81	+ 15.01
	**			- 40.11	- 46.53
	***				- 10.71
Pancreatic CD8 (%)	**Mean ± SEM**	29.20 ±1.46	54.40 ±2.72 [a]	36.77 ±1.84 [b]	31.80 ±1.59 [b]
	*		+ 86.30	+ 25.92	+ 8.90
	**			- 32.40	- 41.54
	***				- 13.51

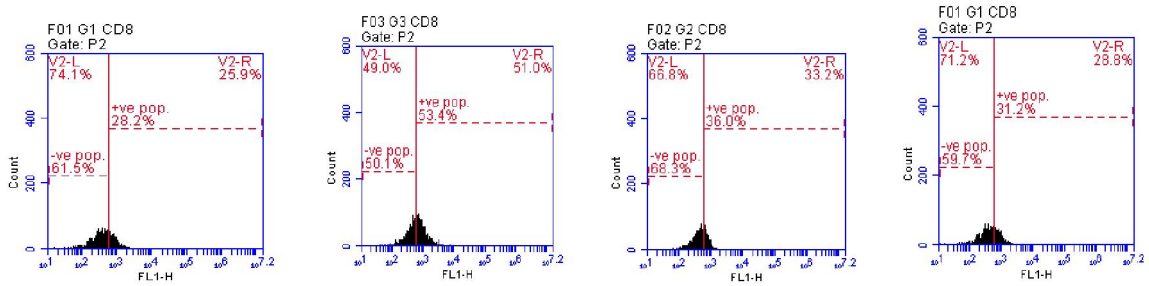
Values expressed as mean ± SEM (n = 6). **a, b** and **c** are Significant differences ($P \leq 0.05$) comparing to control, diabetic and diabetic insulin-treated groups respectively. ***. **** and ******* are % of change comparing to control, diabetic and diabetic insulin-treated groups respectively.

CD4 %



Control group Diabetic group D + Insulin group D + BM-MSCs group

CD8 %



Control group Diabetic group D + Insulin group D + BM-MSCs group

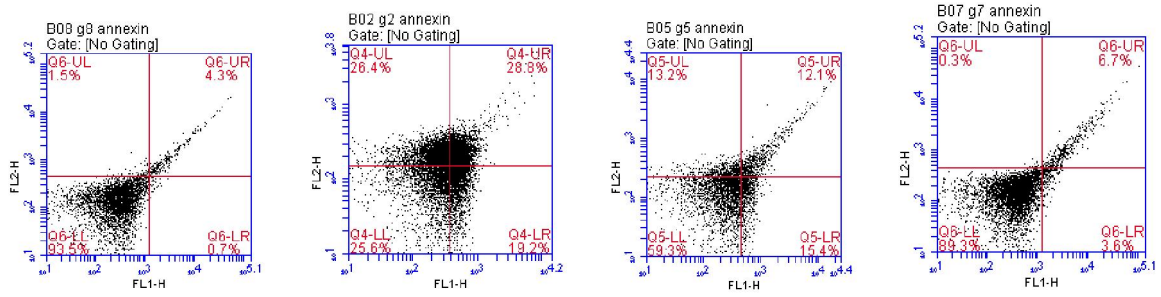
Table 4demonstrated pancreatic annexin, P53, caspase 3 and BCl2 %. Regarding to diabetic group, significant increases in annexin, P53 and caspase 3% while significant decrease in BCl2 % were obtained when compared to normal control one. In contrary, diabetic groups treated with either insulin or BM-MSCs showed significant decreases in annexin, P53 and caspase 3%while significant increase in BCl2 % when compared to the diabetic group, although values of annexin and caspase 3 were still significantly higher while BCl2 level was still significantly lower in diabetic rats treated with insulin; when compared to normal control group. Diabetic rat's treated with BM-MSCs showed a significant improvement in most of the measured parameters compared to diabetic rats treated with insulin except for P53 % where it showed non-significant change.

Table (4): Pancreatic annexin, P53, caspase 3 and BCl2 %.

Group Parameter		Control	Diabetic (D)	D + Insulin	D + BM-MSCs
Annexin (%)	**Mean ± SEM**	4.40 ±0.22	29.64 ±1.48 [a]	11.74 ±0.59 [ab]	6.60 ±0.33 [bc]
	*		+ 573.63	+ 166.81	+ 50.00
	**			- 60.39	- 77.73
	***				- 43.78
P53 (%)	**Mean ± SEM**	29.02 ±1.45	61.34 ±3.07 [a]	35.64 ±1.78 [b]	32.54 ±1.63 [b]
	*		+ 111.37	+ 4.23	+ 12.12
	**			- 41.89	- 46.95
	***				- 8.69
Caspase 3 (%)	**Mean ± SEM**	37.20 ±1.86	72.88 ±3.64 [a]	54.50 ±2.73 [ab]	41.55 ±2.08 [bc]
	*		+ 95.91	+ 46.50	+ 11.69
	**			- 25.21	- 42.98
	***				- 23.76
BCL2 (%)	**Mean ± SEM**	43.18 ± 2.16	14.60 ±0.73 [a]	29.44 ±1.47 [ab]	39.05 ±1.95 [bc]
	*		- 66.18	- 31.82	- 9.56
	**			+ 101.64	+ 167.46
	***				+ 32.64

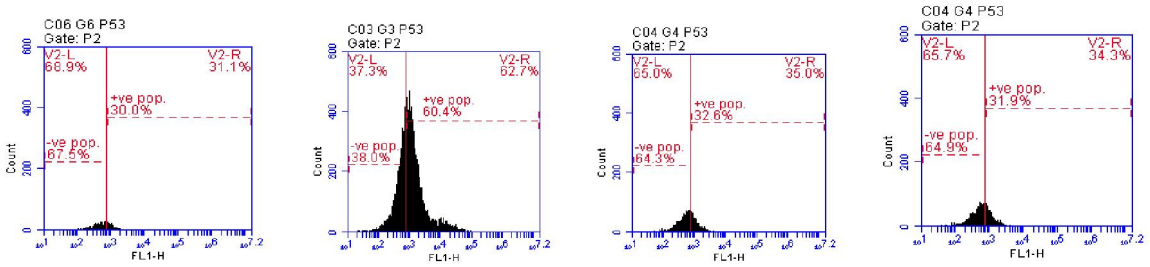
Values expressed as mean ± SEM (n = 6). **a, b** and **c** are Significant differences ($P \leq 0.05$) comparing to control, diabetic and diabetic insulin-treated groups respectively ***.** ** and *** are % of change comparing to control, diabetic and diabetic insulin-treated groups respectively.

Annexin %



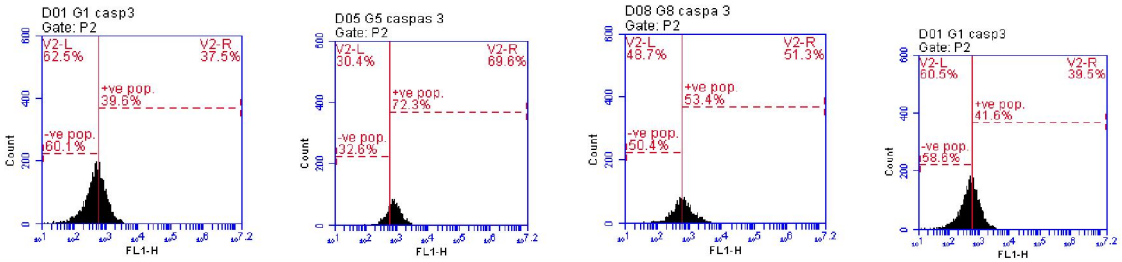
Control group Diabetic group D + Insulin group D + BM-MSCs group

P 53 %



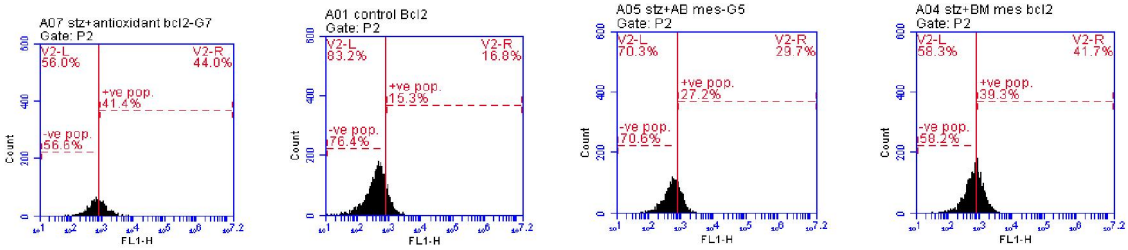
Control group Diabetic group D + Insulin group D + BM-MSCs group

Caspase 3 %



Control group Diabetic group D + Insulin group D + BM-MSCs group

BCl2 %



Control group Diabetic group D + Insulin group D + BM-MSCs group

4. Discussion

There is a great deal of interest in cell therapy for failing tissue and repairing the body. In recent years, MSCs derived from different adult tissues have attracted significant attention for the treatment of DM. Mesenchymal stem cells (MSCs) are multipotent precursor cells having self-renewal ability making them a candidate for use in regenerative medicine**(Ramanathan *et al.*, 2017).** They are known to promote the regeneration of pancreatic islet β cells, protect endogenous pancreatic islet β cells from apoptosis, and ameliorate insulin resistance of peripheral tissues by providing a supportive niche microenvironment driven by the secretion of paracrine factors or the deposition of extracellular matrix **(Zang *et al.*, 2017).**

Oxidative stress plays a major important role in the development of diabetic complications**,** science it is associated with enhanced production of ROS which reacts with lipids, protein, and DNA to promote oxidative stress-induced cellular damage **(Ali *et al.*, 2017). In the present study**, **STZ-induced diabetes in rats** clearly resulted in an increased oxidative stress, as demonstrated by the elevated pancreatic MDA, ROS and XO concomitant to decreased pancreatic antioxidants (GSH, SOD, CAT and GST as well as TAC). These findings are in agreement with **Zang *et al.* (2017)** who reported that the deviations in control of glucose level is sufficient to trigger an array of maladaptive processes including increased generation of ROS and oxidative stress injury; which they were recognized as a major etiological factor in the development of diabetes**.** Similar assumption was

cleared by **Turkmen (2017)** who stated that, the well-established features of STZ-diabetic complications include increased serum glucose levels along with ROS production, leading to considerable cellular damage and to a point of no return in apoptosis when insufficient cytoprotective and ROS scavenging molecules are available**.** However, in DM, protein glycation and glucose auto-oxidation may generate free radicals, which in turn catalyze LPO (**Ghosh *et al*., 2015).**

Regarding antioxidants, **Roslan *et al.* (2017)** showed that STZ increase intracellular glucose levels resulting in an overproduction of LPO and ROS; mostly via mitochondrial electron transport chain; accompanied with the decreased activity of antioxidant enzymes, such as SOD, CAT and GSH-Px; participating in the establishment of oxidative stress in diabetic rats compared to control group**.** Recently, in **Jamshidi *et al.* (2018)** study, the oxidative stress due to STZ was approved via the significant increase in the total oxidative status (TOS) and MDA, decrease in the TAC level and catalase activity.

For insulin and BM-MSCs treatment, the data in the present study showed decreased MDA, ROS and XO levels accompanied by a significant increase in the antioxidant enzyme (SOD, CAT and GST) activities and GSH content in addition to TAC in the pancreas, compared to the diabetic untreated group. The oral treatment with insulin in the form of trimethylchitosan-based nanoparticles and its effect on oxidative stress is shown by the increase in the TAC and the decrease in the total oxidative status (TOS) and MDA levels. These results, besides the decrease in the glucose level after eight weeks post-STZ injection, showed the efficacy of coated insulin in controlling the glucose level and decreasing the oxidative stress related to hyperglycemia **(Jamshidi *et al*., 2018).**

However, in the study by **Chandravanshi and Bhonde (2017),** after 48 h of MSCs transplantation, results revealed reduced levels of ROS, NO, and super oxide ions, suggesting the protective effect of MSCs on islet cells against oxidative stress-mediated cellular injuries. Moreover, MSCs increased expression of renal SOD and CAT, which may associate with detoxifying ROS to prevent oxidative renal damage. **Li *et al.* (2014)** data suggest that the enhanced protective effect of MSCs against DM might be associated with inhibition of oxidative stress-induced renal cell apoptosis and inflammation, in mouse kidneys**,** since it was shown that ROS is a source of cell stress and apoptosis. MSC injection inhibited the activity of renal inducible nitric oxide synthase (iNOS), which is a major source of reactive oxidant stress in murine models of lupus nephritis. They also observed that the expression of GST in MSC-treated mice was accompanied by over-expression of CAT and GPX, which are both potential scavengers of free oxidative radicals.

In this context, according to **Nejad-Moghaddam *et al.* (2016)** study, MSCs administration mitigates oxidative stress and inflammation in sulfur mustard-exposed patients. Expressions of antioxidants genes such as GSH-Rd and GSH-Px were increased after cell therapy. Additionally, a trend for increased value of GSH and decreased levels of MDA was observed from baseline to final evaluation times**.** Given that oxidative stress injury induced by hyperglycemia is recognized as a major etiological factor in the development of diabetes, further investigation of the antioxidant capacity of MSCs for the promotion of islet survival may validate the utility of MSC co-transplantation with islet transplantation **(Zang *et al*., 2017).** Furthermore, **Ramanathan *et al.* (2017)** found a significant decline in hepatic MDA accompanied by marked SOD activity elevation in d-Galactosamine induced acute liver injury in mice compared to control.

Worsening inflammation over time raise concern regarding premature development of DM **(Katz *et al*., 2018). Data in this study showed a** significant elevation in inflammatory markers levels, in CRP, TNF-α, TGF-β and CD95, in pancreas of the diabetic rat group compared with the control group. These results are in accordance with **Rashid and C.Sil (2015)** who reported a proinflammatory cytokines (TNF-α, IFN-γ and IL1-β) levels increase in STZ-induced diabetic male rats serum compared to control group.

In addition to provoking hyperglycemia, STZ elevated serum levels of IL-1β and hyaluronic acid, induced edema in the pancreatic insular tissue and its infiltration by inflammatory cells (neutrophils, lymphocytes, and macrophages) and fibroblasts. Inflammation in pancreatic islets was accompanied by necrotic processes and decreasing counts of insulin producing β-cells **(Dygai *et al*., 2016).** Similar results were declared by the molecular genetics analysis which showed a significant up-regulation of serum and renal IL-8, TGF-β and MCP-1 gene expression level in diabetic group with respect to the control group, attributed to the increased oxidative stress and inflammation due to hyperglycemia **(Hamza *et al*., 2016).** These findings are in great agreement with the present results.

Regarding insulin and BM-MSCs treatment for DM, **the current study showed** a marked reduction in pancreatic levels of various inflammatory markers, such as CRP, TNF-α, TGF-β and CD95, compared with the diabetic group. MSCs have shown particular promise based on their accessibility from

adult tissues and their diverse mechanisms of action including secretion of paracrine anti-inflammatory and cyto-protective factors **(Griffin *et al*., 2016).** MSCs also function as trophic mediators that promote angiogenesis, have anti-apoptotic effects and reduce inflammation **(Ko *et al*., 2015).** These findings are in agreement with those of **Christ *et al.* (2015)** who stated that MSCs, harbor anti-inflammatory properties.

Exposure of MSCs to proinflammatory cytokines such as IFN-g and TNF-a has been reported to induce an anti-inflammatory MSC phenotype **(Davies *et al*., 2016).** Through production of soluble factors, MSCs can alter the secretion profile of dendritic cells resulting in increased production of IL-10, an anti-inflammatory cytokine, and decreased production of IFN-γ and IL-12 **(Davey *et al*., 2014)**. In the study by **Chandravanshi and Bhonde (2017),** after 48 h of MSCs transplantation, lower expression of TGF-β, TNF-α and other pro-inflammatory cytokines was noticed. In another study, **Yu *et al.* (2017)** demonstrated that intravenous administration of MSCs could reduce allergic symptoms significantly and suppress eosinophilic inflammation, in addition to significantly suppressed Th2-associated cytokines production [IL-4, 5 and 13], and improved Th1 cytokine [IFN-γ] production, in a mouse model of allergic airway inflammation**.**

T1DM is a chronic autoimmune disease characterized by absence of insulin secretion due to destruction of the pancreatic β cells**(Ganjali *et al*., 2017).** Thus, **the current results of the flow cytometric data**, showed presence of a marked elevation in pancreatic CD4 and CD8 T-cells, in response to STZ injection in diabetic rats, in comparison to control group. These current data supported the assumption made by **Kuhn *et al*. (2016)** who proposed that progressive defective immune regulation is a hallmark of T1DM; which is certainly one of the prototypic T cell-mediated autoimmune diseases. Failure of immune regulation in T1DM is linked to a faulty sensitivity of pathogenic cells to Treg-mediated suppression**.**

These findings made by the current study were in agreement with **Tai *et al.* (2016)** who cleared that T1DM is an organ-specific autoimmune disease characterized by T cell-mediated destruction of the insulin-producing pancreatic β cells.

In this line, **Kracht *et al.* (2016)** reported that T1DM is characterized by the selective and progressive destruction of insulin-producing β cells by the immune system. An incomplete thymic selection against self-reactive islet antigens partly explains how these T cells reach the periphery and become diabetogenic. However, **Sarikonda *et al.* (2014)** reported that CD4 T-cell reactivity to islet antigens was common in both T1DM and T2DM patients, while the presence of CD8 T-cell autoreactivity was unique to subjects with T1DM.

MSCs-based therapeutic intervention are of particular interest as an emerging strategy for the treatment of autoimmune diseases, owing to their immunosuppressive properties **(Kim *et al*., 2018).** MSCs could control the immune function of most immune cells involved in allergen and antigen recognition of antigen-presenting cells, natural killer cells, T cells, and B cells **(Yu *et al*., 2017). The current study results showed** a significant decrease in both CD4 and CD8 T-cells in pancreas of diabetic rats treated by insulin or BM-MSCs, compared to the untreated diabetic rats. These findings are in agreement with those of **Christ *et al.* (2015)** who stated that MSCsharbor immunomodulatory properties, science their pleiotropic actions include the modulation of immune reactions.

In diabetes, progressive pancreatic β cell loss can be caused by STZ injection, which induces pancreatic β cell apoptosis and decreases insulin secretion, thereby accelerating the hyperglycemic state **(Oh *et al.*, 2015)**. **The present results are in harmony**with these findings as it showed that the annexin %, P 53 % and caspase 3 % were increased in pancreas of STZ diabetic rats along with decreased BCl_2 % in comparison to the control rat group.

Chen *et al.* (2017) demonstrated that β cell death is elevated in high risk DM subjects. After onset, β cell destruction by the ongoing autoimmune infiltration continues and is additionally exacerbated by the increasing metabolic and glycemic overload causing ER stress and apoptosis. Conceivably, as a result from studying mostly severe and early onset cases, near total loss of β cell mass (>80% reduction) was a long held general paradigm for T1DM pathogenesis. DM is associated with enhanced production of ROS which lead to considerable cellular damage and to a point of no return inhibit cell proliferation and initiate cell apoptosis and cell death, when insufficient cytoprotective and ROS scavenging molecules are available **(Ali *et al*., 2017).** In addition to provoking hyperglycemia, STZ induces inflammation in pancreatic islets, accompanied by necrotic processes and decreasing counts of insulin producing β-cells **(Dygai *et al*., 2016).**

Treatment with STZ enhanced levels of signaling molecules of ER stress dependent and independent apoptosis (cleaved caspase-12,9,8 and 3 respectively) in diabetic rats **(Rashid and C.Sil, 2015).** Consistent with these findings, a recent study showed that, STZ could induce pancreatic β-cells apoptosis in diabetic rats, associated with decreased anti-apoptotic BCl_{-2} expression and increased pro-apoptotic Bax expression **(Muruganathan *et al*., 2017).** Previous *in*

vitro study has demonstrated that high glucose levels stimulate TNF-α increased production, which, in turn, induced upregulation of genes that interfere with progression through the cell cycle, leading to caspase-3 activation and apoptosis **(Ko et al., 2015).**

The current study results revealed that, treatment of diabetic rats with either insulin or BM-MSCs down-regulated pancreatic annexin, P53 and caspase 3 levels, while up-regulated BCl_2 expressions at the same time compared to the diabetic-untreated rats. These results indicate that caspase 3 is associated with annexin and P53 in inducing apoptosis in contrast to BCl_2 which responsible for inhibiting this process. **Ning *et al*. (2016)** subsequently clarified that insulin led to a rapid and short-period inhibition of autophagy via stimulation of phosphorylated-Akt and reversed saturated fatty acids-induced up-regulation of caspase-3 and P53 levels in rat's hepatocytes, was insufficient to aggravate saturated fatty acids-induced lipotoxicity.

However, the present research had been focusing on the treatment of diabetes with BM-MSCs. These findings are in agreement with those of **Christ *et al*. (2015)** who stated that MSCs, harbor anti-apoptotic and pro-proliferative properties, causing stimulation of cell proliferation, and the attenuation of cell death responses. In **Chandravanshi and Bhonde (2017)** study**,** after 48 h of MSCs transplantation, islet cells exhibited higher viability and reduced apoptosis as compared with diabetic rats. Biological activity factors, such as VEGF, IGF-1, and β-FGF, secreted by MSCs can regulate the local microenvironment of the damaged tissue, inhibit cell apoptosis, improve the immune defense system, and promote tissue regeneration and revascularization **(Bhansali *et al*., 2015)**. In the process of tissue repair MSC are also able to exert an action on the endogenous cells of the damaged tissue, for example by protecting them from apoptosis or stimulating their proliferation **(Sordia *et al*., 2017)**.

Conclusion

From the above results, it was clear that the future of β cell replacement therapy in DM treatment is very promising; although it is still challenging to protect these cells from autoimmune attack in type 1 diabetic patients. In the coming years, more clinical trials should be launched to move these technologies toward treatments to benefit diabetic patients in a more safe way of recovering.

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