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Investigation of the antioxidant defensive role of both AD-MSCs and BM-MSCs in modulating the alteration in the oxidative stress status in various STZ-diabetic rats' tissues

Shady G. El-Sawah[1,*]; Fayez Althobaiti[2]; Adil Aldhahrani[3]; Eman Fayad[2]; Marwa A. Abdel-Dayem[4]; Rehab M. Amen[5]; El Shaimaa Shabana[6]; Ehab I. El-Hallous[7]; Hanan M. Rashwan[1]

[1] Zoology Department, Faculty of Science, Arish University, North Sinai, Egypt

[2] Biotechnology Department, Faculty of Science, Taif University, Taif, 21944, Saudi Arabia

[3] Clinical Laboratory Science Department, Turabah University College, Taif University, Taif, 21995, Saudi Arabia

[4] Department of Pharmacology and Biochemistry, Faculty of Pharmacy, Horus University-Egypt, New Damietta, 34518, Egypt

[5] Biology Department, College of Science, University of Bisha, Bisha, 61922, Saudi Arabia

[6] Fellow of Biochemistry, Genetic Unit, Children Hospital, Faculty of Medicine, Mansoura University, Mansoura, Egypt

[7] Biology Department, Faculty of Science, Taif University, Taif, Saudi Arabia

Abstract: Diabetes mellitus (DM) could negatively affect patients' health via inducing a lot of serious functional hazards in many tissues' cells at molecular levels. Recently, many scientists had proposed stem cell therapy being an appropriate alternative treatment protocol for numerous health threatening issues including diabetes. Therefore, the current study was designed to investigate the antioxidant potentiality of two MSCs types in alleviating tissues' oxidative stress dramatic elevation resulting as a consequence of Type 1 DM induction. In our 4 weeks study, animals were divided into four groups: control group, STZ-diabetic group (D), D+AD-MSCs group and D+BM-MSCs group. Data reported that diabetic rats treated with either AD-MSCs or BM-MSCs exhibited a marvelous body tissues (Pancreas, Liver and Kidney) enhancing capabilities in attenuating the oxidative stress status; as evidenced by XO, ROS, and MDA levels down-regulation; with a general concomitant elevation in the antioxidants' content; evidenced by many enzymatic and non-enzymatic antioxidants up-regulation; relative to the diabetic untreated group. Interestingly, comparing both treatments with each other and to control group, most of the measured parameters were reverted back to near normal levels after AD-MSCs injection; which clearly point out their stunning health benefits and superiority as anti-diabetic agent in overcoming different tissues' complications; owing to their marked cytoprotective and regenerative potentialities.

Abbreviations

AD-MSCs:	adipose-derived mesenchymal stem cells
AGEs:	advanced glycation end products
BM-MSCs:	bone marrow-derived mesenchymal stem cells
CAT:	catalase
CD:	cluster of differentiation
D:	diabetic
DM:	diabetes mellitus
DMEM:	dulbecco's modified Eagle's medium
FBG:	fasting blood glucose
FBS:	fetal bovine serum
GPx:	glutathione peroxidase
GRd:	glutathione reductase
GSH:	glutathione
GST:	glutathione-S-transferase
H_2O_2:	hydrogen peroxide
HO-1:	heme-oxygenase 1
iNOS:	inducible nitric oxide synthase
IPCs:	insulin producing cells
ISCT:	international Society for Cellular Therapy
LPO:	lipid peroxidation
MDA:	malondialdehyde
MSCs:	mesenchymal stem cells
NO:	nitric oxide
PBS:	phosphate-buffered saline

*Address correspondence to: Shady G. El-Sawah,
dr.shadygamal83@gmail.com

PUFA:	poly-unsaturated fatty acids
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
TBARS:	thiobarbituric acid reactive substances
TOS:	total oxidative status
XO:	xanthine oxidase

Introduction

Diabetes mellitus (DM), a chronic metabolic disorder characterized with persistent elevated blood glucose level, is a growing disease in terms of the number of patients and the percentage of the population. A sudden increase in diabetic patients' number was noticed throughout the world in last decades; particularly in the developing countries, although huge efforts made to suppress outspread of this metabolic disorder. The total number of diabetics worldwide has quadrupled in the last three decades. Interestingly, WHO recent estimations reported that DM prevalence rate among adults over 18 years was increased from 4.7% in 1980 to 8.5% in 2014. Moreover, International Diabetes Federation (IDF) last report expected that diabetic population count will rise from 415 million in 2015 reaching 642 million in 2040 (Peng et al., 2018; Laddha and Kulkarni, 2019; Gharib et al., 2020).

It was well known that pancreatic β-cells' autoimmune destruction could induce Type 1 diabetes mellitus (T1DM) leading to a severe hypoinsulinemia and subsequent blood glucose level elevation. Such events, in turn, could increase advanced glycation end products (AGEs) formation with a marked oxidative stress progression leading to development of many pathological changes and complications in different organs; suggesting this disease one of the major leading causes of mortality worldwide (Laddha and Kulkarni, 2019; Aminzadeh et al., 2020). In patients with T1DM, continuous exogenous insulin therapy through daily injections is the most prominent therapeutic strategy that cannot be avoided. Despite being effective in most patients, sometimes diabetes and its associated complications progression might not be properly managed with insulin injection. Therefore, modern therapeutic approaches as regenerative medicine are advancing rapidly, breaking new ground in DM treatment.

Recently, stem cell therapy technologies; had gained a huge popularity as a promising therapeutic strategy for many degenerative disorders, including T1DM, owing to their self-renewal ability, multipotentiality, and immunomodulatory and regenerative capabilities (Päth et al., 2019; Aminzadeh et al., 2020; Bani Hamad et al., 2021). Since mesenchymal stem cells (MSCs) had taken the lead in β-cells regeneration, both adipose-derived (AD-MSCs) and bone-marrow (BM-MSCs) stem cells were proposed as more readily available types of stem cells that could be used in diabetes treatment (Brovkina et al., 2019). According to the minimal criteria laid down by Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) (Dominici et al., 2006), MSCs were defined by three parameters: the cells (1) they must be plastic-adherent and can be maintained in standard culture conditions; (2) express cluster of differentiation (CD105, CD73 and CD90 without expressing other lineage markers CD45 (panleukocyte), CD34 (hematopoietic and endothelial), CD14/CD11b (monocytic), CD79a/CD19 (B cell), or human leukocyte antigen (HLA) class II); and (3) show the classical trilineage differentiation into osteoblasts, adipocytes, and chondroblasts in vitro.

AD-MSCs success in translational medicine applications had been attributed to many reasons; such as their self-renewal, multipotency properties and the relatively simple and safe cell isolation protocol (Argentati et al., 2018; Si et al., 2019). However, hematopoietic stem cells and mesenchymal stem cells; are two distinct populations found in BM-MSCs; of which the later was considered more successful in adopting a pancreatic fate (Carlsson et al., 2015). Collectively, since T1DM was suggested as a probable candidate which might benefit from stem cell therapy procedure, this review outline the possible therapeutic benefits of both AD-MSC and BM-MSCs for the treatment of T1DM and its related complications via exploring their antioxidant capacity functionality in regulating and alleviating tissues' oxidative stress status resulting as a consequence of DM induction in rats.

Materials and Methods

Chemicals
Streptozotocin (STZ) and culture media constituents of both BM-MSCs and AD-MSCs were purchased from Sigma Aldrich Co. (St. Louis, Mo 6, USA); and were of pure chemical gradient.

Biochemical assays
Serum glucose concentration was estimated by Trinder (1969) method using SPINREACT diagnostics kit, Spain. Insulin measurement occurred by ELISA kit purchased from Boehringer Mannheim, Germany, according to the method of Flier et al. (1976) using Boehringer Analyzer ES 300. C-peptide measurement occurred by enzyme immunoassay (EIA) kit purchased from Bio Vision, USA, according to the method of Flier et al. (1976). Glycosylated hemoglobin (HbA1c) was estimated according to the method of Gonen and Rubenstein (1978) by using glycosylated hemoglobin kit obtained from Teco Diagnostics, USA. Bio Diagnostic Company (Egypt) kits were used in estimating tissue levels of some oxidative stress markers [Xanthine oxidase (XO) and Reactive oxygen species (ROS) according to Young (2001), Malondialdehyde (MDA) according to Ohkawa et al. (1982)] in addition to many antioxidant markers [Catalase (CAT) according to Bock et al. (1980), Glutathione-S-transferase (GST) according to Habig et al. (1974), Superoxide dismutase (SOD) according to Nishikimi et al. (1972), Glutathione (GSH) according to Prins and Loose (1969), Heme-oxygenase 1 (HO-1) according to Gonen and Rubenstein (1978) and Total antioxidant capacity (TAC) according to Koracevic et al. (2001)], all according to the instructions of the supplier. Meanwhile, tissue G0/G1% in addition to surface markers % (for characterization of the

isolated BM-MSCs and AD-MSCs phenotyping) was determined via Sigma Aldrich Company (St. Louis, Mo 6, USA) kits through flow cytometric analysis. Cultured cells were harvested and stained with antibodies against CD44, CD73, CD90, CD45, CD11b and CD31. using FACS caliber flow cytometer (Becton Dickinson, Sunnyvale, CA, USA), equipped with a compact air cooked low power 15 mW argon-iron laser beam (488 nm). Average evaluated nuclei per specimen are 20.000 (120 nuclei/s). Dean and Jett computer program for mathematical analysis is used to obtain the DNA histograms (Dean and Jett, 1974).

BM-MSCs and AD-MSCs preparation

Fresh bone marrow and subcutaneous adipose tissues were obtained from male 6–8-week-old rats and used to isolate BM-MSCs and AD-MSCs, respectively. To prepare BM-MSCs, each end of the femur and tibia was cut to expose the marrow cavity, then washed three times with phosphate-buffered saline (PBS). Fresh bone marrow was collected and centrifuged at 2000 rpm for 10 min. Pelleted cells were suspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 U/mL penicillin with 100 µg/mL streptomycin as an antibiotic at 37°C in a 5% CO_2 atmospheric state with 95% humidity, then centrifuged at 2000 rpm for 10 min (Hamza et al., 2017). On the other hand, 1–2 mg fresh rats' subcutaneous adipose tissues (epididymal fat) were harvested by lipoaspiration, minced, washed extensively three times in PBS and incubated into a digestion solution containing 0.075% collagenase Type I (prepared in PBS) at 37°C for 3 h, then centrifuged for 10 min at 2000 rpm. After discarding the supernatant, cells were collected as a pellet and suspended in DMEM (10% FBS, 2 mM L-glutamine, and 100 U/mL penicillin with 100 µg/mL streptomycin as an antibiotic) at 37°C in 5% CO_2 with 95% humidity (Chen et al., 2020). The growth mediums of both MSCs types were changed every 3 days, and non-adherent cells were removed. All MSCs used in this study, were from passage 3–4, and transferred chilled for transplantation within 2 h.

BM-MSCs and AD-MSCs characterization

The inverted microscope has been used to perform both BM-MSCs and AD-MSCs morphological characterization for confirming their identity. Moreover, stemness of cultured cells should be confirmed by positive and negative surface markers (CD11b, CD34, CD45, CD73, CD90 and CD105) by flow cytometry to confirm retaining of their phenotype before performing the animal study; according to the minimal criteria defined by ISCT.

Experimental animals and maintenance

In this experimental study, twenty-four Rattus rattus male albino rats (6–8 weeks, 100–120 g) were kept throughout the study under a conventional laboratory conditions (a 12/12-h light/dark cycle, humidity of 50 ± 5%, and room temperature of 22 ± 2°C) in an aerated stainless cage. All rats were allowed free access to bottles of water, and standard pellet chow-containing plates. Following two weeks of acclimatization, four rat groups have been established randomly (n = 6). The study

was continued for 4 consecutive weeks. All experimental procedures were approved and performed in accordance with the guidelines of the Animal Ethics Committee of the Faculty of Science, Arish University, North Sinai, Egypt. All efforts were made to minimize animal suffering.

Diabetes induction

A single STZ solution dose (45 mg/kg dissolved in citrate buffer, pH 4.6) were injected intraperitoneally in overnight fasting rats, while control rats received the vehicle alone. Animals were allowed to drink 5% glucose solution overnight to overcome drug-induced hypoglycemia. Diabetes was confirmed 3 days after induction via detecting level of blood glucose from tail vein, using Glukotest of diagnosis glucose level by ACCU–CHEKGo apparatus (Roche Company, Germany). Rats having fasting blood glucose (FBG) above 250 mg/dL were confirmed as diabetic and used for further experimentation (Kodidela et al., 2020).

Experimental design

1. Control group: Injected intraperitoneally with a single dose of sodium citrate buffer (pH 4.5).
2. Diabetic (D) untreated group: Injected intraperitoneally with a single dose of STZ (45 mg/kg bw) dissolved in sodium citrate buffer (pH 4.5).
3. Diabetic AD-MSCs treated group: Injected intravenously with a single dose of AD-MSCs (1×10^6 cell/rat).
4. Diabetic BM-MSCs treated group: Injected intravenously with single dose of BM-MSCs (1×10^6 cell/rat).

Sample collection

Before being dissected, diethyl ether was used to anesthetize rats and tissues' specimens (pancreas, liver, and kidney) were quickly separated. An appropriate part was weighed and homogenized in cold distilled water forming 10% (w/v) homogenate, then labeled and kept at −20°C for latter biochemical estimations. However, the remnant parts were 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 (n = 6). The significance between groups was considered at the $P \leq 0.05$.

Results

Figs. 1A and 1B demonstrated the characterization of BM-MSCs and AD-MSCs based on the expression of their specific surface markers by fluorescence-activated cell sorting flow cytometry analysis. Data indicating that detected MSCs surface markers were highly expressed by both BM-MSCs and AD-MSCs. CD73, CD90 and CD105 expression were very high and considered positive. By contrast, CD11b, CD34 and CD45 expression were very low
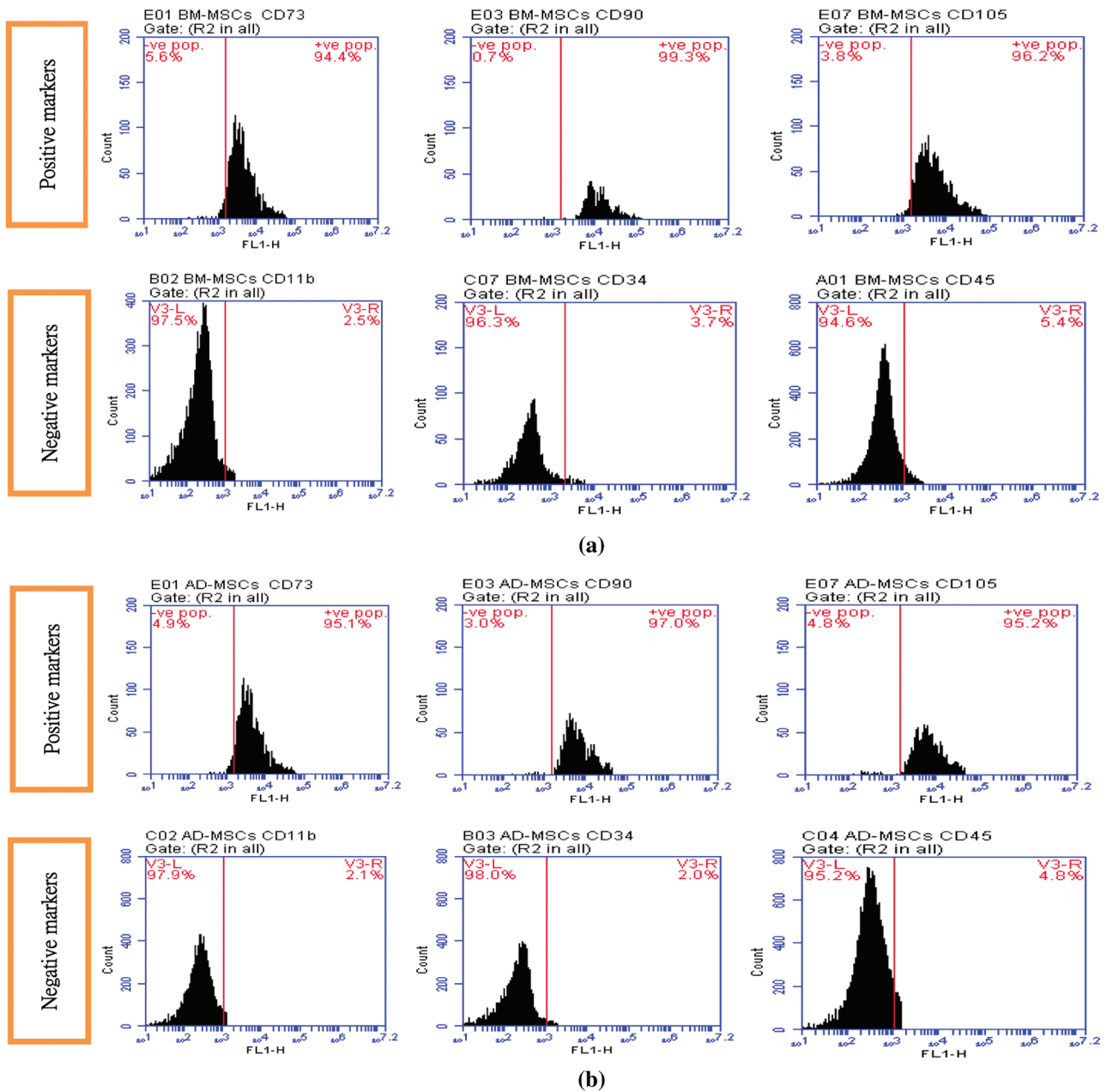
(a)

(b)

FIGURE 1. (A) BM-MSCs positive surface markers (CD 73: 94.4%, CD 90: 99.3%, CD 105: 96.2%) BM-MSCs negative surface markers (CD 11b: 2.5%, CD 34: 3.7%, CD 45: 5.4%), (B) AD-MSCs positive surface markers (CD 73: 95.1%, CD 90: 97.0%, CD 105: 95.2%). AD-MSCs negative surface markers (CD 11b: 2.1%, CD 34: 2.0%, CD 45: 4.8%)

and considered negative. Such results are concomitants with the minimal criteria defined by ISCT.

Tab. 1 showed a marked serum glucose and HbA1c elevation coupled with a significant insulin and C-peptide levels decline in diabetic group; compared to control. Regarding both MSCs-treated groups, all measured parameters of the diabetic rats revealed an obvious enhancement relative to diabetics, although values of BM-MSCs were still significantly variable relative to control. However, data of diabetic rats injected with AD-MSCs detected a marked hypoglycemic superiority when compared to those of AD-MSCs.

Tab. 2 illustrated that diabetic group exhibited significant increases in pancreatic oxidative stress markers (MDA, ROS, and XO) levels coupled with a marked decline in all

antioxidant markers (GSH, SOD, CAT, GST, TAC and HO-1) levels in addition to pancreatic viable cells (%G0/G1), when compared to control group. However, present results revealed that diabetic rats treated with either AD-MSCs or BM-MSCs showed marked decrease in oxidative stress markers, while a significant increase in both antioxidant markers levels and G0/G1%; when compared to the diabetic group. On the other hand, AD-MSCs cleared non-significant changes in all tested parameters compared to control group; in contrast to BM-MSCs values which were still significantly variable. Interestingly, all results of diabetic rats treated with AD-MSCs recorded a much more enhancement than BM-MSCs treatment.

Tab. 3 summarized data indicated a significant increase in all hepatic oxidative stress parameters

TABLE 1

Serum glucose, insulin, C-peptide and HbA1c levels

	Control	Diabetic (D)	D+AD-MSCs	D+BM-MSCs
Glucose (mg/100 mL)	93.91 ± 4.42	405.40 ± 16.46 [a]	96.75 ± 5.70 [b]	160.50 ± 5.22 [abc]
Insulin (μIU/mL)	17.15 ± 0.66	8.02 ± 0.33 [a]	16.90 ± 0.34 [b]	14.32 ± 0.57 [abc]
C-peptide (ng/mL)	0.84 ± 0.03	0.30 ± 0.03 [a]	0.81 ± 0.01 [b]	0.75 ± 0.02 [abc]
HbA1c (%)	2.95 ± 0.11	4.90 ± 0.20 [a]	2.98 ± 0.14 [b]	3.29 ± 0.10 [abc]

Note: Values expressed as mean ± SEM (n = 6). a, b, c are significant differences ($P \leq 0.05$) compared to control, diabetic and diabetic AD-MSCs treated groups, respectively.

TABLE 2

Pancreatic oxidative stress, antioxidant markers levels and viable cells (%G0/G1)

	Control	Diabetic (D)	D+AD-MSCs	D+BM-MSCs
MDA (nmol/g)	10.40 ± 0.43	30.33 ± 1.05 [a]	10.65 ± 1.03 [b]	15.50 ± 1.76 [abc]
ROS (nmol/g)	0.79 ± 0.03	2.12 ± 0.08 [a]	0.80 ± 0.03 [b]	1.02 ± 0.02 [abc]
XO (nmol/mL)	21.50 ± 1.03	50.00 ± 2.67 [a]	22.25 ± 1.08 [b]	29.50 ± 1.10 [abc]
GSH (mg/gm)	53.00 ± 2.05	21.30 ± 1.34 [a]	50.50 ± 2.64 [b]	39.06 ± 2.87 [abc]
SOD (U/gm)	10.22 ± 0.32	4.50 ± 0.13 [a]	10.11 ± 0.43 [b]	8.60 ± 0.43 [abc]
CAT (U/gm)	0.60 ± 0.04	0.32 ± 0.03 [a]	0.62 ± 0.03 [b]	0.49 ± 0.02 [abc]
GST (mmol/gm)	6.30 ± 0.21	2.70 ± 0.16 [a]	6.22 ± 0.22 [b]	4.50 ± 0.24 [abc]
TAC (mg/gm)	1.89 ± 0.09	0.53 ± 0.03 [a]	1.85 ± 0.07 [b]	1.40 ± 0.06 [abc]
HO-1 (pmol/mg)	309.00 ± 13.42	110.00 ± 4.26 [a]	304.00 ± 11.23 [b]	235.00 ± 13.09 [abc]
%G0/G1	93.50 ± 2.23	55.40 ± 1.37 [a]	92.50 ± 2.73 [b]	75.00 ± 2.22 [abc]

Note: Values expressed as mean ± SEM (n = 6). a, b, c are significant differences ($P \leq 0.05$) comparing to control, diabetic and diabetic AD-MSCs treated groups, respectively.

TABLE 3

Hepatic oxidative stress, antioxidant markers levels and viable cells (%G0/G1)

	Control	Diabetic (D)	D+AD-MSCs	D+BM-MSCs
MDA (nmol/g)	80.00 ± 4.23	161.00 ± 8.54 [a]	81.00 ± 4.08 [b]	102.00 ± 4.65 [abc]
ROS (nmol/g)	0.57 ± 0.04	2.70 ± 0.19 [a]	0.59 ± 0.04 [b]	0.75 ± 0.03 [abc]
XO (nmol/mL)	43.00 ± 2.14	90.00 ± 5.54 [a]	46.00 ± 2.78 [b]	57.50 ± 2.32 [abc]
GSH (mg/gm)	80.00 ± 4.28	35.22 ± 1.65 [a]	80.30 ± 3.87 [b]	62.59 ± 3.38 [abc]
SOD (U/gm)	22.22 ± 1.03	10.20 ± 0.42 [a]	20.50 ± 1.02 [b]	16.07 ± 0.76 [abc]
CAT (U/gm)	0.98 ± 0.03	0.20 ± 0.01 [a]	0.94 ± 0.03 [b]	0.68 ± 0.04 [abc]
GST (mmol/gm)	10.90 ± 0.44	5.40 ± 0.53 [a]	10.70 ± 0.40 [b]	8.20 ± 0.42 [abc]
TAC (mg/gm)	3.20 ± 0.12	1.20 ± 0.06 [a]	3.15 ± 0.15 [b]	2.75 ± 0.13 [abc]
HO-1 (pmol/mg)	425.00 ± 17.13	210.50 ± 13.33 [a]	416.66 ± 15.25 [b]	365.30 ± 18.65 [abc]
%G0/G1	95.00 ± 2.47	60.10 ± 1.23 [a]	93.00 ± 1.42 [b]	78.50 ± 1.82 [abc]

Note: Values expressed as mean ± SEM (n = 6). a, b, c are significant differences ($P \leq 0.05$) compared to control, diabetic and diabetic AD-MSCs treated groups, respectively.

accompanied with a significant decrease in all antioxidant markers levels and %G0/G1 in diabetic group, regarding normal control one. On the other hand, relative to diabetic group, both treated groups' results showed a significant enhancement in all tested parameters. Surprisingly, obtained results of the diabetic rats injected with AD-MSCs displayed an obvious improvement variation in all mentioned parameters, compared to those treated with BM-MSCs.

Tab. 4 obtained results revealed a marked oxidative stress status development in kidney tissues, while a marked antioxidant capacity decline in diabetic rats, in regard to

TABLE 4

Renal oxidative stress, antioxidant markers levels and viable cells (%G0/G1)

	Control	Diabetic (D)	D+AD-MSCs	D+BM-MSCs
MDA (nmol/g)	28.00 ± 1.30	65.53 ± 2.87 [a]	30.16 ± 1.34 [b]	39.15 ± 1.73 [abc]
ROS (nmol/g)	0.45 ± 0.03	1.60 ± 0.09 [a]	0.50 ± 0.03 [b]	0.65 ± 0.04 [abc]
XO (nmol/mL)	32.30 ± 1.34	70.00 ± 3.33 [a]	33.00 ± 2.12 [b]	45.00 ± 1.54 [abc]
GSH (mg/gm)	48.24 ± 2.65	20.87± 1.75 [a]	49.73 ± 2.27 [b]	36.20 ± 2.29 [abc]
SOD (U/gm)	11.24 ± 0.38	4.65 ± 0.23 [a]	11.70 ± 0.24 [b]	9.40 ± 0.33 [abc]
CAT (U/gm)	0.59 ± 0.04	0.30 ± 0.02 [a]	0.59 ± 0.02 [b]	0.43 ± 0.03 [abc]
GST (mmol/gm)	5.50 ± 0.36	2.20 ± 0.14 [a]	5.50 ± 0.20 [b]	4.12 ± 0.23 [abc]
TAC (mg/gm)	1.30 ± 0.05	0.40 ± 0.03 [a]	1.20 ± 0.03 [b]	0.97 ± 0.04 [abc]
HO-1(pmol/mg)	255.00 ±10.30	110.50 ± 6.34 [a]	249.00 ± 11.54 [b]	210.00 ± 13.54 [abc]
%G0/G1	94.50 ± 2.73	59.60 ± 2.24 [a]	94.00 ± 2.21 [b]	79.50 ± 2.26 [abc]

Note: Values expressed as mean ± SEM (n = 6). a, b, c are significant differences ($P \leq 0.05$) comparing to control, diabetic and diabetic AD-MSCs treated groups, respectively.

control rats. Both MSCs treated groups tested indicators clearly demonstrated a significant improvement, when compared to the diabetic untreated group. Notably, diabetic rats injected with AD-MSCs recorded a great amelioration in all tested parameters; comparing to BM-MSCs treated rats.

Discussion

DM impact on the human body is not restricted to a respective organ, since severe acute or chronic co-morbidities could take place if hyperglycemia persists and improperly controlled (Rogal et al., 2019). Since, current and traditional diabetes treatments only focused on to regulate the insulin level without alleviating the diabetic complications (Jamshidi et al., 2018), nowadays treatment protocols focus on the antioxidant protection of the pancreatic β-cells and other tissues' cells; in order to facilitate their repair. In this line, cell-based therapy, in particular stem cells injection, was suggested as a promising therapeutic candidate against diabetic complications; owing to their regeneration potential and multilineage differentiation (Aminzadeh et al., 2020).

Course STZ administration was found to selectively targets and kills pancreatic β cells, causing a reduction in insulin production and subsequent prolonged hyperglycemia inducing an experimental model of T1DM; possibly via induction of cellular stress leading to excessive ROS production resulting in generation of lipid peroxidation (LPO) and DNA breaks, which plays a vital role in pancreatic dysfunction via causing inflammation, pancreatic islet cell death and diabetes (Hamza et al., 2017; Jamshidi et al., 2018). Herein, the increase in fasting blood glucose (FBG) in diabetic rats is well expected and agrees with earlier reports that cleared that diabetic subjects exhibited a huge serum glucose and HbA1c levels, coupled with a marked C-peptide and insulin levels decline; regarding control subjects (Amer et al., 2018; Samaha et al., 2020). These findings are also in agreement with Zang et al. (2017) and Nia et al. (2018) and their teams who reported that glucose level control deviation is sufficient to initiate a series

of maladaptive processes resulting in an oxidative stress injury and elevated ROS and MDA production; which they were recognized as the major etiological factor in the development of diabetes; which might be a reflection of decreased antioxidant capability of the defensive systems and/or glucose oxidation. This would cause an inner endothelial tissue damage; that might eventually be directly responsible for the blood glucose level elevation. Similar assumption has been documented by Turkmen (2017) who stated that, the well-established features of STZ-diabetic complications include elevated blood glucose concentration accompanied by increased overproduction of LPO and ROS; mostly via mitochondrial electron transport chain; participating in the establishment of oxidative stress in many diabetic rats' tissues compared to control. Many researchers attributed these disturbances to the marked decreased activities of many antioxidant enzymes, such as GSH-Px, SOD and CAT; suggesting that these antioxidants are exhausted to combat the deleterious effects of the increased oxidative stress (Mayyas et al., 2018; Kodidela et al., 2020). Such events could result in a notable cellular injury and to a point of no return in apoptosis when ROS scavenging and cytoprotective molecules are insufficiency present. Similar results were reported by El-Kholy et al. (2018) and El-Sawah et al. (2020).

On the other hand, owing to their ability to stimulate the damaged pancreatic β-cells proliferation and regeneration, MSCs hypoglycemic therapeutic effects on diabetes have been confirmed by many reports. Surprisingly, insulin producing cells (IPCs) differentiated from either AD-MSCs or BM-MSCs injection could obviously lower glucose and HbA1c serum levels and increase both C-peptide and insulin release; resulting in marked glucose and HbA1c reduction in diabetic rats (Gabr et al., 2017; El-Kholy et al., 2018) and in diabetic mice (Kono et al., 2014) as well as in diabetic patients (Thakkar et al., 2015); confirming MSCs hypoglycemic activity. In this regard, Amer et al. (2018) found that STZ-diabetic rats received IPCs differentiated from AD-MSCs; showed an apparent islet cells proliferation

and regeneration that leads to a marked C-peptide increase accompanied with insulin secretion elevation; in glucose dependent manner. In this line, Zang *et al.* (2017) also reported that BM-MSCs showed a powerful glycemic control indicated by the 50% decline in insulin requirements; through restoring islet function, endogenous islet cells protection, induction of islet cell regeneration and differentiation into IPCs; followed by marked restoration of β-cell mass and significant hypoglycemia in STZ-diabetic mice.

Many reports attributed the obvious hypoglycemic capability of MSCs to their marked antioxidant activity in alleviating the resultant oxidative stress status due to prolonged hyperglycemia. Interestingly, according to Fattah *et al.* (2020) and Aminzadeh *et al.* (2020) administration of MSCs extremely ameliorated the elevated MDA and ROS levels while increasing the mRNA expression levels of many antioxidants, such as GSH, SOD-1 and 3, CAT, GSH-Px-1, 3 and 4, and TAC; in STZ-diabetic rats' various tissues including pancreas; relative to diabetic untreated rats. In this regard, Chandravanshi and Bhonde (2017) also suggested a 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 were found to suppress oxidative stress-induced renal damage through detoxifying ROS and increasing renal GST, GPx, SOD and CAT expression; which are considered as potential scavengers of free oxidative radicals; associated with renal cell inflammation and apoptosis inhibition in diabetic mouse kidneys. According to Chen *et al.* (2020), a marked ROS production decrease with a HO-1 increase were found in pancreas of STZ-diabetic rats after injection with AD-MSCs (1×10^6 cells/rat) for 2 months; compared to the untreated rats. Also, El-Kholy *et al.* (2018) and El-Sawah *et al.* (2020) results showed significant improvement in glucose, insulin, TAC, and MDA levels in STZ-diabetic rats injected with BM-MSCs (1×10^6 cell/rat); compared to the diabetic untreated rats.

Conclusion

Since our data reported the superiority of AD-MSCs injection over BM-MSCs in mitigating the prolonged hyperglycemic condition in diabetic subjects via increasing their tissues' protective antioxidant contents; and thus, lowering the progress of the oxidative stress status; we could strongly propose AD-MSCs administration as a promising therapeutic strategy for T1DM; that may validate its co-transplantation utility with islet transplantation in managing diabetic complications.

Authors' Contribution: SGE and FA conceived and designed the study. Experiments and lab work were done by SGE, MAA, HR, EIH, RMA and ES, while data tabulating and acquisition, searching for literature and preparing the first draft of the manuscript were performed by SGE, MAA, AA and EF. All authors have read and agreed to the published version of the manuscript.

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