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RESEARCH ARTICLE

BM-MSCS' IMMUNOMODULATORY, ANTI-INFLAMMATORY, ANTI-APOPTOTIC AND ANTIOXIDANT CAPACITY ROLES IN MODULATING THE ALTERED TISSUES' OXIDATIVE STRESS STATUS IN STZ-DIABETIC RATS: IN COMPARISON TO THE STANDARD INSULIN TREATMENT

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Abstract

Purpose: The present study was designed to investigate the probable hypoglycemic, antioxidant, anti-inflammatory, immuno-modulatory as well as anti-apoptotic protective capacities of bone marrow derived mesenchymal stem cells (BM-MSCs) in comparison with the standard insulin treatment in diabetic rats.

Methods: Animals were divided into four groups; control group, STZ-diabetic group (D), D+Insulin group and D+BM-MSCs group; and the study continued for four consecutive weeks.

Results: Insulin as well as BM-MSCs administration significantly improved the hyperglycemic status resulting from diabetes induction; as evidenced by lowered blood glucose, HbA1c and AGEs levels, while enhanced serum insulin, C-peptide and HO-1 levels, compared to the diabetic group. Regarding oxidative stress and antioxidants, both insulin or BM-MSCs injection significantly attenuate the oxidative stress status resulting from diabetes induction, as evidenced by down-regulating MDA, ROS and XO levels, accompanied by up-regulating the antioxidants' markers content as GSH, SOD, CAT, GST, TAC and HO-1 in different body tissues (Pancreas, Liver, Kidney and Heart) compared to the diabetic group. Also, treatment of diabetic rats with insulin or BM-MSCs significantly ameliorated the inflammatory disorders as indicated via 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 V, P53 and caspase-3 levels accompanied by Bcl-2 level elevation; compared to the diabetic group. Comparing both treatments together, most of the measured parameters were reverted back to near normal levels after BM-MSCs treatment confirming its anti-diabetic potency, in addition to their tissue protective and regenerative capabilities.

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Conclusion: Current findings clearly point out the health benefits of BM-MSCs; more than insulin; therapy in ameliorating various metabolic disorders including diabetic complications; owing to their marked antioxidant, anti-inflammatory, immunomodulatory and anti-apoptotic characteristics.

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Introduction:-

Recent times have witnessed large-scale human dramatic progress in diabetes mellitus (DM) patients' count; making it the most devastating, chronic, common non-communicable disease; that has become a major health concern and a serious problem across the globe for the developed and developing nations as well; particularly in the Middle East. The incidence of diabetes is advancing at an alarming rate, since around 400 million people worldwide suffer from DM; and this growing disease accounts annually for approximately 5% of all deaths around the world; and the number is rapidly increasing **(1).**

Type 1 diabetes mellitus (T1DM) is a pancreas chronic, autoimmune, multifactorial disorder causing irreversible progressive destruction of insulin-producing islets β-cells that eventually leads to loss of insulin production and absolute insulin deficiency, resulting in hyperglycemia and increased blood glucose levels as a result of glucose regulation failure. This form of diabetes is caused when the susceptible population is exposed to environmental factors; where T-cells alter their function and secrete large amounts of cytokines such as interleukin-2 (IL-2) and γ-interferon (IFN-γ) which trigger an inflammatory response in the pancreatic islets, which damages β cells, resulting in dysfunction and insulin secretion deficiency **(2).**

Diabetic patients are subjecting to a variety of serious chronic complications which significantly limiting diseased individual's regular productivity and activity, and decrease their life quality; creating marked social and economic burdens. T1DM is insulin-dependent, and it is generally lethal unless treated with daily exogenous insulin injection; which still the gold standard and the primary treatment of for these diabetic patients to replaced missing hormone in order to alleviate the symptoms, and nor diet neither exercise can prevent or reverse this type **(2).** However, insulin injections do not adequately mimic β cell function; sometimes resulting in hypoglycemia, ketosis and coma; as it 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 **(3).**

With no current cure, this disease management focusing nowadays on limiting complications via optimizing blood glucose control **(4).** Because in T1DM patients, β cells majority are lost by an autoimmune attack; injection of insulin only focuses in reversing hyperglycemia, not to increase β cells count **(5).** However, blood glucose optimal control alone could not prevent complications; therefore, to overcome diabetes, the best strategy might be through β cell mass replenish**;** which promote essentiality using an alternative treatment approach. New β cells generation is an important target in T1DM treatment**;** thus, stem cells usage development could be the ideal choice for this disease therapy **(6).**

Mesenchymal stem cells (MSCs) exist in almost all tissues; with marked differentiation capability into several cell type; holding notable promise in repairing of tissues in a cell replacement manner **(7).** A plenty of evidence illustrated that MSCs can delay T1DM onset and relieve hyperglycemia via improving pancreatic β cell regeneration, differentiating into IPCs, ameliorating insulin resistance, increase insulin production and promoting the conversion of α cells to β; thus preventing a lot of long-term complications, improving life quality and minimizing immunosuppression-related side effects **(8).** 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 **(9).**

Hence, in this review, we discuss BM-MSCs as an alternative cell source for DM treatment in alleviation and suppression of the tissue experimentally-induced diabetic oxidative stress and apoptotic status; owing to their immunomodulating potency and trans-differentiating capacity into IPCs; that may encourage the further future study of using stem cell therapy in treatment of other different tissue complications' diseases.

Materials And Methods:-

Chemicals

STZ was purchased from MP-Biomedicals Company (Bp 50067, Lllkrich, France). While, Insulinagypt containing insulin (100 IU/ml) produced and supplied by Medical Union Pharmaceuticals Company, Egypt; was purchased from a local pharmacy in El-Mansoura city, Ciro, Egypt. BM-MSCs Culture media constituents were purchased from Sigma Aldrich Company, Egypt; and were of pure chemical gradient**.** Fasting serum glucose concentration were estimated using SPINREACT diagnostics kit, Spain. Meanwhile, HbA1c, AGEs and HO-1were measured by using kits obtained from Teco Diagnostics, USA. Serum insulin was assessed by ELISA kit purchased from Boehringer Mannheim, Germany, using Boehringer Analyzer ES 300; while C-peptide measurement occurred by enzyme immunoassay (EIA) kit purchased from Bio Vision, USA. Tissue levels of Malondialdehyde (MDA), Glutathione (GSH) and Total antioxidant capacity (TAC) as well as the activities of Superoxide dismutase (SOD), Catalase (CAT) and Glutathione-S-transferase (GST) were estimated by using kits from Bio Diagnostic Company (Egypt) according to the instructions of the supplier. On the other hand, Heme-oxygenase 1 (HO-1) were estimated by using kits obtained from Teco Diagnostics, USA. However, tissue Reactive oxygen species (ROS) content was measured by kit purchased from AMSBIO, UK.; while Xanthine oxidase (XO) activity measurement occurred by using XO–kit from Bio Vision Company, USA. Meanwhile, flow cytometric analysis of tissue TNF-α, TGF-β, $CD95^+$, IL-6, $CD4^+$, $CD8^+$, annexin V, P53, caspase-3, Bcl-2 and G0/G1 % were determined via Sigma Aldrich Company (St. Louis, Mo 6, USA) kits'**;** using FACS caliber flow cytometer (Becton Dickinson, Sunnyvale, CA, USA)**.**

BM-MSCs

Preparation of BM-MSCs:

Isolated BM-MSCs were obtained from 6–8-week-old male rats (femurs and tibias) and suspended in Dulbecco's modified Eagle's medium (DMEM) media (contain streptomycin/penicillin as an antibiotic (100 µg/ml and 100 U/ml respectively) and 10% fetal bovine serum (FBS), at -22 ℃); in an atmospheric state of 5% carbon dioxide. Then, morphological characterization was carried out using an inverted microscope to confirm the BM-MSCs identity **(10).**

Characterization of BM-MSCs via flow cytometry:

BM-MSCs flow cytometric analysis was performed to verify retaining of their phenotype following their expanding in the cell culture. $CD44^+$, $CD45^+$ and $CD90^+$ antibodies were placed against their surface markers: 30 min for the $CD45^+$ antibody and 4 min for both $CD44^+$ and $CD90^+$ antibodies, all at -20°C; before flow cytometry was assessed **(10).**

Animals

100-120 g *Rattus rattus* male albino rats obtained from the National Research Center, Dokki, Giza, Egypt were housed in plastic cages and were maintained under conventional laboratory conditions throughout the study (22°C under 12 h light/12 h darkness photoperiod). Rats were fed standard pellet chow and water *ad libitum*. After acclimatization for one week, rats were divided into 4 groups each of 6 animals. All experimental procedures were approved by the Animal Ethics Committee of the Faculty of Science, Mansoura University, Mansoura, Egypt.

Experimental design

1. **Control group:** Rats received intraperitoneal single dose of citrate buffer (2 ml/kg b.w.), pH 4.6.
2. **Diabetic untreated group:** Rats received intraperitoneal single dose of STZ (45 mg/kg b.w.) dissolved in citrate buffer (2 ml/kg b.w.), pH 4.6.
3. **Diabetic insulin-treated group:** Diabetic rats received subcutaneous insulin injection dose (0.75 IU/100 g b.w.)**,** once daily for 4 weeks.
4. **Diabetic BM-MSCs-treated group:** Diabetic rats received intravenous single dose of BM-MSCs ($1x10^6$ cell/rat).

Induction of diabetes

Overnight fasting rats were injected intraperitoneally with a single dose of freshly prepared STZ solution (45 mg/kg b.w.) dissolved in citrate buffer, pH 4.6; while control rats received the vehicle alone. Three days after induction, diabetes was confirmed by examining blood glucose level from tail vein using Glukotest of diagnosis glucose level by ACCU–CHEKGo apparatus (Roche Company, Germany). Rats with fasting blood glucose level over 200 mg/dl were selected for randomized grouping and considered as diabetic **(11).**

Samples collection

At the end of the experimentation period (4 weeks), overnight fasted rats were anesthetized using diethyl ether before being dissected and blood samples were immediately withdrawn directly from the heart. Only few droplets of blood samples were placed in clean heparinized tubes for measuring glycosylated hemoglobin (HbA1c). In clean non-heparinized centrifuge tubes, the remaining of blood samples were collected and let to stand for 15 min, after which they were centrifuged at 3000 rpm for 15 min. Blood sera were carefully separated, labeled and kept at -20 °C for subsequent biochemical analysis. On the other hand, either pancreas, liver, kidney and heart specimens were quickly separated and an appropriate part was weighed and homogenized forming 10% (w/v) homogenate in cold distilled water, labeled and kept at -20 °C for subsequent biochemical examinations; while the remnant parts labeled and kept at -80 °C for subsequent flowcytometric analysis.

Statistical analysis

Obtained data were statistically evaluated with ANOVA followed by Post-Hoc Tukey multiple range tests using Statistical Package for the Social Sciences (SPSS/17.5 software version) for Windows. All the results were expressed as the mean ± SEM for 6 animals in each group. *P* values equal or less than 0.05 were considered the minimal level of significance. Additionally, percentage of change in the treated groups was calculated **(12).**

Results:-

Table 1 demonstrate serum glucose, insulin, C-peptide, HbA1c, AGEs and HO-1 levels. Diabetic group showed a significant increase in serum glucose, HbA1c and AGEs while showed a marked decline in insulin, C-peptide and HO-1 levels when compared to normal control one. The results revealed that treatment of diabetic rats with either insulin or BM-MSCs showed significant amelioration in all tested parameters; except insignificant increase in C-peptide in case of insulin treatment; when compared to the diabetic group. While non-significant changes compared to control group were observed except for C-peptide and HO-1 levels in diabetic rats treated with insulin which were still significantly lower than control. There were no remarkable changes between the results of insulin and BM-MSCs treatments of diabetic rats except for C-peptide and HO-1 which showed a marked enhancement with BM-MSCs than insulin treatment.

Table (1):- Serum glucose, insulin, C-peptide, HbA1c, AGEs and HO-1 levels.

		Control	Diabetic (D)	D + Insulin	D + BM-MSCs
Glucose (mg/ 100 ml)g)	**Mean ± SEM**	92.83 ± 4.64	395.20 ± 19.76 [a]	115.00 ± 5.75 [b]	108.30 ± 5.41 [b]
	*		+ 325.72	+ 23.88	+ 16.66
	**			- 70.90	- 72.59
	***				- 5.82
Insulin (µ I U/ml)	**Mean ± SEM**	17.10 ± 0.86	9.22 ± 0.46 [a]	17.00 ± 0.85 [b]	16.97 ± 0.76 [b]
	*		- 46.08	- 0.58	- 0.76
	**			+ 84.38	+ 84.05
	***				- 0.17
C-peptide (ng/ml)	**Mean ± SEM**	0.85 ± 0.04	0.31 ± 0.02 [a]	0.37 ± 0.02 [a]	0.79 ± 0.04 [bc]
	*		- 63.52	- 56.47	- 7.05
	**			+ 19.35	+ 154.83
	***				+ 113.51
HbA1c (%)	**Mean ± SEM**	2.92 ± 0.15	4.94 ± 0.25 [a]	3.40 ± 0.17 [b]	3.10 ± 0.16 [b]
	*		+ 69.17	+ 16.43	+ 6.16
	**			- 31.17	- 37.24
	***				- 8.82
AGEs	**Mean**	2.86	8.58	3.48	3.24

	± SEM	± 0.14	± 0.43 [a]	± 0.19 [b]	± 0.16 [b]
	*****		+ 200	+ 21.67	+ 13.28
	******			- 59.44	- 62.23
	*******				- 6.89
HO-1 (P mol/mg)	**Mean ± SEM**	270.60 ± 13.53	72.00 ± 3.60 [a]	185.20 ± 9.26 [ab]	251.00 ± 12.55 [bc]
	*****		- 73.39	- 31.55	- 7.24
	******			+ 157.22	+ 248.61
	*******				+ 35.52

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 of hepatic MDA, ROS, XO, GSH, SOD, CAT, GST, TAC and HO-1levels were summarized in **table (2).** The obtained data indicated a significant increase in oxidative stress parameters (MDA, ROS and XO) coupled with a significant decrease in all antioxidant markers (GSH, SOD, CAT, GST, TAC and HO-1) levels in diabetic group; when compared to normal control one. On the other hand, diabetic rats treated with either insulin or BM-MSCs showed a significant decrease in oxidative stress markers accompanied by a marked elevation in the antioxidants level; when compared to the diabetic group. While ROS and XO levels in case of insulin treatment still significantly higher and GST level (in both treated diabetic groups) and TAC (in insulin-treated group) still significantly lower; when compared to the control group. Meanwhile, diabetic rat's treatment with BM-MSCs obtained results displayed non-significant improvement variations in the mentioned parameters compared to diabetic rats treated with insulin.

Table (2):- Hepatic oxidative stress and antioxidants markers.

		Control	**Diabetic (D)**	**D + Insulin**	**D + BM-MSCs**
MDA (n mol/g)	**Mean ± SEM**	80.80 ± 4.04	156.80 ± 7.84 [a]	98.50 ± 4.93 [b]	88.20 ± 4.41 [b]
	*****		94.05+	21.90+	0.67-
	******			37.18-	- 43.75
	*******				10.45-
ROS (n mol/g)	**Mean ± SEM**	0.56 ± 0.03	2.66 ± 0.13 [a]	1.00 ± 0.05 [ab]	0.78 ± 0.04 [b]
	*****		+ 375	+ 78.57	+ 39.28
	******			- 62.40	- 70.67
	*******				- 22.00
XO (nmol/ml)	**Mean ± SEM**	42.00 ± 2.10	89.00 ± 4.45 [a]	54.25 ± 2.71 [ab]	48.00 ± 2.40 [b]
	*****		+ 111.90	+ 29.16	+ 14.28
	******			- 39.04	- 46.06
	*******				- 11.52
GSH (mg/gm)	**Mean ± SEM**	81.50 ± 4.08	36.02 ± 1.80 [a]	77.02 ± 3.85 [b]	76.53 ± 3.83 [b]
	*****		- 55.80	- 5.49	- 6.09
	******			+ 113.82	+ 112.46
	*******				- 0.63
S O D (u/	**Mean ± SEM**	21.86 ± 1.09	10.30 ± 0.52 [a]	20.30 ± 1.02 [b]	19.65 ± 0.98 [b]

	*		- 52.88	- 7.13	- 10.10
	**			+ 97.08	+ 90.77
	***				- 3.20
CAT (u/gm)	Mean ± SEM	0.99 ± 0.05	0.29 ± 0.01 [a]	0.83 ± 0.04 [b]	0.92 ± 0.05 [b]
	*		- 70.70	- 16.16	- 7.07
	**			+ 186.20	+ 217.24
	***				+ 10.84
GST (Mmol/gm)	Mean ± SEM	10.86 ± 0.54	6.48 ± 0.32 [a]	8.44 ± 0.42 [ab]	8.80 ± 0.44 [ab]
	*		- 40.33	- 22.28	- 18.96
	**			+ 30.24	+ 35.80
	***				+ 4.26
TAC (mg/gm)	Mean ± SEM	3.16 ± 0.16	1.30 ± 0.07 [a]	2.64 ± 0.13 [ab]	2.86 ± 0.14 [b]
	*		- 58.86	- 16.45	- 9.49
	**			+ 103.07	+ 120.00
	***				+ 8.33
HO-1 (P mol/mg)	Mean ± SEM	423.00 ± 21.15	213.70 ± 10.68 [a]	361.00 ± 18.05 [b]	405.30 ± 20.27 [b]
	*		- 49.47	- 14.65	- 4.18
	**			+ 68.92	+ 89.65
	***				+ 12.27

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.

Table 3 represents renal MDA, ROS, XO, GSH, SOD, CAT, GST, TAC and HO-1levels. The obtained results revealed that significant increases of oxidative stress status with a marked decline in the antioxidant capacities; were seen in diabetic group when compared to normal control one. However, diabetic insulin or BM-MSCs treated groups showed significant decreases in oxidative stress parameters level accompanied by a marked elevation in antioxidants levels; when compared to the diabetic group. Non-significant changes were shown in the above tested parameters in case of diabetic rats treated with BM-MSCs when compared to the control group; except the still presence of a higher ROS and lower TAC levels. For diabetic rats treated with insulin, results of ROS and XO were still significantly higher, while SOD and TAC levels still significantly lower; in comparison with the control rats. No detectable changes were recorded in all tested parameters level between the two diabetic rats-treated groups; except for ROS level which showed a much more enhancement in BM-MSCs treated group comparing to insulin-treated one.

Table (3):- Renal oxidative stress and antioxidants markers.

		Control	Diabetic (D)	D + Insulin	D + BM-MSCs
MDA (n mol/g)	Mean ± SEM	28.08 ± 1.40	53.68 ± 2.68 [a]	32.00 ± 1.60 [b]	32.13 ± 1.61 [b]
	*		+ 91.16	+ 13.96	+ 14.42
	**			- 40.38	- 40.14
	***				+ 0.40
ROS (n mol/g)	Mean ± SEM	0.42 ± 0.02	1.56 a ± 0.08 [a]	0.84 ab ± 0.04 [ab]	0.62 ± 0.03 [abc]
	*		+ 271.42	+ 100.00	+ 47.61

	**			- 46.15	- 60.25
	***				- 26.19
XO (nmol/ml)	Mean ± SEM	32.20 ± 1.61	69.40 ± 3.47 [a]	42.80 ± 2.14 [ab]	37.40 ± 1.87 [b]
	*		+ 115.52	+ 32.91	+ 16.14
	**			- 38.32	- 46.10
	***				- 12.61
GSH (mg/gm)	Mean ± SEM	47.34 ± 2.37	22.92 ± 1.15 [a]	49.80 ± 2.49 [b]	44.23 ± 2.21 [b]
	*		- 51.58	+ 5.19	- 6.56
	**			+ 117.27	+ 92.97
	***				- 11.18
SOD (u/gm)	Mean ± SEM	11.56 ± 0.58	4.84 ± 0.24 [a]	9.60 ± 0.48 [ab]	10.53 ± 0.53 [b]
	*		- 58.13	- 16.95	- 8.91
	**			+ 98.34	+ 117.56
	***				+ 9.68
CAT (u/gm)	Mean ± SEM	0.58 ± 0.03	0.31 ± 0.02 [a]	0.51 ± 0.03 [b]	0.51 ± 0.03 [b]
	*		- 46.55	- 12.06	- 12.06
	**			+ 64.51	+ 64.51
	***				0.00
GST (Mmol/gm)	Mean ± SEM	5.40 ± 0.27	3.30 ± 0.17 [a]	4.70 ± 0.24 [b]	5.14 ± 0.26 [b]
	*		- 38.88	- 12.96	- 4.81
	**			+ 42.42	+ 55.75
	***				+ 9.36
TAC (mg/gm)	Mean ± SEM	1.28 ± 0.06	0.42 ± 0.02 [a]	0.86 ± 0.04 [ab]	0.98 ± 0.05 [ab]
	*		- 67.18	- 32.81	- 23.43
	**			+ 104.76	+ 133.33
	***				+ 13.95
HO-1 (P mol/mg)	Mean ± SEM	254.00 ± 12.70	112.50 ± 5.63 [a]	213.00 ± 10.65 [b]	242.00 ± 12.10 [b]
	*		- 55.70	- 16.14	- 4.72
	**			+ 89.33	+ 115.11
	***				+ 13.61

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.

Table 4 shows cardiac MDA, ROS, GSH and TAC levels and XO, SOD, CAT, GST and HO-1activities. Results of this study showed that regarding to diabetic group, significant increases in MDA, ROS and XO levels were obtained, while significant decrease in antioxidants levels; when compared to normal control one. In contrary, both diabetic treated groups showed significant decline in oxidative stress status with marked elevation in all antioxidant parameters; when compared to the diabetic group. However. values of MDA, XO, SOD and CAT in insulin-treated

group and ROS in both treated groups still significantly variable in comparing to normal control group. The results reveal non-significant changes between both diabetic treated groups, except for CAT activity in BM-MSCs treated rats which was significantly higher compared to insulin-treated rats.

Table (4):- Cardiac oxidative stress and antioxidants markers.

		Control	Diabetic (D)	D + Insulin	D + BM-MSCs
MDA (n mol/g)	**Mean ± SEM**	67.62 ± 3.38	162.3 ± 8.11 [a]	88.98 ± 4.45 [ab]	80.84 ± 4.04 [b]
	*		+ 140.01	+ 31.58	+ 19.55
	**			- 45.17	- 50.19
	***				- 9.14
ROS (n mol/g)	**Mean ± SEM**	0.42 ± 0.02	2.16 ± 0.11 [a]	0.72 ± 0.04 [ab]	0.68 ± 0.03 [ab]
	*		+ 414.28	+ 71.42	+ 61.90
	**			- 66.66	- 68.51
	***				- 5.55
XO (nmol/ml)	**Mean ± SEM**	40.60 ± 2.03	89.40 ± 4.47 [a]	55.40 ± 2.77 [ab]	44.60 ± 2.23 [b]
	*		+ 120.19	+ 36.45	+ 0.00
	**			- 38.03	- 50.11
	***				- 19.49
GSH (mg/gm)	**Mean ± SEM**	51.18 ± 2.56	24.13 ± 1.21 [a]	49.16 ± 2.46 [b]	48.54 ± 2.43 [b]
	*		- 52.85	- 3.94	- 5.15
	**			+ 103.72	+ 101.16
	***				- 1.26
SOD (u/gm)	**Mean ± SEM**	5.76 ± 0.29	1.70 ± 0.09 [a]	4.84 ± 0.24 [ab]	5.03 ± 0.25 [b]
	*		- 70.48	- 15.97	- 12.67
	**			+ 184.70	+ 195.88
	***				+ 3.92
CAT (u/gm)	**Mean ± SEM**	0.66 0.03±	0.22 ± 0.01 [a]	0.45 ± 0.02 [ab]	0.62 ± 0.03 [bc]
	*		- 66.66	- 31.81	- 6.06
	**			+ 104.54	+ 181.81
	***				+ 37.77
GST (Mmol/gm)	**Mean ± SEM**	5.00 ± 0.25	2.10 ± 0.11 [a]	4.66 ± 0.23 [b]	4.70 ± 0.24 [b]
	*		- 58.00	- 6.80	- 6.00
	**			+ 121.90	+ 123.80
	***				+ 0.85
TAC (mg/gm)	**Mean ± SEM**	2.36 ± 0.12	0.76 ± 0.04 [a]	2.04 ± 0.10 [b]	2.06 ± 0.10 [b]
	*		- 67.79	- 13.55	- 12.71
	**			+ 168.42	+ 171.05
	***				+ 0.98

HO-1 (P mol/mg)	**Mean ± SEM**	293.00 ± 14.65	146.00 ± 7.30 [a]	270.50 ± 13.53 [b]	281.50 ± 14.08 [b]
	*		- 50.17	- 7.67	- 3.92
	**			+ 85.27	+ 92.80
	***				+ 4.06

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.

Table 5 illustrate pancreatic MDA, ROS, XO, GSH, SOD, CAT, GST, TAC and HO-1 levels. The diabetic group showed a significant increase in pancreatic MDA, ROS and XO while showed a marked decline in GSH, SOD, CAT, GST, TAC and HO-1 levels when compared to normal control one. 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, TAC and HO-1 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 treatment results of insulin and BM-MSCs diabetic rats except for MDA which showed a marked enhancement in treatment with BM-MSCs than insulin treatment.

Table (5):- Pancreatic oxidative stress and antioxidants markers.

		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/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 (	**Mean**	0.58	0.34	0.49	0.52

	± SEM	± 0.03	± 0.02 [a]	± 0.02 [b]	± 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
HO-1 (P mol/mg)	**Mean ± SEM**	309.00 ± 15.45	116.50 ± 5.83 [a]	273.50 ± 13.68 [b]	294.00 ± 14.70 [b]
	*		- 62.29	- 11.48	- 4.85
	**			+ 134.76	+ 152.36
	***				+ 7.49

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.

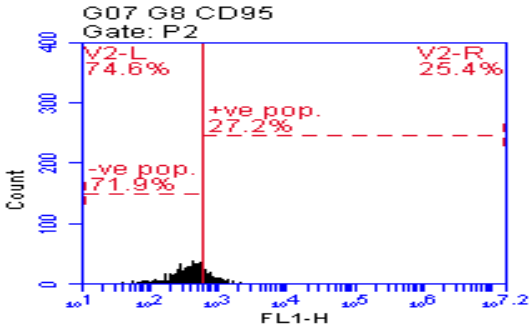
Table 6 and figures 1, 2 and 3 illustrate that significant increases in all inflammatory markers % were seen in diabetic group; when compared to control one. Concerning treatment, both treated groups showed significant decreases in all inflammatory markers % compared to the diabetic group, while markedly declined values; for CRP % in both groups in addition to TGF-β, $CD95^+$ and IL-6 % in case in insulin treated group; still significantly higher when compared to the control group. No detectable changes were recorded in CRP, TNF-α, $CD95^+$ and IL-6 % levels between the two diabetic rats-treated groups, while TGF-β % level exhibited a marked enhancement in BM-MSCs treated rats, compared to insulin group.

Table (6):- Pancreatic CRP, TNF-α, TGF-β, CD95 and IL-6 levels.

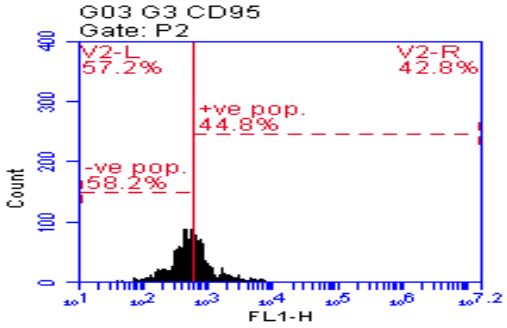
		Control	**Diabetic (D)**	**D + Insulin**	**D + BM-MSCs**
CRP (g/dl)	**Mean ± SEM**	3.60 ± 0.18	18.20 ± 0.91 [a]	7.75 ± 0.39 [ab]	7.33 ± 0.37 [ab]
	*		+ 405.55	+ 115.27	+ 103.61
	**			- 57.41	- 59.72
	***				- 5.41
TNF-α (%)	**Mean ± SEM**	27.80 ± 1.39	44.02 ± 2.20 [a]	33.70 ± 1.69 [b]	30.00 ± 1.50 [b]
	*		+ 58.34	+ 21.22	+ 7.91
	**			- 23.44	- 31.84
	***				- 10.97
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

$CD95^+$ (%)	**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
IL-6 (%)	**Mean ± SEM**	38.12 ± 1.92	77.04 ± 2.49 [a]	55.46 ± 2.14 [ab]	42.13 ± 1.38 [b]
	*		+ 102.09	+ 45.48	+ 10.51
	**			- 28.01	- 45.31
	***				- 24.03

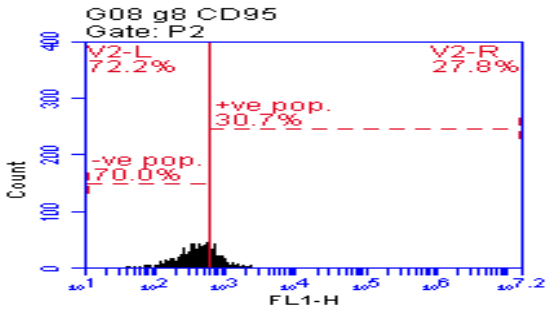
Values expressed as mean ± SEM (n = 6). **a, b** and **c** are Significant differences ($P \leq 0.05$) comparing to control, diabetic and diabetic insulin-treated groups respectively. ***. **** and ******* are % of change comparing to control, diabetic and diabetic insulin-treated groups respectively.



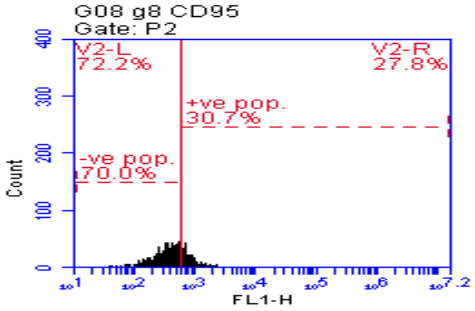
1 (a): Control group



1 (b): Diabetic group

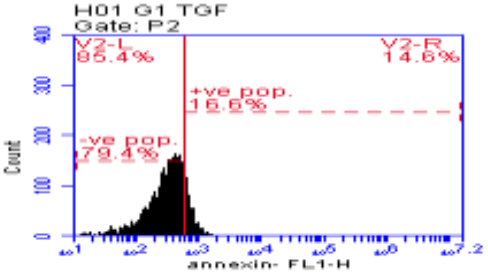


1 (c): D + Insulin group

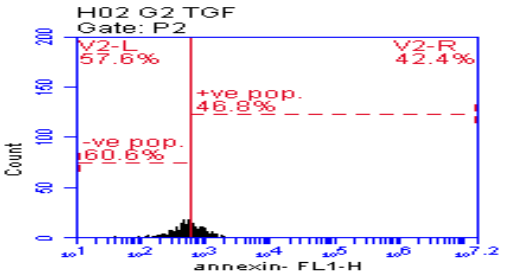


1 (d): D + BM-MSCs group

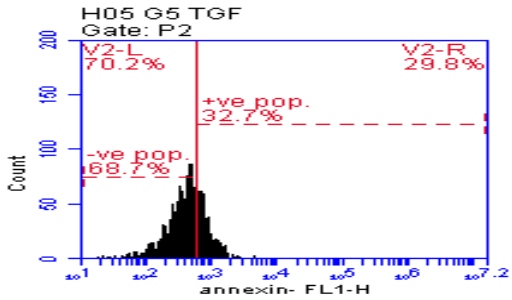
Figure 1 (a, b, c and d): Pancreatic TNF-α levels.



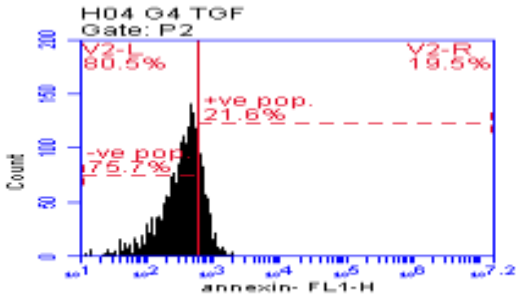
2 (a): Control group



2 (b): Diabetic group

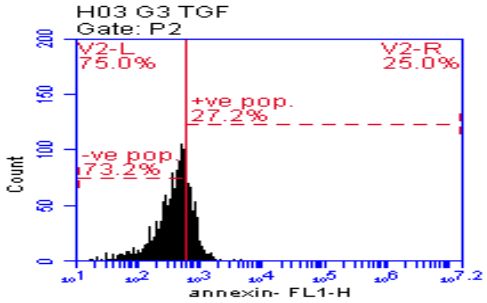


2 (c): D + Insulin group

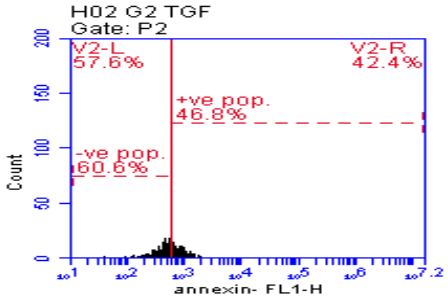


2 (d): D + BM-MSCs group

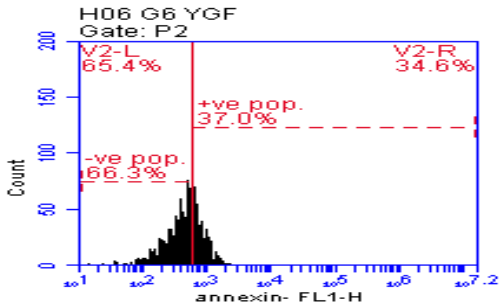
Figure 2 (a, b, c and d): Pancreatic TGF-β levels.



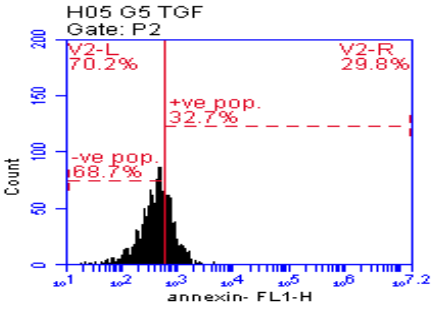
3 (a): Control group



3 (b): Diabetic group



3 (c): D + Insulin group



3 (d): D + BM-MSCs group

Figure 3 (a, b, c and d): Pancreatic CD 95^{+} levels.

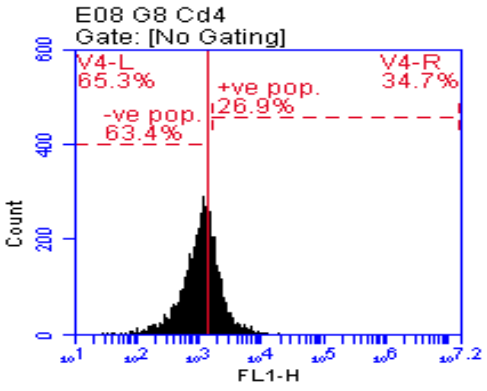
Table 7 and figures 4 and 5:
results showed that significant increases in CD4^{+} and CD8^{+} % were seen in diabetic group when compared to control. On the other hand, both diabetic treated groups showed significant decreases in both parameters compared to diabetic group; while a non-significant elevation when compared to the control. The results reveal non-significant changes between insulin and BM-MSCs treated diabetic rats.

Table (7):- Pancreatic T-Helper (CD 4^{+}) and T-Cytotoxic (CD 8^{+}) %.

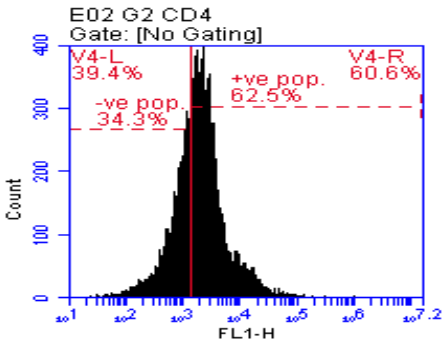
		Control	Diabetic (D)	D + Insulin	D + BM-MSCs
T-Helper CD 4^{+} (%)	**Mean ± SEM**	27.97 ± 1.40	60.17 ± 3.01 [a]	36.03 ± 1.80 [b]	32.17 ± 1.61 [b]
	*		+ 115.12	+ 28.81	+ 15.01
	**			- 40.11	- 46.53

	***				- 10.71
T-Cytotoxic CD 8^+ (%)	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

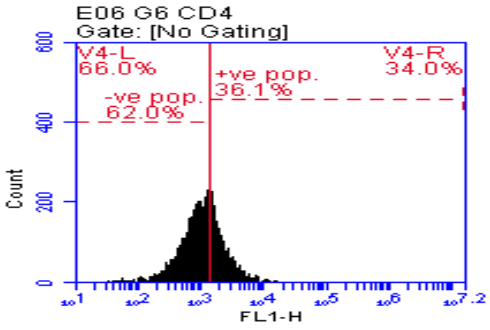
Values expressed as mean ± SEM (n = 6). **a, b** and **c** are Significant differences ($P \leq 0.05$) comparing to control, diabetic and diabetic insulin-treated groups respectively. ***. **** and ******* are % of change comparing to control, diabetic and diabetic insulin-treated groups respectively.



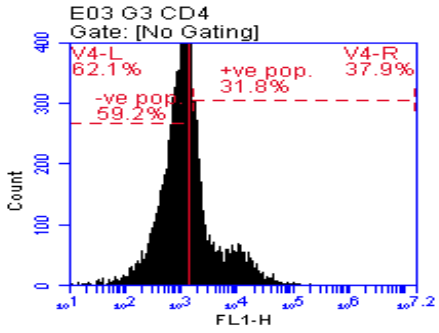
4 (a): Control group



4 (b): Diabetic group

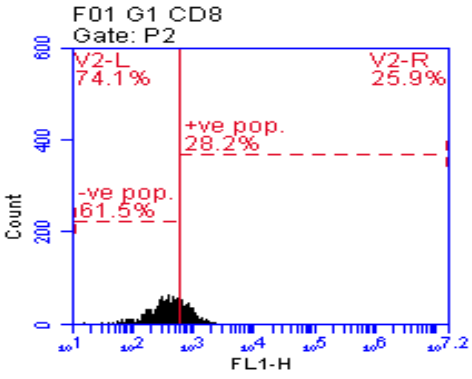


4 (c): D + Insulin group

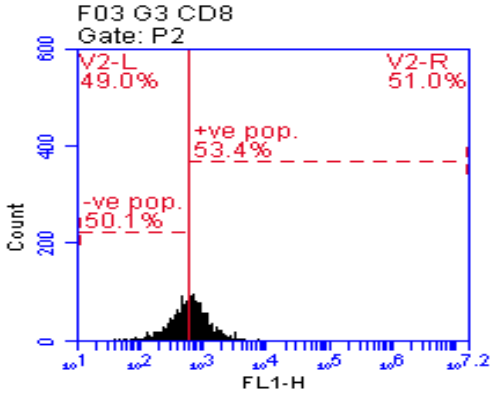


4 (d): D + BM-MSCs group

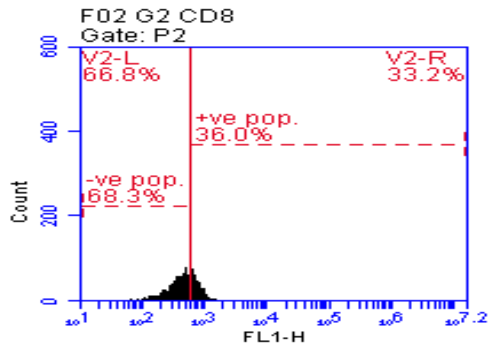
Figure 4 (a, b, c and d): Pancreatic T-Helper ($CD4^+$) %.

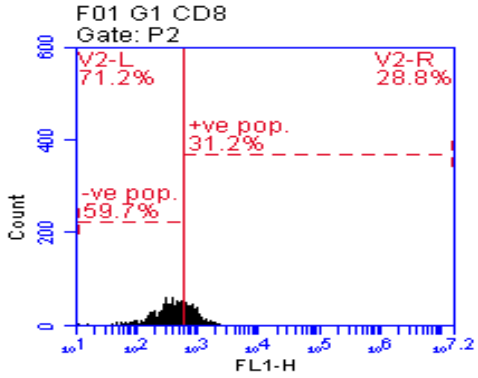


5 (a): Control group



5 (b): Diabetic group





5 (c): D + Insulin group **5 (d): D + BM-MSCs group**

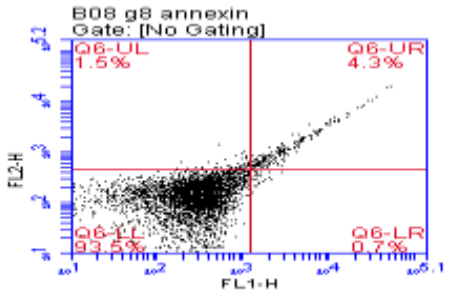
Figure 5 (a, b, c and d):- Pancreatic T-Helper ($CD8^+$) %.

Table 8 and figures 6, 7, 8 and 9 demonstrated that, regarding to diabetic group, significant increases in annexin V, P53 and caspase 3 % while significant decrease in Bcl-2 % were obtained when compared to normal control one. In contrary, diabetic groups treated with either insulin or BM-MSCs showed significant decreases in annexin V, P53 and caspase 3 %while significant increase in Bcl-2 % when compared to the diabetic group, although values of annexin V and caspase 3 were still significantly higher while Bcl-2 level was still significantly lower in diabetic rats treated with insulin; when compared to normal control group. Diabetic rat's treatment with BM-MSCs showed a significant improvement in almost mentioned parameters compared to diabetic rats treated with insulin except for P53 % where it resulted in a non-significant change.

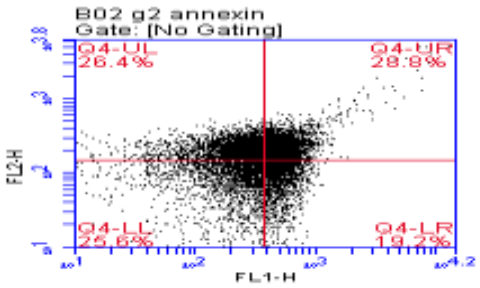
Table (8):- Pancreatic annexin, P53, caspase 3 and Bcl-2 %.

		Control	Diabetic (D)	D + Insulin	D + BM-MSCs
Annexin V (%)	**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
BcL-2 (%)	**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

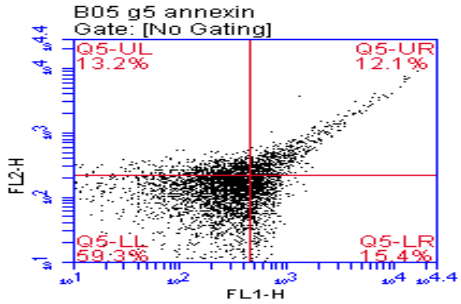
Values expressed as mean ± SEM (n = 6). **a, b** and **c** are Significant differences ($P \leq 0.05$) comparing to control, diabetic and diabetic insulin-treated groups respectively ***. **** and ******* are % of change comparing to control, diabetic and diabetic insulin-treated groups respectively.



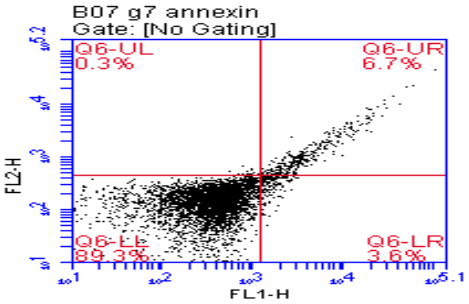
6 (a): Control group



6 (b): Diabetic group

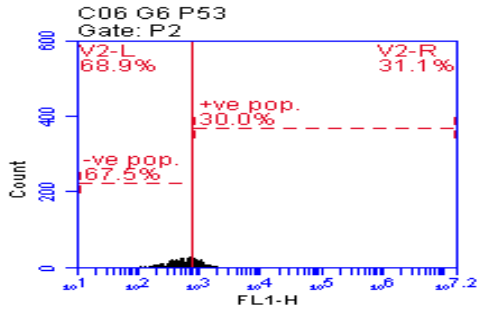


6 (c): D + Insulin group

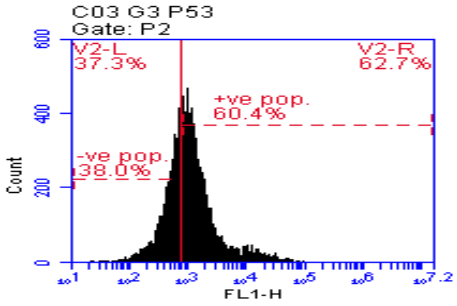


6 (d): D + BM-MSCs group

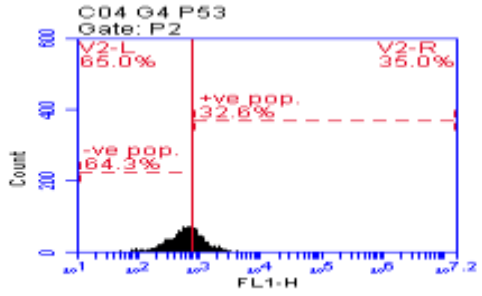
Figure 6 (a, b, c and d): Pancreatic annexin V %.



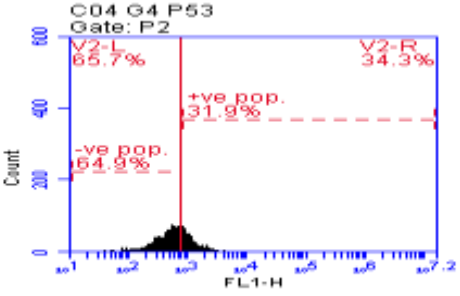
7 (a): Control group



7 (b): Diabetic group

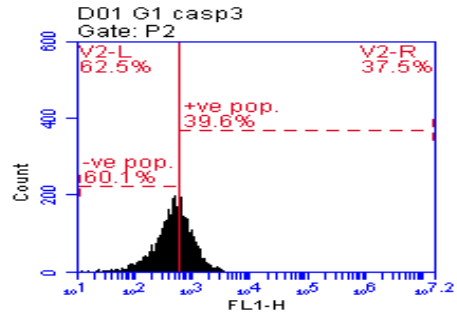


7 (c): D + Insulin group

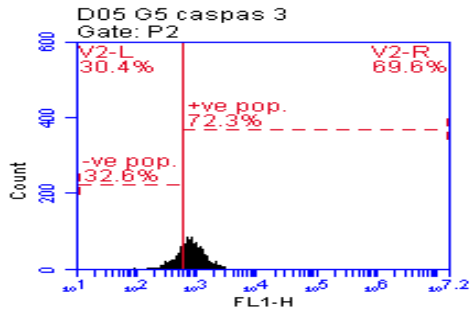


7 (d): D + BM-MSCs group

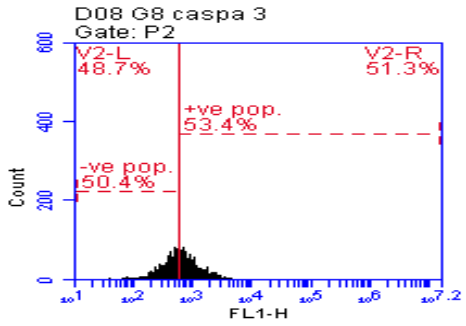
Figure 7 (a, b, c and d): Pancreatic P53 %.



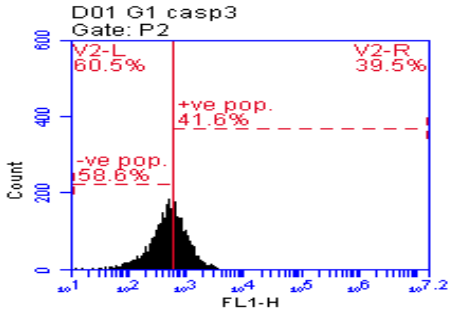
8 (a): Control group



8 (b): Diabetic group

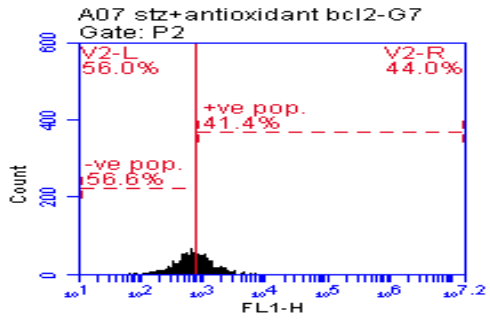


8 (c): D + Insulin group

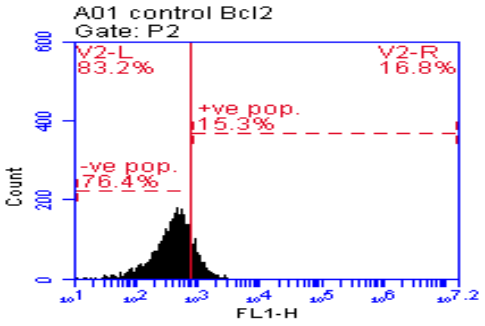


8 (d): D + BM-MSCs group

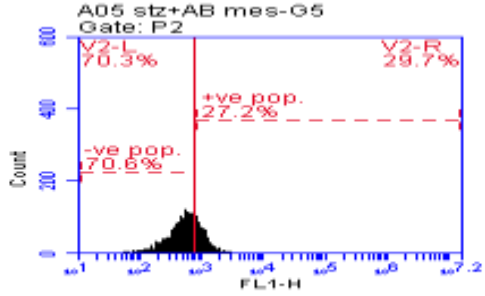
Figure 8 (a, b, c and d): Pancreatic caspase 3 %.



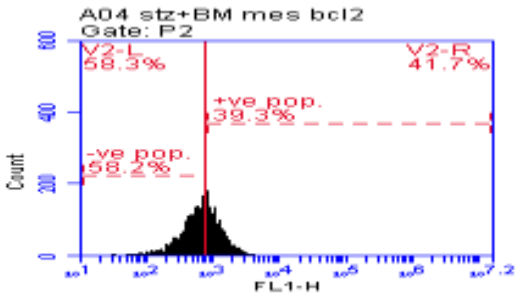
9 (a): Control group



9 (b): Diabetic group



9 (c): D + Insulin group



9 (d): D + BM-MSCs group

Figure 9 (a, b, c and d):- Pancreatic Bcl-2 %.

A significant decline in viable cells (G0/G1) % in pancreas, liver, kidney and heart tissues were reported in **table 9 and figure 10** in diabetic group; when compared to control one. However, both treated groups showed marked elevation in viable cells % in all tissues compared to the diabetic group, while values; in case of insulin treated group; still significantly lower when compared to the control group. Interestingly, all tissues viable cells % showed a notable increase in BM-MSCs treated rats, compared to insulin group.

Table (9):- Viable cells (G0/G1) % in pancreas, liver, kidney and heart tissues.

		Control	Diabetic (D)	D + Insulin	D + BM-MSCs
Pancreas	**Mean ± SEM**	93.40 ± 2.26	62.70 ± 178 [a]	77.30 ± **1**.53 [ab]	91.80 ± 2.30 [bc]
	*		- 32.86	- 17.23	- 1.71
	**			+ 23.28	+ 46.41
	***				+ 18.75
Liver	**Mean ± SEM**	95.20 ± 2.25	68.20 ± 1.07 [a]	77.10 ± 1.28 [ab]	91.20 ± 1.68 [bc]
	*		- 28.36	- 19.01	- 4.20
	**			+ 13.04	+ 33.72
	***				+ 18.28
Heart	**Mean ± SEM**	96.50 ± 2.36	60.20 ± 0.83 [a]	77.60 ± 1.26 [ab]	91.70 ± 1.85 [bc]
	*		- 37.61	- 19.58	- 4.97
	**			+ 28.90	+ 52.32
	***				+ 18.17
Kidney	**Mean ± SEM**	94.70 ± 2.86	62.30 ± 2.68 [a]	74.10 ± 2.33 [ab]	89.40 ± 2.78 [bc]
	*		- 34.21	- 21.75	- 5.59
	**			+ 18.94	+ 43.49
	***				+ 20.64

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.

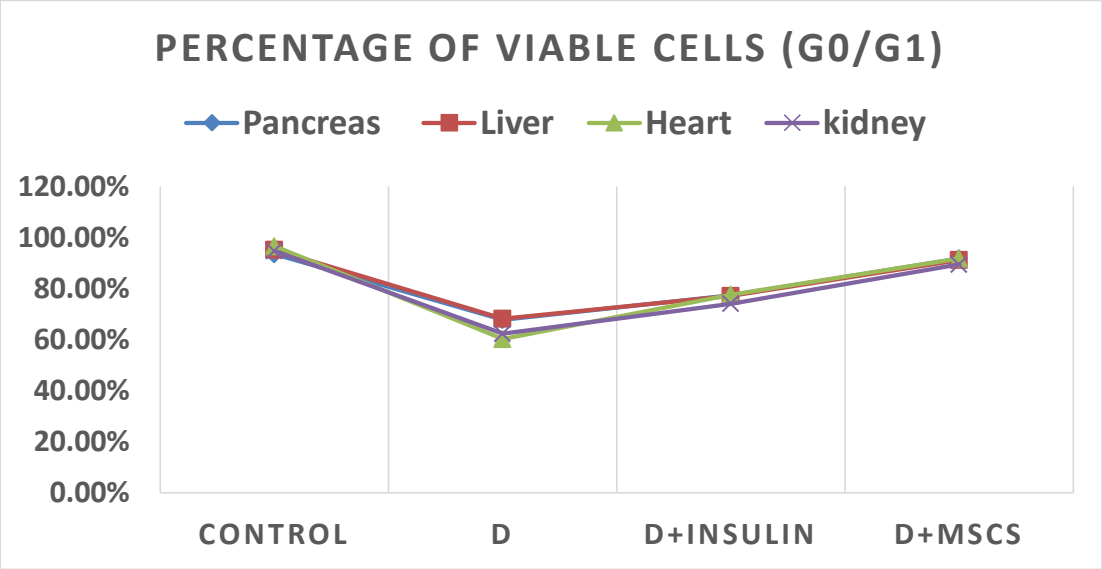


Figure (10):- Viable cells (G0/G1) % in pancreas, liver, kidney and heart tissues.

Discussion:-

DM is considered the world' dominant metabolic disease; with a next 20 years expected prevalence elevation of over 50%; representing a global health problem**.** T1DM is a heterogeneous disorder that characterized by insulin-secreting pancreatic β cells autoimmune destruction; hence, exogenous insulin injection is crucial for metabolic optimization **(13).** Despite extensive research to develop alternative treatments; from the early days to the current time; β-cell damage leading to insulin decline or impairment production eventually leads to the need for exogenous insulin **(14)**; making it the most reliable way for serum glucose concentration control**.** Current DM traditional injection method treatment has caused a heavy burden on the patient **(15).** Thus**,** stem cell therapy holds great promise for the damaged tissues and organs, that was considered one of the most promising therapies for DM**.**

Carbohydrate and glycemic control

The results of the present study showed that a single injection of 45 mg/kg bw of STZ to the rats caused a significant increase in fasting blood glucose (FBG) and blood HbA1c levels as well as in serum advanced glycation end products (AGEs) in contrast to significant decrease in serum insulin and C-peptide levels as well as serum heme oxygenase-1 (HO-1) as compared to the control group. Similarly, participants with diabetes presented with significantly higher FBG and HbA1c, and lower insulin and C-peptide when compared with control subjects **(6,16).** These results are, also, in accordance with the findings of **Mayyas *et al*. (17)** and **Nia *et al*. (18).**

In addition to provoking hyperglycemia, **Jaen *et al*. (3)** cleared that in diabetic dogs there was total or near total loss of insulin secretion, as number of cells producing insulin were reduced. The experiment done by **Ghosh *et al*. (19)** suggested DNA alkylation as the major cause for β-cell death induced by STZ resulting in significant insulin decrease and marked elevation in serum glucose level. Such suggestion was in harmony with the study of **Adam *et al*. (20)** who observed that deviations in control of glucose level, due to STZ injection, is sufficient to trigger an array of maladaptive processes including decreased serum insulin level with huge signs of pancreatic destruction and glucose transporter-4 (GLUT-4) depletion in the pancreas of diabetic rats compared to control group**.** However, C-peptide is excised from proinsulin to generate biologically active insulin; it is used to assess endogenous insulin secretion, as its decline is indicative for the insulin production decrease and DM progression **(21).**

HbA1c and AGEs elevated levels; in the present study; in STZ-induced diabetic rats are in accordance with the results revealed a significant HbA1c elevated levels with a marked decrease in the total Hb, which may be attributed to the higher blood glucose levels and its impaired utilization; as HbA1c is produced in a non-enzymatic glycation manner when Hb exposed to excess glucose, serving as an average blood glucose levels marker; and the rate of glycation is proportional to the blood glucose concentration **(17,22).**

It was clear from **Hamza *et al*. (10)** data that extended hyperglycemia causes blood and tissue AGEs accumulation in diabetic rats**;** which may be due to increased ROS production; having a pivotal role in the long-term diabetic complications' development**.** In hyperglycemia, oxidative stress increased production leads to tissues increase of AGEs and their receptors (RAGE) formation and deposition; inducing NFκB activation and IL increased production **(23).** However, there was a significant decrease in the serum HO-1activity; a rate-limiting enzyme catalyzes heme breakdown yielding cytoprotective products including carbon monoxide, ferritin and bilirubin; in the diabetic group relative to control **(10,24).**

On the other hand; in the current study; marked hypoglycemic effects were shown by either insulin or BM-MSCs treatments in STZ-diabetic rats. Also, their administration raised serum insulin and C-peptide levels as well as HO-1 levels, while significantly lowered blood glucose, HbA1c and AGEs levels to reach nearly normal control values in comparison with diabetic rats. The results, herein, are in harmony with the findings devoted that insulin treatment clearly reversed serum glucose and HbA1c to normal levels in STZ-treated mice **(25)**, in dogs **(3)** and in diabetic patients **(26);** causing recurrent hypoglycemia**.** It was known that, insulin lowers the increased blood glucose level by increasing glycolysis and glucose uptake by insulin-sensitive peripheral tissues like muscle, liver and fat cells; inhibits glycogenolysis and gluconeogenesis in the liver; and inhibits lipolysis in adipose tissue **(3).** Furthermore, insulin injection could directly affects glucose metabolism in STZ-diabetic rats via glucokinase and pyruvate kinase gene expressions up-regulation; the key rate-limiting enzymes mediating glucose oxidation and ATP generation **(15).**

There is no doubt that BM-MSCs have therapeutic effects on diabetes; as they are able to stimulate damaged pancreatic β-cells regeneration and becoming an alternative β cell source after induction; making them an ideal

choice for DM treatment**. Wang *et al*. (27) and Sood *et al*. (28)** proved that BM-MSCs treatment resulted in HbA1c levels and insulin requirement reductions in diabetic rats, and in 9 out of 11 diabetic patients **(29).** Hypoglycemic therapeutic potential of MSC transplantation may be a direct effect of their intrinsic regenerative capacity and differentiation into IPCs, insulin release in a glucose-dependent manner, improving diabetic symptoms in T1DM animal (less likely) and preserve residual β-cell mass; or an indirect effect of immunomodulators secretion, thus, arresting autoimmune T cells from inducing destruction to the pancreatic β-cell **(7).** This is confirmed, also, by **Zang *et al*. (8)** who reported that MSCs exert beneficial effects on glycemic control as insulin requirements decreased by 50%; by ameliorating insulin resistance and restoring islet function; through promotion of islet cell regeneration, differentiation into IPCs, protection of endogenous islet cells and promote trans-differentiation of α cells into β cells followed by β cell mass restoration and dramatic hyperglycemia amelioration, in mice with STZ-induced T2DM**.**

Recently confirming this regard**, Amer *et a*l. (6)** revealed that transplanted IPCs differentiated from Ad-MSCs in STZ-diabetic rats; showed marked pancreatic β cell markers expression, apparent islet cells regeneration and proliferation and significant increase in C-peptide with increased insulin secretion in glucose dependent manner. Interestingly, MSCs infusion during the early phase (7 days) could ameliorate pancreatic islets destruction, restore β-cell function, reduce insulin resistance and promote MSCs recruitment to the damaged tissues; whereas late phase infusion (21 days) merely ameliorated insulin resistance **(30).** Meanwhile, FBG, insulin requirement and HbA1c levels were decreased while C-peptide level was increased, in diabetic rats after BM-MSCs therapy for 3 months **(31),** 6 months **(32)** or 12 months **(33),** compared with the diabetic control therapy; due to prevent islet cell loss, elevated insulin secretion from existing β-cells and insulin biosynthesis marked increase**,** suggesting improvement in the islet β cells number and/or function **(34).**

Herein, the insignificant increase of serum C-peptide level in insulin treated group, in contrast to its huge significant increase in BM-MSCs treated group, clearly confirms the importance of BM-MSCs as a therapeutic adjunct for diabetes cure. The most probable explanation for this was that MSCs rapid infusion could improve remnant β cell regeneration and proliferation, leading to an endogenous insulin secretion elevation; minimizing the exogenous insulin injection need.

Oxidative stress and antioxidants

It is well known that oxidative stress exhibits a basic principle role in the development of diabetic complications **(18),** since DM is associated with enhanced production of ROS; which reacts with protein, lipids and DNA; resulting in a huge oxidative stress-induced cellular damage **(35).** In the present study, STZ-induced diabetes in rats clearly resulted in an increased oxidative stress condition; as demonstrated by the elevated tissue MDA, ROS and XO concomitant to decreased levels of various antioxidant markers (GSH, SOD, CAT, GST, TAC as well as HO-1).

These notes are consistent with **Xie *et al*. (36)** and **Nia *et al*. (18)** findings**,** who noticed a marked increase in serum MDA level in diabetic rats; which might be a reflection of decreased antioxidant capability of the defensive systems and/or glucose oxidation; leading to increased free radicals production that react with cell membranes poly-unsaturated fatty acids (PUFA) causing lipid peroxidation (LPO); which will in turn result in more free radicals production and diabetic complications development. Another possible mechanism for MDA elevation was postulated by **Singh and Kakkar (37)** who noticed that low levels of lipooxygenase peroxides could stimulate insulin secretion, while its increase might initiate uncontrolled LPO; which impairs membrane functions by changing the membrane-bound receptors and enzymes activity with increasing membrane fluidity; causing islet cell damage as well as cellular infiltration in T1DM. These two mentioned factors lead to cellular oxidation/reduction imbalance that may be related to the association of STZ destruction of islet's β-cells and free radicals generation with the oxidative stress markers rise **(11).** This would cause an inner endothelial tissue damage; that might eventually be directly responsible for the blood glucose level elevation.

These findings are in agreement with **Zang *et al*. (8)** 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 were recognized as the major etiological factor in DM development**.** Similar assumption has been documented by **Turkmen (38)** who stated that, the well-established features of STZ-diabetic complications include elevated blood glucose concentration accompanied by increased ROS production; resulting in a notable cellular injury and to a point of no return in apoptosis; when ROS scavenging and cytoprotective molecules insufficiency are present**.** Moreover, high glucose level could induce the monocyte chemoatractant-1(MCP-1) gene expression; a causal factor in DM vascular complications facilitation; and increase ROS formation as well; via the transcription

factor NF-B activation **(39).** Increased MDA level indicates a highly destructive LPO products accumulation as consequences of necrotic tissue progressive degradation; that induces structural and functional cellular membrane alterations and fatty acids oxidation, leading to hepatic and pancreatic injury **(36).** However, in DM, glucose auto-oxidation and protein glycation, also, might stimulate free radical's production; catalyzing LPO in turn **(19).**

Regarding antioxidants, **Adam *et al*. (20)** and **Roslan *et al*. (40)** showed that STZ increased intracellular glucose levels resulting in an overproduction of LPO and ROS; mostly via mitochondrial electron transport chain; accompanied with marked antioxidant enzymes' decreased activity, such as glutathione peroxidase (GPx), SOD and CAT; participating in the establishment of oxidative stress in diabetic rats compared to control group**.** In this context, other researchers also revealed increased levels of MDA and lowered activities of antioxidants defense systems; both non-enzymatic (GSH) and enzymatic (GST, GPx, SOD, CAT); in STZ-diabetic rats' plasma, pancreas, kidney, liver and heart after 60 days of diabetes induction, suggesting that these antioxidants are exhausted to combat the deleterious effects of increased oxidative stress (**41,42).** Additionally, **Mayyas *et al*. (17)** revealed a significant increase in MDA level, in contrast to a marked GSH, SOD and CAT activities decrease in STZ-diabetic rats' heart; compared to normal rats. Recently, in ***et al*. (15)** study, the STZ-induced oxidative stress was approved through the total oxidative status (TOS) and MDA level marked elevation with the significant CAT activity and TAC level decline. However, hyperglycemia effect on the cell membrane non-enzymatic glycosylation besides the NADH/NAD+ disbalance, might be another possible oxidative stress cause**.**

Similar results were obtained by **Singh and Kakkar, (37) and Koroglu *et al*. (11**) who demonstrated a marked decrease in GSH levels in liver after STZ treatment as compared to normal control animals; which represents an increased glutathione oxidation and utilization due to oxidative stress; that might be attributed to its regenerating enzyme (glutathione reductase, GRd) inhibition and also to the antioxidant recycling mechanism regression in the diabetic rats. Furthermore, SOD and CAT activities were significantly lower in DM than that of normal, probably due to their inactivation by H_2O_2 and superoxide radical respectively, and/or glycosylation through combination with the higher blood glucose levels; which may be responsible for antioxidant defenses inadequacy in combating damage and deleterious effects **(19).** Taken together, all pervious results confirm the amplification of the oxidative stress status and the inhibitory action of STZ on both enzymatic and non-enzymatic antioxidants in various tissues.

On the other hand, for insulin or BM-MSCs treatment, the data in the present study showed decreased MDA, ROS and XO levels accompanied by a marked elevation in antioxidant enzyme (SOD, CAT, GST and HO-1) activities as well as GSH content in addition to TAC in diabetic tissues, compared to the diabetic untreated group. ***et al*. (15)** showed the efficacy of oral coated insulin; in the form of trimethylchitosan-based nanoparticles; in managing the elevated oxidative stress status and blood glucose level related to hyperglycemia; after 8 weeks post-STZ injection; as evidenced via the marked TOS and MDA levels decline coupled with the significant TAC incline, in addition to an obvious serum glucose concentration down-regulation.

However, **Chandravanshi and Bhonde (43)** suggested the MSCs-protective effect on the pancreatic islet cells of diabetic rats against oxidative stress-mediated cellular injuries; as indicated by the reduced levels of ROS, nitric oxide (NO), and super oxide ions; after 48 h of MSCs transplantation. In addition, MSCs increased expression of renal SOD and CAT, which may associate with detoxifying ROS to prevent oxidative renal damage. **Li *et al*. (44)** data suggested that the enhanced protective effect of MSCs against DM might be associated with the 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. Furthermore, MSCs injection inhibited the renal inducible nitric oxide synthase (iNOS) activity; which is a major source of reactive oxidant stress in murine models of lupus nephritis. They also observed that the increased 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*. (45)**, MSCs injection mitigated inflammation and oxidative stress, while antioxidants genes expressions; such as GRd and GPx; were increased after cell therapy, with a trend for decreased MDA levels and increased GSH contents was observed; in most tissues; from baseline to final evaluation times**.** Furthermore, **Ramanathan *et al*. (46)** 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.

Inflammation markers

Worsening inflammation over time raise concern regarding premature development of DM **(26).** 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 (47)** who reported a proinflammatory cytokines (TNF-α, IFN-γ and IL1-β) levels increase in STZ-induced diabetic male rats serum. 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 **(48).** 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 **(10).**

In STZ-diabetic rats, hyperglycemia enhanced increased oxidative stress and proinflammatory cytokines; such as CRP, TNF-α, IFN-γ, IL1-β and IL-6; which are positively correlated with measures of insulin deficiency, suggesting that oxidative stress promotes a state of low-grade systemic inflammation in diabetic subjects **(49).** Interestingly, **Elmarakby *et al*. (50)** findings confirming a close association between oxidative stress and inflammation in diabetes, as it was hypothesized that an increase in oxidative stress-derived inflammation is a major mechanism in the pathogenesis and progression of diabetic nephropathy. In addition, an increase in inflammatory cytokine levels in diabetes may drive a further increase in oxidative stress**.**

Regarding insulin and BM-MSCs treatment for DM, our results declared 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 **(51).** MSCs also function as trophic mediators that promote angiogenesis, have anti-apoptotic effects and reduce inflammation **(52).** These findings are in agreement with those of **Christ *et al*. (53)** 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 **(9).** 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 **(54).**

In the study by **Chandravanshi and Bhonde (43),** after 48 h of MSCs transplantation, lower expression of TGF-β, TNF-α and other pro-inflammatory cytokines was noticed. Moreover, mice injected with MSCs showed alleviated renal inflammatory infiltration of lymphocytes and macrophages and reduced expression of IL-1β and IL-6, compared with controls; further confirming that suppression of oxidative stress can ameliorate inflammatory damage **(44).** Furthermore, BM-MSCs reversed excessive expression of proinflammatory cytokines in parenchymal cells, and regulated proliferation and survival signaling in the liver, after 8 weeks of treatment in high fat diet- and STZ-diabetic mice **(55).** Meanwhile, diabetic group treated with MSCs showed significant down-regulation in the gene expression levels of serum TGF-β, IL-8 and MCP-1, in comparison with the corresponding untreated groups. Hence, it could be deduced that the mechanisms mediate the protective effects of MSCs most probably, paracrine **(10).**

Immune system

The flow cytometric data, herein, 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 data support the assumption made by **Kuhn *et al*. (56)** who proposed that progressive defective immune regulation is a hallmark of T1DM; which is certainly one of the prototypic T cell-mediated autoimmune diseases. However, in T1DM, β cells are highly sensitive to selective damage and recruit immune cells by chemokine production. The autoimmune destruction of the pancreatic islet β cells is due to a targeted lymphocyte attack. These immune cells directly damage β cells and induce enzymes and cytokines that cause free radical and cytokine-induced apoptosis. Damaged islets express innate immune receptors, engagement of which may amplify β cell destruction contributing to their own destruction **(57).** In this line, **Kracht *et al*. (58)** 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*. (59)** 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 **(60).** 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 **(61).** Our 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. In fact, sensing damaged signals, both endogenous and exogenous MSCs migrate to the damaged site where they involve in the reconstitution of the immune microenvironment and empower tissue stem/progenitor cells and other resident cells, whereby facilitate tissue repairs. In this process, MSCs have been found to exert extensive immunosuppression on both innate and adaptive immune response, while such regulation needs to be licensed by inflammation **(62).**

MSCs have shown suppressive effects on many types of immune cells *in vitro* and *in vivo*. For example, it has been demonstrated that MSCs directly suppress T cell activation/proliferation and induce T cell apoptosis by expressing nitric oxide (NO), indoleamine 2,3, dioxygenase (IDO), programmed death ligand 1 (PD-L1), or Fas ligand. Also, MSCs have been shown to affect differentiation, maturation, and function of antigen-presenting cells (APCs); including dendritic cells and macrophages; which results in conversion of APCs into a suppressive or tolerogenic phenotype **(63).** Hence, hypoglycemia of MSC transplantation is an indirect effect of secretion of immunomodulators, which prevent T cells from eliciting pancreatic β-cell destruction, **(64).** The starting hypothesis is that an increase in the number of circulating MSCs would provide immunomodulation, and then would be able to interrupt the immune process that causes β cell death in T1DM **(65).**

Apoptosis

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 **(66).** The present results are in consistence with these findings as it showed that the annexin V %, P53 % 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*. (21)** 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. Injection 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 **(47).** 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 **(22).** 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 **(52).**

Our ongoing study revealed that, treatment of diabetic rats with either insulin or BM-MSCs down-regulated pancreatic annexin V, P53 and caspase 3 levels, while up-regulated Bcl-2 expressions at the same time compared to the diabetic-untreated rats. Such 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*. (67)** 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, these findings are in agreement with those of **Christ *et al*. (53)** 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 (43)** study**,** after 48 h of MSCs transplantation, islet cells exhibited higher viability and reduced apoptosis. 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 **(29).** 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 **(65).** MSCs also function as trophic mediators that promote angiogenesis, have anti-apoptotic effects and reduce inflammation **(52).** Such suggestion was supported by **Li *et al*. (44)** findings which stated that the percentage of renal apoptotic cells was significantly lower in H_2O_2-induced glomerulonephritis mice treated with MSCs. In addition, Bcl-2 and CD40lg were up-regulated, contributing to the antiapoptotic effect of MSCs in mouse kidney**;** that could possibly attributable to their ability to erase oxidative radicals by detoxifying ROS and superoxide**.**

Conclusion:-

Taken together, these studies indicated that MSCs therapy of diabetes metabolic abnormalities in addition to markedly hepato-renal dysfunction was superior to insulin treatment; which might have glycemic and metabolic control but was less effectively improve diabetic complications. BM-MSCs beneficial effects may involve individual or combinatorial effects of various protective processes, e.g., cells differentiation and regeneration, anti-inflammatory potency, immune modulation and protection capacity and control of hyperglycemia; however, the complete derivation of the exact mechanisms of action have yet to be elucidated.

The use of a cell-based therapy and in this particular case MSCs, is a global reality with benefits for both patients and health care systems. The issues associated with the scale up production, hamper the exploration of stem cells to be used as a choice of therapeutic resources. Given that hyperglycemia-induced oxidative stress has been well recognized as the major DM development etiological factor, further MSCs' antioxidant capacity role investigation for the pancreatic islets and various tissues survival promotion may validate its co-transplantation utility with islet transplantation in managing diabetic complications. However, the future years may come up with the solutions to use stem cells as a therapeutic agent to cure diabetes.

From our results, the future of β cell replacement therapy in DM treatment is very promising; more than insulin injection; although it is still challenging to protect these cells from autoimmune attack in type 1 diabetic patients. In the coming years, we recommended more clinical trials to move these technologies toward treatments of DM and other diseases.

List of abbreviations

AGEs: Advanced glycation end products, **BM-MSCs:** Bone marrow derived mesenchymal stem cells**, CAT:** Catalase, **D:** Diabetic, **DM:** Diabetes mellitus**, DMEM:** Dulbecco's modified Eagle's medium, **FBG:** Fasting blood glucose, **FBS:** fetal bovine serum, **GLUT-4:** glucose transporter-4, **GPx:** Glutathione peroxidase, **GRd:** Glutathione reductase, **GSH:** Glutathione**, GST:** Glutathione-S-transferase, **HbA1c:** Glycosylated hemoglobin, **H&E:** Hematoxylin and Eosin, **HO-1:** Heme-oxygenase 1, **Inos:** inducible nitric oxide synthase, **IPCs:** Insulin producing cells, **LPO:** Lipid peroxidation, **MCP-1:** Chemoatractant-1**, MDA:** Malondialdehyde, **MSCs:** Mesenchymal stem cells, **NO:** Nitric oxide, **PUFA:** Poly-unsaturated fatty acids, **RAGE:** Receptor of AGEs, **ROS:** Reactive oxygen species, **SEM:** Standard error of mean**, SOD:** Superoxide dismutase, **SPSS:** Statistical Package for Social Scientists, **STZ:** Streptozotocin, **T1DM:** Type 1 diabetes mellitus, **TAC:** Total antioxidant capacity, **TOS:** Total oxidative status, **XO:** Xanthine oxidase.

Declarations

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Authors' contributions

SGE, AN, HR and DYK conceived and designed the study. Experiments and lab work were done by SGE, HR and DYK, while tabulating and acquisition the data, searching for literature and preparing the first draft of the manuscript were performed by SGE, HR and AN. Meanwhile, histology and pathology work were carried out by SGE and RYK. All authors have read and approved the final state of the manuscript.

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Availability of data and materials

The data-sets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

All experimental procedures were done according to research protocols approved by the Animal Care and Bioethics Committee of the Faculty of Science, El-Arish University, El-Arish, Egypt.

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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