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Therapeutic Effects of Bone Marrow Stem Cells in Diabetic Rats

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Abstract

Background: Currently, diabetes mellitus, specifically, Type 2 diabetes is a multifactorial metabolic disorder that affects more than 348 million people worldwide. It is considered to be one of the main causes of mortality. The pathway of type 2 diabetes is characterized both by insulin resistance in muscle, fat, and liver and a relative failure of the pancreatic β cell. Despite extensive study, yet no unifying hypothesis exists to explain these defects and the proper treatment. The key goal of diabetes treatment is to prevent complications because over time, diabetes can damage the heart, blood vessels, eyes, kidneys, and nerves. Therefore there is a great need to develop new and effective therapies for treating diabetic complications early before it cause irreparable tissue damage. Recently, advances experimental evidence empowers the idea that diabetic patients may greatly benefit from cell-based therapies, which include the use of adult stem and/or progenitor cells in disease therapy. In particular, therapeutic effect of bone marrow stem cell in treating the type 2 diabetic patients.

Motivation: Mesenchymal stem cells (MSCs) are adherent and pluripotent and non-hematopoietic progenitor cells. Human bone marrow MSCs have been shown to inhibit antigen-dependent CD4+ and CD8+ T cell proliferation in an allogeneic setting *in vitro*. They have been found to reside in most organs and tissues investigated to date, including bone marrow, adipose, dermis, muscular tissue, hair follicles, the periodontal ligament and the placenta. In addition, recent studies have shown that adult bone marrow stem cells can differentiate into several types such as blood, liver, lung, skin, muscle, neuron and insulin producing cells. This has motivated us to explore potentials of their therapeutic applications in treating diabetes mellitus or type-2 diabetes. This article proposes a novel mechanism to isolate adult rat bone marrow stem cells and test their ability to treat diabetic rats. The main focus of this research is to investigate the therapeutic effect of mesenchymal stem cells on the diabetic rats.

Experimental methods, data and results: The experimental studies were carried out based on twelve-week old healthy Sprague Dawley (S.D) rats and it was used for isolation and transplantation of stem cells. We carried a total number of 40 Sprague Dawley (S.D) male rats; 12-14 weeks old age and weighting 180-250 gm were used in the experimental study. Rats were obtained from Animal House of Nile Center for Experimental Researches, Mansoura, Egypt. Animals were housed in separate metal cages, fresh and clean drinking water was supplied adlibtium through specific nipple. The animals were anesthetized by halothane, and then the skin was sterilized with 70% ethyl alcohol before cutting the skin. The femurs and tibia were carefully dissected from adherent soft tissues. Then they were placed into sterilized beaker containing 70% ethyl alcohol for 1-2 min. The bones were put in Petri dish contain Phosphate buffer saline 1X (PBS) (Hyclone, USA) for wash. The bones were taken to laminar air flow (unilab biological safety cabinet class II, china) to extract the BM. The two ends of the bones were removed using sterile scissors.

Conclusion and future work: Currently, the obtained results revealed that diabetes caused bad effects on the blood picture, pancreas and kidney functions, as well as the immune system represented by TNF. Treatment the diabetic rats by MSCs engrafting improved the tested parameters towards the stats of normal case. Nevertheless, the wide application of the stem cells engrafting still needs more investigations to be assured.

Keywords: Stem cell; Therapeutic; Diabetes progression and complications; Bone marrow

Introduction

The worldwide increase in the prevalence of diabetes mellitus reinforces the search for solutions to prevent it as well as to oppose the development and the progression of its complications. Particularly, the increasing prevalence of diabetes mellitus (DM) now affects adolescents and younger adults, thus promoting an earlier development of invalidating chronic diseases [1-4]. Experimental evidence suggests that cell-based therapies might represent a new and promising strategy for the treatment of diabetic vascular complications, and growing interest has recently been focused on mesenchymal stem cells and endothelial progenitor cells. Both cells types not only act against the mechanisms underlying diabetic complications but also rescue the abnormalities that stem cells present in diabetic patients, which contribute to the vascular complications. Notably, these cells avoid the ethical issues relating to the use of the embryonic cells. However, there are concerns about how the diabetic environment affects these cells. So, additional challenges for these cells include making them resistant to the diabetic environment and thus increasing their clinical efficacy [2]. On these premises, we will here review the evidence suggesting why adult stem/progenitor cells should be used in diabetic patients, the

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therapeutic benefits that these cells seem to offer for treating macro vascular and microvascular complications, and the challenges that cell-based therapies in DM present.

Stem cells

Adult stem cells comprise of roughly three different groups: the bone marrow stem cells (BM-SC), the circulating pool of stem or progenitor cells (which are also derived from the bone marrow), and the tissue resident stem cells. BM-SC can be further categorized into multipotent adult progenitor cells, mesenchymal stem cells (MSC), and hematopoietic stem cells. The circulating pool of stem/progenitor cells includes different types of cells, among which the most studied for the setting of vascular complications are the endothelial progenitor cells (EPC). EPC were identified by the authors Calcutt et al., Jarajapu et al., Asahara et al. and Bernardi et al. the search for circulating angiogenic cells [1-4]. They observed that these cells were able to form new blood vessels and promote neovascularisation after ischemia. Therefore, these cells seem to be the most promising in the setting of DM because of their potential utility in therapeutic neovascularisation and vascular repair. This paper will be focused on BM-SC, since this BM-SC is the most studied in the field of the cell-based therapies for DM and for diabetic complications.

Bone marrow-derived mesenchymal stem cells (BM-MSCs) are multi-potent stroma cells which can provide a potential therapy for diabetes mellitus, but the mechanism is still controversial [5]. Bone marrow (BM) is a mixture of cells containing hematopoietic progenitor cells and a connective-tissue network of stromal cells. Marrow stroma includes a subpopulation of undifferentiated cells. These cells are referred to as mesenchymal stem cells (MSCs), since they have the capacity of proliferation and differentiation into the mesenchymal lineage. Mesenchymal stem cells are capable of becoming one of a number of phenotypes, including muscle, bone, cartilage, tendon, fat, and marrow stromal connective tissue. Due to their potential for differentiation into different tissues, MSCs have emerged as a promising tool for clinical applications such as tissue engineering and cell therapy [6].

Diabetes mellitus is a multi-metabolic disorder that influences more than 348 million people worldwide [7]. A key goal of diabetes treatment is to prevent complications because over time, diabetes can damage the heart, blood vessels, eyes, kidneys, and nerves [8]. Consequently there is an incredible need to develop new and successful therapies for treating diabetic complications early before it causes irreparable tissue damage. Recent studies has shown that adult bone marrow stem cells can differentiate into several types such as blood, liver, lung, skin, muscle, neuron and insulin producing cells [9]. Mesenchymal stem cells (MSCs) are adherent, fibroblast- like, pluripotent and non-hematopoietic progenitor cells. They have been found to reside in most organs and tissues investigated to date, including bone marrow, adipose, dermis, muscular tissue, hair follicles, the periodontal ligament and the placenta. Previous study has demonstrated that MSCs can be differentiated into chondrogenic, osteogenic, adipogenic, myogenic, cardiomyogenic, and hematopoietic potential stromal cells. In addition, apart from their differentiate abilities; MSCs play a supportive role in organ regeneration processes [10]. Moreover, several studies by Ju et al. have postulated that the use of MSCs *in vivo* should be safer than that of embryonic stem cells due to their higher chromosomal stability and lower tendency to form teratoma in the recipient host [11].

The intensive insulin therapy is generally the best option for the treatment of diabetes mellitus (DM); however, this approach does not overcome serious microvascular complications. Therefore, pancreas and pancreatic islet transplantation are considered an effective approach for the treatment of diabetes. Currently, a limited supply of donor pancreatic islets and the risk of immunological rejection prevent widespread use of these approaches [12]. In recent years, researchers have tried to identify a substitute to pancreatic islet transplantation [13,14].

A promising alternative for the generation of pancreatic islets are stem cells located in embryonic and adult tissues. Many researchers have become interested in adult stem cells because these cells have sufficient plasticity and there fewer ethical problems. Bone marrow derived mesenchymal stem cells (BMSCs) offer significant benefits for clinical application, because they can be easily harvested and, when autologous transplanted, there is no immunological rejection [15]. Moreover, BMSCs can differentiate into a wide variety of cell types. Here, we focused on bone marrow-derived mesenchymal stem cells. Other authors have demonstrated that mesenchymal stem cells (MSCs) can transdifferentiate into insulin-producing cells (IPC) under defined conditions and normalize the glucose level of streptozotocin (STZ)-induced diabetic rats. Aim of this research: To study the therapeutic effects of the bone marrow stem cells (BMSCs) in treatment of diabetes mellitus (DM) in experimentally induced diabetic rats. This study will be done using immunological, biochemical and histopathological investigations to prove the promising effect of the BMSCs in cure of or improving the diabetic rats' condition.

This paper is organized as follows: Section 2 gives a detailed materials and methods with experimental data. Section 3 proposes results and detailed discussions. Section 4 proposes the historical analysis with the detailed histopathological changes of both pancreas and kidney. Section 5 proposes deep comparative studies and benchmarks with brief discussions about the obtained results. Finally, the conclusions and future outlooks with our recommendations are drawn in Section 6.

Materials and Methods

Experimental animals

Animals used in this work were male Sprague Dawley (S.D) rats, aged 12-14 weeks, weighing 180-250 grams. Rats were obtained from Animal House of Nile Center for Experimental Researches, Mansoura, Egypt. After the two weeks of acclimatization the rats were divided randomly to four groups each contains 10 rats as follow:

Group 1 (Control group): 10 healthy rats did not receive any treatment and served as control group.

Group 2 (stem cells group): 10 rats received mesenchymal stem cells (2×10^6 cells /rat), first dose (10^6 cells/ rat) and after one week, rats received the second dose of cells (10^6 cells/ rat).

Group 3 (Diabetic group): 10 rats in which type 2 diabetes was induced by a single intra-peritoneal injection of streptozotocin (STZ) 40 mg/kg body weight dissolved immediately before administration in freshly prepared 0.2 ml citrate buffer (pH 4.5). Diabetes was defined as a random blood glucose reading of >300 mg/dl after 72 hours of STZ injection.

Group 4 (treated group):10 diabetic rats, these diabetic rats received (2×10^6 cells /rat) mesenchymal stem cells (MSCs) by intravenous injection through penial vein per rat, first dose (10^6 cells/ rat) in 0.2 ml Dulbecco's modified Eagles medium (DMEM) and after one week rats received the second dose of cells (10^6 cells/ rat) in 0.2 ml DMEM.

Isolation and preparation of Mesenchymal stem cells (MSCs): Isolation and culturing of MSCs were done according to the method given by Abdel Aziz et al. [16].

Isolation and preparation of Bone Marrow (BM): The animals were anesthetized by halothane, and then the skin was sterilized with 70% ethyl alcohol before cutting the skin. The femurs and tibia were carefully dissected from adherent soft tissues. Then they were placed into sterilized beaker containing 70% ethyl alcohol for 1-2 min. The bones were put in Petri dish contain Phosphate buffer saline 1X (PBS) (Hyclone, USA) for wash. The bones were taken to laminar air flow (Unilab biological safety cabinet class II, china) to extract the bone marrow (BM). The two ends of the bones were removed using sterile scissors. Bone marrow was harvested by flushing the tibiae and femurs of 12- weeks old (S.D) male rats with (DMEM) (lonza, Belgium) supplemented with 10% fetal bovine serum (FBS) (lonza, Belgium) and 1% Antibiotic, Antimicotic (*penicillin streptomycin*) (lonza, USA). The marrow plugs were cultured in 20 ml complete media and incubated at 37°C in 5% humidified CO_2 incubator (shellab, USA) for 7-10 days as primary culture or upon formation of large colonies.

Biochemical measurements

Lymphocyte issolation and counting: Lymphocyte was isolated according to the method previously described by Maisel et al. [17].

Principle: Lymphocyte was isolated using a standard density gradient separation method from whole blood using commercially available ficoll. The procedure consists of layering whole blood over the density gradient ficoll, centrifugation, separation of lymphocyte layer, and lysis of residual erythrocytes. Cells were then washed, counted, and resuspended in buffer to the desired concentration.

Neutrophil counting: Neutrophil was calculated by auto-analyser of blood picture (coulter counter model s-coultronics, France S.A.) Neutrophil count = count/cmm.

Quantitative detection of rat tumor necrosis factor alpha (TNF-α): Serum TNF-α was determined according to Chen et al. by solid phase Enzyme Linked Immuno Sorbent Assay (ELISA) using rat TNF-α kits (eBioscience, Austria) and a microtiter plate reader capable of reading at 450 nm [18].

Glucose tolerance: Glucose tolerance was done according to the method described that was determined in serum by using specific kit (SPINREACT, S.A. / S.A.U.) [19].

Determination of glucose-6-phosphate dehydrogenase: Glucose-6-Phosphate dehydrogenase was determined in whole blood using a specific kit (span diagnostics Ltd. Surat, India) and according to the method previously described by Muller [20].

Glycosylated hemoglobin test: Glycosylated haemoglobin was done according to the method previously described, Bunn by using specific kit (VITRO SCIENT, Germany) [21].

Determination of total cholesterol concentration: Serum total cholesterol concentration was determined according to the method described by NCEP expert panel [22].

Determination of triglycerides concentration: Serum triacylglycerols concentration was determined according to the method described by Stein [23].

Determination of serum high density lipoprotein-cholesterol (HDL-C) concentration: The concentration of serum HDL-C was estimated by colorimetric kit as of method of Finley et al. [24], using HDL-C kit purchased from SPINREACT, S.A. Ctra, Santa Coloma, Spain [25].

Determination of serum low density lipoprotein cholesterol (LDL-C): The (LDL-C) concentration in serum was calculated from the total cholesterol concentration, the (HDL-C) concentration and triglycerides concentration according to Friedewald et al. [22].

Determination of serum creatinine: Serum creatinine was determined by a modified rate Jaffe method described by Schirmeister, using kits obtained from (Beckman, USA), following the instructions of the manufacturer.

Determination of serum uric acid: Serum uric acid concentration was determined according to the method described by Fossati et al. [26].

Determination of serum total proteins: Total proteins were determined in serum sample by the method of Gornall et al. [27], using a commercially available assay kit (Egyptian American company for Laboratory Services, Egypt).

Determination of serum albumin: The concentration of albumin was determined in serum samples using binding method of Doumas et al. [28], which is commercially available as an assay kit (Egyptian American Company for Laboratory Services, Egypt).

Determination of serum globulin: The concentration of albumin was determined in serum samples using binding method of Goldenberg and Drewes [29].

Histology analysis

The pancreatic and renal tissues were prepared for light microscopy as described by Moussa et al. [30]. For light microscopy, pancreas and kidney were taken from rats in different groups and fixed in 10% neutral formalin. Then washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at a thickness of 4 µm by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin stain for routine examination then examination was done through the light electric microscope.

Statistical analysis

Statistical analysis was carried out between the control and each of the experimental groups. Results were expressed as mean ± S.E and analysed for statistical significance based on *p*-values and *t*- test using SPSS V. 20 software and Intel® core™ i7-4700MQ CPU @2.4 GHz and 64-bit operating system.

Results and Discussion

Results for both control and experimental groups

Based on the collected whole blood samples from all animal groups (control and experimental groups), below are the obtained results for the utilized parameters: Lymphocyte, neutrophil, tumor necrosis factor alpha, glucose tolerance, glucose-6-Phosphate dehydrogenase, glycosylated hemoglobin, total cholesterol, triglycerides, serum high and low Density lipoprotein- cholesterol, serum creatinine, uric acid, total proteins, albumin, and globulin.

We have achieved the statistical analysis of the 14 experimental tests using the IBM SPSS software. The complete visualizations and

interpretations of the results were summarized in Tables and graphs. The following symbols are utilized to show the significance differences between the means of each test for abbreviations:

- ***a***: means the significance as compared with the control group;
- ***b***: means the significance as compared with the diabetic group;

where, ***a*** and ***b*** means that there is a significance difference, which means ***p*-value ≤ 0.05**. If ***p*-value is** greater than **0.05**, then the test is not significant.

Lymphocyte: The effect of mesenchymal stem cell transplantation on lymphocyte count of diabetic and nondiabetic rats are represented in Table 1 and illustrated in Figure 1. The mean count of blood lymphocyte decreased significantly in diabetic group (+ve) comparing to control group (-ve), increased significantly in diabetic + MSCs group comparing to diabetic group (+ve) but slightly decreased comparing to that in control group (-ve).

By looking at Table 1, we observe that the data are presented as mean ± standard error (s.e.), and comparative studies are carried to compare the differences between means using ANOVA and independent sample *t*-test based on the IBM SPSS software; then the significance differences indicators are recorded in bold.

Neutrophil: The effect of mesenchymal stem cell transplantation on neutrophil counts of diabetic and nondiabetic rats are represented in Table 2 and illustrated in Figure 2. The mean count of blood neutrophil is increased highly significant in diabetic group (+ve) comparing to control group (-ve), highly decreased in diabetic + MSCs group comparing to diabetic group (+ve) but increased slightly significant comparing to control group (-ve).

Tumor necrosis factor alpha (TNF- α): The effect of stem cell transplantation on TNF-α of diabetic and nondiabetic rats are represented in Table 3 and illustrated in Figure 3. The mean level of serum TNF-α is increased highly significant in diabetic group (+ve) comparing to control group (-ve), decreased significantly in diabetic + MSCs group but still higher than that in control group and decreased significantly comparing to diabetic group (+ve).

Glucose tolerance: The effect of stem cell transplantation on glucose tolerance of diabetic and nondiabetic rats are represented in Table 4 and illustrated in Figure 4. The mean blood glucose tolerance is increased highly significant in diabetic group (+ve) comparing to control group (-ve), decreased in diabetic + MSCs group but still significantly higher than that in control group (-ve) and decreased significantly in diabetic + MSCs group comparing to diabetic group (+ve).

Glucose-6-phosphate dehydrogenase: The effect of mesencymal stem cell transplantation on glucose-6-phosphate dehydrogenase of diabetic and nondiabetic rats are represented in Table 5 and illustrated in Figure 5. The mean blood glucose-6-phosphate dehydrogenase is decreased highly significant in diabetic group (+ve) comparing to control group (-ve) and increased significantly in diabetic + MSCs group but still significantly lower than that in control group (-ve). Also, the mean blood glucose-6-phosphate dehydrogenase level is increased significantly in diabetic + MSCs group comparing to diabetic group (+ve).

Glycosylated hemoglobin (HBA1C): The effect of mesencymal stem cell transplantation on HBA1C counts of diabetic and nondiabetic rats are represented in Table 6 and illustrated in Figure 6. The mean blood HBA1C is increased highly significant in diabetic group (+ve) comparing to control group (-ve), and decreased highly significant in diabetic + MSCs group comparing to diabetic group (+ve).

Total cholesterol concentration: The effect of mesencymal stem cell transplantation on total cholesterol concentration of diabetic and

Lymphocytes test (No. of cells/ ml)	Index	Control (-ve)	MSCs	Diabetic (+ve)	Diabetic + MSCs
	Mean	2,121,666	2,273,333	986,666[a]	1,910,000 [a,b]
	± S.E.	176,151	205,437	55,537	170,450

where [a] and [b] indicate the significant changes from Control (-ve), Diabetes (+ve), and Diabetes + mesenchymal stem cells groups, respectively, and $p<0.05$.

Table 1: The effect of mesenchymal stem cell transplantation on blood lymphocyte counts in diabetic and nondiabetic rats.

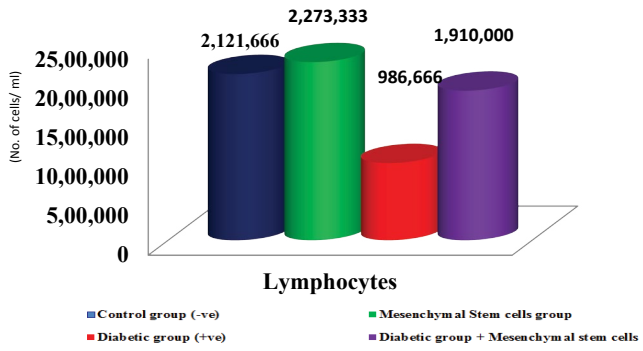

Figure 1: Effect of mesenchymal stem cell transplantation on blood lymphocyte counts in diabetic and nondiabetic rats.

Neutrophil test (Absolute value/ul)	Index	Control (-ve)	MSCs	Diabetic (+ve)	Diabetic + MSCs
	Mean	2,234	1,690	493,333 [a,b]	3,185 [a,b]
	± S.E.	271.33	205.99	82,589.21	210.70

where [a] and [b] indicate the significant changes from Control (-ve), Diabetes (+ve), and Diabetes + mesenchymal stem cells groups, respectively, and $p<0.05$.

Table 2: The effect of mesenchymal stem cell transplantation on blood neutrophil counts in diabetic and non-diabetic rats.

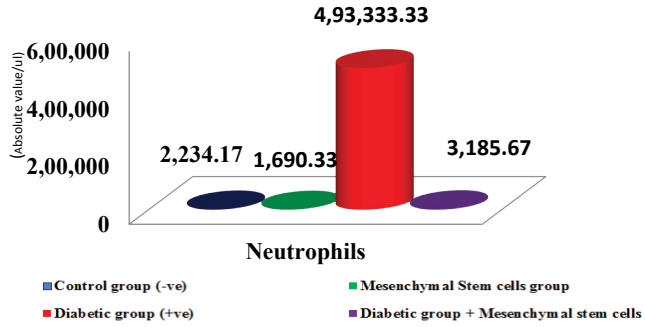

Figure 2: Effect of mesenchymal stem cell transplantation on blood neutrophil count in diabetic and nondiabetic rats.

Tumor necrosis factor alpha (TNF- α) (pg/ml)	Index	Control (-ve)	MSCs	Diabetic (+ve)	Diabetic + MSCs
	Mean	43.17	47.15	377.90 [a]	105.20 [a,b]
	± S.e.	1.10	2.81	29.19	2.71

where [a] and [b] indicate the significant changes from Control (-ve), Diabetes (+ve), and Diabetes + mesenchymal stem cells groups, respectively, and $p<0.05$.

Table 3: The effect of stem cell transplantation on blood TNF-α in diabetic and nondiabetic rats.

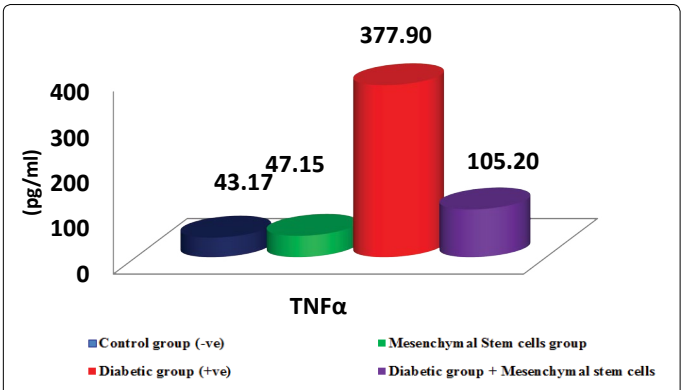

Figure 3: Effect of mesenchymal stem cell transplantation on blood TNF-α in diabetic and nondiabetic rats.

Glucose test (mg/dl)	Index	Control (-ve)	MSCs	Diabetic (+ve)	Diabetic + MSCs
	Mean	102.22	94.80	550.67 [a]	239 [a,b]
	± S.e.	4.18	4.95	17.17	24.97

where [a] and [b] indicate the significant changes from Control (-ve), Diabetes (+ve), and Diabetes + mesenchymal stem cells groups, respectively, and $p<0.05$.

Table 4: The effect of mesenchymal stem cell transplantation on blood glucose tolerance in diabetic and nondiabetic rats.

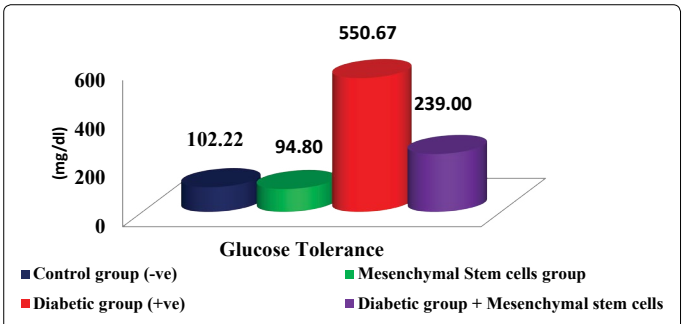

Figure 4: Effect of mesencymal stem cell transplantation on blood glucose tolerance in diabetic and nondiabetic rats.

Glucose -6-phosphate dehydrogenase Test (u/g Hb)	Index	Control (-ve)	Stem cells	Diabetic (+ve)	Diabetic + Stem cells
	Mean	116.16	102.62	57.85 [a]	88.2 [a,b]
	± S.e.	7.77	2.93	2.26	3.07

where [a] and [b] indicate the significant changes from Control (-ve), Diabetes (+ve), and Diabetes + mesenchymal stem cells groups, respectively, and $p<0.05$.

Table 5: The effect of mesencymal stem cell transplantation on blood glucose-6-phosphate dehydrogenase in diabetic and nondiabetic rats.

nondiabetic rats are represented in Table 7 and illustrated in Figure 7. The mean total cholesterol concentration is increased highly significant in diabetic group (+ve) comparing to control group (-ve), and decreased significantly in diabetic + MSCs group comparing to that in diabetic

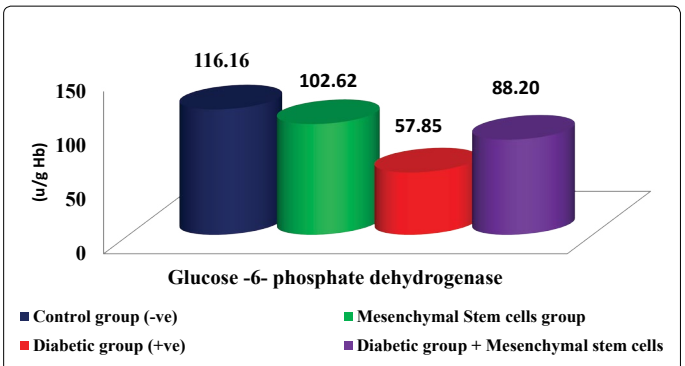

Figure 5: Effect of mesencymal stem cell transplantation on blood glucose-6-phosphate dehydrogenase in diabetic and nondiabetic rats.

HBA1C test (%)	Index	Control (-ve)	Stem cells	Diabetic (+ve)	Diabetic + Stem cells
	Mean	2.93	2.88	5.02 [a]	3.40 [b]
	±S.e.	0.09	0.17	0.36	0.10

where [a] and [b] indicate the significant changes from Control (-ve), Diabetes (+ve), and Diabetes + mesenchymal stem cells groups, respectively, and $p<0.05$.

Table 6: The effect of mesencymal stem cell transplantation on blood HBA1C in diabetic and nondiabetic rats.

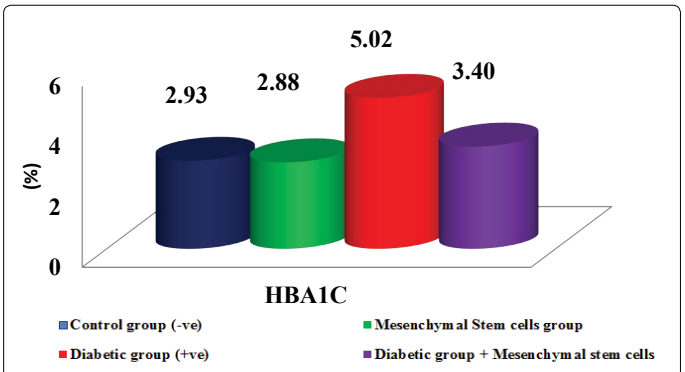

Figure 6: Effect of mesencymal stem cell transplantation on blood HBA1C in diabetic and nondiabetic rats.

Total Cholesterol Concentration test (mg/dl)	Index	Control (-ve)	MSCs	Diabetic (+ve)	Diabetic + MSCs
	Mean	59.98	62.02	117.33[a]	78.54 [a,b]
	± S.e.	1.31	1.89	9.93	3.01

where [a] and [b] indicate the significant changes from Control (-ve), Diabetes (+ve), and Diabetes + mesenchymal stem cells groups, respectively, and $p<0.05$.

Table 7: The effect of mesenchymalstem cell transplantation on serum total cholesterol in diabetic and nondiabetic rats.

group (+ve). Also, the mean total cholesterol concentration is decreased in diabetic + MSCs but still significantly higher than that in control group (-ve).

Triglycerides concentration: The effect of mesencymal stem cell transplantation on blood triglyceride concentration of diabetic and nondiabetic rats are represented in Table 8 and illustrated in Figure 8. The mean serum triglyceride concentration is increased highly significant in diabetic group (+ve) comparing to control group (-ve), decreased highly significant in diabetic + MSCs group comparing to that in diabetic group (+ve), is decreased in diabetic + MSCs group but still significantly higher than that in control group (-ve) and is increased in MSCs group comparing to that in control group (-ve).

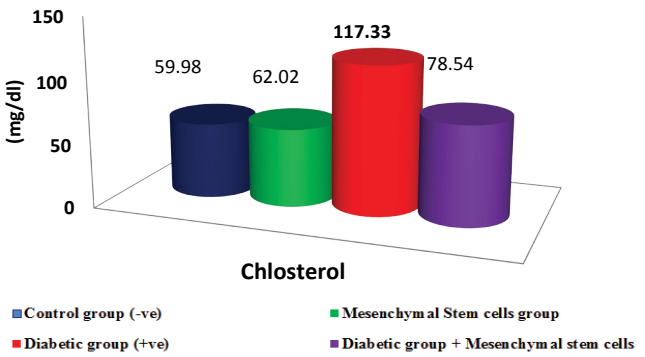

Figure 7: Effect of mesenchymal stem cell transplantation on blood total cholesterol concentration in diabetic and nondiabetic rats.

Triglycerides Concentration test (mg/dl)	Index	Control (-ve)	MSCs	Diabetic (+ve)	Diabetic + MSCs
	Mean	34.81	44.34	90.78 [a]	57.62 [a,b]
	± S.e.	3.31	2.14 [a]	6.22	5.05

where [a] and [b] indicate the significant changes from Control (-ve), Diabetes (+ve), and Diabetes + mesenchymal stem cells groups, respectively, and $p<0.05$.

Table 8: The effect of mesenchymal stem cell transplantation on serum triglyceride concentration in diabetic and nondiabetic rats.

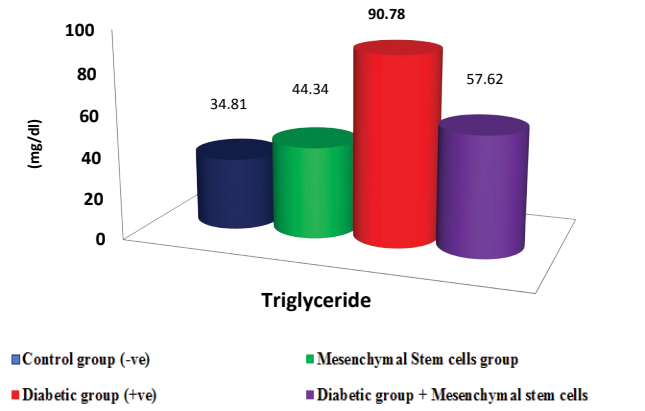

Figure 8: Effect of mesenchymal stem cell transplantation on blood triglyceride concentration in diabetic and non-diabetic rats.

Serum high density lipoprotein-cholesterol (HDL-C) concentration: The effect of mesenchymal stem cell transplantation on serum HDL-C of diabetic rats are represented in Table 9 and illustrated in Figure 9. The mean serum HDL-C is decreased significantly in diabetic group (+ve) comparing to control group (-ve), increased significantly in diabetic + MSCs group comparing to diabetic group (+ve) and slightly decreased in diabetic + MScs group comparing to that in control group (-ve).

Serum low density lipoprotein cholesterol (LDL-C) concentrations: The effect of mesenchymal stem cell transplantation on serum LDL-C of diabetic rats are represented in Table 10 and illustrated in Figure 10**.** The mean serum LDL-C increased significantly in diabetic group (+ve) comparing to control group (-ve), increased significantly in diabetic + MSCs group comparing to control group (-ve), but decreased significantly comparing to diabetic group (+ve)

HDL- C test (mg/dl)	Index	Control (-ve)	MSCs	Diabetic (+ve)	Diabetic + MSCs
	Mean	36.30	37.83	27.67 [a]	31.58 [a,b]
	± S.e.	2.64	2.59	1.97	0.98

where [a] and [b] indicate the significant changes from Control (-ve), Diabetes (+ve), and Diabetes + mesenchymal stem cells groups, respectively, and $p<0.05$.

Table 9: The effect of mesenchymal stem cell transplantation on serum HDL-C in diabetic and nondiabetic rats

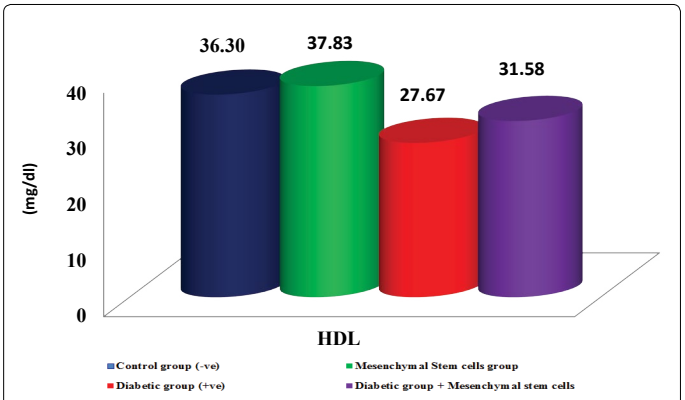

Figure 9: Effect of mesenchymal stem cell transplantation on serum HDL-C concentration in diabetic and nondiabetic rats.

LDL-C test (mg/dl)	Index	Control (-ve)	MSCs	Diabetic (+ve)	Diabetic + MSCs
	Mean	21.59	15.32 [a]	71.56 [a]	30.36 [a,b]
	± S.e.	0.88	1.22	8.81	2.36

where [a] and [b] indicate the significant changes from Control (-ve), Diabetes (+ve), and Diabetes + mesenchymal stem cells groups, respectively, and $p<0.05$.

Table 10: The effect of mesenchymal stem cell transplantation on serum LDL-C in diabetic and nondiabetic rats.

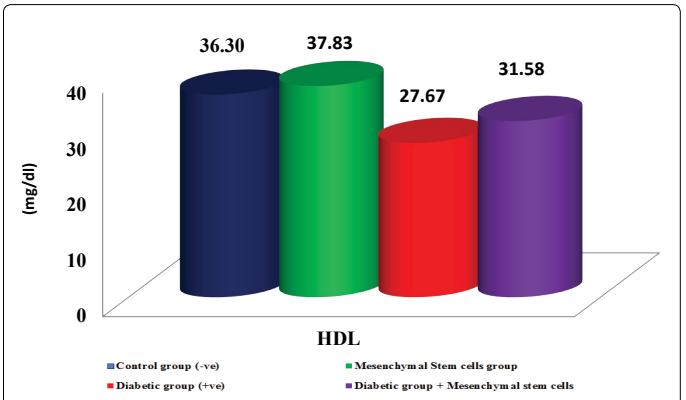

Figure 10: Effect mesenchymal of stem cell transplantation on serum LDL-C concentration in diabetic and nondiabetic rats.

and decreased significantly in MSCs group comparing to that in control group (-ve).

Serum creatinine: The effect of mesenchymal stem cell transplantation on serum creatinine of diabetic rats are represented in Table 11 and illustrated in Figure 11. The mean serum creatinine increased significantly in diabetic group (+ve) comparing to control group (-ve), decreased significantly in diabetic + MSCs group but

still slightly higher than that in control group (-ve) and decreased significantly comparing to diabetic group (+ve) and slightly decreased in MSCs group comparing to that in control group (-ve).

Serum uric acid: The effect of mesenchymal stem cell transplantation on serum uric acid of diabetic rats are represented in Table 12 and illustrated in Figure 12. The mean serum uric acid increased significantly in diabetic group (+ve) comparing to control group (-ve), decreased significantly in diabetic + MSCs group comparing to diabetic group (+ve) and increased significantly in diabetic + MSCs group comparing to that in control group (-ve).

Serum total proteins: The effect of mesenchymal stem cell transplantation on serum total proteins of diabetic rats are represented in Table 13 and illustrated in Figure 13. The mean serum total proteins decreased significantly in diabetic group (+ve) comparing to control group (-ve), increased significantly in diabetic + MSCs group comparing to that in diabetic group (+ve) and no changes in MSCs group that still similar to that in control group (-ve).

Serum albumin: The effect of mesenchymal stem cell transplantation on serum albumin of diabetic rats are represented in Table 14 and illustrated in Figure 14. The mean serum albumin decreased significantly in diabetic group (+ve) comparing to control group (-ve), increased slightly in diabetic + MSCs group comparing to

Creatinine test (mg/dl)	Index	Control (-ve)	MSCs	Diabetic (+ve)	Diabetic + MSCs
	Mean	0.52	0.48	0.80 [a]	0.62 [a,b]
	± S.e.	0.03	0.03	0.04	0.03

where [a] and [b] indicate the significant changes from Control (-ve), Diabetes (+ve), and Diabetes + mesenchymal stem cells groups, respectively, and $p<0.05$.

Table 11: The effect of mesenchymal stem cell transplantation on serum creatinine in diabetic and nondiabetic rats.

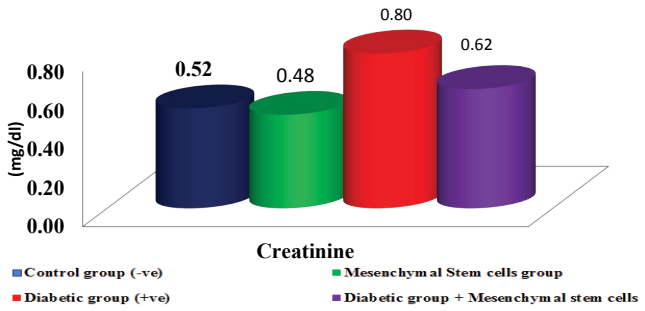

Figure 11: Effect of mesenchymal stem cell transplantation on serum creatinine concentration in diabetic and nondiabetic rats.

Uric acid test (mg/dl)	Index	Control (-ve)	MSCs	Diabetic (+ve)	Diabetic + MSCs
	Mean	1.93	1.95	3.63 [a]	2.2 [a,b]
	± S.e.	0.20	0.24	0.18	0.15

where [a] and [b] indicate the significant changes from Control (-ve), Diabetes (+ve), and Diabetes + mesenchymal stem cells groups, respectively, and $p<0.05$.

Table 12: The effect of mesenchymal stem cell transplantation on serum uric acid in diabetic and nondiabetic rats.

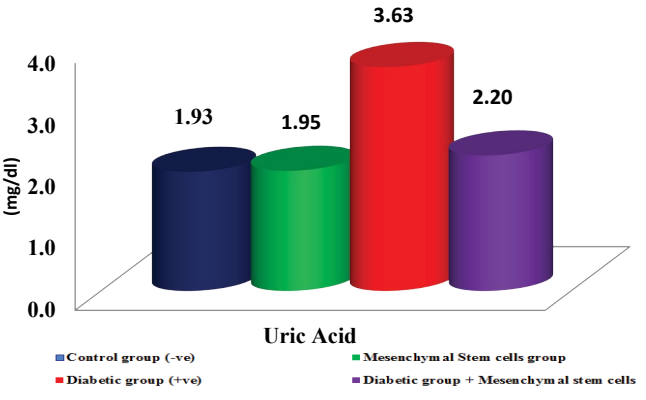

Figure 12: Effect of mesenchymal stem cell transplantation on serum uric acid concentration in diabetic and nondiabetic rats.

Total proteins test (g/dl)	Index	Control (-ve)	MSCs	Diabetic (+ve)	Diabetic + MSCs
	Mean	6.48	6.32	4.64 [a]	5.52 [b]
	± S.e.	0.27	0.20	0.24	0.08

where [a] and [b] indicate the significant changes from Control (-ve), Diabetes (+ve), and Diabetes + mesenchymal stem cells groups, respectively, and $p<0.05$.

Table 13: The effect of mesenchymal stem cell transplantation on serum total proteins in diabetic and nondiabetic rats.

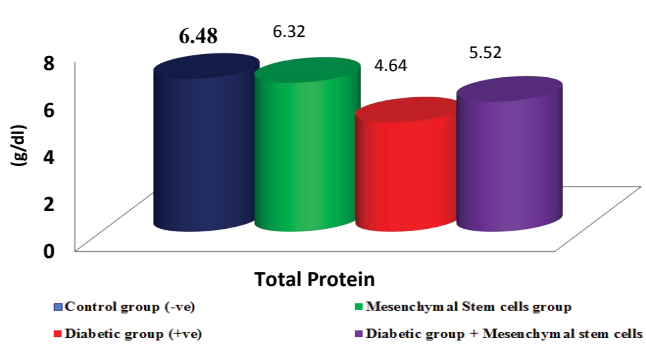

Figure 13: Effect of mesenchymal stem cell transplantation on serum total proteins concentration in diabetic and nondiabetic rats.

Albumin test (g/dl)	Index	Control (-ve)	MSCs	Diabetic (+ve)	Diabetic + MSCs
	Mean	3.12	3.12	2.32 [a]	2.73[b]
	± S.e.	0.05	0.09	0.09	0.12

where [a] and [b] indicate the significant changes from Control (-ve), Diabetes (+ve), and Diabetes + mesenchymal stem cells groups, respectively, and $p<0.05$.

Table 14: The effect of mesenchymal stem cell transplantation on serum albumin in diabetic and nondiabetic rats.

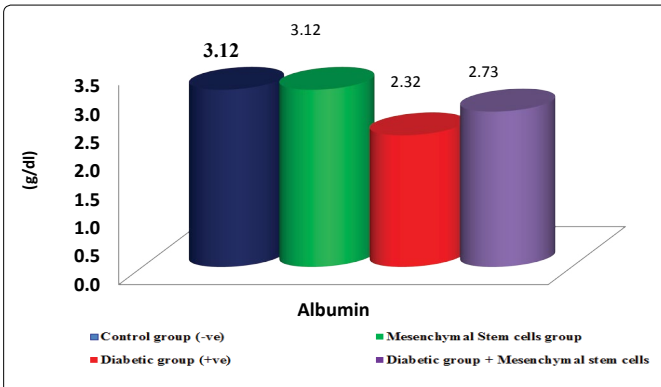

Figure 14: Effect of mesenchymal stem cell transplantation on serum albumin concentration in diabetic and nondiabetic rats.

diabetic group (+ve) and not changed in MSCs group and still similar to that in control group (-ve).

Histopathological changes of pancreas: Light microscopy of the control pancreas showed normal islet of Langerhanz with normal β cell in the center, α- cell in pancreas and normal capillaries The pathology of pancreas in normal rats transplanted with mesenchymal stem cells showed normal islet of Langerhanz with normal β cell in the center and α- cell in pancreas. The pathology of pancreas in rats injected with STZ showed necrosis in β cell of islets of Langerhanz, and normal exocrine portion. The pathology of pancreas in rats injected with STZ and transplanted with mesenchymal stem cells showed mild atrophy of islets of Langerhanz with normal β cells and normal exocrine portion of pancreas.

Histopathological changes of kidney: Light microscopy of the control kidney showed normal renal glomeruli and normal renal tubules. The pathology of kidney in normal rats transplanted with mesenchymal stem cells showed normal renal glomeruli and normal renal tubules. The pathology of kidney in rats injected with STZ showed dissolution, proliferation of mesangial cells in the glomeruli, necrosis in renal tubular epithelium, and hemorrhage in interstitial tissue with hemosiderosis. The pathology of kidney in rats injected with STZ and transplanted with mesenchymal stem cells showed congestion in glomerular tuft, vacuolation in renal tubular epithelium and mild hemorrhage in interstitial tissue.

Histological Analysis

Histopathological changes of pancreas

Light microscopy of the pancreas of the control group (-ve) showed normal islet of Langerhanz (L) with normal β cell (B) in the center, α- cell in pancreas (A) and normal capillaries (C) (Figure 15). The pathology of pancreas in MSCs group showed normal islet of Langerhanz (L) with normal β cell (B) in the center and α- cell in pancreas (A) (Figure 16). The pathology of pancreas in diabetic group (+ve) showed necrosis (N) in β cell of islets of Langerhanz (L), and normal exocrine portion (E) (Figure 17). The pathology of pancreas in diabetic + MSCS group showed mild atrophy of islets of Langerhanz with normal β cells (B) and normal exocrine portion of pancreas (E) (Figure 18).

Histopathological changes of the kidney

Light microscopy of the kidney in control group (-ve) showed normal renal glomeruli (G) and normal renal tubules (Figure 19). The pathology of kidney in MSCs group showed normal renal glomeruli (G) and normal renal tubules (Figure 20). The pathology of kidney in diabetic group showed dissolution, proliferation of mesangial cells in the glomeruli (G), necrosis in renal tubular epithelium (N), hemorrhage in interstitial tissue (H) with hemosiderosis (S) (Figure 21). The pathology of kidney in diabetic + MSCs group showed congestion (C) in glomerular tuft (G), vacuolation (V) in renal tubular epithelium and mild hemorrhage in interstitial tissue (H) (Figure 22).

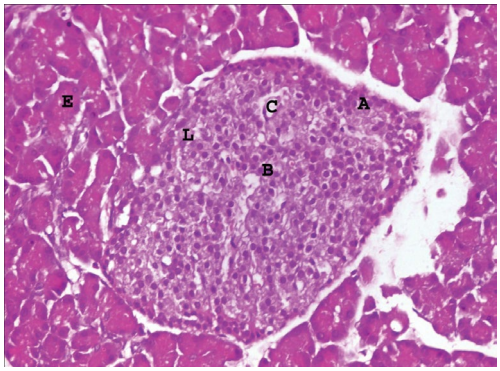

Figure 15: Light microscopy of the pancreas of control group (-ve) showing normal islet of Langerhanz (L) with normal β cell (B) in the centre, α- cell in pancreas (A) and normal capillaries (C) (HE, 400X).

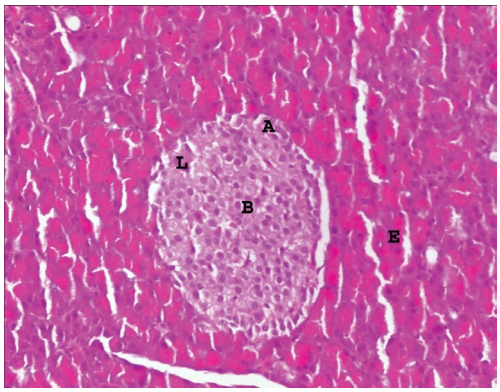

Figure 16: Light microscopy pancreas in MSCs group showed normal islet of Langerhanz (L) with normal β cell (B) in the centre and α- cell in pancreas (A) (HE, 400X).

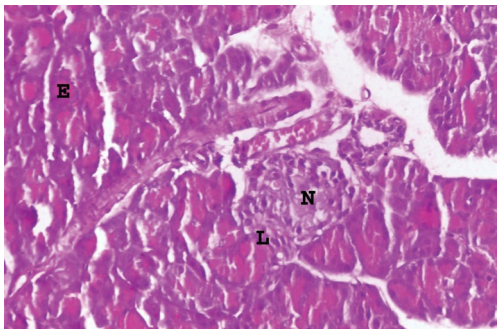

Figure 17: Light microscopy of pancreas in diabetic group (+ve) showed necrosis (N) in β cell of islets of Langerhanz (L), and normal exocrine portion (E) (HE, 400X).

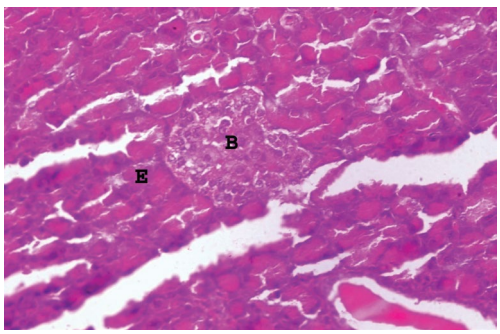

Figure 18: Light microscopy of pancreas in diabetic + MSCs group showed mild atrophy of islets of Langerhanz with normal β cells (B) and normal exocrine portion of pancreas (E) (HE, 400X).

Comparative Studies and Brief Discussion

It is well known that the diabetes mellitus can be characterized as a

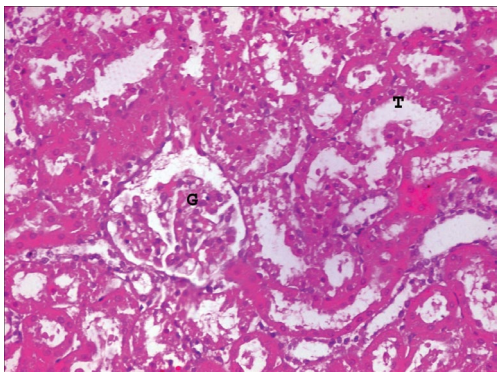

Figure 19: Light microscopy of kidney in control group (-ve) showed normal renal glomeruli (G) and normal renal tubules (HE, 400X).

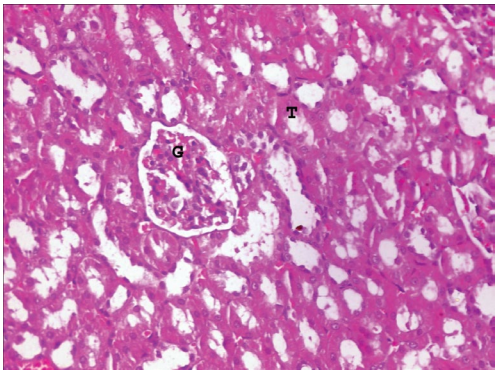

Figure 20: Light microscopy of kidney in MSCs group showed normal renal glomeruli (G) and normal renal tubules (HE, 400X).

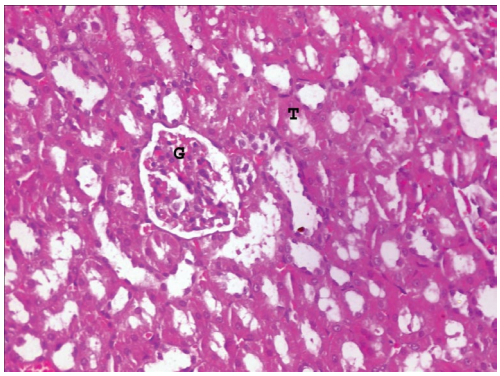

Figure 21: Light microscopy of kidney in diabetic group (+ve) showed dissolution, proliferation of mesangial cells in the glomeruli (G), necrosis in renal tubular epithelium (N), hemorrhage in interstitial tissue (H) with hemosiderosis(S) (HE, 400X).

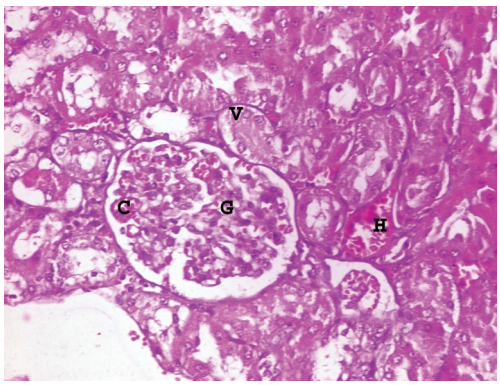

Figure 22: Light microscopy of kidney in diabetic + MSCs group showed congestion (C) in glomerular tuft (G), vacuolation (V) in renal tubular epithelium and mild hemorrhage in interstitial tissue (H).

gathering of metabolic maladies described by perpetual hyperglycemia coming because of imperfections in insulin release, insulin activity or both, bringing about impeded function in lipid, carbohydrate and protein digestion metabolism [31]. In this study, the results showed that rats injected with STZ developed significant hyperglycemia and STZ-treated rats were improved significantly by single infusion of MSCs into STZ-treated group. These results are in agreement with other study [32]. MSCs have been indicated to have direct immunosuppressive properties by repressing the actuation and expansion of effector T cells (both CD4+ and CD8+) through cell-to-cell contact and the elaboration of different solvent variables [33]. Hess et al. utilized bone marrow cells, and they concluded that the transplanted bone marrow cells in all likelihood empowered endogenous pancreatic tissue recovery instead of contributing specifically to beta cell neogenesis [34]. Another study reported that bone marrow derived cells could differentiate into beta-cells *in vivo* and demonstrated that fusion is not a mechanism in beta-cell regeneration [35].

In our study, we observed that there are significant increase of serum triglycerides and cholesterol levels in streptozotocin diabetic animals and it is comparable with what it has been proposed by Wasan et al. [36]. The abnormally high concentration of serum lipids in diabetes mellitus is mainly due to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase [37]. As of HMG Co-A reductase enzyme is responsible for the synthesis of cholesterol and insulin has an inhibitory effect on HMG-Co-A reductase, it is obvious that deficiency of insulin will enhance the generation of cholesterol [38]. The increase in serum LDL-C level may result from nonenzymatic glycosylation of LDL-C and may result in decreased LDL clearance [39]. We observed that the mean value of serum creatinine and uric acid concentrations were significantly increased in diabetic rats and that agreed with some studies reported that there is a positive association between elevated serum uric acid levels and diabetes, which is coincided with the obtained results of Bandaru and Shankar [40]. Galkina and Ley mentioned that macrophage accumulation and activation are associated with prolonged hyperglycemia so activated macrophages produce pro-inflammatory factors such as TNF-α which act as a mediator of inflammatory tissue damage because TNF-α is cytotoxic to renal cells and able to induce direct renal injury [41].

In our study, we observed that the mean value of serum total protein concentration is significantly decreased in diabetic rats compared to control group. These results are agreed with some studies reported that there is a reduction in total protein concentration in diabetic rats, which is very close to what it has been proposed in Changrani et al. who demonstrated that the diminishment in serum total protein due to several changes including a disturbance in the protein rate of catabolism and anabolism, pancreatic atrophy and derangement in gene expression for protein synthesis [42]. It was obtained that the mean serum albumin was decreased significantly in diabetic group

(+ve) comparing to control group (-ve). Furthermore, the obtained new results were in agreement with previous study by Sun et al. [43]. Supporting these data, it was demonstrated that the decrease in serum albumin concentration occurred in chronic inflammatory diseases [44]. Diabetes mellitus is an inflammatory disease due to its increase in serum concentration of several acute-phase markers [45]. While the serum albumin concentration in diabetic rats treated with MSCs was increased slightly comparing to diabetic group (+ve) but still less than that in control group. In another study it was reported that stem cells ameliorate the levels of serum albumin [43].

In the present study, utilizing of STZ which makes pancreas swell and causes degeneration in Langerhans islet beta cells and induces experimental diabetic rats which have significant increase in blood glucose level [46]. Our finding is in agreement with previous studies which included that high glucose impairs glucose-6-phosphate dehydrogenase activity in endothelial and kidney cells and that leads to decreased cell survival [47]. Recognition of high blood level of HBA1C in diabetic rats has been mentioned by Guzik et al. who stated that Glycosylated hemoglobin is a relatively stable ketonamine formed from the condensation of glucose with hemoglobin amino group and that form advanced glycation end products (AGEs). The pathogenicity of AGEs is related to their ability to generate oxygen derived free radicals and further increase of the oxidative stress [48].

On other hand, diabetes mellitus is an inflammatory disease and it does not act directly against cell or organs but through activation of the immune system, especially through monocytes and macrophages with the release of a range of pro-inflammatory such as tumor necrosis factor (TNF) [49]. In present study, there is a significant increase in serum TNF-α in diabetic group compared to control group these results are in conformity with another study [33]. In addition, there is a significant decrease in lymphocyte count and a significant increase in neutrophil count in diabetic group compared to control group. These results are in accordance with Claes et al. who stated that diabetes causes inflammation [50].

Conclusion and Future Outlook

We know that the diabetes mellitus can be characterized as a gathering of metabolic maladies described by perpetual hyperglycemia coming because of imperfections in insulin release, insulin activity or both, bringing about impeded function in lipid, carbohydrate and protein digestion metabolism. This paper has proposed new results revealed that diabetes caused bad effects on the blood picture, pancreas and kidney functions, as well as the immune system represented by TNF. Treatment the diabetic rats by MSCs engrafting improved the tested parameters towards the stats of normal case. Nevertheless, the wide application of the stem cells engrafting still needs more investigations to be assured. They observed that there are significant increase of serum triglycerides and cholesterol levels in streptozotocin diabetic animals and it is comparable with what it has been proposed in literature. In addition, the abnormally high concentration of serum lipids in diabetes mellitus is mainly due to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Furthermore, we conclude that the increase in serum LDL-C level may result from nonenzymatic glycosylation of LDL-C and may result in decreased LDL clearance.

We observed that the mean value of serum creatinine and uric acid concentrations were significantly increased in diabetic rats and that agreed with some studies reported that there is a positive association between elevated serum uric acid levels and diabetes, which is coincided with the obtained results of the published literature; especially, the activated macrophages produce pro-inflammatory factors such as TNF-α which act as a mediator of inflammatory tissue damage because TNF-α is cytotoxic to renal cells and able to induce direct renal injury, which is similar to what it has proposed in published literature. Moreover, the obtained results have the mean value of serum total protein concentration is significantly decreased in diabetic rats compared to control group. These results are agreed with some studies reported that there is a reduction in total protein concentration in diabetic rats, which is very close to what it has been proposed in published literature.

We concluded that the mean serum albumin was decreased significantly in diabetic group (+ve) comparing to control group (-ve). Furthermore, the obtained new results were in agreement with previous study. In addition, the supporting these data, it was demonstrated that the decrease in serum albumin concentration occurred in chronic inflammatory diseases. Furthermore, the diabetes mellitus is an inflammatory disease due to its increase in serum concentration of several acute-phase markers. In conclusion, the consequences of the present work uncovered that rodent bone marrow harbors cells that have the ability to recover the islets of Langerhanz and differentiate into useful insulin-secreting cells fit for controlling hyperglycemia, hyperlipidemia, and diverse adjusted parameters in diabetic rats. This may be useful in the avoidance of diabetic complications.

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