INNOVARE JOURNAL OF SCIENCES

Vol 6, Issue 1, 2018

ISSN - 2321-5496

Research Article

ANTIDIABETIC AND ANTIOXIDANT EFFICACY OF BONE MARROW STEM CELLS ON STZE-INDUCED MALE ALBINO WISTAR RATS

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Received:04 January 2018, Revised and Accepted:20April 2018 May 2017

ABSTRACT

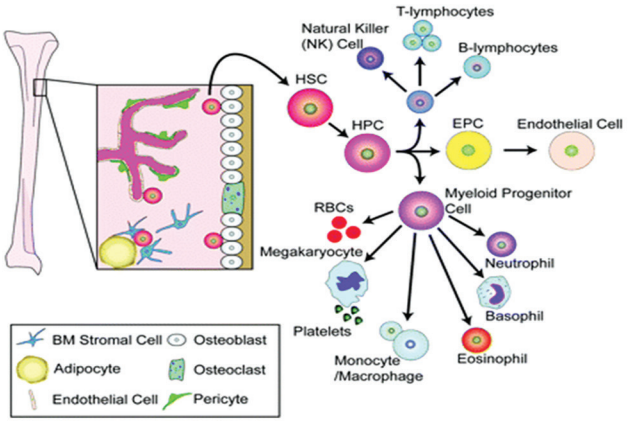
Diabetes mellitus is a multimetabolic disorder that influences more than 348 million people worldwide. A key goal of diabetes treatment is to prevent complications because over time, diabetes can damage the heart, blood vessels, eyes, kidneys, and nerves. Consequently, there is an incredible need to develop new and successful therapies for treating diabetic complications early before it causes irreparable tissue damage. 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. Moreover, BMSCs can differentiate into a wide variety of cell types. Here, we focused on BMSCs can transdifferentiate into insulin-producing cells under defined conditions and normalize the glucose level of streptozotocin (STZ)-induced diabetic rats. The main objective of the study was to evaluate the antidiabetic activity of the dental pulp cells in STZ-induced diabetic Wistar albino rats. To calculate the biochemical estimation of both normal and treated groups. To study the effects of dental pulp cells on the morphological characterization of normal and treated groups. To determine the enzymatic activity and non-enzymatic activity of both normal and treated groups.

Keywords: Streptozotocin, Wistar albino rat, Insulin, Stem cell, Fibroblast.

INTRODUCTION

Diabetes has become one of the most serious threats to global public health, with an estimated worldwide prevalence of 171 million cases among the population [1]. Diabetes mellitus (DM) is a serious and complex chronic condition and the metabolic disorder is characterized by hyperglycemia and disturbance of carbohydrates, proteins, and fat metabolism, secondary to an absolute or relative lack of hormone insulin. DM may be suspected or recognized clinically by the onset of one or more of characteristic symptoms such as polyuria, polydipsia, and polyphagia and weight loss. When the insulin deficiency is extreme, it leads to the development of ketoacidosis. Development of ketoacidosis is the major cause of death in men with diabetes.

In the WHO classification, 2 major types of diabetes; type 1 (insulin dependent) and type 2 (non-insulin dependent). In type 1 diabetes, the body's immune system attacks its own cells and destroys them. As a result, the pancreatic islet cells, which normally produce insulin, are destroyed so glucose cannot enter cells and remains in the blood. Type 1 diabetes is the most common and the type on which most current research is being carried out. Type 2 diabetes is on the increase in obese humans. There is no enough data to confirm if overweight humans have overweight pets, but certainly there is a problem as specialist obesity referral clinics for animals are becoming increasingly common. Type 2 diabetes occurs when the body cannot use insulin effectively but can often be controlled with a combination of diet, exercise, and oral medication to stabilize blood glucose concentrations; however, the disease may progress to the point at which only insulin therapy controls blood glucose levels. According to the development stage, stem cell can be dividing into embryonic stem cell and adult stem cell. During embryonic development in mammals begins with series of cleavage division to generate a population of equivalent blastomeres. An adult stem cell is an undifferentiated cell found among differentiated cells in a tissue can renew itself and can differentiate to yield the major specialized cell types of the tissue.



Adult stem cells of the bone marrow

Adult stem cells comprise of roughly three different groups: The bone marrow-derived mesenchymal stem cells (BMSCs), the circulating pool of stem or progenitor cells (which are also derived from the BM), and the tissue-resident stem cells. BMSC can be further categorized into multipotent adult progenitor 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 (EPCs). EPCs were identified by the authors Calcutt *et al.* [2], the search for circulating angiogenic cells. They observed that these cells were able to form new blood vessels and promote neovascularization after ischemia. Therefore, these cells seem to be the most promising in the setting of DM because of their potential utility in therapeutic neovascularization and vascular repair. This paper will be focused on BMSC, since this BMSC is the most studied in the field of the cell-based therapies for DM and for diabetic complications. BMSCs are multipotent stromal cells which can provide a potential therapy for DM, but the mechanism is still controversial [3]. 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 MSCs since they have the capacity of proliferation and differentiation into the mesenchymal lineage. MSCs are capable of becoming one of a number of phenotypes, including muscle, bone, cartilage, tendon, fat, and marrow stromal connective tissue.

Objective of the study

The objective of the study was to evaluate the antidiabetic activity of the dental pulp cells in streptozotocin (STZ)-induced diabetic Wistar albino rats, to calculate the biochemical estimation of both normal and treated groups, to study the effects of dental pulp cells on the morphological characterization of normal and treated groups, and to determine the enzymatic activity and non-enzymatic activity of both normal and treated groups.

METHODS

Stem cell

Human BMSCs isolated, cultured, and identified in Mother Cell Regenerative Centre, Trichy, was used for the entire studies.

Experimental model

Healthy adult male Wistar albino rats weighing 150–220 g were chosen as an experimental animal. They were purchased from TANUVAS, Madhavaram, Chennai, Tamil Nadu, India, were used for this study and they were maintained under laboratory conditions of temperature with 12 h day and 12 h night cycle in different cages and feed up with standard pellet diet and water *ad libitum* (Sai Enterprises). The animals used for the experiment were approved by the Animal Ethical Committee and by the regulatory body of the government (Reg.No.07/2013).

Induction of diabetes

Freshly prepared STZ at the dosage of 60 mg/kg body weight was injected intraperitoneally to the rats. STZ was dissolved in 0.1M sodium citrate buffer at the pH 4.5. The normal control group received only the citrate buffer alone. Then, the states of diabetes were confirmed by measuring the blood glucose at regular intervals. After a week, rats with diabetes (with blood glucose levels of above 250 mg/dl) were chosen for the experiment.

Experimental design

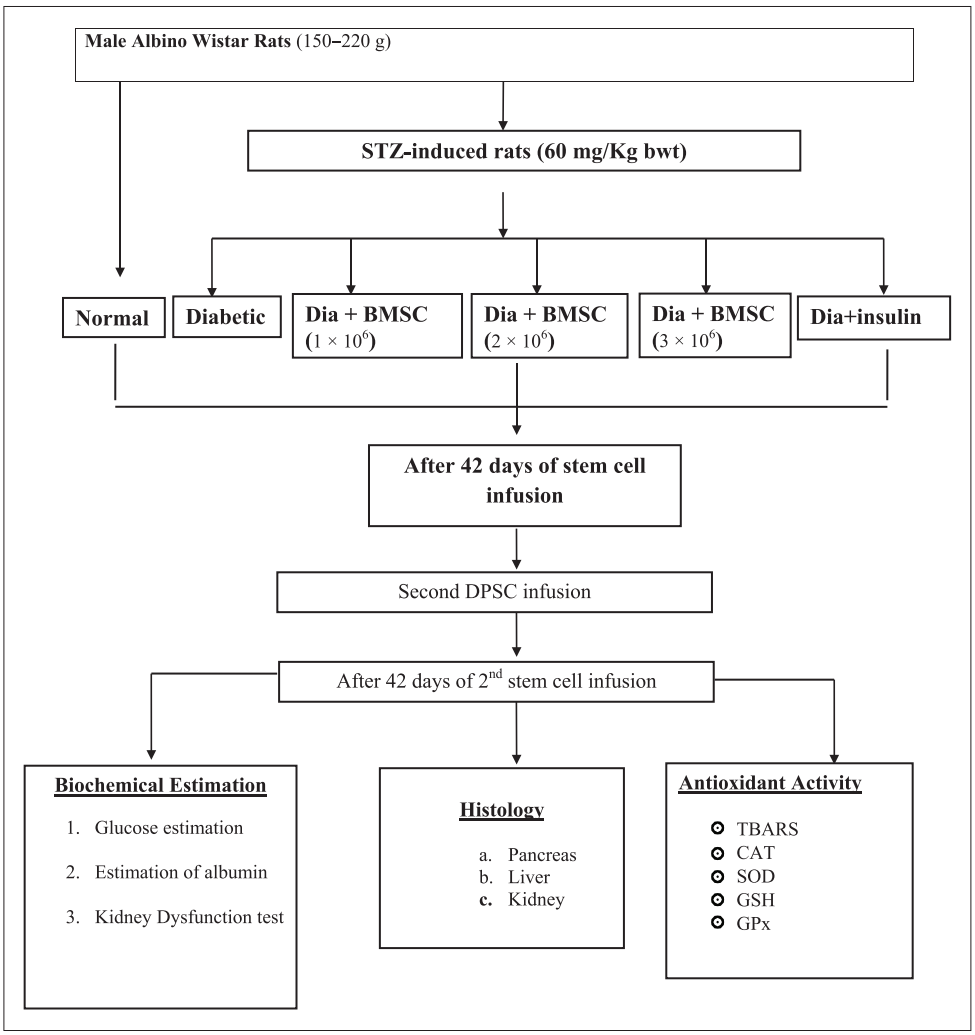
The rats were divided into four groups.

Group I: Normal rats (0.1% carboxymethyl cellulose) orally.

Group II: Diabetic control rats (rats injected with STZ at a dose of 60 mg/kg bwt).

Group III: Diabetic rats treated with BM cells at different concentration (1×10^6, 2×10^6, 3×10^6, respectively).

Group IV: Diabetic with insulin-injected rats (rats injected with insulin at a dose of 3 IU/Kg bwt). BMSC was infused in rats at different doses of stem cells (1×10^6, 2×10^6, 3×10^6) in 0.2 mL of physiological saline through the tail vein at 7 (early phase) days after STZ injection. The BMSCs-treated rats at 7 days were then infused with the same dose of



Flowchart 1:

BMSCs at 42-day post-STZ administration. The untreated control rats were infused with 0.2 mL physiological saline (Flowchart 1).

Biochemical estimations

Estimation of glucose (Diagnostic Kit–Reddy's Laboratories, Bachupally, Hyderabad, India).

Principle

Glucose was oxidized by the enzyme glucose oxidase to give D-gluconic acid and hydrogen peroxide. Hydrogen peroxide, in the presence of the enzyme peroxidase oxidized phenol, combine with 4-aminoantipyrine to produce a pink-colored quinone imine dye. The intensity of the color produced was proportional to glucose concentration in the sample.

D–glucose + H_2O+O_2 Glucose oxidase D-gluconic acid + H_2O_2

H_2O_2 + 4–Aminoantipyrine + Phenol Peroxidase Quinoneimine dye + Phenol + H_2O

Reagents

1. Glucose reagent
2. Standard (100 mg/dL)

Procedure

For the estimation of glucose, 10 μL each of serum and working standard were incubated with 1 ml of the reagent for 15 min at 37°C and the absorbance at 505 nm was measured against a reagent blank. For reagent blank, 10 μL of distilled water was added to 1 ml of the reagent. The concentration of glucose in serum samples was calculated as:

$$\frac{\text{Absorbance of the sample}}{\text{Absorbance of the standard}} \times 100 = {}^{\text{mg}}\!/_{\text{dl}}$$

Estimation of albumin

Principle

Albumin in the serum was estimated by Biuret method. Proteins from a purple-colored complex with cupric ions in alkaline solutions. The reactions take its name from the simple compound biuret which reacts in the same way. The intensity of the purple color is proportional to the amount of protein present in the sample.

Reagent

(i) Stock solution: 45 g of sodium potassium tartrate was dissolved in 400 ml of 0.2N sodium hydroxide and 15 g of copper sulfate was added and stirred. 5 g of potassium iodide was then added, dissolved, and made up to 1.0 L with 0.2N sodium hydroxide.
(ii) Dilution biuret reagent: 200 mL of stock biuret reagent was diluted to 1 L with 0.2N sodium hydroxide containing 5.0 g potassium iodide/L.
(iii) Standard egg albumin: 500 mg/100 mL distilled water (small quantity of alkali was added to dissolve albumin).
(iv) Sodium sulphite solution: 28%

Procedure

0.5 mL of serum was taken in a test tube and 6 mL of sodium sulfite solution was added and mixed. To mixture, 3.0 mL of ether was added, stoppered, shaken well for 20s, and then centrifuged for 5 min. 3.0 mL of the clear supernatant was taken for the estimation of albumin and treated with 5 mL of biuret reagent simultaneously, 2.0 mL of standard egg albumin were mixed with 1.0 mL of H_2O and treated with 5.0 mL of biuret reagent. The purple color developed was read at 540 nm after 15 min using reagent blank. Values were expressed as g/dl of serum.

Kidney function tests

Estimation of protein [4]

Principle

The aromatic amino acids such as tyrosine and tryptophan present in protein reacted with Folin–Ciocalteu reagent to give a dark blue color. The intensity of the color obtained was directly proportional to the amount of protein present in the sample.

Reagents

1. 5% trichloroacetic acid.
2. Reagent A – 2 g of sodium carbonate in 100 ml of 0.1N sodium hydroxide.
3. Reagent B – 1 g of copper sulfate in 100 ml of distilled water.
4. Reagent C – 2 g of sodium potassium tartrate in 100 ml of distilled water.
5. Reagent D – Reagents B and C mixed in 1:1 ratio.
6. Reagent mixture – Reagents A and D in 50:1 ratio.
7. Folin–Ciocalteu's reagent – Folin phenol reagent diluted 1:1 with distilled water.

Procedure

0.5 mL of the serum was mixed with 1 mL of 5% trichloroacetic acid and centrifuged to precipitate the protein. The precipitate was dissolved in 1N sodium hydroxide and made up to 10 mL. To 1 mL of the sample, 5 mL of reagent D was added, and after 10 min incubation, 0.5 mL of Folin–Ciocalteu's reagent was added and mixed. After 30 min, the intensity of the blue color was read at 620 nm against a reagent blank. Protein content of serum sample was determined from a standard curve. Standard curve was prepared using bovine serum albumin prepared at a stock concentration of 1 mg/ml and diluted to obtain serial dilutions at 50, 100, 150, 200, and 250 μg/ml.

Estimation of plasma insulin

Radioimmunoassay kit – Diasorin, Italy.

Principle

The principle of the assay was based on the competition between labeled insulin and insulin contained in standards or specimens to be assayed for a fixed and limited number of antibody binding sites. After the incubation, the amount of labeled insulin bound to the antibody was inversely related to the amount of unlabeled insulin present in the sample. The method adopted for separation was based on the use of a precipitating reagent, in which a second antibody is pre-precipitated and in excess.

Reagents

- 125 I-labeled insulin.
- Insulin standards.
- Insulin antiserum.
- Precipitating reagent.
- Control sample.

Procedure

- Standards: 100 μl of the standard from the respective standard bottles (0–5) was pipetted out into different vials and 100 μl of tracer and 100 μl of antiserum were added to it.
- Sample: 100 μl of plasma sample was pipetted out into a vial, 100 μl of tracer and 100 μl of antiserum were added to it.
- Total activity: To measure the total activity, 100 μl of tracer was pipetted out into a vial.

The contents of the above vials were mixed with a vortex and incubated for 1.5 h at room temperature. The bottle of precipitating reagent was allowed to reach the room temperature and mixed well by repeated tilting. 1 mL of precipitating reagent was dispensed into all vials (except total activity vial). The contents of all the vials were

again vortexed and the vials were allowed to stand for 15 min at room temperature. The vials were centrifuged at 1500 rpm for 15 min. The supernatant was discarded. The radioactivity of the precipitate was measured.

The mean net counts for each group of tubes were computed.

The binding ability was evaluated as follows:

$$(B/T)\ 0\% = \frac{\text{Zero standard mean counts}}{\text{Total activity mean counts}} \times 100$$

The mean counts for each standard and unknown sample was expressed as a percentage of zero standard mean counts.

$$B/B_0\% = \frac{\text{Standardorsample mean counts}}{\text{Zero standard mean counts}} \times 100$$

The percent values of each standard versus the insulin amount expressed as μU/mL was plotted in linear-linear or semi-log coordinates to obtain a calibration curve. By interpolation of the calibration curve, the insulin level of the samples was obtained.

Estimation of plasma C-peptide

Radioimmunoassay kit – Missouri, USA.

Principle

The principle of the assay was based on the competition between a fixed concentration of labeled tracer antigen and constant dilution of antiserum such that the concentration of antigen binding sites on the antibody is limited. Thus, the amount of tracer bound to antibody will decrease as the concentration of unlabeled antigen increases. This can be measured after separating antibody bound from free tracer and counting one or other or both fractions.

Reagents

- Assay buffer.
- Rat C-peptide antibody.
- 125 I – Rat C-peptide standards.
- Labeled hydrating buffer.
- Rat C-peptide standards.
- Control.
- Precipitating reagent.

Procedure

300 μl of assay buffer was pipetted out to the non-specific binding tubes. 200 μl of assay buffer was pipetted out in the reference tube. 100 μl assay buffer pipetted out to all the other tubes (sample). These tubes are vortexed and incubated for 20–24 h at 4°C. After 24 h of incubation, 100 μl of 125I – Rat C-peptide tracer pipetted out to all the tubes. These tubes are vortexed and incubated for 22–24 h at 4°C. 1 mL of cold precipitating reagent is added to all the tubes except total count tubes. All the tubes are vortexed and incubated for 20 min at 4°C. Tubes are centrifuged at 4°C for 20 min at 2000–3000× *g*. Supernatant was decanted from all centrifuged tubes except total count tubes, and all the tubes were counted in a gamma counter for 1 min.

The percentage of tracer bound,

$$\frac{\text{Total binding counts}}{\text{Total counts}} \times 100$$

The percentage of total binding (%B/B0) for each standard and sample,

$$\%B/B0 = \frac{\text{Sample or standard}}{\text{Total binding}} \times 100$$

Histological studies

Light microscopic studies–Paraffin method [5]

Following solutions were used:

- Physiological saline (0.9%)
- Bouin–Hollande fixative
- Ehrlich's hematoxylin
- Eosin procedure.

The pancreas, liver, and kidney from untreated and parallel experimental groups were blotted free of mucus, washed in physiological saline, cut into pieces of desired size, and fixed in Bouin–Hollande fixative for 72 h. After fixation, the tissues were washed in 70% alcohol for 2 or 3 days to remove the excess picric acid and dehydrated in graded series of alcohol. The tissues were cleared using xylene. The cleared tissues were infiltrated with molten paraffin at 58–60 through three changes (20–30 min each) and finally embedded in paraffin. 3–5 μm thick sections of all the tissues were obtained using a rotary microtome (Leica, Germany) and stained in Ehrlich's hematoxylin with eosin as the counterstain. The slides were mounted using DPX mountant.

Enzyme activity

Estimation of thiobarbituric acid reactive substances (TBARS)

Reagent

1. TCAL: 15%
2. HCL: 0.025N
3. TBA: 0.375% in hot distilled water
4. TBA – TCA – HCL reagent: Solutions 1, 2, and 3 mixed in the ratio of 1:1:1 were freshly prepared before use.
5. Stock standard: 4:8 molar solution of the stock was prepared from 1,1,3,3 tetramethoxypropane.
6. Working standard: Stock solution was diluted to get a concentration of 48 nmol/ml.

Procedure

- 0.5 mL of homogenate was diluted to with to 0.5 mL with double-distilled water and mixed well, and then 2.0 mL of TBA – TCA – HCL reagent was added.
- The mixture was kept in boiling water bath for 15 min, after cooling, the tubes were centrifuged at 1000 g for 10 min and the supernatant was estimated.
- Series of standard solution in the concentration of 2–10 mol was treated in a similar manner.
- The absorbance of the chromophore was read at 535 nm against reagent blank.
- The valves were expressed as mmol/100 g of tissue.

Reduced of glutathione(GSH)

1. Phosphate buffer: 0.1M pH 8.0.
2. TCA 50%.
3. Elman's reagent: 34 mg of DTNB in control of 0.1% sodium citrate.
4. Disodium hydrogen phosphate: 0.3M.
5. Standard glutathione solution: 100 mg GSH in 100 ml H_2O.
6. Working standard: Stock was diluted to get a concentration of 100 mg/ml.

Procedure

- Tissue was homogenized used in PO_4 buffer (0.1 m pH 7.0).
- 0.5 mL of homogenate (or) plasma was pipette out and precipitated with 2.0 mL of 5% TCA.
- 2.0 mL of supernatant was taken after centrifugation and 1.0 mL of Elman's reagent in 4.0 mL of 0.3M disodium hydrogen phosphate were added.
- The yellow color developed was read in a spectronic 20 at 412 nm.
- A series of standard (20–100 mg) was reacted in a similar manner along with a blank containing 1.0 ml of buffer.
- The amount of glutathione was expressed as μ/mol of tissue.

Non-enzymatic activity

Superoxide dismutase (SOD)

1. Sodium pyrophosphate buffer: 0.025 M, PH 8.3
2. Absolute ethanol
3. Chloroform and n–butanol phenazine methosulfate: (186 µl/mol)
4. Nitroblue tetrazolium (300 µl/mol)
5. Nicotinamide adenine dinucleotide (NADH): 780 µl mol

Procedure

- Tissue was homogenized using sodium pyropo4 buffer.
- 0.5 mL of tissue homogenate (or) 0.5 mL of serum was diluted to 1.0 mL with water followed by addition of 2.5 mL of ethanol and 1.5 mL of chloroform. This mixture was taken for incubation 90 min at 4°C and then centrifuged.
- The enzyme activity in the supernatant was determined. The assay mixture contained 1.2 mL of sodium pyropo 4 buffer.
- 0.1 mL of phenazine methosulfate and 0.3 mL of nitroblue tetrazolium of appropriately diluted enzyme preparation in a total volume of 3 ml the reaction of 0.2 mL NADH.
- After incubation at 30°C for 90°C s, the reaction was stopped by the addition of 1 mL glacial acid. The reaction mixture was stirred vigorously and shaken with 4ml n-butanol layer was separated.
- The color density of the chromofenin n-butanol was measured in a spectrophotometer at 520 nm.
- The enzyme concentration required to inhibit the chromogen produced by 50% in one minute under standard condition was taken as one unit.
- The specific activity of the enzyme was expressed as unit/min/mg of tissue.

Catalase (CAT) activity

1. Phosphate buffer: 0.01M, PH 7.0
2. Hydrogen peroxide: 0.2M
3. Potassium dichromate: 5%
4. Dichromate – acetic acid reagent
5. 1:3 ratio of potassium dichromate was mixed with glacial acetic acid. From this, 1ml was diluted again with 4 ml of acetic acid.
6. Standard hydrogen peroxide: 0.2 mM.

Procedure

- Tissue homogenate was prepared using PO_4 buffer (0.01M, PH 7.0).
- To 0.9 mL of PO_4 buffer, 0.1 ml tissue homogenate (or) 0.1 mL of serum of 0.4 mL of hydrogen peroxide were added.
- The reaction was arrested after 30 s interval by adding 2.0 mL of dichromate – acetic acid mixture.
- The tubes were kept in a boiling H_2O both for 10 min, cooled and color developed was read at 620 nm.
- Standard in the concentration range of 20–100 µ/M were taken and processed as for the test.
- The specific activity was expressed as µ mol of H_2O_2 consumed/min/mg of tissues.

Glutathione peroxidase (GPx)

1. Tris buffer: 0.4M PH 7.0
2. Sodium azide solution: 10 mM
3. TCA: 10%
4. EDTA: 0.4 Mm
5. H_2O_2 solution: 0.2 Mm
6. Glutathione solution: 2 Mm

Procedure

- The tissue was homogenized using Tris buffer to 0.2 mL of tris buffer.
- 0.2 mL of EDTA 0.1 mL of sodium azide 0.5 mL of tissue homogenate was added.
- To the mixture, 0.2 mL of GSH followed by 0.1 ml of H_2O_2 was added
- The contents were mixed well and incubation at 37°C for 10 min, along with a control containing all reagent except homogenate.
- After 10 min, the reaction was arrested by the addition of 0.5 mL of 10% TCA.
- The tubes were centrifuged and the supernatant was assayed for GSH by the method of Ellman's.
- The activity was expressed as the of HSH utilized/min/mg of tissues.

Statistical analysis

All data were expressed as means±standard deviation for control and experimental groups. The data were analyzed using one-way analysis of variance on Statistical Package for the Social Sciences (Version 17.0) and

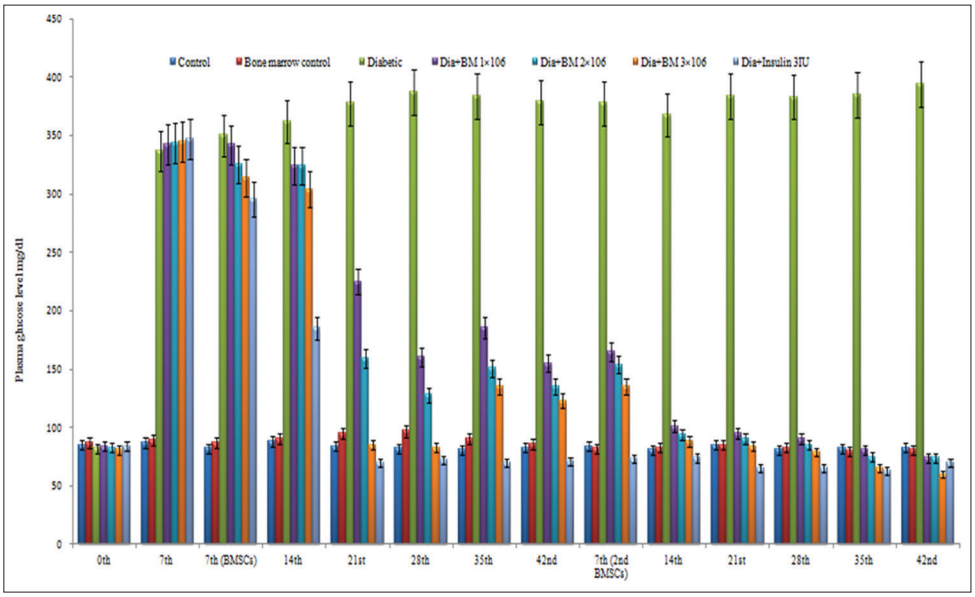


Fig. 1: Effect of bone marrow stem cells on plasma glucose levels in normal and streptozotocin-induced diabetic male Wistar rats. Each value is mean±standard deviation for 6 rats in each group. Significance at: p<0.05. Values not sharing a common superscript differ significantly at p<0.05, Duncan's multiple range test

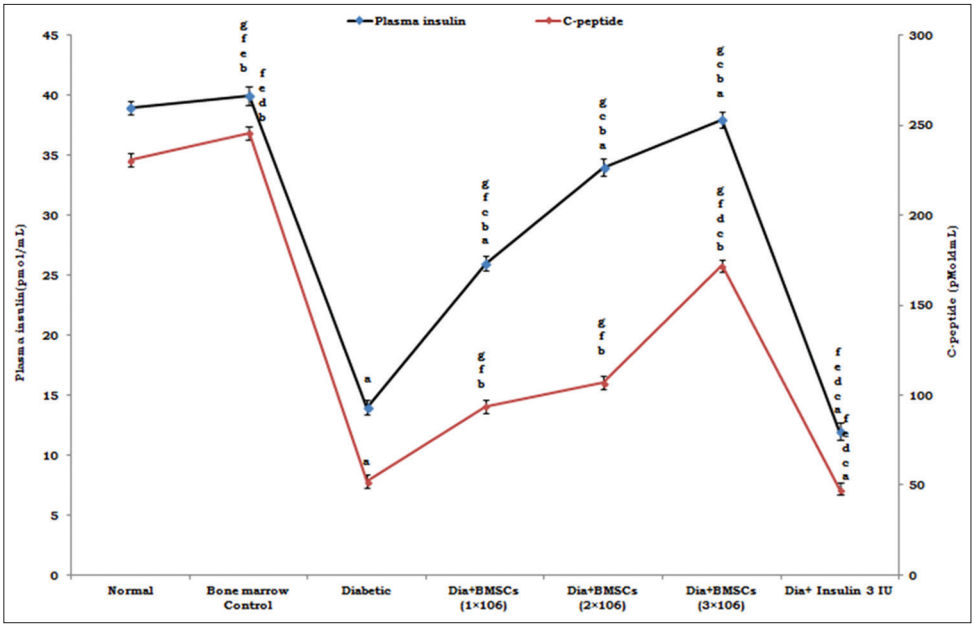


Fig. 2: Effect of bone marrow stem cells on plasma insulin and C-peptide levels in normal and streptozotocin-induced diabetic male Wistar rats. Each value is mean±standard deviation for 6 rats in each group. Significance at: p<0.05. Values not sharing a common superscript differ significantly at p<0.05, Duncan's multiple range test

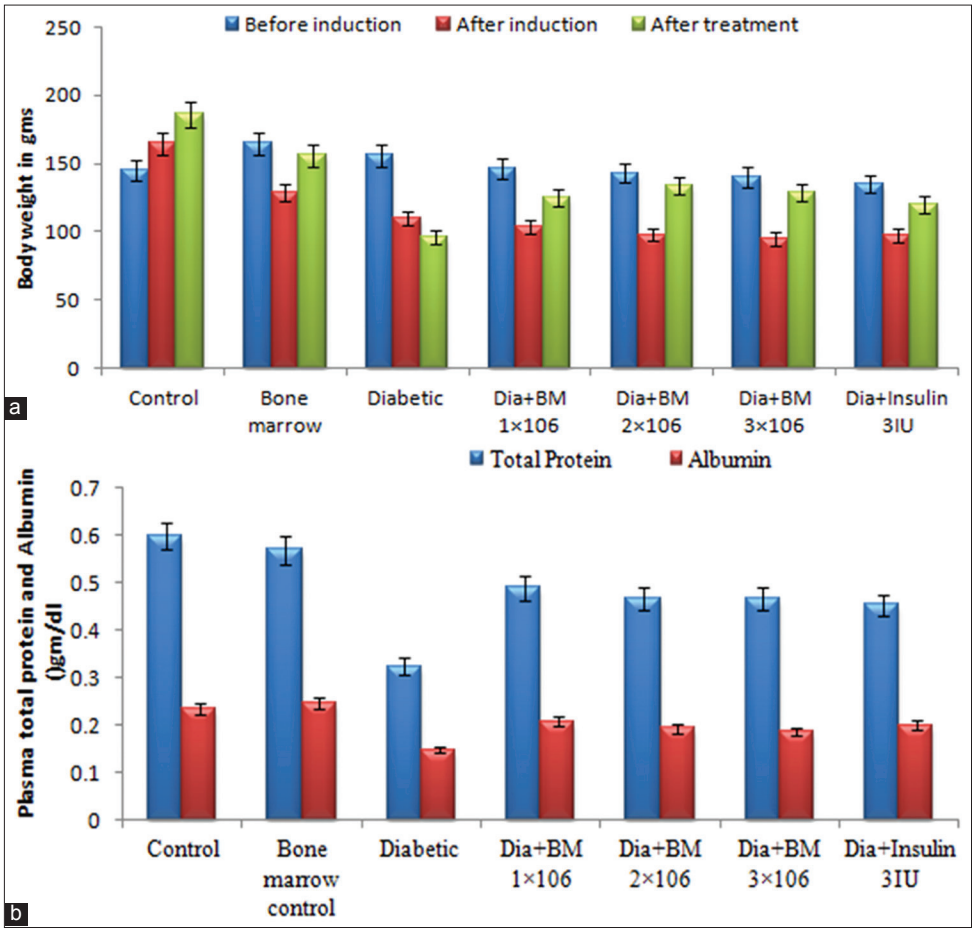


Fig. 3 (a) Effect of bone marrow stem cells on body weight levels in normal and streptozotocin (STZ)-induced diabetic male Wistar rats. (b) Effect of bone marrow stem cells on plasma total protein and albumin levels in normal and STZ-induced diabetic male Wistar rats. Each value is mean±standard deviation for 6 rats in each group. Significance at: p<0.05. Values not sharing a common superscript differ significantly at p<0.05, Duncan's multiple range test

the group means were compared by Duncan's multiple range test [6]. The results were considered statistically significant if the calculated $p<0.05$.

RESULTS

Biochemical estimations

Blood glucose

Blood glucose levels in the normal untreated rats did not show any significant variation throughout the experimental period. Administration of STZ (60 mg/kg) led to an elevated blood glucose level, almost 5-fold increase than the normal untreated group. Blood glucose levels measured in normal and other experimental group rats at the end of 7, 14, 21, 28, 35, and 42 days of 1st and 2nd BMSC infusion is given in Fig. 1. The decrease in blood glucose in the BMSC-treated groups could be observed from the 21st day onwards, registering a significant decrease on the 35th day, while a near normal level comparable to the BMSCs-treated and untreated were observed after the 21st day of BMSC treatment of 2nd infusion. BMSC treatment for 84 days made a significant reduction in the blood glucose levels.

In the STZ diabetic rats, the level rose sharply. In case of diabetic rats treated with the BMSCs, a significant decrease in the blood glucose level was observed with promising outcome.

Insulin and C-peptide levels

Fig. 2 depicts the insulin and C-peptide levels of normal, diabetic, and BMSC-treated groups. The decreased level of plasma insulin and C-peptide was recorded in diabetic rats. The insulin (reference drug) treatment did not restore the plasma insulin and C-peptide. Other treatment groups after the treatment with BMSC for 84 days had significantly increased the level of C-peptide which was near normal level, but the increase in insulin level did not match the normal. There was a concentration-dependent normalization of insulin and C-peptide and 3×10^6 was found to be the effective.

Body weight

Fig. 3 show the body weight of the rats in all groups which were taken in the beginning and at the end of the experiment. No significant change was observed in the normal untreated rats. A drastic reduction in the body weight was recorded in STZ-induced diabetic group at the end of 84 days. A gradual restoration could be seen in the BMSC-treated rats and was equal to normal untreated rats at the end of the treatment. Similar trend was seen in insulin-treated rats.

Histological studies

Hematoxylin and eosin-stained sections

Hematoxylin and eosin-stained sections of the pancreas, kidney, and liver of untreated rats revealed that each islet of Langerhans is formed of numerous compactly arranged cells occurring as dense cords (Fig.4a-f). The islets appeared lightly stained when compared with the surrounding acinar tissue. The islet cells were round to ovoid with round vesicular nuclei and pale pink cytoplasm. Capillaries were found in between the islet cells. Islets from the pancreas of diabetic control rats showed an entirely different picture in hematoxylin and eosin-stained sections. Most of the cells in the islets possessed pyknotic nuclei, whereas some cells contained dark nuclei. Islets from the pancreas of insulin-treated rats showed similar picture of diabetic control in hematoxylin and eosin-stained sections. Hematoxylin and eosin-stained section of the pancreas of diabetic rats treated with the BMSC showed several islet cells to contain vesicular nuclei. Restoration of normal islet cells was noticed with ovoid nuclei.

The tissue architecture of kidney was found to be normal in the control rats with normal glomeruli and capsule (Fig. 5a-f). In diabetic rats, both the capsule and glomeruli were found to be disrupted, whereas such

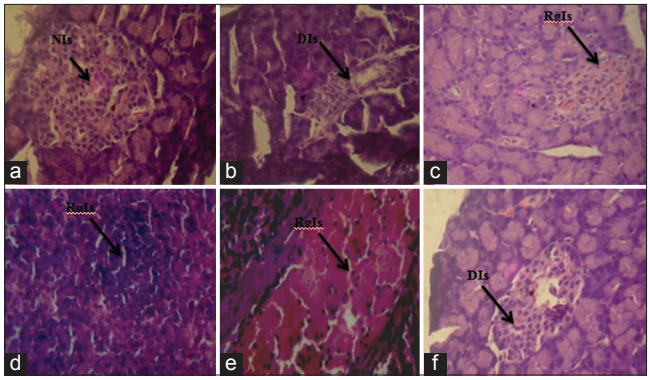


Fig. 4: Effect of bone marrow-derived mesenchymal stem cells (BMSCs) on pancreas of the streptozotocin-induced male albino Wistar rats.(a) Photograph of islet of normal rat (200×) (I - Islets of Langerhans). (b) Islets of Langerhans of diabetic control. Cellular boundary has been disrupted in islet cells (×200).(c): Islets of BMSCs (1×10^6)-treated diabetic rats (200×) (I - Islets of Langerhans).(d) Islets of BMSCs (2×10^6)-treated diabetic rats (200×) (I - Islets of Langerhans). (e) Islets of BMSCs (3×10^6)-treated diabetic rats (200×) (I - Islets of Langerhans).(f) Islets of insulin-treated diabetic rats (×200)

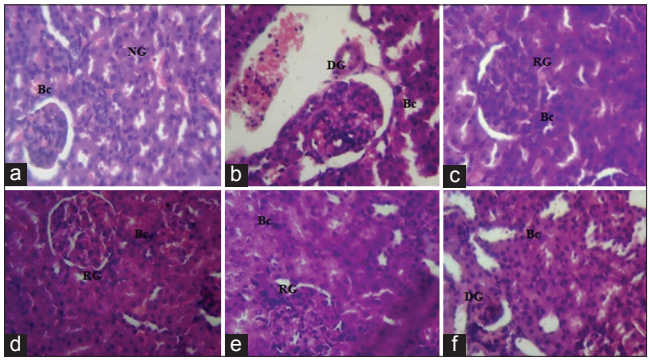


Fig. 5: Effect of bone marrow-derived mesenchymal stem cells (BMSCs) on kidney of the streptozotocin-induced male albino Wistar rats. (a) Photograph of kidney of normal rat. (200×) (Bc - Bowman's capsule, G: Glomeruli). (b) Paraffin section of kidney of diabetic control. Destruction of the capsule (200×). (c) Kidney section of BMSCs (1×10^6)-treated diabetic rats (200×) (Bc - Bowman's capsule, G: Glomeruli). (d) Kidney section of BMSCs (2×10^6)-treated diabetic rats (200×) (Bc - Bowman's capsule, G - Glomeruli). (e) Kidney section of BMSCs (3×10^6)- treated diabetic rats (200×) (Bc - Bowman's capsule, G - Glomeruli).(f) Paraffin section of insulin-treated diabetic rat kidney (200×) (Bc - Bowman's capsule, G - Glomeruli)

condition was not found in the BMSC-treated diabetic rats. Insulin-treated rats had kidney architecture similar to normal.

The liver tissue of the normal control rats was found distributed with hepatocytes, whereas in diabetic rats, the cells were found to be disrupted (Fig. 6a-f). On BMSC treatment, the hepatocytes were found to be as in the normal. Insulin-treated diabetic rats also possessed normal liver histology.

Antioxidant analysis

Effect of BMSCs on lipid peroxidation

Effect of BMSC on lipid peroxidation in STZ-induced rats determined is depicted in Fig. 7, respectively. An increased level of TBARS was

estimated in liver, pancreas, kidney, lungs, and heart tissues. BMSC treatment showed remarkably low levels of TBARS in STZ-induced animals which was identified to be similar to normal.

Effect of BMSCs on enzymatic antioxidants

Variations in the activities of enzymatic antioxidants SOD, CAT, and GPx in the liver, pancreas, kidney, lungs, and heart tissues of the experimental groups with and without BMSC treatment estimated as determined are presented in Figs. 8-10. Significant reduction in the activity of all the enzymatic antioxidants was observed in diabetic groups when compared to normal. Besides, a significant increase in the activity of antioxidant enzymes was noted in BMSC-treated diabetic groups. On contrary, increase in the activity of these antioxidant enzymes was identified in BMSC-treated STZ-induced rats and was restored near to normal, whereas STZ-induced rats had reduction in these enzymes.

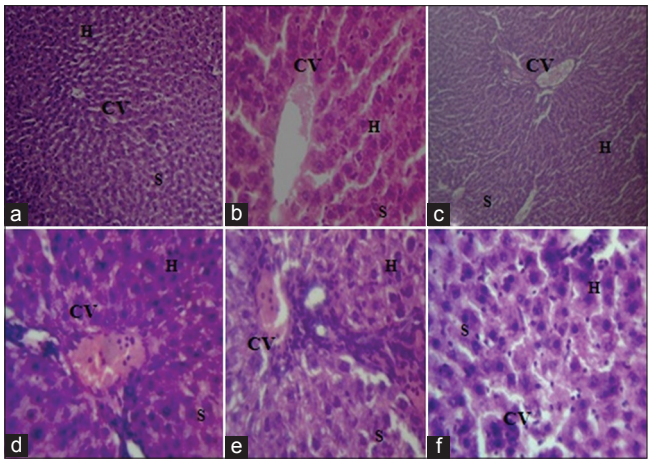


Fig. 6: Effect of bone marrow-derived mesenchymal stem cells (BMSCs) on liver of the streptozotocin-induced male albino Wistar rats. (a) Photomicrograph of liver of normal Wistar rat (200×). (b) Paraffin section of liver of diabetic control. Destruction of liver cells (200×) (H - hepatocytes). (c) Liver section of BMSCs (1×10^6)-treated diabetic rats (200×) (H - hepatocytes) (d) Liver section of BMSCs (2×10^6)-treated diabetic rats (200×) (H - hepatocytes). (e) Liver section of BMSCs (3×10^6)-treated diabetic rats (200×) (H - hepatocytes). (f) Paraffin section of insulin-treated diabetic rat liver (200×) (H - hepatocytes)

Effect of BMSCs on reduced glutathione

The non-enzymatic antioxidant GSH levels in liver, pancreas, kidney, lungs, and heart tissues are depicted in Fig. 11. Normal levels of GSH were observed in the normal control rats and the GSH levels in the STZ-induced diabetic rats were found have a marked decrease in their level. BMSC treatment increased the levels of GSH to near normal. Insulin-treated rats also maintained a normal level of GSH.

DISCUSSION

Diabetes has become one of the most serious threats to global public health, with an estimated worldwide prevalence of 171 million cases among the population [1]. DM is a serious and complex chronic condition and the metabolic disorder is characterized by hyperglycemia and disturbance of carbohydrates, proteins, and fat metabolism, secondary to an absolute or relative lack of hormone insulin. DM may be suspected or recognized clinically by the onset of one or more of characteristic symptoms such as polyuria, polydipsia, and polyphagia and weight loss. When the insulin deficiency is extreme, it leads to the development of ketoacidosis.

The main challenge for successful stem cell therapy to treat T1DM lies in producing functional β-cells and overcoming the autoimmune response. The β-cell mass and function could be preserved and/or restored in at least three different ways: replacing damaged β-cells by direct stem cell differentiation, modifying the pancreatic microenvironment allowing endogenous β-cell regeneration, and abrogating the autoimmune response to β-cells. Multipotent mesenchymal stromal cells (also referred to as MSCs), a heterogeneous adult stem cell population, seems to represent an ideal tool since they can be easily isolated from bone marrow and other mesenchymal tissues, such as adipose tissue, dental pulp, placenta, Wharton's jelly, and umbilical cord. MSCs are hypoimmunogenic, allowing allogeneic transplant without histocompatibility or recipient conditioning being required.

In the present study, we observed the reduced rate of weight loss and increased the level of glucose in STZ-induced diabetic rat and also decrease the level of food and water intake. After the injection of

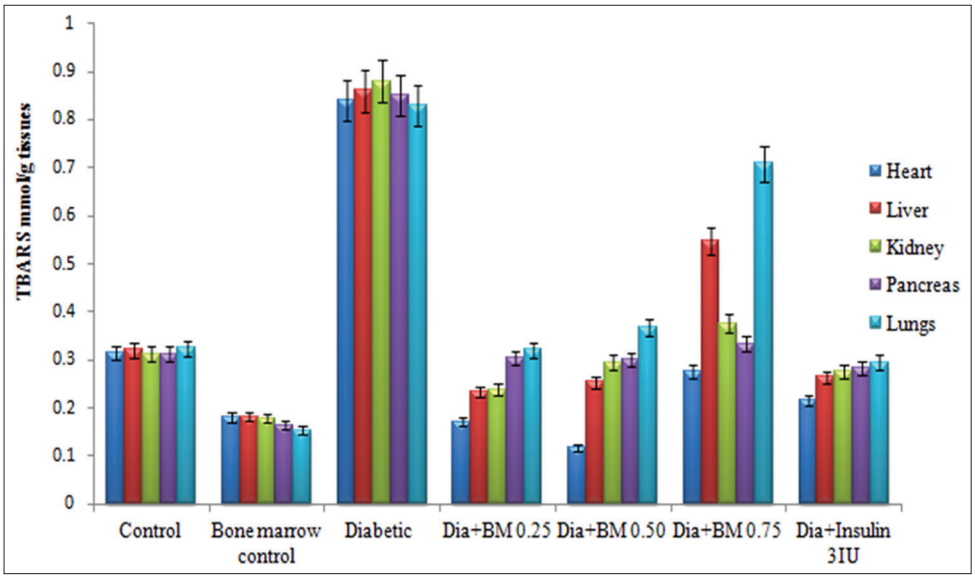


Fig. 7: Effect of bone marrow stem cells on tissue lipid peroxidation in normal and streptozotocin-induced diabetic male Wistar rats. Each value is mean±standard deviation for 6 rats in each group. Significance at: $p<0.05$. Values not sharing a common superscript differ significantly at $p<0.05$, Duncan's multiple range test

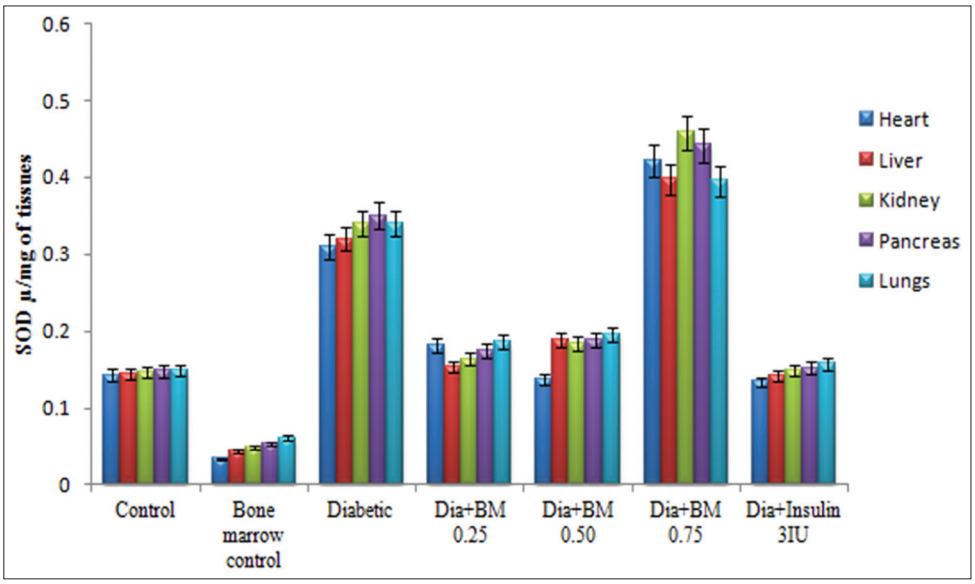


Fig. 8: Effect of bone marrow stem cells on tissue Superoxide dismutase in normal and streptozotocin-induced diabetic male Wistar rats. Each value is mean±standard deviation for 6 rats in each group. Significance at: p<0.05. Values not sharing a common superscript differ significantly at p<0.05, Duncan's multiple range test

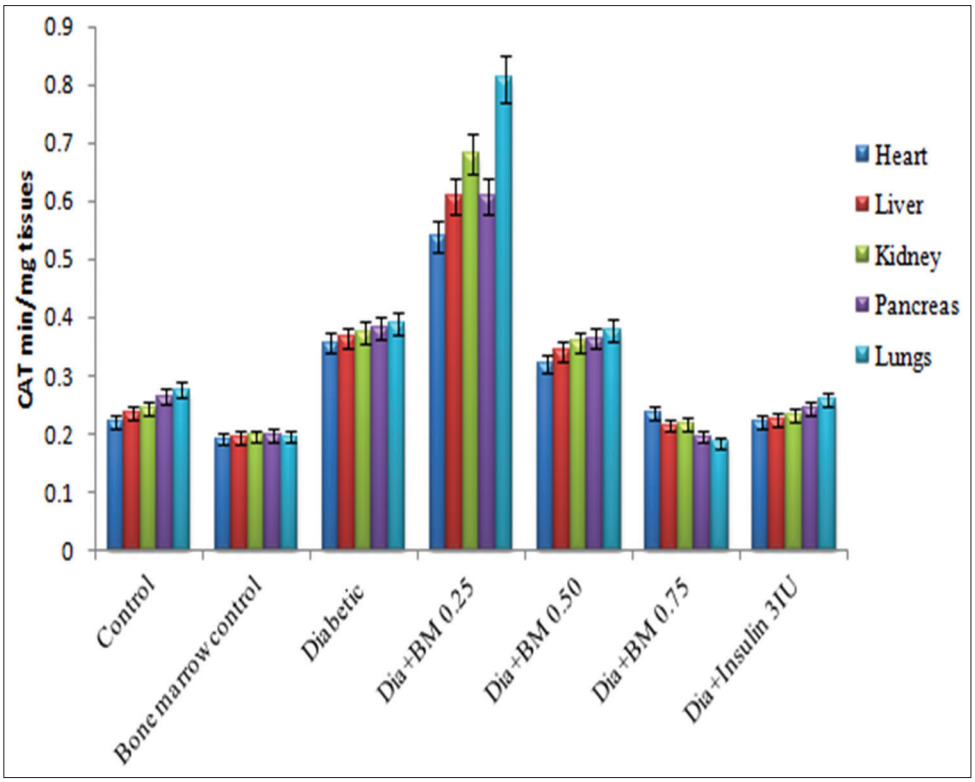


Fig. 9: Effect of bone marrow stem cells on tissue catalase in normal and streptozotocin-induced diabetic male Wistar rats. Each value is mean±standard deviation for 6 rats in each group. Significance at: p<0.05. Values not sharing a common superscript differ significantly at p<0.05, Duncan's multiple range test

BMSCs to STZ diabetic rat, the condition was reversed which means diabetic rat blood glucose level got reduced to that of control rat. Here, it proves that the stem has increased the secretion of insulin from the regenerated β-cells of the pancreas.

Insulin plays a very vital role in lowering blood glucose level by enhancing glycogenesis and inhibiting glycogenolysis. In DM, the normal capacity of the liver to synthesize glycogen is impaired. Liver plays a very important role in buffering the post-prandial hyperglycemia and is involved in the synthesis of glycogen from glucose. The decrease observed in the liver and skeletal muscle glycogen content of STZ diabetic rats is due to the lack of insulin, which results in the inactivation of glycogen synthase system.

Glutathione is an important inhibitor of free radical-mediated lipid peroxidation group possesses antioxidant property and GSH is the major source of thiol groups in the intracellular compartment. GSH reacts with aldehydes produced during lipid peroxidation protecting the thiol groups of membrane proteins.

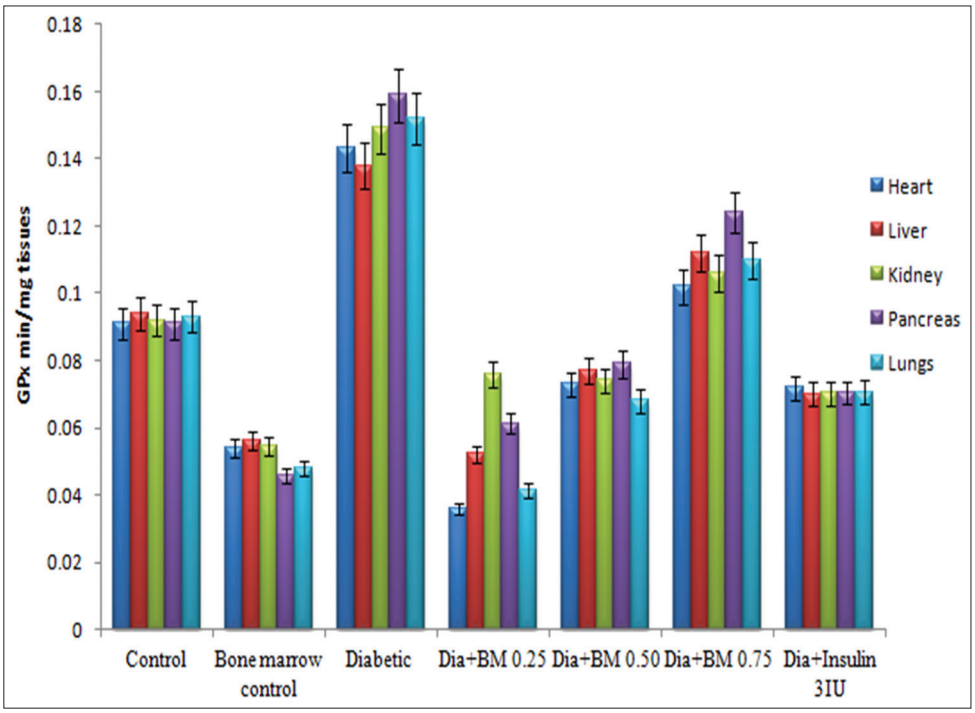


Fig. 10: Effect of bone marrow stem cells on tissue glutathione peroxidase in normal and streptozotocin-induced diabetic male Wistar rats. Each value is mean±standard deviation for 6 rats in each group. Significance at: p<0.05. Values not sharing a common superscript differ significantly at p<0.05, Duncan's multiple range test

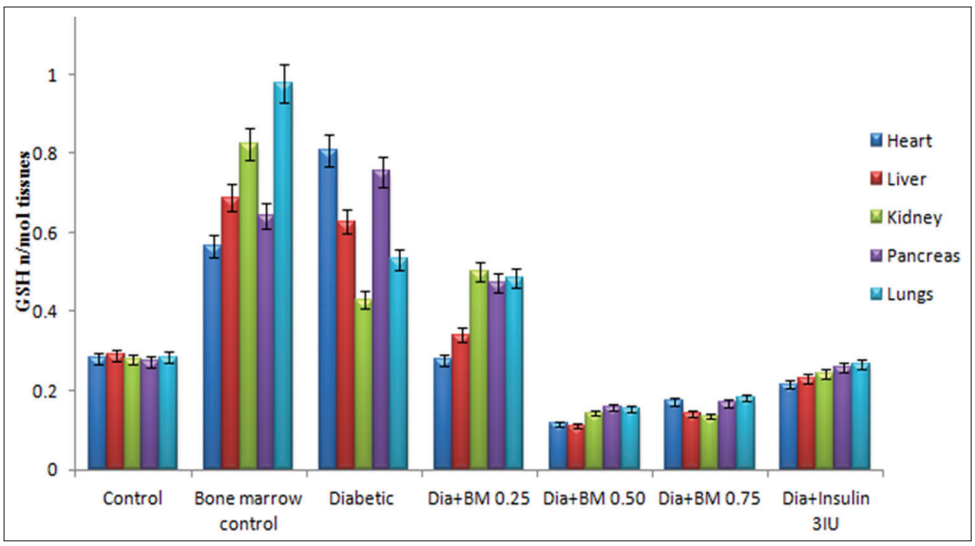


Fig 11. Effect of bone marrow stem cells on tissue reduced glutathione in normal and streptozotocin-induced diabetic male Wistar rats. Each value is mean±standard deviation for 6 rats in each group. Significance at: p<0.05. Values not sharing a common superscript differ significantly at p<0.05, Duncan's multiple range test

Endogenous antioxidant enzymes, namely, GPx, CAT, SOD, and glutathione reductase level decreases in diabetic rat [7]. GPx plays a pivotal role in H_2O_2 catabolism. CAT is a heme protein which catalyzes the reduction of hydrogen peroxides and protects the tissues from hydroxyl radicals [8]. Hence, the present study confirms the β-cell regenerative efficacy of BMSCs in diabetic effects by several parameters such as a decrease in blood glucose levels, normal insulin and C-peptide levels, normal histology of pancreas with regenerated β-cells, possessing antioxidant effects by restoring the normal levels of TBARS, SOD, CAT, and GPx levels in diabetic rats. Thus, BMSCs could be used as an effective therapy for diabetes by regenerating the destructed pancreatic β-cells. Finally, BMSC is giving a better result for the treatment of type 1 DM.

SUMMARY AND CONCLUSION

- DM is a serious and complex chronic condition and the metabolic disorder is characterized by hyperglycemia and disturbance of carbohydrates, proteins, and fat metabolism, secondary to an absolute or relative lack of hormone insulin. T1DM is undoubtedly a terrible, lifelong disease, involving substantial short-term and long-term complications such as diabetic polyneuropathy (DPN), atherosclerosis, and myocardial infarction.
- Oxidative stress is known to increase as a consequence of hyperglycemia and is responsible for the development of various diabetes-associated secondary complications. Oxidative stress has been shown to have a role in diabetes and some antioxidant also have a role in reduction of diabetes and some related problems.

- In DM, the normal capacity of the liver to synthesize glycogen is impaired. Liver plays a very important role in buffering the post-prandial hyperglycemia and is involved in the synthesis of glycogen from glucose. The free radicals produce various byproducts plays a crucial role to regulate the progression of liver disease.
- Nowadays, stem cell therapy is used as treatment for diabetes. MSCs have been indicated as a novel emerging regenerative therapy for diabetic neuropathy because of their multipotency. In BMSCs, cells have the ability to prevent DPN in STZ-induced diabetic rat.
- The blood glucose level was measured in normal diabetic and BMSCs-treated group of rats, significantly decrease in blood glucose level in the BMSC-treated groups compared to insulin-treated group. Serum protein concentration decreases in STZ-induced diabetic rats. The decreased protein concentration was restored on administration of BMSCs compared to insulin.
- BMSCs were found to be normalized all the parameters studies from the diabetic abnormal levels. BMSCs infusion was found to regenerate the β-cells of the pancreatic islets and thereby decrease blood glucose level with increase in insulin and C-peptide levels.
- The BMSCs were also found to have hepatic and renal protective effects on the diabetic animals. BMSCs showed potential antioxidant effects by normalizing TBARS, GSH, SOD, CAT, and GPx levels in the diabetic groups.
- It is clearly evident from the present study that BMSCs infusion to the diabetic animals may potential normalize the diabetic conditions such as blood glucose, insulin, c-peptide, and tissue morphology. It was also found that BMSCs were efficient antioxidants as they restored normal TBARS, GSH, SOD, CAT, and GPx levels in diabetic animals. Hence, the BMSCs can be used as a potential therapy to treat diabetic patients as they regenerate the destructed β-cells.

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