Therapeutic Efficacy of Bone Marrow- Derived Mesenchymal Stem Cells Plus Zinc Oxide Nanoparticles Against Streptozotocin- Induced Type 2 Diabetes in Albino Rats.

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ABSTRACT

Diabetes mellitus (DM) is characterized by hyperglycemia which is often related with long-term complications, including retinopathy, nephropathy, and neuropathy, macro vascular and micro vascular damage. The current study was designed to evaluate the ability of bone marrow stem cells and ZnO nanoparticles to ameliorate the disturbances in some physiological parameters induced by DM in rats. Histological studied on the pancreas were also involved. Forty-two male albino rats were divided into seven groups, (n = 6 per group). Four of the groups were induced with diabetes by a single intraperitoneal injection of freshly prepared streptozotocin at a dose of 50 mg/kg body weight. After the induction of diabetes, the rats were treated with MSCs a single dose of (10^6cells per rat) and ZnO nanoparticles (10mg/kg) separately and in combination daily for 4 weeks, whereas, one of the diabetic groups served as a positive control. The blood glucose, serum insulin, pancreatic malondialdehyde (MDA), total antioxidant capacity (TAC) and interleukin-10(IL-10) levels were determined. Diabetic rats demonstrated a significant increase in plasma levels of glucose, pancreatic MDA and IL-10. In contrast, levels of plasma insulin and TAC in pancreas decreased significantly in diabetic rats compared to the control rats. Hence, diabetic rats treated with MSCs and ZnONPs in combination showed maximum improvements with respect to the indices in addition to a significant recovery observed by histopathological study when compared to the diabetic group.

Conclusions: These results revealed that treatment with MSCs in combination with ZnONPs in diabetic rats was more effective than treatment with either of MSCs or ZnONPs alone to alleviate the symptoms of diabetic mellitus

Keywords: Type 2 diabetes mellitus, bone marrow mesenchymal stem cells, ZnO nanoparticles, streptozotocin, Pancreas

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INTRODUCTION

Diabetes mellitus (DM), a life threatening single-cell metabolic disorder resulting from deficiency in insulin secretion or action or both as a result of damage or defective pancreatic beta cells (β-cell) (Tariq *et al.,* 2013). The occurrence of diabetes is rapidly increasing all over the world possibly due to population growth, ageing, increasing prevalence of obesity and sedentary lifestyle. According to the most recent report of the International Diabetes Federation (IDF), Diabetes affects 382 million people through the world and this number will rise to 592 million by 2035 (Nazarizadeh and Asri-Rezaie, 2016)

A combination of insulin resistance and pancreatic β-cell disorders appear to be involved in the progress of type 2 diabetes mellitus (T2DM). It is attributed to glucotoxicity, oxidative stress; free radical- mediated injury and possibly enhanced programmed β-cell (Rother, 2007).

The physiological control of blood glucose levels can only be restored efficiently by replacing the β-cell mass (Kim *et al.,* 2007). β-cells in the pancreatic islets of Langerhans are responsible for the secretion of insulin and much of the pathology of diabetes losses may be due to the loss of β-cell number and function. It was stated that the β-cell mass is unable to meet the increased insulin demands of the body, in patients with type 2 diabetes (ADA, 2013). Eventually, the β-cell mass in type 2 diabetes also declines to 40–60% of the normal range. Several hypoglycemic drugs, such as sulfonylurea and metformin are being used for the treatment of diabetes but their use is limited because none of the anti-diabetic drugs could give a long term glycaemic control without causing any adverse side effects. Also, the insulin therapy is the current mainstream treatment for diabetes, but unfortunately, even with improved insulin formulations, the use of new infusion systems as well as continuous glucose monitoring, keeping tight control in the normal range is impossible (El-Badri and Ghoneim, 2013). Moreover, Dominique Farge (2008) recorded that the alternate method to treat diabetes are transplantation of whole pancreas and/or pancreatic islets cells. But it is associated with several restrictions, such as major surgery, and organ rejection after transplantation (Azarpira *et al.,* 2015).

Hence, much effort has been occurred to generate β cell mass either by stimulating endogenous regeneration of islets or in vitro differentiation of islet-like cells (Bonner-Weir *et al.,* 2000, Schmied *et al.,* 2001 , Abraham *et al.,* 2002 and Godfrey *et al.,* 2012). Stem cells are characterized by their potential of self-renewal and differentiation into one or more cell types. Therefore, they represent an alternative and unlimited source for differentiation toward islet cells. Amongst stem cells, mesenchymal stem cells (MSCs) have numerous advantages for therapeutic use such as ability to migrate to the sites of tissue injury, strong immunosuppressive effects (Abdi *et al.,* 2008 and Volarevic *et al.,* 2010), and better safety after infusion of allogeneic MSCs (Hare *et al.,* 2009).

MSCs are multi-potent, self-renewing cells that are isolated from different tissues, such as the bone marrow, adipose tissue (Zuk *et al.,* 2001), amniotic fluid (**In 't Anker** *et al.,* 2003), and umbilical cord blood (Reddi *et al.,* 2010). The cells have great multiplication potency and can be expanded in culture for several passages without losing their properties (Polak and Bishop, 2006). MSCs can

differentiate into several cell types, including osteoblasts, cardiomyocytes, adipocytes, vascular endothelial cells, neurons, hepatocytes, epithelial cells, insulin-producing cells and making them a potentially important source for the treatment of debilitating human diseases (Muñoz-Elias *et al.,* 2004 and Chen *et al.,* 2008). Such multipotent differentiation characteristics coupled to their capacity for self-renewal and capability for the regulation of immune responses, described MSCs as potentially new therapeutic agents for treatment of the complications of DM (Pittenger *et al.,* 1999 and Ryan *et al.,* 2005). Several evidences suggested that due to the ability of MSCs to differentiate into many cells and tissues, MSCs are responsible for pancreatic endocrine differentiation and help in proliferation and vascularization (Hess *et al.,* 2003 and Mathews *et al.,* 2004).

In any case, the viability role of stem cells in homing, differentiation and their paracrine action, it is important to state that, oxidative stress could have a negative effect on the survival of transplanted MSCs. This is mostly attributed to the inflammatory and oxidative stress environment at the site of injury. It has been suggested that endogenous antioxidant level of stem cells might impact their destiny after transplantation at damaged host sites (El-Badri and Ghoneim, 2013).

Zinc (Zn) is a necessary micronutrient for pancreatic function through its effect on insulin stability and disturbance in Zn metabolism related to diabetic complications (Wolfgang, 2017). It is also a paramount player in the intracellular antioxidant mechanization through its contribution in the main antioxidant enzymes with free radical scavenging effect such as catalase and superoxide dismutase *(Masaki et al., 2007).* Hussein *et al.* (2014) stated that the reduction of Zn may exacerbate the oxidative stress-mediated complications of diabetes.

Zn has been reported to play a straight role in glucose homeostasis through enhancing hepatic glycogenesis through its actions on the insulin signaling pathway and thus it recovers glucose utilization (Jansen *et al.,* 2009); it inhibits intestinal glucose absorption (Ueda *et al.,* 2005) and increases glucose uptake in skeletal muscle and adipose tissue (Jansen *et al.,* 2009). Moreover, Zn is reported to inhibit glucagon secretion (Egefjjord *et al.,* 2010), thus reducing gluconeogenesis and glycogenolysis; it also enhances the structural integrity of insulin (Sun *et al.,* 2009). Lower level of zinc in pancreatic tissues associated with lower insulin synthesis through β- cells (Huang *et al.,* 2011). Elevated ROS can induce oxidative damage in pancreatic tissues with subsequent increased hyperglycemia *(*Meyer and Spence, 2009). Several Zn complexes have been synthesized and proven to be effective in rodent models of diabetes (Karmaker *et al.,* 2009). Recently there has been a huge development of nanotechnology in the science and technology field; metallic nanoparticles, like gold, silver, iron, Zn, and metal oxide nanoparticles, have shown great challenges in the field of medicine and its applications (Hirst *et al.,* 2013). The aim of the study is to demonstrate ameliorative effect of bone marrow -derived mesenchymal stem cells and zinc oxide nanoparticles in streptozotocin-induced type 2 diabetes mellitus.

MATERIAL AND METHODS

Animals
Fourty two adult male albino rats were employed in the present study. They were obtained from the animal house of Biology Department, College of Education, university of Salahaddin. Their weight ranged between 200-250g representing 8-9 weeks of age. Animals were allowed to adapt for one week pre-experimentation in order to avoid any complications along course of the experiment. They were housed in metabolic cages and received food and water ad-libitum with fresh supplies presented daily.

Ethics statement
This study was performed in accordance to the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH publication No. 85–23, revised 1996).

Chemicals
Streptozotocin (STZ), N (nitro derivative of glucosamine (2deoxy2 ({[methyl (nitroso) amino] carbonyl} amino) was purchased from Sigma chemical company (USA). Zinc oxide nanoparticles (ZON) were obtained from Sigma-Aldrich, in the form of dispersion of the following properties, concentration 20 wt% in H_2O, the average nanoparticle size < 40 nm, the particle size distribution (hydrodynamic diameter) < 100 nm using dynamic light scattering (DLS) technique, pH 7.5 ± 1.5 (for aqueous systems), and density 1.7 g/mL ± 0.1 g/mL at 25 °C.

Preparation of bone marrow -derived mesenchymal stem cells from rats
Bone marrow was collected by flushing the tibiae and femurs of 7-week-old male albino rats with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine serum (GIBCO/BRL). Nucleated cells were seperated with a density gradient [Ficoll/ Paque (Pharmacia)] and resuspended in complete culture medium supplemented with 1% penicillin–streptomycin (GIBCO/BRL). Cells were incubated at 37°C in 5% humidified CO2 for 12–14 days as primary culture or upon formation of large colonies. When large colonies developed (80–90% confluence), cultures were washed twice with phosphate buffer saline(PBS) and the cells were trypsinized with 0.25% trypsin in 1 mM EDTA (GIBCO/BRL) for 5 min at 37°C. After centrifugation, cells were resuspended in serum supplemented medium and incubated in 50 cm2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures (Abdel Aziz *et al.,* 2007). Cells were recognized as being MSCs by their morphology, adherence, and their power to differentiate into osteocytes and chondrocytes. Differentiation into osteocytes was achieved by adding 1-1000 nM dexamethasone, 0.25 mM ascorbic acid, and 1-10 mM beta glycerophosphate to the medium. Differentiation of MSCs into osteoblasts was definited by morphological

changes, Alzarin red staining of differentiated osteoblasts. Differentiation into chondrocyte was achieved by adding 500 ng/mL bone morphogenetic protein-2 (BMP-2;R&D Systems, USA) and 10 ng/ml transforming growth factor b3 (TGFb3) (Peprotech, London) for 3 weeks. In vitro differentiation into chondrocytes was confirmed by morphological changes, Alcian blue staining of differentiated chondrocytes.

Experimental design

Rats were randomly assigned into two main groups as following

Group(1) (Control group): 18 healthy male albino rats.

Group(2)(Diabetic group):Diabetes was induced according to Tamaddonfard *et al.* (2013) in 24 adult male albino rats. Briefly, the rats in the diabetic groups were fasted overnight, then injected intraperitoneally (i.p.) with freshly prepared STZ in citrate buffer (0.1 M, pH 4.5) at a single dose of 50 mg/kg, body weight. Hyperglycemia was confirmed by the elevated glucose levels in serum via a commercial glucometer (BIONIME GmbH, Switzerland), determined at 72 h after injection of the STZ. The rats were considered diabetic if the blood glucose level was >250 mg/dl.

Group 1 (Control group) was further divided into three subgroups each group contains (6) rats:

Subgroup 1a: left as normal control rats..

Subgroup 1b: considered as positive stem cell which received MSCs (which were processed and cultured for 14 days), in a single dose of (10^6cells) per rat by intravenous injection in rat tail vein (Abdel Aziz *et al.,* 2011).

Subgroup 1c: considered as positive zinc oxide nanoparticles which injected with 10 mg/kg orally by gavage (Nazarizadeh *et al.,* 2016)

Group 2 (Diabetic group) was also divided into four subgroups each group contain (6) rats:

Subgroup 2a: considered as diabetic control they did not receive any treatment.

Subgroup 2b: Diabetic+ MSCs the rats in this group received MSCs (which were processed and cultured for 14 days), in a single dose of (10^6cells) per rat by intravenous injection in rat tail vein.

Subgroup2c: Diabetic+ ZnONPs -rats injected with 10 mg/kg orally.

Subgroup2d: Diabetic+ MSCs + ZnONPs were injected with in a single dose of (10^6cells) per rat i.v and 10mg/kg ZnONPs orally.

Four weeks after MSCs and ZnONPs administration, the animals were euthanized under slight ether anesthesia. The animals of all groups were sacrificed; then blood samples were collected, allowed to stand for half an hour, and then centrifuged at 5000 rpm for 15 min at 4 °C to separate serum which stored at − 20 °C for the different biochemical measurements. Pancreas was dissected out and cut into pieces for the different studies. Pancreas was weighed and homogenized immediately to give 10%(w/v) homogenate in ice-cold medium containing 50mM Tris–HCl, pH 7.4. The homogenate of the pancreas was centrifuged at 5000 rpm for 10 min at 4 °C. The supernatant was stored at − 70 °C until used for the various biochemical investigations.

Biochemical Investigations

Determination of blood glucose level

Serum glucose level was determined by glucose oxidase method according to the method of Trinde (1969) using a serum glucose assay kit.

Determination of serum insulin level

Quantitative measurement of serum insulin was carried out adapting to ELISA technique using kits specific for rats purchased from Bio Vendor (Gunma, Japan) according to the protocol provided with the kit.

Determination of lipid peroxidation (MDA) and total antioxidant capacity (TAC) levels

Homogenates of the pancreas were used to determine lipid peroxidation (LPO) according to the procedure of Ohkawa *et al.* (1979). Similarly, Total antioxidant capacity (TAC) was determined using commercial kits obtained from Cell Biolabs' OxiSelect™, according to the method described by Allard (1998)

Determination of pancreatic interleukin 10 (IL-10)

Levels of interleukin 10 (IL-10) were determined using the commercial ELISA kits (R&D Systems, USA).

Histological investigation

The histological study of the pancreas sections were aimed to support the biochemical investigation.

Statistical analysis

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 25 (IBM Corp., Armonk, NY, USA). Data was summarized using mean and standard error of the mean (SEM) for quantitative variables. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test for comparison between each 2 groups (Chan, 2003). P-values less than 0.05 were considered as statistically significant.

RESULTS

Effect on plasma glucose level

The obtained data of the level of glucose in the plasma are given by table (1). Normal rats showed more or less constant levels during the course of the study. Moreover, no remarkable changes were reported after rats were treated with either MSCs or ZnONPs throughout the experimental duration.

The statistical analysis confirmed the induction of diabetes in the diabetic rats. As presented in table 1, Fig. 1, the level of glucose in the plasma was significantly increased to 247 mg/dl in comparison to that in the control rats (105.5 mg/dl). Treatment of diabetic rats with MSCs only, ZnONPs only, or the combination of both MSCs and ZnONPs for 4 weeks was found to significantly attenuate (P < P<0.05) this elevation of plasma glucose level (decrease of -33.4, -38.32,and -44.4% respectively) in comparison to the non-

treated diabetic rats. but, the maximum correction in the plasma glucose level was recorded in the diabetic rats group which were treated with both MSCs and ZnONPs.

Effect on plasma insulin level
The statistical analysis revealed that the plasma insulin concentration of the diabetic rats was significantly decreased (P <0.05) by -84.6%when compared to the control rats at 4weeks after the diabetes induction (table 1, Fig. 2). Interestingly, differently treated diabetic rats (DM+ MSCs, DM + ZnONPs, DM + MSCs + ZnONPs) had significantly

restored insulin level (233.3%, 291.6% and 410.4%, respectively) in comparison to the non-treated diabetic rats. Treatment by MSCs in combination with ZnONPs (DM + MSCs + ZnONPs) was found to significantly restore the insulin level (2.45 ng/ml) to that of the control rats (3.12 ng/ml), while rats treated with either MSCs or ZnONPs only regained their level of insulin to 1.6 and 1.88 respectively in comparison to the control (3.12 ng/ml). These results remarkably revealed that the treatment by MSCs in combination with ZnONPs (DM + MSCs + ZnONPs) was better than either that of MSCs or

Table 1: Effect of MSCs only, ZnONPs only, or the combination of both MSCs and ZnONPs s on plasma glucose and insulin levels in diabetic rat for 4 weeks.

	Normal control (NC)	Positive control (MSCs)	Positive control (ZnONPs)	Diabetic group (DM)	Diabetic group + MSCs (DM+ MSCs)	Diabetic group + ZnONPs (DM + ZnONPs)	Diabetic group + MSCs + ZnONPs (DM + MSCs + ZnONPs)
Insulin (ng/ml)	3.12±0.6	4.17±0.7 *	4.38±0.42 *	0.48±0.07 *#$	1.6±0.29 *#$	1.88±0.12 *#$@	2.45±0.39 #$@&
		33.6%	40.38%	-84.6%	233.3%	291.6%	410.4%
Glucose (mg/dl)	105.5±5.01	98.5±2.26*	94.67±2.8*	247±13.39 *#$	164.5±8.89 *#$@	152.33±2.07 *#$@	137.17±5.27 *#$@&%
		-6.63%	-10.26%	134.1%	-33.4%	-38.32%	-44.4%

Values are presented as mean ±SD*: statistically significant compared to corresponding value in group I (P<0.05),#: statistically significant compared to corresponding value in group II (P<0.05),$: statistically significant compared to corresponding value in group III (P<0.05),@: statistically

significant compared to corresponding value in group IV (P<0.05),&: statistically significant compared to corresponding value in group V (P<0.05),%: statistically significant compared to corresponding value in group VI (P<0.05)
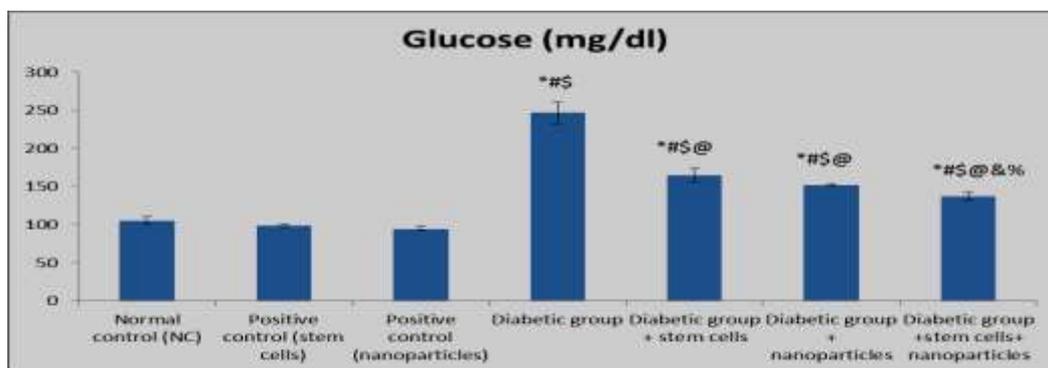

Fig. 1: Effect of MSCs only, ZnONPs only, or the combination of both MSCs and ZnONPs s on plasma glucose level in diabetic rat for 4 weeks.
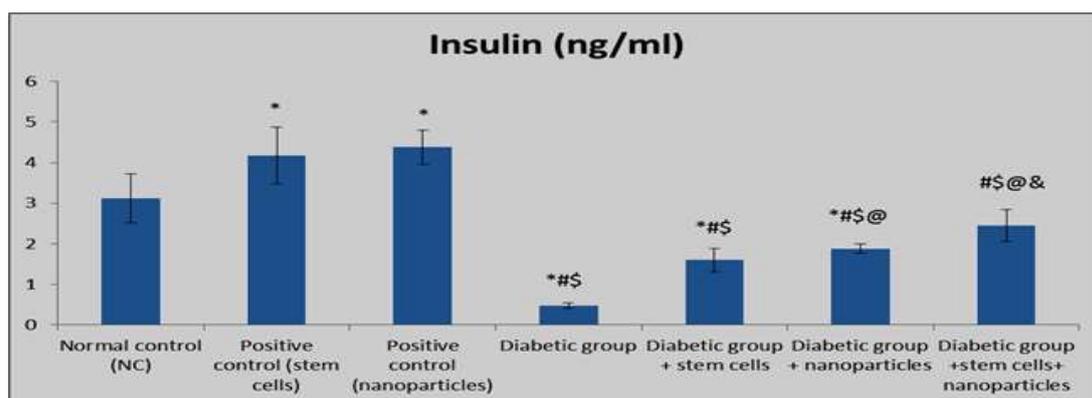
Fig. 2: Effect of MSCs only, ZnONPs only, or the combination of both MSCs and ZnONPs s on plasma insulin level in diabetic rat for 4 weeks.

Pancreatic oxidative stress

Lipid peroxidation (MDA)level

As expected, diabetes significantly (P <0.05) impaired oxidative stability because MDA level was found to substantially increase by -44% compared to that of control rats. Treatment of diabetic rats with MSCs, ZnONPs, or both considerably restored the levels of MDA to control values, especially in DM + MSCs + ZnONPs rats (0.09 mol/l) in comparison to that of control rats(0.07 mol/l) (table 2, Fig. 3).

Level of the total antioxidant capacity (TAC)

On the opposite side, the antioxidant TAC levels of diabetic control rats were found to significantly decrease (P <0.05) in comparison with control values. Treatment of diabetic rats (DM+ MSCs, DM + ZnONPs, DM + MSCs + ZnONPs) significantly (P <0.05) improved the concentrations of the antioxidant TAC level in comparison to the diabetic control values of this parameter. Statistical analysis showed that DM + MSCs + ZnONPs had superior improvements, returning to that of control levels (table 2, Fig. 4)

Table 2: Effect of MSCs only, ZnONPs only, or the combination of both MSCs and ZnONPs s on MDA and TAC levels in diabetic rat for 4 weeks.

	Normal control (NC)	Positive control (MSCs)	Positive control (ZnONPs)	Diabetic group (DM)	Diabetic group + MSCs (DM+ MSCs)	Diabetic group + ZnONPs (DM + ZnONPs)	Diabetic group + MSCs + ZnONPs (DM + MSCs + ZnONPs)
MDA (m. mol/l)	0.07±0.01	0.04±0.01	0.03±0.02	0.56±0.05 *#$	0.28±0.02 *#$@	0.16±0.03 *#$@&	0.09±0.01 #$@&%
		-42.8%	-57.1%	-44%	-50%	71.4%	83.9%
TAC (m. mol/l)	45.97±1.15	54.63±2.7 *	57.87±1.99 *	10.13±1.17 *#$	34.26±3.17 *#$@	38.88±1.18 *#$@&	43.75±1.55 #$@&%
		18.83%	25.9%	-141%	99.16%	116.6%	137.2%

Values are presented as mean ±SD*: statistically significant compared to corresponding value in group I (P<0.05),#: statistically significant compared to corresponding value in group II (P<0.05),$: statistically significant compared to corresponding value in group III (P<0.05),@: statistically significant compared to corresponding value in group IV (P<0.05),&: statistically significant compared to corresponding value in group V (P<0.05),%: statistically significant compared to corresponding value in group VI (P<0.05)
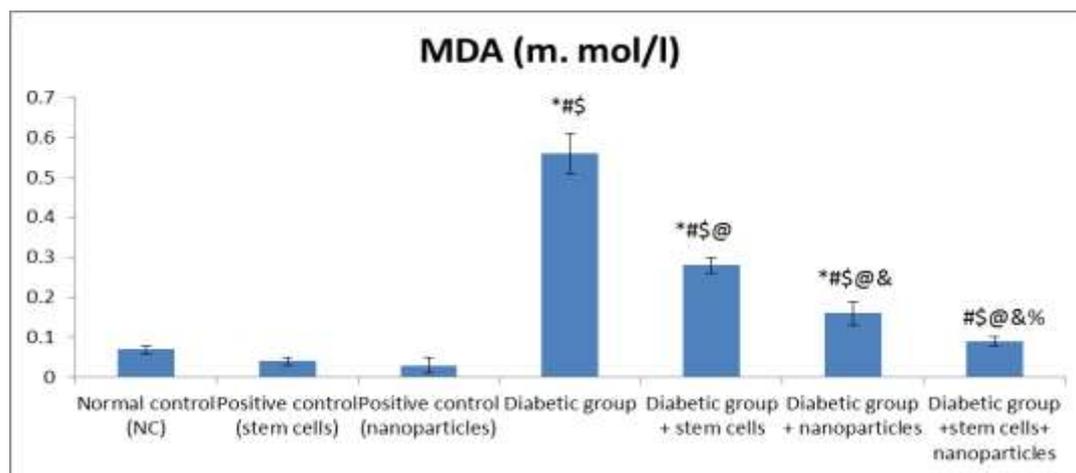
Fig. 3: Effect of MSCs only, ZnONPs only, or the combination of both MSCs and ZnONPs s on MDA level in diabetic rat for 4 weeks.
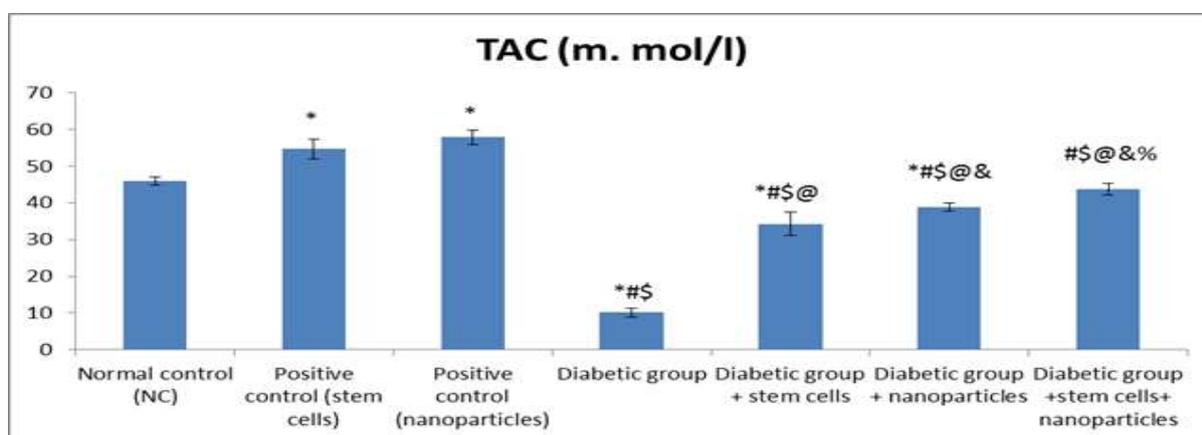

Fig. 4: Effect of MSCs only, ZnONPs only, or the combination of both MSCs and ZnONPs on TAC level in diabetic rat for 4 weeks.

Cytokine interleukin -10(IL-10) levels

Next, we estimated the levels of anti-inflammatory (IL-10) cytokines (table 3,Fig. 5). Diabetes resulted in significantly (P <0.05) decreased the level of IL-10 to 67% of the control value. Treatment of diabetic rats, notably DM + MSCs + ZnONPs, significantly (P <0.05) improved the level of IL-10 (181pg/mL) in comparison to that of control rats (184 pg/mL).

Table 3: Effect of MSCs only, ZnONPs only, or the combination of both MSCs and ZnONPs on interleukin-10 (IL-10) level in diabetic rat for 4 weeks.

Group / Parameter	IL-10 (pg/ml)
Normal control (NC	184±4.2
Positive control (MSCs)	192.17±8.38 4.44%
Positive control (ZnONPs)	196.33±4.03 * 6.7%
Diabetic group (DM)	76.32±2.3 *#$ -58.5%
Diabetic group + MSCs (DM+ MSCs)	152.33±2.07 *#$@ 99.5%
Diabetic group + ZnONPs(DM + ZnONPs)	165.33±3.5 *#$@& 116.6%
Diabetic group + MSCs + ZnONPs (DM + MSCs + ZnONPs)	181±7.64 #$@&% 137.1%

Values are presented as mean ±SD*: statistically significant compared to corresponding value in group I (P<0.05),#: statistically significant compared to corresponding value in group II (P<0.05),$: statistically significant compared to corresponding value in group III (P<0.05),@: statistically significant compared to corresponding value in group IV (P<0.05),&: statistically significant compared to corresponding value in group V (P<0.05),%: statistically significant compared to corresponding value in group VI (P<0.05)
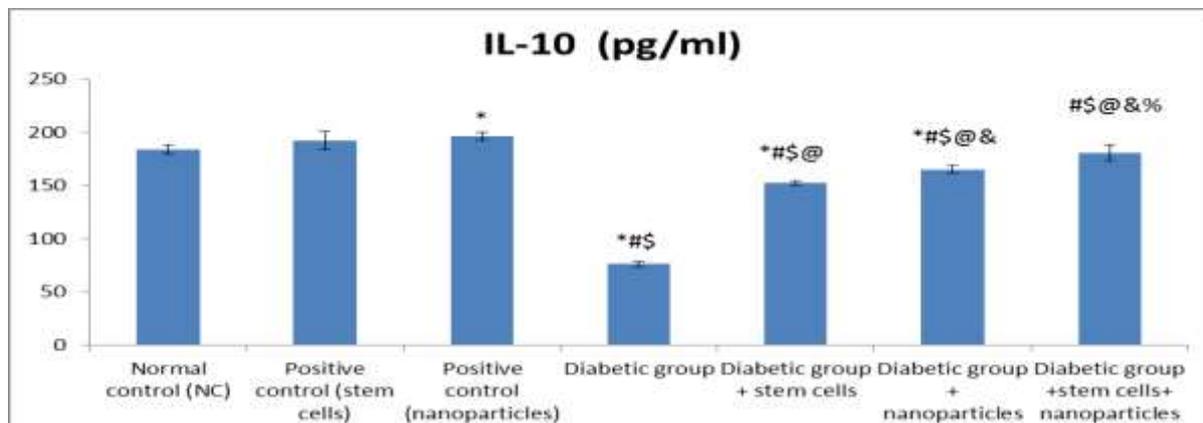

Fig. 5: Effect of MSCs only, ZnONPs only, or the combination of both MSCs and ZnONPs on TAC level in diabetic rat for 4 weeks.

Histological studies

Usually histological section of pancreas consists of pancreatic acini (P.A), islets of Langerhans (IL) and connective tissue. Pancreatic tissue in control rats showing normal pancreatic islet of Langerhans; Scattered as spheroid mass; β- cells occupies the core of the islets. Alpha and delta cells form the periphery of the Langerhans islet (Fig.a), While the islets of Langerhans of diabetic rats showed signs of completely distortion and also pyknotic and haemorrhage was evident (Fig.b). As shown in Fig.c, treatment of the diabetic rats with ZnONPs regenerated the islets of Langerhans and nearly restored the normal shape. Pancreas of rats treated with MSCs showed moderate restoration in Langerhans islet cells, limited pyknotic, and few vacuoles still detected in some cells (Fig.d) pancreatic tissue of diabetic group showing treated with MSCs and nanoparticles showing restoration of some Langerhans islets appearing with normal architecture (Fig.f).
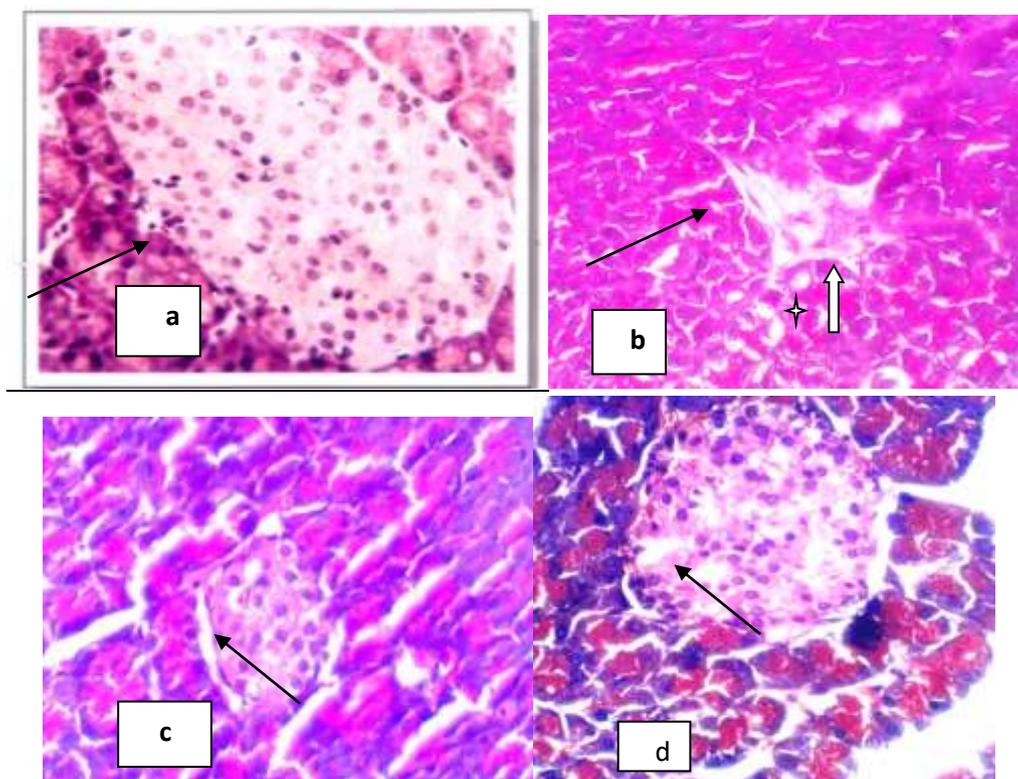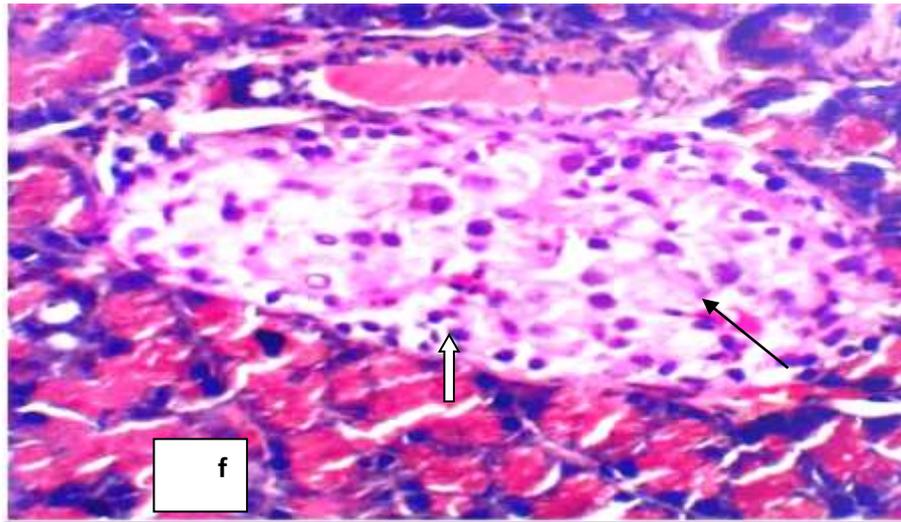
Fig. 6: Histopathology of the pancreatic tissues of the studied groups. a control (NC) group, showing normal cells in the islet of Langerhans. b Diabetic untreated control (DC) group, showing pyknotic ( ) and haemorrhage ( )in islet of Langerhans. c. MSCs treated diabetic group, showing moderate restoration in Langerhans islet cells. d ZnONPs treated diabetic group, ZnONPs protected the majority of cells in the islet of Langerhans. f representative section of the pancreas of diabetic rats treated with MSCs and ZnONPs showing restoration of some Langerhans islets appearing with normal architecture Sections stained with haematoxylin and eosin.

Immunohistochemical results

Anti-PCNA stained pancreatic sections:

Sections of control group revealed no immunoreactivity (Fig. 7a) while sections of diabetic group revealed few PCNA immune reactive cells of islets of Langerhans, (Fig. 7b). Sections of diabetic rat treated with ZnONPs revealed PCNA immune reactive cells (Fig. 7c). Sections of diabetic rat treated with MSCs revealed nuclear immunoreactivity in more islet cells(Fig. 7d). Sections of diabetic group treated with MSCs and ZnONPs showing widespread expression of PCNA of islets of Langerhans (Fig. 7f).
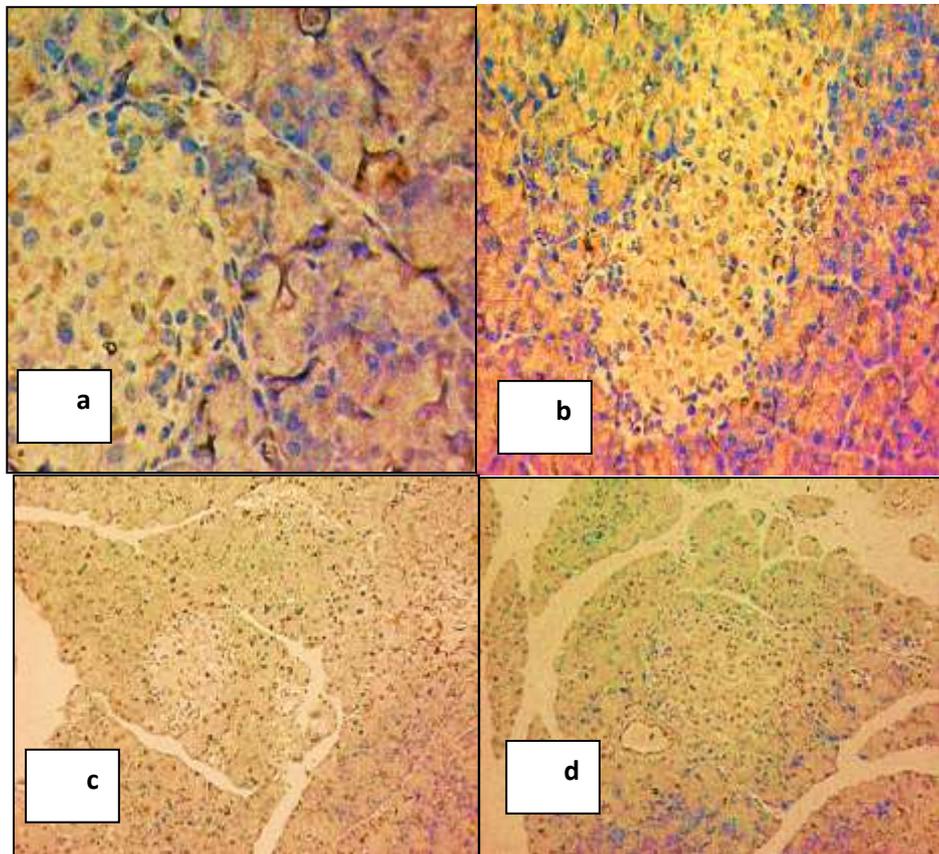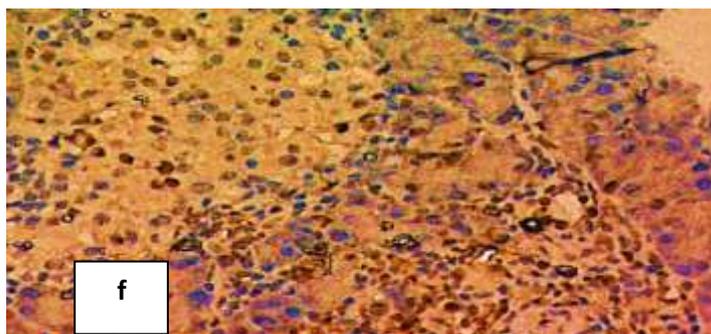
Fig. 7: Immunohistochemistry stain of the pancreatic islets of the different groups. sections of the pancreatic tissue of albino rats showing negative nuclear immunoreactivity in all islet cells in NC (a). Very few immunoreactivity nuclei (arrows) are seen in islet cells in group DM (b). Some islet cells show moderate immune reactive nuclei (arrows) in diabetic rats treated with ZnONPs (c) while in diabetic group treated with MSCs (d) the immunoreactivity is detected in most islet (arrows). Sections of diabetic group treated with MSCs and ZnONPs(f) showing widespread expression of PCNA of islets of Langerhans (Anti-PCNA Immunostaining x400)

DISCUSSION

The goal of the present investigation was to determine the efficacy of a bone marrow-derived mesenchymal stem cells and ZnO nanoparticles combination treatment to recover endogenous β cells and to reduce oxidative stress in a STZ-induced diabetic rat model. Our findings are consistent with previous observations (Bhansali *et al.,* 2015 and Othman *et al.,* 2019) demonstrating that MSCs and ZnONPs, exhibit anti-inflammatory activities and can remarkably improve glucose levels and restored levels of antioxidant enzymes. The results in present study showed a significant elevation in glucose and a significant decrease in the concentration of insulin in blood. These results are attributed to the toxic effect of STZ on the beta cells of pancreatic islets which led to inhibition of insulin synthesis and increased blood glucose level due to decrease entry of glucose into peripheral tissues, muscles and adipose tissue (Itoh *et al.,* 2002), increased glycogen breakdown (Geethan and Prince, 2008). The increased of hepatic glucose output either by enhanced glycogenolysis and/or gluconeogenesis (Eliza *et al.,* 2009). On the other hand, diabetic rats treated with the combination of MSCs and ZnONPs, exhibited a significant improvement in glucose and insulin levels. Glucose is one of the body's main sources of energy. In normal physiology, the body maintains blood glucose levels within a narrow range (70-130mg/dl). The body regulates the processes that control the production and storage of glucose by secreting the endocrine hormone, insulin, from the pancreatic B-cells. Insulin facilitates anabolic metabolism throughout the body. Studies with and mesenchymal stem cells have revealed encouraging results in rats (Si *et al.,* 2012). These studies exhibited that there was an improvement in the glucose profile and development of new islet on histology. Azab *et al.* (2011) reported that after injection and treatment with the haematopoietic stem cells and MSCs derived from human bone marrow separately in Alloxan induced diabetic rats, there was a significant reduction in blood glucose level in these rats and trans-differentiation of MSCs into insulin producing cells *in vitro.*

Umrani and Paknikar (2014) stated the ability of ZnONPs for improve the blood glucose in STZ-diabetic rats. This

observed a great antidiabetic activity of zinc oxide nanoparticles. Because zinc has been clarified to be a potent metal which improves glucose utilization and metabolism through its potent influence on enhancement of hepatic glycogenesis through actions on the insulin signaling pathway (Jansen *et al.,* 2009).

Moreover, Rutter *et al.* (2016) reported that Zn is essential cation not only for insulin synthesis, storage, and structural stability, but also protects against oxidative stress that seen in DM. This mechanism may be due to inhibition of intestinal α-glucosidase enzyme by Zn thereby reduce glucose absorption, Zn raises glucose uptake in the liver and then, glycogenesis (Amiri *et al.,* 2018). Zn is also involved in glucose uptake by the GLUT and it acts as an inhibitor of glucagon secretion, which leads to a reduction in glycogenolysis and gluconeogenesis. In addition, Zn has a proliferative and protecting effect on the pancreatic islets and plays a significant role in insulin synthesis, storage, and secretion (Jayawardena *et al.,* 2012).

Oxidative stress is defined as an imbalance of free radicals and antioxidants in the body, which can lead to cell and tissue damage. (Ahrén, 2005). Continued oxidative stress leads to the development of chronic diseases, such as diabetes mellitus, cancer, neuro-degeneration, cardio vascular and metabolic disease (Elahi *et al.,* 2009). Here in, we found that induction of STZ resulted in an noticeable increase in lipid peroxidation marker MDA levels and a significant decrease in the total antioxidant capacity (TAC) in pancreas as compared to the control rats.

Measurement of lipid peroxidation is an important marker of oxidative damage caused by ROS, and the assessment of MDA is a dependable method to gain such determination (Duzguner and Kaya, 2007 and Özcelik *et al.,* 2012). Highly reactive oxygen metabolites, especially hydroxyl radicals, act on unsaturated fatty acids of phospholipid components of membranes to produce malondialdehyde, a lipid peroxidation product (Mansour and Mossa, 2009). Such damage to cell membrane lastly can lead to cell death. Our results of diabetic rats were in agreement with those of current studies that recorded increase in MDA concentrations by hyperglycemia induced glucose auto-

oxidation and glycation of proteins (El-Missiry and El Gindy, 2000 and Tang *et al.,* 2010).

Tiwari *et al.* (2013) reported that antioxidant capacity of serum is the primary measure to evaluate the status and potential of oxidative stress in the body. In fact, the capacity of known and unknown antioxidants and their synergistic interaction is consequently assessed, thus giving an insight into the delicate balance in vivo between oxidants and antioxidants (Ghiselli *et al.,* 2000). Our findings showed that the serum TAC of diabetic rats was considerably lower than the healthy control.These results are in harmony with (Lodovici *et al.,* 2003, Hisalkar *et al.,* 2012 and Catanzaro *et al.,* 2013). On contrast, a significant reduction in the serum MDA and TAC of STZ-diabetic male rats that injected intravenous with bone marrow mesenchymal stem cells after four weeks. The obtained data were in agreement with *(Fang et al., 2012)* they reported that treatment diabetic rats with mesenchymal stem cells significantly inhibited the increment of MDA in comparison to the diabetic animals. Moreover, it has been well established that the combination of MSCs and ZnONPs is a potent combination that can help to maintain oxidative stability because it was reported by **Bettger and O'Dell (1981***)* that Zinc has also been proposed to interact with cell membranes to stabilize them against oxidative damages.

IL-10 is anti-inflammatory cytokine that have relates with the severity of pancreatitis. Also, IL-10 has the capacity to suppress several inflammatory events including the production of other cytokines and signal molecules [e.g., IL-1, IL-6, and TNF-α, interferon-α (IFN-α)] in macrophages and monocytes. In this study, pancreatic IL-10 level was reduced after administration with STZ. Treatment with BMSCs in was associated with significant elevation of serum IL-10 24h and on 4thday after AP induction as compared with AP group. This was in accordance with Tu *et al. (2012)* who found that treatment with BMSCs suppressed the high level of TNF and IL-6 but increased that of IL-10 in severe acute pancreatitis (SAP).

CONCLUSION

Bone marrow-derived mesenchymal stem cell and ZnO nanoparticles have been shown to improve insulin levels and glucose concentration in type 2 diabetic rats. Also, it ameliorates oxidative induced pancreatic damage by lowering levels of lipid peroxidation and stimulating the antioxidant defense system.

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