Al Azhar Bulletin of Science Vol. 29, No. 1, (June) 2018, pp. 1-14

THERAPEUTIC ROLEOF MESENCHYMAL STEM CELLS AND VITAMIN D ON SOME BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS INSTREPTOZOTOCIN-INDUCED DIABETIC MALE ALBINO RATS.

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ABSTRACT

Background: *Diabetes is one of the most important causes of mortality and morbidity all over the world. Renewal of functional pancreatic islets has been a goal of stem cell biologists since early 2000. Since that time, many studies have reported successful creation of glucose-responsive pancreatic beta-cells.* ***Aim of work:*** *This work aimed to study the effect of MSC.s alone and/or in combination with vitamin D_3in Streptozotocin (STZ)-induced diabetic male albino rats to detect its potential therapeutic effect and its possible application to humans.* ***Material and methods:*** *Twenty-four male albino rats (150 – 170 grams) were included in this study. They were divided into four equal groups; each group have six rats: Group I (Normal: control of healthy), group II (STZ: control of diabetes), group III (diabetic group post-treated with MSC.s) and group IV (diabetic group post-treated with MSC.sin combination with vitamin D_3). Diabetes was induced by intraperitoneal injection of STZ (50 mg/kg); MSC.s were injected intravenously into the rat tail vein in group III and group IV then left for sixweeks; vitamin D (cholecalciferol) was administered orally at 150 ng (500 IU/kg) each other day at three times per week for a long 6 weeks.Blood glucose leveland body weight were measured weekly for all groups at the beginning of the study at the intervalsixweeks. While, haematological parameters were measured after six weeks.* ***Results:*** *Diabetic group (group II) showed significant higher glucose levels while there was a significant lower body weight levels compared to control of non-diabetic group. Group IIIas well as group IV showed significant elevation of body weightand reduction of blood glucose level as well as amelioration of haematological parameters in compared to group II.****Conclusion:*** *treatment with MSC.sand/or in combination with vitamin D_3 showed significant lower levels of glucose and higher body weight levels as compared to diabetic group.*

Key Words: STZ (Streptozotocin), MSC.s (Mesenchymal stem cells) and Vit D (Vitamin D_3).

1. INTRODUCTION

Diabetes is a major health problem in different societies. Specifically, in Egypt, the published figures showed that the prevalence of diabetes in persons over 20 years is increasing from 9.9% in 1995 to 10.2% in 2000 and expected to reach 13.3% in 2025 **(Ahmed *et al.*, 2017).** It is estimated that diabetic patients will be 439 million worldwide by 2030 **(Tran *et al.*, 2014).**

Diabetes mellitus (DM) is a major and rapidly growing public health concern. The prevalence of diabetes in all age groups worldwide was estimated to be 2.8% in 2000 and is estimated to be 4.4% in 2030. The total number of people with diabetes is expected to increase from 171 million in 2000 to 366 million in 2030 **(Rathmann and Giani., 2004).**The complications of diabetes mellitus have significant health, economic and social impacts on individuals, families, health systems and countries **(Lv*et al.*, 2014).**

Diabetes is a group of diseases characterized by abnormally high levels of the sugar glucose as well as lack of insulin leads to hyperglycemia in the bloodstream and this excess glucose is responsible for devastating complications of diabetes, which include blindness, kidney failure, cardiovascular disease, stroke, neuropathy and amputations **(Liao *et al.*, 2007).**

Diabetes mellitus type 1 is a form of diabetes mellitus that results from the autoimmune destruction of insulin-producing beta cells in the pancreas. Type 1 diabetes (previously called juvenile-onset or insulin-dependent IDDM) is primarily due to autoimmune pancreatic islet β-cell destruction **(Rhabasa*et al.*, 2004).** Experimental induction of DM in animal models is essential for the understanding of the various aspectsof its pathogenesis and for screening potential therapies for the treatment of this condition. Induction of experimental diabetes in rats using streptozotocin (STZ) is a very convenient and simple technique. STZ (*N*-nitro derivative of glucosamine) is a naturally occurring broad-spectrum antibiotic and cytotoxic chemical that is particularly toxic to the pancreatic insulin-producing β cells in mammals **(Abeeleh*et al.*, 2009).**

There is currently no cure for diabetes. People with type 1 diabetes must take insulin severaltimes a day and test their blood glucose concentrationthree to four times a day throughout their entire lives **(Bonner *et al.*, 2000).** Insulin replacement represents the current therapy for type 1 diabetes. However, its metabolic control remains difficult, as exogenous insulin cannot precisely mimic the physiology of insulin secretion. Exogenous insulin supply is not fully capable of achieving tight control of glucose regulation, leading to long-term complications **(Hori, 2009).**

Over the past several years, doctors have attempted to cure diabetes by injecting patients with pancreatic islet cells of the pancreas that secrete insulin and other hormones. However, the requirement for steroid immunosuppressant therapy to prevent rejection of the cells increases the metabolic demand on insulin-producing cells and eventually they may exhaust their capacity to produce insulin. The deleterious effect of steroids is greater for islet cell transplants than for whole-organ transplants **(Itken*et al.*, 2001).**The current gold standard therapy for pancreas transplantation has limitations because of the long list of waiting patients and the limited supply of donor pancreas **(Hantuchova*et al.*, 2015).**

Mesenchymal stem cells (MSC.s) are multipotent and can differentiate into bone, cartilage, fat and connective tissue; capacity for self-renewal, and differentiation into a wide range of tissues that are most frequently isolated from bone marrow but can generally be derived from any organ and have anti-inflammatory and immunomodulatory properties **(Abdi *et al.*, 2008).** The ability of MSC.s to differentiate into several cell types including muscle, brain, vascular, skin, cartilage, and bone cells makes them attractive as therapeutic agents for many diseases including DM **(Volarevic*et al.*, 2011).** Recently, some studies have shown that MSCs can improve the metabolic profiles of diabetic animal models, providing evidence for the potential therapeutic efficacy of MSC therapy in diabetes **(Wagner *et al.*, 2010).**

Stem cell therapy can be an effective therapeutic approach in type 1 diabetes (T1D); which characterized by the deficiency of endocrine β cells in the pancreatic islets of Langerhans. Based on the generation of insulin-producing cells (IPCs) derived from MSCs, represents an attractive possibility. Based on the characterization of MSC immunomodulatory effects, and present the current experimental evidence for the potential therapeutic efficacy of MSC.s transplantation in diabetes **(Vija*et al.*, 2009).**

Focusing on MSCs therapy in most clinical applications they are isolated from bone marrow (BM) (**Kern *et al.*, 2006).** Depending on their intended purpose, experimental or therapeutic use, the main functional characteristics of MSCs are their immune-modulatory abilitymake them a promising therapeutic tool for severe refractory autoimmune diseases. They suppress T-cell proliferation and significantly reduce the expression of certain activation markers on stimulated lymphocytes **(Abdi *et al.*,2008)**, the other main functionsare self-renewal, and differentiation into tissues of mesodermal origin **(Addi*et al.*,2004).**

MSC.s can be differentiated into IPCs by using a specific culture medium enriched with insulin-promoting factors (mainly glucose and

nicotinamide). Several lines of evidence suggest that in vivo hyperglycemia is an important factor for bone marrow-derived MSC.s differentiation into IPCs capable of normalizing hyperglycemia in diabetic rats, including those with chronic hyperglycemia **(Tang *et al.*, 2004).**

There is a possible therapeutic effect of MSC.s in diabetes suggested by their capacity to generate insulin-producing cells (IPCs) **(Nautta and Febbe., 2007).** These IPCs express multiple genes related to the development or function of pancreatic beta cells, including high expression of insulin **(Volarevic*et al.*, 2010)** and were able to release insulin in a glucose-dependent manner that led to amelioration of diabetic conditions in streptozotocin (STZ)-treated rats **(Xie*et al.*, 2009).**

2. AIM OF THE WORK:

The study was carried out to investigate the therapeutic effect of MSC.s and/or vitamin D_3in the recovery of STZ-induced DM in adult male albino rats, monitored physically, blood glucoselevel and haematological parameters.

3. MATERIALS AND METHODS:

Animal: The study was carried out on twenty-four (12 weeks old) adult male albino rats (*Rattus*norvegicus) were included in the present study. Their weights ranged from 150 to 180 g ± 20 g (mean ± SD: 160 ± 1.11) were obtained from the Al_Nile Company of Pharmaceutical Products (Cairo, Egypt). They were housed in a temperature at 25 ± 2°C and light-controlled room (12-h light/dark cycle) with free access to standard diet pellets (El-Nasr, Cairo, Egypt), and tap water. Animals were housed in metallic cages and left to acclimatize for one week before starting the experiment. The study was conducted at the animal house at faculty of science, Al-Azhar University according the Guidelines of Ethics for the Care and Use of Laboratory Animals.

Chemical: Streptozotocin (STZ) was purchased from Sigma Chemical Company (St Louis, Missouri, USA) in the form of powder. Vitamin D was purchased from local market, Elnasr, co, Cairo, Egypt.

Research design and methods

Thetwenty-fourrats were randomly divided into four groups, each group has six rats as the following:

Group I: Non- diabetic rats (Served as control of healthy). This group included 6 rats, they were injected intraperitoneally with citrate buffer and were sacrificed along with the experimental group for 6 weeks.

Group II: Diabetic non-treated group (Control of diabetes) using Streptozotocin (STZ). This group included 6 rats that were fasted for 12 h before induction of diabetes. Diabetes was induced by means of a single intraperitoneal injection of STZ at a dose of 50 mg/kg body weight **(Bhansali*et al.*, 2015)**for 6 weeks.

Group III: Diabetic post-treated group (STZ +MSC.s). This group included 6 rats in which diabetes was induced by means of a single intraperitoneal injectionof STZ; followed by intravenous injection in a single dose of 0.5×10^6 MSC.s (which were processed and cultured for 14 days) per rat through the tail vein**(El Aziz *et al.*, 2011)** for 6 weeks.

Group IV: Diabetic post-treated group (STZ +MSC.s + Vitamin D). This group included 6 rats in which diabetes was confirmed; they were injected with MSC.s and their administrated vitamin D3 per oral; cholecalciferol (Doxercalciferol) was administered orally at 150 ng (500 IU/kg) each other day at three times per week for a long 6 weeks **(Choi *et al.*, 2011).**

The rats were observed daily for signs of STZ-toxicity and body weight was recorded weekly during the interval sixweeks of the experiment. Rats from all groups were sacrificed at weeks 6^{th} post- first week of streptozotocin-induced diabetic rats.

Dose titration of STZ in induction of hyperglycemia:

After fasting rats for 18 h, Streptozotocin (STZ) was purchased from Sigma Chemical Company (St Louis, Missouri, USA) was freshly dissolved in 0.1 M citrate buffer (pH = 4.5) and administrated at the dose of 50 mg/kg B.W. intraperitoneally within 15 minutes of dissolution **(Bhansali*et al.*, 2015).** The non-diabetic control rats (group I) also received an injection of the citrate buffer. Following the injections, the rats had free access to (5%) glucose solutions for 24 hours in order to avoid the anticipated hypoglycemic shock. 72 hours following the injection, tail blood samples from overnight fasting rats were obtained to measure blood glucose level **(Montilla*et al.*, 1998).** Diabetes was confirmed by the determination of fasting glucose concentration on the third day post administration of streptozotocin. Rats with glucose levels was higher than 200 mg/dl were considered to be diabetic and chosen for the experiment while those with blood glucose level outside this range were excluded **(Afifi, 2012& Furman, 2015).**

Preparation of bone marrow- derived mesenchymal stem cells:

The six-week-old male white albino rats were sacrificed after administration of sodium pentobarbital intraperitoneally at a dose of 30 mg/kg. Bone marrow was harvested by flushing the tibiae and femurs of rats with Dulbecco's modified Eagle's medium (DMEM, Gibco/BRL, Gibco BRL, Karlsruhe, Germany) supplemented with 10% fetal bovine serum (Gibco/BRL). Nucleated cells were isolated with a density gradient (Ficoll/Paque; Pharmacia) and re-suspended in complete culture medium supplemented with 1% penicillin–streptomycin (Gibco/BRL). Cells were incubated at 37°C in 5% humidified CO_2 for 12–14 days until formation of large colonies (80–90% confluence). The culture was washed with PBS and released with 0.25% trypsin in 1 mM/l EDTA (Gibco/BRL) for 5 min at 37 °C. After centrifugation, the cells were re-suspended with serum- supplemented medium and incubated in a 50-cm^2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures **(Alhadlaq& Mao., 2004).** MSCs in culture were characterized by their adhesiveness and fusiform shape **(Rochefort *et al.*, 2005).**

Treatment of diabetes mellitus by mesenchymal stem cells:

Blood samples were obtained from the retro-orbital veins plexus into capillary tubes after 48 h to confirm that the animals had become diabetic. Thereafter, MSCs were injected after diabetes confirmation by injecting one million units of cells per animal through the tail vein**(El Aziz *et al.*, 2011).**

Laboratory investigations

Measurements of blood glucose level:

Blood samples were drawn from allexperimental diabetic groups at 1, 2, 3, 4, 5 and 6 weeks over the period of experiment. blood drop was taken from the distal end of the tail, applied to a test strip, and analyzed immediately via a blood glucose monitoring system with a blood glucose monitoring device (Accu-Check Active, Roche Diagnostics, Mannheim, Germany) **(Brăslasu*et al.*, 2007).**

Body weight gain determination:

A triple electronic compact scale beam balance (OHAUS MACRO REG –made in Boland 1995) was used for the determination of the animal's body weight each week.

Preparation of Biological Samples:

At the end of the experiment, rats were fasted for 12 hr, weighed and the blood samples were collected from each animal under diethyl ether anesthesia from retro-orbital venous plexus puncture using blood capillary tube. Blood samples were collected on EDTA (Ethylene Diamine Tetra Acetic Acid) for hematological study.

Hematological parameters

Hematological parameters; Red blood corpuscles (RBCs), White blood cells (WBCs), Differential leukocytes count, Hemoglobin concentration, Hematocrit value (Hct)/ (PCV) packed cell volume, blood

Indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) as well as platelets counts were measured by using CBC analyzer **(Sino thinker. sk9000, U.S)** at physiology lab, faculty of science, Al-Azhar University, Cairo, Egypt.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS, version 22) was used in data analysis. Data were expressed as mean ± S.E.M One-way analysis of variance (ANOVA) test according to **Roa*et al.* (1985**) was used to compare between groups followed by Fisher's least significant difference (LSD) analysis. *P* values less than 0.05 were considered significant **(Armitage & Berry., 1994).** Data were tabulated asit was represented.

4. RESULTS:

Change in the Body Weight:

Body weight change was represented as in table (1) according to the interval weeks of treatment. The body weight at baseline in all the groups was similar. Administration of STZ in diabetic-untreated rats resulted in significant decrease in the final body weight at day 42 (week six) when compared with the corresponding value in control group. While, there was no statically significant change found (P<0.05) in thediabetic rats post-treated with mesenchymal stem cells alone and/or in combination with vitamin D respectively, over six weeks of treatment as compared with the corresponding value of non-diabetic rats served as the normal control group.

On the other hand, the results revealed that, a very high significant increase (P<0.001) in the percentage of body weight gain in diabetic rats post-treated with mesenchymal stem cells and/or in a combination with vitamin D in compared with the corresponding values of diabetic-untreated rats after six weeks of treatment.

The percentage of body weight gain showed a significant elevation in diabetic rats post-treated with MSC.s and/or in a combination with vitamin D on 2nd week at 12.4% and 19.1% and 3rd week at 12.3% and 17.9% respectively as compared with the corresponding values of its initial body weight at zero day. While, the percentage of change in body weight showed a significant decrease of -7.9% and -13.5% respectively on 5th and 6thweeks in diabetic-untreated rats in compared with initial body weight.

On the other hand, the percentage of change showed a significant decrease at 9.8% and 14.6% in diabetic rats post-treated with MSC.s and in diabetic rats post-treated with MSC.s in a combination with Vitamin D raised about 11.2% and 15.5% respectively on 5th and 6th weeks in compare with the initial body weight of their experimental rats. Furthermore, the percentage of final body weight showed a significant decrease of -9.6% in diabetic-untreated rats, while the percentage of body weight gain showed a significant elevation of 36.5% and 36.2% in diabetic rats post-treated with mesenchymal stem cells and/or in a combination with vitamin D at the end of treatment in compared with the corresponding values of initial body weight (baseline).

Blood glucoselevel:

All rats fulfilled the criteria of diabetes after streptozotocin induced-diabetic rat model as defined by an increase in non-fasting blood glucose level more than 300 mg/dl on multiple occasions. Moreover, the blood glucose level at baseline in all diabetic treated and un-treated groups was similarafter administration of STZ, blood glucose level significantly increased from normal to hyperglycaemic level as in table (2).

However, blood glucose level in STZ-induced diabetic rats showed a significant elevation (P<0.05) on the interval data of 1st, 2nd, 3rd, 4th, 5th and 6th weeks respectively as compared with the corresponding values of non-diabetic rats. The percentage of change of blood glucose level in diabetic-untreated rats showed a very high significant elevation at 12.0%, 25.5%, 26.4%, 21.7%, 37.0% and 34.7% on 1st, 2nd, 3rd, 4th, 5th and 6th weeks respectively when

compared with the corresponding values of interval data in diabetic-untreated rats at baseline.

However, this level showed significant decrease (P<0.05) on 1st, 2nd, 3rd, 4th, 5th and 6th weeks respectively in diabetic rats post-treated with MSC.s. and/or in a combination with vitamin D to be around the normal levels as compared with the corresponding values of diabetic-untreated (control) rats. Also, the percentage of change of blood glucose level in diabetic rats post-treated with MSC.salone showed significant decrease about -18.5%, -38.3%, -49.0%, -35.2%, -49.0% and -50.9% respectively in compared with the corresponding values of interval data in diabetic rats post-treated with MSC.s at baseline. Additionally, the percentage of change of blood glucose level in diabetic rats post-treated with MSC.s in combination with vitamin D showed a significant decrease at -17.3%, -26.2%, -40.4%, -42.9%, -53.1% and -54.8% respectively of the interval data at 1st, 2nd, 3rd, 4th, 5th and 6th weeks as compared with the corresponding values of baseline.

Hematological Parameters:

Hematological parameters were represented in table (3 and 4) The data obtained revealed that RBC.s count showed significant decrease at weeks 6th post- first week of streptozotocin-induced diabetic un-treated rats when compared with non-diabetic (control) rats. On the other hand, RBC.s count in diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D showed significant increase (P<0.05) ascompared with diabetic-untreated rats.

The results of HGB concentration showed a very high significant decrease (P<0.001) in diabetic-untreated ratswhen compared with the corresponding value of non-diabetic rats. Moreover, there was no statically significance difference in diabetic-untreated rats, diabetic rats post-treated with MSC.s and/or in a combination with vitamin D in compared with the corresponding value of non-diabetic rats.Otherwise, in diabetic rats post-treated with MSC.s in combination with vitamin D showed a significant increase (P<0.05)incompared with the corresponding value of diabetic-untreated rats after six weeks of the treatment.

HCT % showed a significant decrease (P<0.05) in diabetic-untreated rats and diabetic rats post-treated with MSC.s and/or in a combination with vitamin Dwhen compared with the corresponding value of non-diabetic rats. Otherwise, HCT % in diabetic rats post-treated with MSC.s showed a significant increase at (P<0.05)incompared with the corresponding value of diabetic-untreated rats.

The results of MCV in diabetic rats post-treated with MSC.s alone and/or in combination with Vitamin D showed a significance decrease (P<0.05) while, in MCHCshowed a significant increase (P<0.05) as compared with the corresponding values of non-diabetic rats.However, erythrocytes indices in diabetic-untreated rats showed no statically significance difference in compared with the corresponding values of non-diabetic rats. In addition, there was no statically significance difference in MCV, MCH and MCHCin diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D incompared with the corresponding value of diabetic-untreated rats.

The results of WBC.s count after six weeks of treatment showed significant decrease (P<0.05) after six weeks of STZ-administration in diabetic-untreated and diabetic post-treated rats when compared with the corresponding value of non-diabetic rats. Otherwise, WBC.s count showed a very high significant elevation at(P<0.001) in diabetic rats post-treated with MSC.salone and/or in combination with vitamin D as compared with the corresponding value of diabetic-untreated rats.

The results of lymphocyte, monocyte and neutrophil percentage showed a significant decrease (P<0.05) in diabetic-untreated rats as well as in diabetic rats post-treated rats compared with the values of non-diabetic rats.On the other hand, monocyte as well as neutrophil percentage after six weeks of treatment in diabetic rats post-treated with

MSC.s alone and/or in combination with vitamin D showed a very high significant increase (P<0.001) when as compared with the corresponding value of diabetic-untreated rats.

The results of platelets count showed a significant decrease (P<0.05) in diabetic-untreated when compared with the corresponding value of non-diabetic rats. While, platelets count in diabetic rats post-treated with MSC.s showed a significant elevation *(*P<0.05) near to be restored to normal rat when as compared with the corresponding value of diabetic-untreated rats after six weeks of treatment.

5. DISCUSSION:

Previous Studies have shown an association between hyperglycemia and decreased body weight of diabetic animals. The study was aimed to observe the effects of streptozotocin (STZ)-induced diabetes and to find an association between the reduction in the weights of animals and glucose levels in albino rats. In the same way, diabetic rats in the study observed the clinical manifestations, glucose, body weight using a 50 mg/kg dose of Streptozotocin ensured induction of diabetes in rats. Hyperglycemia, hypo-insulinemia, polyphagia, polyuria and polydipsia accompanied by weight loss were seen in adult rats within two days of Streptozotocin treatment which indicates irreversible destruction of Langerhans islets cells. In comparison with diabetic rats, there was significant in the states of polyphagia, polydipsia and body weight in diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D as compared with diabetic untreated rats **(Tögel*et al.*, 2007).**

STZ-induced diabetes at 50 mg/kg body weight in diabetic untreated rats shows that there was a significant reduction (P<0.001) in the body weight at week 6 as compared to normal and diabetic post treated rats **(Nagarchi*et al.*, 2015).** This was probably due to dehydration and excessive breakdown of tissue proteins, and protein wasting due to unavailability of carbohydrate as an energy source **(Kamalakkannan& Prince., 2006).**

The results also are in harmony with the study of **Zafar *et al.* (2010)** was observed streptozotocin dose was as 50 mg/kg body weight showed highly significant decrease (P<0.001) in body weight when compared with initial body weight. The loss in the body weight of the diabetes untreated rats agrees with the finding of **Oyedemi*et al.* (2011)** observed similar effect on diabetic animals induced with streptozotocin. This reduction of body weight has been linked to degradation of structural proteins and muscle degenerative. Weight loss during diabetes is also related to urinary glucose excretion because cells begin to use glucose due to the defect in glucose metabolism and excessive breakdown of tissue protein which is a characteristic condition of diabetics (**Swanston-Flat *et al.*, 1990).**

The results are in accordance with the study of **Oyedemi*et al.* (2011)** observed a significant decrease in the body weights of diabetic rats was observed 10 days after induction of streptozotocin. Moreover, the animals treated with STZ seemed very week with loss of their body weights because of adverse effects of STZ which caused alkylation of DNA and produced hyperglycaemia and necrotic lesions. Present observations are in accordance with the findings of **Habibuddin*et al.* (2008)** and **Lee *et al.* (2009).**

After administration of STZ, blood glucose level showed a significant elevation in STZ-induced diabetic rats (P<0.001) after 1st, 2nd, 3rd, 4th, 5th and 6th weeks as compared with the corresponding values of non-diabetic rats as shown in table (1). The results are in accordance with **Mohammed *et al.* (2013)** reported that the blood glucose level was a statistically significant (P< 0.001) increase in diabetic control group after day 14, 21 and 28 of inductions with STZ when compared to non-diabetic/control group.

However, transplantation of MSC.s in diabetic rats post-treated with M.S. Cs alone and/or in combination with Vitamin D showed a very high significant decrease at (P<0.001) blood glucose level to be around the normal

levels on 1st, 2nd, 3rd, 4th, 5th and 6th weeks as compared with the corresponding values of STZ induced diabetic-untreated rats Table (2). The results suggested that, recovery of the pancreatic β-cells and controlling the blood glucose level in cases of diabetes could be achieved by transplantation of BMSCs**(Oh *et al.*, 2004).** Furthermore, the ability of transplanted MSCs are attracted by pancreatic islet both *in vivo* to 'home in' at the site of tissue injury. Therefore, the ability of pancreatic islets to allure MSCs suggests a potential role for these cells in β-cells replacement therapy **(Sordi*et al.*, 2005).**

The results in agreement with **Bhansali*et al.* (2015)** demonstrated that allogenic MSC.s transplantation significantly decreased in the blood glucose level on days 17 and 24 in diabetic post-treated rats in compared with STZ induced diabetic untreated rats. Furthermore, the results are in accordance with the study of **Si *et al.* (2012)** has shown encouraging results which showed that there was an improvement in the glucose profile and development of new islet cells after mesenchymal stem cells transplanted in rats. Finally, the results are in a harmony with the results of **Itkin-Ansari (2001)** suggested that, mesenchymal stem cells, may be a new procedure for clinical diabetes stem -cell therapy, as they can control blood glucose level in the diabetic rats, by islet differentiation to produce normal amounts of insulin.

Chronic hyperglycemia and other metabolic disturbances of diabetes mellitus lead to long-term tissue and organ damage as well as dysfunction involving kidneys, nervous and vascular systems **(ADA, 1998).**Anaemia has been severally reported as a complication of diabetes mellitus **(Kotharia and Bokariya, 2012).** It results due to the increase in non-enzymatic glycosylation of Red blood cells (RBC.s) membrane proteins. The oxidation of these proteins and in the presence of hyperglycemia as obtainable in diabetes results to lyses of the blood cells and so anaemia ensues **(Oyedemi*et al.*, 2011).** Meanwhile, the link between chronic diseases and anemia is well characterized **(Weiss and Goodnough, 2005).**Streptozotocin is a well-known chemical that suppresses the immune system by damaging WBC and certain organs in the body **(Oyedemi*et al.*, 2011).**

In diabetes, the value of RBC.s, HGB and HCT were significantly decreased as compared with the corresponding value of non-diabetic rats. This reduction may be due to lyses of blood cells caused by reactive oxygen species (ROS) and the resulting oxidative stress (**Mohammad *et al.*, 2013 and Uko*et al.*, 2013).** Those results observed in diabetic un-treated rats in compared with non-diabetic rat implication is an accompanying anaemia in diabetes. This. However, after six weeks of treatment there was no statically significance difference in MCV, MCH and MCHC in diabetic rats post-treated with MS C.s as compared with the corresponding values of non-diabetic rats. These parameters are used mathematically to define the concentration of haemoglobin and to suggest the restoration of oxygen carrying capacity of the blood. Moreover, the non– significant change ($P<0.05$) in the MCV and MCH values indicate absence of macrocytic anemia since increased in MCV an MCH values are known to be indicative of macrocytic anaemia **(Mohammed *et al.*, 2013).**

Furthermore, the diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D caused significant ($P<0.05$) changes in the value of these parameters such that it brought about a significant increase in the HGB and MCH value, a factor that measures the rate of erythrocyte synthesis. Though, the action mechanism of this MSC.s transplantation and/or a combination with vitamin D administration it may be attributed to the ability to lower lipid peroxidation level that causes haemolysis of erythrocytes **(Ashafa*et al.*, 2009).** It therefore can be deduced that MSC.s therapy and vitamin D supplement were able to reverse the lytic effect of ROS and so reduced or rather completely prevent oxidative stress

thereby giving room for the regeneration of erythropoietic cells, a process mediated by erythropoietin secretion from the bone marrow **(Ohission*et al*., 2006).**

Streptozotocin a well-known chemical has been reported to suppress the immune system by destroying white blood cells and certain organs in the body **(Oyedemi*et al*., 2011)** as was observed in this present study. STZ-induced diabetic untreated rats showed significantly reduced blood levels of total white blood cell count, neutrophils, lymphocytes, and monocytes when compared to the non-diabetic rats. The intraperitoneal injection of streptozotocin into rats significantly reduced the WBC count and its differentials such as monocytes, lymphocytes and neutrophils.

The reduction of these parameters could be linked to suppression of leukocytosis from the bone marrow which may account for poor defensive mechanisms against infection **(Oyedemi*et al*., 2010).**Consequentially, they might have effects on the immune system and phagocytic activity of the animals **(Torel*et al*., 1986).** Additionally, the reduction of these parameters after six weeks of treatment could be attributed to suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection, thus may have consequential effects on the immune system and phagocytic activity of the diabetic untreated rats **(Afolayan and Yakubu, 2009; Oyedemi*et al*., 2010).**

On the other hand, after six weeks of treatment, Table (4) shows the level of WBC.s as well as lymphocyte, monocytes and neutrophil percentage in diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D showed significant increase as compared with the corresponding value of diabetic-untreated rats. Meanwhile, neutrophils ingest and kill bacteria; have been called the body's first defense line against bacterial infections **(Ganong, 1991).**

The values of white blood cells and its related indices were significantly restored to near normal after MSC.s transplantation at both times. The presence of MSC.s in combination with vitamin D with ability to stimulate the production of white blood count in the extract could be responsible for the observed result in the post-treated rats after six weeks of treatment. Additionally, this increase of monocyte and neutrophil percentage may be due to fight an infection upon MSC.s transplantation which are multipotent; they have angiogenic, anti-apoptotic, anti-inflammatory and immunomodulatory effects **(Cao *et al*., 2015).**

Platelet aggregation ability has been shown in diabetic patient with long term poor glycaemic control due to lack or deficiency of insulin **(Jarald*et al*., 2008).** Platelets known as thrombocytes help to mediate blood clotting, which is a meshwork of fibrin fibers. The fibers adhere to any vascular opening and thus prevent further blood clot. It plays a crucial role in reducing blood loss and repairing of vascular injury **(Oyedemi*et al*., 2010).** The reduction of platelets levels ($P<0.001$) in diabetic untreated rats induced with streptozotocin was confirmed in this study rather than in diabetic rats post-treated with MSC.s alone and/or in combination with vitamin D showed no statically significance differences in relation to the non-diabetic rats. Long term reduction of this parameter may result in internal and external haemorrhage and finally leads to death.

However, platelets count showed a very high significant elevation ($P<0.001$) in diabetic rats post-treated with MSC.s after six weeks of treatment as compared with the corresponding value of diabetic-untreated rats. This effect indicated the ability of MSC.s alone and/or in combination with vitamin D to stimulate the biosynthesis of clotting factors **(Adebayo *et al*., 2005).** Also, the results confirmed that due to the presence of active compounds that might help to precipitate blood coagulation or clotting, especially during severe bleeding or haemorrhage **(Dahlbäck, 2008).**

Table (1): Effect of mesenchymal stem cells alone and/or in combination with vitamin D on body weight in STZ-induced diabetic rats at the interval six weeks of treatment.

Period of treatment	% change of body weight per week (g)						
Experimental groups	**Baseline (g)**	**1st week (g)**	**2nd week (g)**	**3rd week (g)**	**4th week (g)**	**5th week (g)**	**6th week (g)**
	Mean ±SE	**Mean±SE**	**Mean±SE**	**Mean±SE**	**Mean±SE**	**Mean±SE**	**Mean±SE**
Group I Normal (Control of healthy)	154.0±2.3	165.9±2.1	176.4±2.7	189.9±2.2 [b]	200.6±3.1 [b]	209.7±2.9 [b]	217.1±2.9 [b]
% change		**7.7%**	**14.6%**	**23.3%**	**5.6%**	**10.5%**	**14.3%**
Group II (Diabetic: STZ)	159.5±2.4	172.4±2.5	173.3±2.9	166.8±3.1[a]	160.5±2.9 [a]	153.5±2.5 [a]	144.2±2.3 [a]
% change		**8.1%**	**8.7%**	**4.6%**	**-3.8%**	**-7.9%**	**-13.5%**
Group III (STZ+M.S. Cs)	154.4±2.1	165.0±2.2	173.5±2.6	183.9±2.8[b]	192.7±2.8 [b]	201.8±2.8 [b]	210.8±2.7 [b]
% change		**6.9%**	**12.4%**	**19.1%**	**4.8%**	**9.8%%**	**14.6%**
Group IV (STZ+MSC.s + Vit D)	157.2±1.9	166.7±2.5	176.5±2.6	185.3±2.8 [b]	195.4±2.8 [b]	206.0±2.6 [b]	214.1±2.5 [b]
% change		**6.0%**	**12.3%**	**17.9%**	**5.5%**	**11.2%**	**15.5%**
F-Probability	**N.S.**	**N.S.**	**N.S.**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**
F-value	**1.32**	**2.07**	**0.40**	**12.02**	**39.14**	**99.75**	**189.76**

Table (2): Effect of mesenchymal stem cells alone and/or in combination with vitamin D on blood glucose level in STZ-induced diabetic ratsat the interval six weeks of treatment.

Period of treatment	% change of body weight per week (g)						
Experimental groups	**Baseline (g)**	**1st week (g)**	**2nd week (g)**	**3rd week (g)**	**4th week (g)**	**5th week (g)**	**6th week (g)**
	Mean ± SE	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Group I Normal (Control of healthy)	89±2.07	87±3.21	90±4.00	84±2.06	87±2.03	89±4.55	89±4.65
Group II (Diabetic: STZ)	417±41.7	467±39.30 [a]	524±25.24 [a]	527±37.05 [a]	508±28.67 [a]	562±26.47 [a]	571±29.61 [a]
% change		**12.0%**	**25.5%**	**26.4%**	**21.7%**	**34.7%**	**36.9%**
Group III (STZ+M.S. Cs)	424±31.94	346±35.73[a, b]	262±31.36[a, b]	216±33.28[a, b]	275±37.50 [a, b]	214±17.65 [a, b]	208±23.34[a, b]
% change		**-18.5%**	**-38.3%**	**-49.0%**	**-35.2%**	**-49.0%**	**-50.9%**
Group IV (STZ+MSC.s + Vit D)	449±38.16	371±33.49 [a, b]	331±26.59 [a, b]	268±22.92[a, b]	256±17.47[a, b]	211±15.86 [a, b]	203±14.99[a, b]
% change		**-17.3%**	**-26.2%**	**-40.4%**	**-42.9%**	**-53.1%**	**-54.8%**
F-Probability	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**
F-value	**13.8**	**14.3**	**35.0**	**31.8**	**31.0**	**88.8**	**84.6**

Each value represents mean of 12 records ± S.E.

Means with dissimilar superscript letter are significantly different at (P < 0.05), where: [a] significance vs. control group; [b] significance vs. STZ group.

Percent of changes (%) are calculated by comparing the interval weeks of experimental diabetic groups with the baseline (post- first week of streptozotocin induction)

STZ= Streptozotocin;**MSC.s=** Mesenchymal stem cells;**Vit-D=** Vitamin D

Table (3): Effect of mesenchymal stem cells alone and/or in combination with vitamin D on Erythrocyte count and Erythrocyte indicesin STZ-induced diabetic rats.

parameters / Experimental groups	T. RBC.S/Erythrocytes(10^6/mm^3)		HGB (g/dl)		PCV (HCT) %		Erythrocyte indices					
							MCV(fl)		MCH (pg)		MCHC (g/dl)	
	Mean ± SE	%change	Mean ± SE	%change	Mean ± SE	%change	Mean ± SE	%change	Mean ± SE	%change	Mean ± SE	%change
Group I: Normal (Control)	6.6±0.2		15.9±0.2		44.0±1.2		67.0±3.2		24.2±0.9		36.3±1.3	
Group II: (Diabetic control: STZ)	4.5±0.3[a]	**-32.4%**	10.8±1.2[a]	**-32.2%**	27.4±1.5[a]	**-37.7%**	62.3±3.2	**-7.1%**	23.9±1.8	**-1.0%**	39.3±4.1	**8.3%**
Group III (STZ+ MSC.s)	5.2±0.3[a]	**-21.6%**	15.0±0.9[b]	**-5.4%**	35.9±1.8[a,b]	**-18.4%**	69.5±1.7	**3.7%**	28.9±0.6[a,b]	**19.8%**	41.8±1.3	**15.2%**
GroupIV (STZ+MSC.s + Vit D)	5.6±0.3[a,b]	**-14.6%**	15.6±0.4[b]	**-2.0%**	37.1±1.7[a,b]	**-15.7%**	65.9±2.2	**-1.7%**	28.0±1.5[a,b]	**15.8%**	42.6±2.5	**17.5%**
F-Probability	**p<0.001**		**p<0.001**		**p<0.001**		**N.S.**		**p<0.05**		**N.S.**	
F-value	**11.8**		**9.9**		**18.6**		**1.3**		**4.0**		**1.2**	

Table (4): Effect of mesenchymal stem cells alone and/or in combination with vitamin D on leucocyte count, platelets count and a differential leucocyte (lymphocyte, neutrophil and monocyte percentage in STZ-induced diabetic rats.

Parameters / Experimental groups	T. WBC.s/leucocytes (10^3 /mm^3)		Differential leucocyte						Platelets (10^3/mm^3)	
			Lymphocyte Percentage (%)		Monocyte Percentage (%)		Neutrophil Percentage (%)			
	Mean±SE	%change	Mean±SE	%change	Mean±SE	%change	Mean±SE	%change	Mean±SE	%change
GroupI: Normal (Control)	6.8±0.3		67.7±1.7		3.6±0.4		21.4±1.0		259.1±9.7	
GroupII: (Diabetic control: STZ)	11.4±0.7[a]	**67.3%**	42.2±1.6[a]	**-37.7%**	5.4±0.3[a]	**49.0%**	32.6±1.0[a]	**51.8%**	145.1±29.8[a]	**-44.0%**
GroupIII (STZ+MSC.s)	7.6±0.4[b]	**10.6%**	55.8±8.3[b]	**-17.6%**	4.8±0.7	**32.0%**	21.4±0.5[b]	**-0.1%**	229.9±23.6[b]	**-11.3%**
GroupIV (STZ+MSC.s + Vit D)	7.9±0.6[b]	**15.7%**	56.0±3.2[b]	**-17.2%**	4.2±0.5	**15.4%**	21.4±1.0[b]	**-0.3%**	218.7±15.0[b]	**-15.6%**
F-probability between groups	**p<0.001**		**p<0.001**		**N.S.**		**p<0.001**		**p<0.001**	
F-value	**15.5**		**5.2**		**2.3**		**37.4**		**5.3**	

Each value represents mean of 5 records ± S.E.

Means with dissimilar superscript letter are significantly different at (P < 0.05), where: [a] significance vs. control group; [b] significance vs. STZ group.

Means, which have the same superscript symbol (N.S.), are not significantly different.

Percent of changes (%) are calculated by comparing treated groups with normal control group.

STZ=Streptozotocin;**MSC.s=** Mesenchymal stem cells;**Vit-D=** Vitamin D

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الملخص العربي

الدور العلاجى للخلايا الجزعيه وفيتامن د على بعض المعايير البيوكميائية والقياسات الدمويه فى ذكور الجرذان البيضاء المصابه بداء السكرى المستحدث بواسطة الإستربتوزوتوسين.

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المقدمة : السكربمرض مزمن وغير معد ويصيب جميع الأشخاص بمختلف الأعمار وتزداد معدلات الإصابة بتقدم العمر وهو مرض شائع يؤثر على معظم سكان العالم وتمتلك مصر نسبة كبيره من المرض على طول الإحصائيات المعدة ، وهو عبارة عن ارتفاع معدل السكر الطبيعي في الدم ، نتيجة لنقص نسبي أو كامل في الأنسولين أو لخلل في تأثير الأنسولين على الأنسجة ، مما ينتج عنه مضاعفات مزمنة في أعضاء مختلفة من الجسم.
وفى الاونة الاخيرة .. وفيما اطلق عليه ثورة فى علاج مرض السكر،تم الاعلان عن علاج جديد من شأنه القضاء على المرض وو الخلايا الجزعيةتز
الهدف من الدراسه: أجريت هذه الدراسة لتقييم الأضرار الناجمة عن مرض السكرى كما تهدف أيضاً هذه الدراسة إلي إلقاء الضوء علي الدور العلاجى للخلايا الجزعيه والوقائى لفيتامين د ضد التغيرات البيوكيميائية والفسيولوجية في دم ذكور الجرذان البيضاء المصابه بمرض السكرى المستحدث بواسطة الإستربتوزوتوسين على مدار ٦ أسابيع من العلاج.
المواد والطرق المستخدمه:الدراسة الحالية، استخدمت ٢٤عدد من ذكور الجرذان البيضاء وزنها ١٥٠-١٨٠جم تم تقسيم الفئران إلى أربع مجموعات. (تحتوي على عدد ٦جرذ في كل مجموعة) وصممت على النحو التالي:
١- **المجموعة الأولى:** (مجموعة التحكم)، المجموعة الضابطة: تم تجريع الجرذان على ما يعادل ١مل / كجم يوميا من المحلول الملحى الفسيولوجي (٠,٩٪ كلوريد الصوديوم) عن طريق الفم لمدة ٤٢ يوما.
٢- **المجموعة الثانية:** (مجموعة السكر)، المجموعة الضابطة حقنت جرذان هذه المجموعة بمادة الإستريبتوزيتوسينداخل التجويف البريتوني مره واحده (٥٠ ملجم / كجم من وزن الجسم) طوال فترة التجربة لمدة ٦ أسابيع.
٣- **المجموعة الثالثه:** مجموعة (الخلايا الجذعيه)، تم حقن الجرذان المصابه بمرض السكرى فى هذه المجموعة بالخلايا الجذعيه بجرعه مليون خليه/ ٠,٥ مل مره واحده لكل جرذ من جرذان المجموعه على طول مدة التجربه.
٤- **المجموعة الرابعه:**مجموعة (الخلايا الجذعيه وفيتامين د)، حقنت الجرذان المصابه بمرض السكرى فى هذه المجموعة بالخلايا الجزعيه كما هو الحال في المجموعة الثالثه وقد تم إعطاء فيتامن د كعامل وقائى بجرعه ١جم / كجم من وزن الجسم عن طريق الفم يوم بعد يوم طوال فترة التجربة.
النتائج: لوحظ انخفاض أوزان الجرذانفي مجموعة السكربخلال الأسبوع الرابع والخامس والسادس من التجربه في حين مستوى السكر في الدم ارتفع بنسبه كبيرهعلى عكس الحال فيفي المجموعات المعامله بالخلايا الجذعيه على حده أو مع فيتامين دبالموازنة مع المجموعة الضابطة.في حين أظهرت نتائج القياسات الدمويه تحسنا ملحوظاًفي كلا من تلك القيم السابقه في المجموعات المعامله كما زادت أوزان الجرذان وانخفض مستوى سكر الدم بالموازنه معمجموعه السكرى.

الخلاصه:بناءعلى ماتوصلت إليهنتائجالدراسةالحالية،فقد أظهرتالنتائجأناستخداممادة الإستربتوزوتوسين المحدثه لمرض السكرى أدتإلىنقص في وزن الجسم علاوة على الخلل الناجم في القياسات الدمويه وكذا بالأحرى مستوى السكر في الدم مقارنة بنظائرها من المجموعات المختلفه. في حين أن العلاج بالخلايا الجذعيه على حده أو مع فيتامين د ظهرت النتائج إيجابيه لحد كبير لنفس القياسات السابقه وذلك عند مقارنتها بمجموعة السكر الضابطه.