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Antidiabetic and Antioxidant Efficacy of Bone Marrow Stem Cells on Stze-Induced Male Albino Wistar Rats

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Abstract: Diabetes mellitus is a multi-metabolic disorder that influences more than 348 million people worldwide [5]. 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 [1,2]. Here, we focused on bone marrow-derived 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. The main objective of the study was To evaluate the antidiabetic activity of the Dental pulp cells in Streptozotocin (STZ)-induced diabetic Wistar albino rats and To calculate the biochemical estimation of both normal and treated groups for drug preparation against diabetic in pharma industries.
Keywords: Streptozotocin.,Wistar albino rat.,Insulin.,stemcell.,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 [9]. Diabetes mellitus 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. Diabetes mellitus may be suspected or recognized clinically by 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 [1, 5].

In the WHO classification, 2 major types of diabetes type1 (insulin dependent) type2 (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. [5, 6] Type 2 diabetes is on the increase in obese humans. There is not enough data to confirm if overweight humans have overweight pets, but there is certainly a problem as specialist obesity referral clinics for animals are 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 progresses to the point at which only insulin therapy controls blood glucose levels [2]. According to development stage, stem cell can be dividing into embryonic stem cell and adult stem cell.

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 morphological characterization of normal and treated groups.To determine the enzymatic activity & non-enzymatic activity of both normal and treated groups.

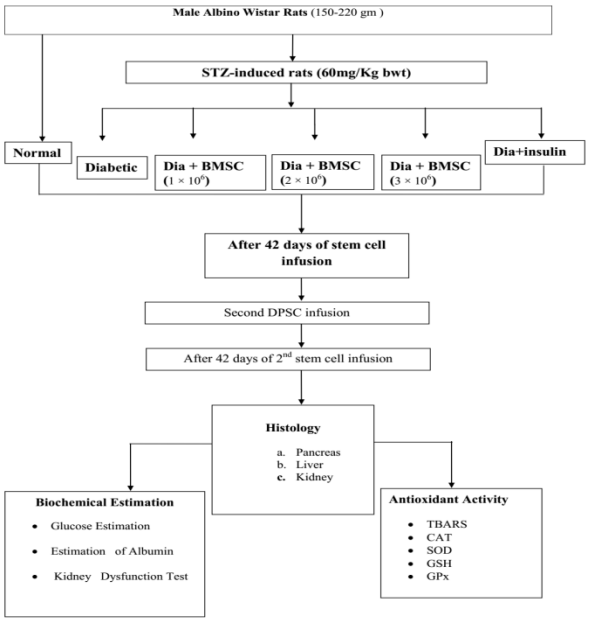
MATERIALS AND METHOD

Stem Cell Collection

Human Bone marrow stem cell (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 gm 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 hours day and 12 hours night cycle in different cages and feed up with standard pellet diet and water and libitum(SaiEntreprisei). The animals used for the experiment were approved by Animal Ethical Committee (ACE) and by the regulatory body of the government (Reg.No.07/2013).



Induction of Diabetes

Freshly prepared streptozotocin at the dosage of 60 mg/kg body weight was injected intraperitonally to the rats. Streptozotocin 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 250mg/dl) were chosen for the experiment.

Experimental Design

The rats were divided into four groups.

Group I: Normal rats (0.1% Carboxy Methyl Cellulose) orally.

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

Diabetic rats treated with bone marrow 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). BMSCs infusion 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 BMSCs at 42 days post-STZ administration. The untreated control rats were infused with 0.2 mL physiological saline [8].

Biochemical Estimations

Estimation of glucose (Diagnostic Kit–Reddy's Laboratories, Bachupally, Hyderabad, India) For the estimation of glucose, 10µL each of serum and working standard were incubated with 1ml of the reagent for 15 min at 37°C and the absorbance at 505nm was measured against a reagent blank. For reagent blank, 10µL of distilled water was added to 1ml of the reagent. The concentration of glucose in serum samples was calculated as:

Estimation of Albumin

Procedure

0.5mL of serum was taken in a test tube and 6 mL of sodium sulphite solution was added and mixed. To mixture, 3.0 mL of ether was added, stoppered, shaken well for 20s and then centrifuged for 5min. 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 H2O and treatedwith 5.0 mL of biuret reagent. The purple color developed was read at 540nm after 15min using reagent blank. Values were expressed as g/dl of serum.

Kidney Function Tests

Estimation of protein [13]

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 upto 10 mL. To 1 mL of the sample, 5 mL of reagent D was added, and after 10min 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 620nm 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 1mg/ml and diluted to obtain serial dilutions at 50, 100, 150, 200 and 250µg/ml.

Estimation of plasma insulin (Radioimmunoassay kit – Diasorin, Italy)

- **Standards:** 100µl of the standard from the respective standard bottles (0-5) was pipetted out into different vials and 100µl of tracer and 100µl of antiserum were added to it.
- **Sample:** 100µl of plasma sample was pipetted out into a vial; 100µl of tracer and 100µl of antiserum were added to it.
- **Total activity:** To measure the total activity, 100µl 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. 1mL 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.

Estimation of plasma C-peptide (Radioimmunoassay kit – Missouri, USA)

300µl of assay buffer was pipetteed out to the non specific binding tubes. 200µl of assay buffer was pipetted out in the reference tube. 100µl assay buffer pipetted out to all the other tubes (sample). These tubes are vortexed and incubated for 20-24 hours at 4˚C. After 24 hours of incubation 100µl of 125I – Rat C-peptide tracer pipetted out to all the tubes. These tubes are vortexed and incubated for 22-24 hrs at 4˚C. 1mL 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 2000-3000xg. Supernatant was decanted from all centrifuged tubes except total count tubes, and all the tubes were counted in a gamma counter for 1 min.

Enzyme Activity

Estimation of Tbars

Procedure

0.5mL 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 both for 15min, after cooling, the tubes were centrifuged at 1000g for 10min & the supernatant was estimated. Series of standard solution in the concentration of 2-10mol was treated in a similar manner. The absorbance of the chromophore was read at 535nm against reagent blank. The valves were expressed as mmol/100g of tissue.

Gsh (Reduced Of Glutathone)

Tissue was homogenized used in PO_4 buffer (0.1m 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 colour developed was read in a spintronic 20 at 412nm. A series of standard (20 – 100mg) was reacted in a similar manner along with a blank containing 1.0ml of buffer. The amount of glutathione was expressed as µ/mol of tissue.

Non-Enzymatic Activity

Tissue was homogenized by 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 pyropo4 buffer. 0.1 mL of phenazinemethosulphate and 0.3 mL of nitrobluetetrazolum of appropriately diluted enzyme preparation in a total volume of 3ml the reaction of 0.2 mL NADH. After incubation at 30°C for 90°C sec, the reaction was stopped by the addition of 1 mL glacial acid. The reaction mixture was stiread vigorously and shaken with 4ml n – butanol layer was separated. The color density of the chromogenic n-butanol was measured in a spectrophotometer at 520nm.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 Activity

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

The tissue was homogenized using Tries buffer to 0.2 mL of tries 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 0.1ml of H_2O_2 was added .The contents were mixed well and incubation at 37°C for 10min, along with a control containing all reagents except homogenate? After 10min, 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 Elman's. The activity was expressed as the of HSH utilized / min / mg of tissues.

STATISTICAL ANALYSIS

All data were expressed as means ± SD for control and experimental groups. The data were analyzed using one way Analysis of Variance (ANOVA) on Statistical Package for Social Sciences (SPSS) (Version 17.0) and the group means were compared by Duncan's Multiple Range Test [12]. The results were considered statistically significant if the calculated 'p' value was less than 0.05.

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 (60mg/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, 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 21st day of BMSC treatment of 2nd infusion. BMSC treatment for a period of 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.

RESULTS

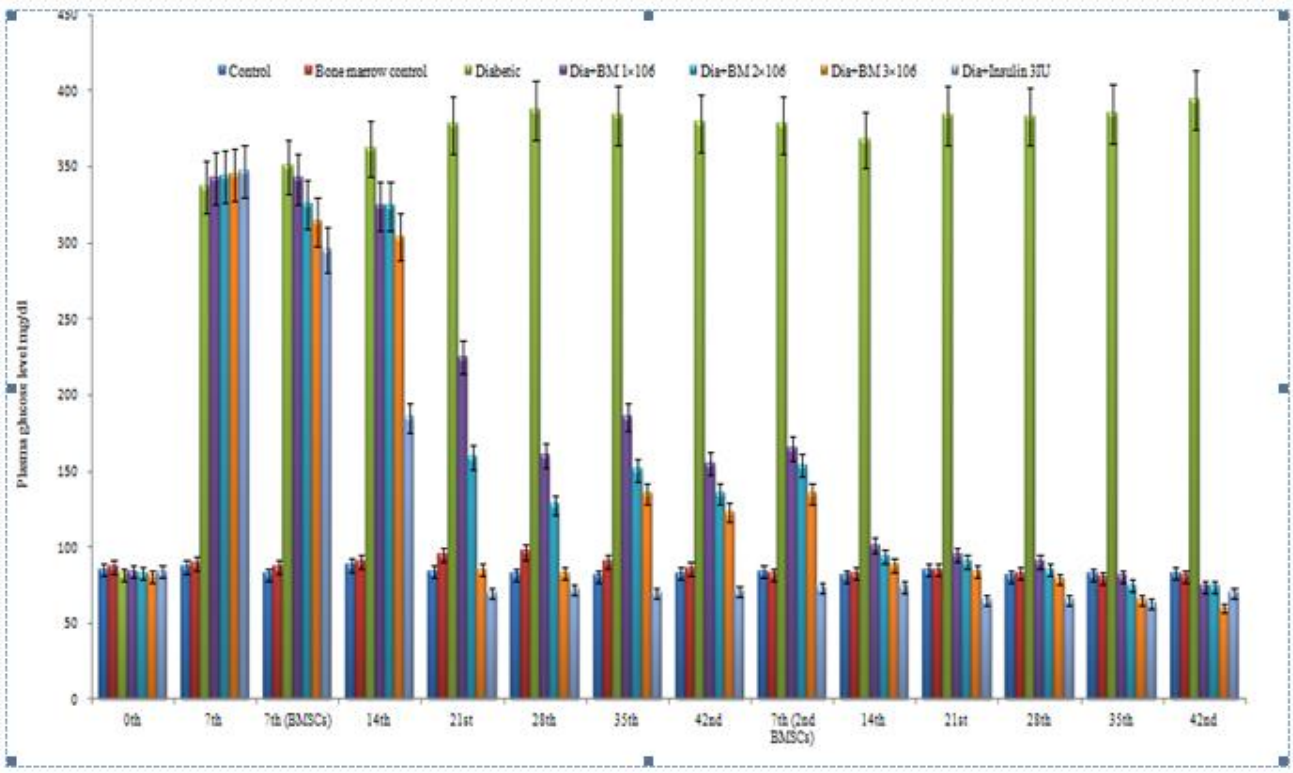


Fig-1: **Effect of Bone marrow stem cells on plasma glucose levels in normal and STZ-induced diabetic male Wistar rats**

Each value is mean ± S.D. 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 (DMRT).

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 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.

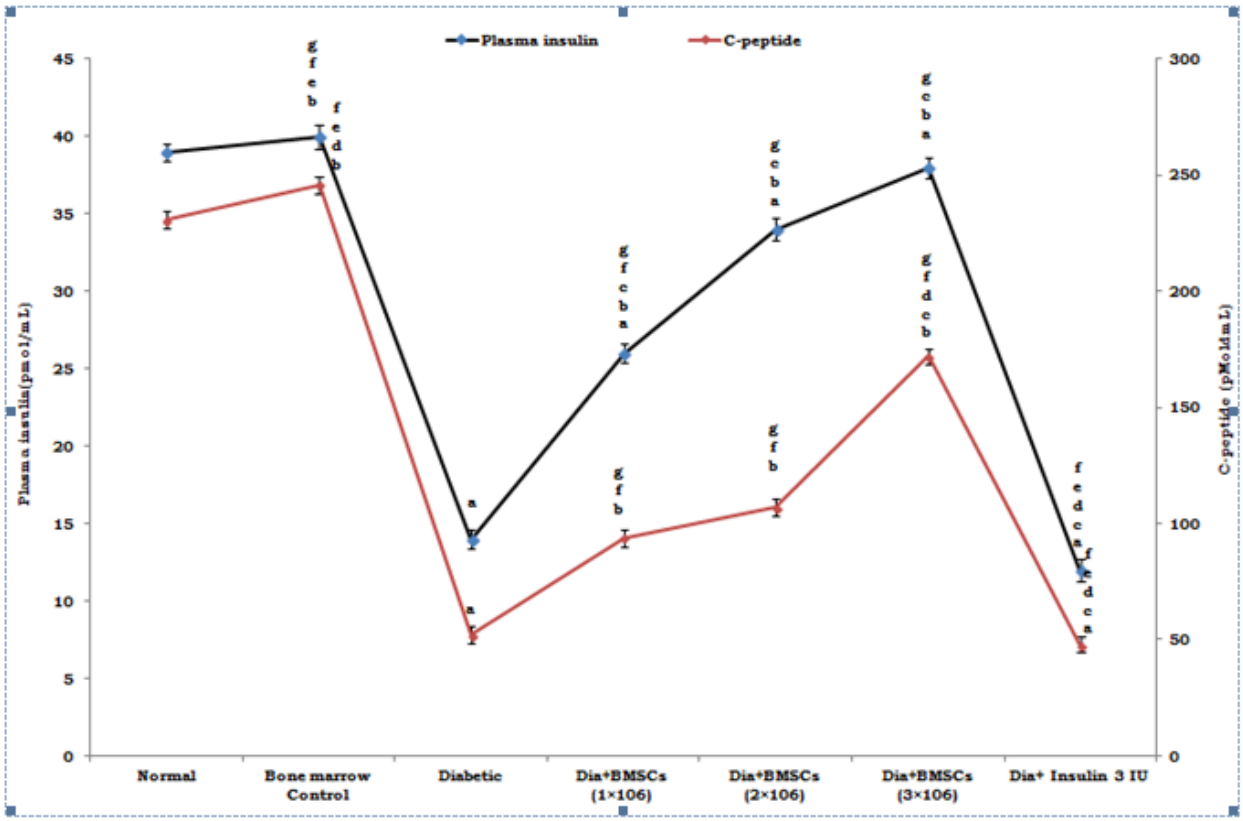


Fig-2: **Effect of Bone marrow stem cells on Plasma insulin and C-Peptide levels in normal and STZ--induced diabetic male Wistar rats**

Each value is mean ± S.D. 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 (DMRT).

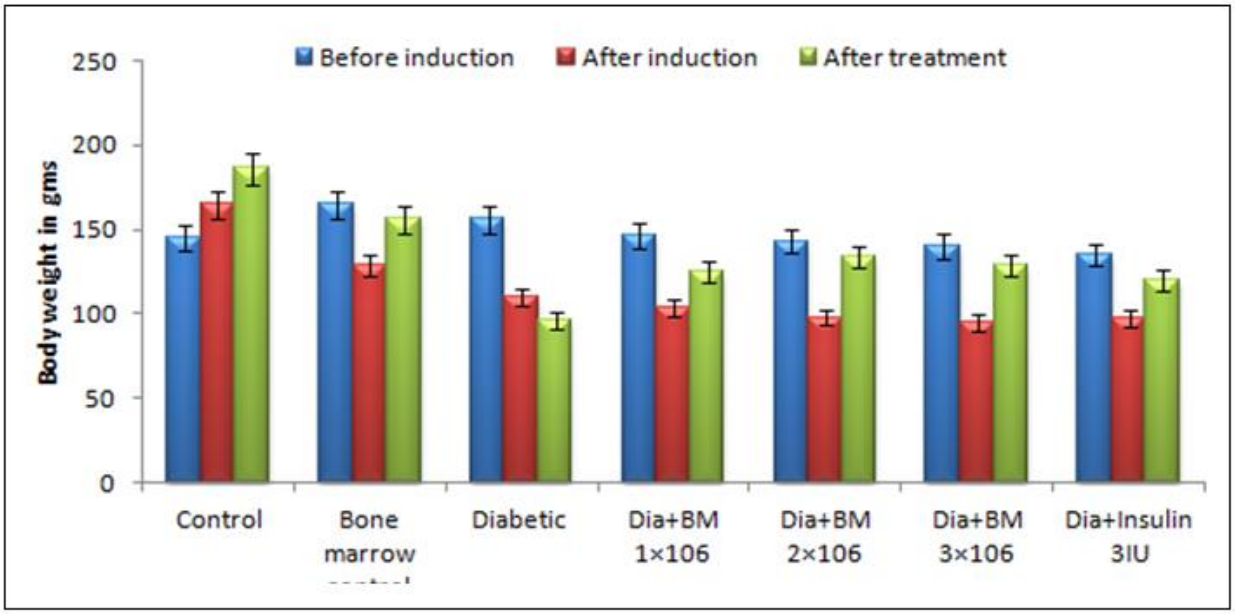


Fig-3: **Effect of Bone marrow stem cells on body weight levels in normal and STZ--induced diabetic male Westar rats**

Each value is mean ± S.D. 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 (DMRT).

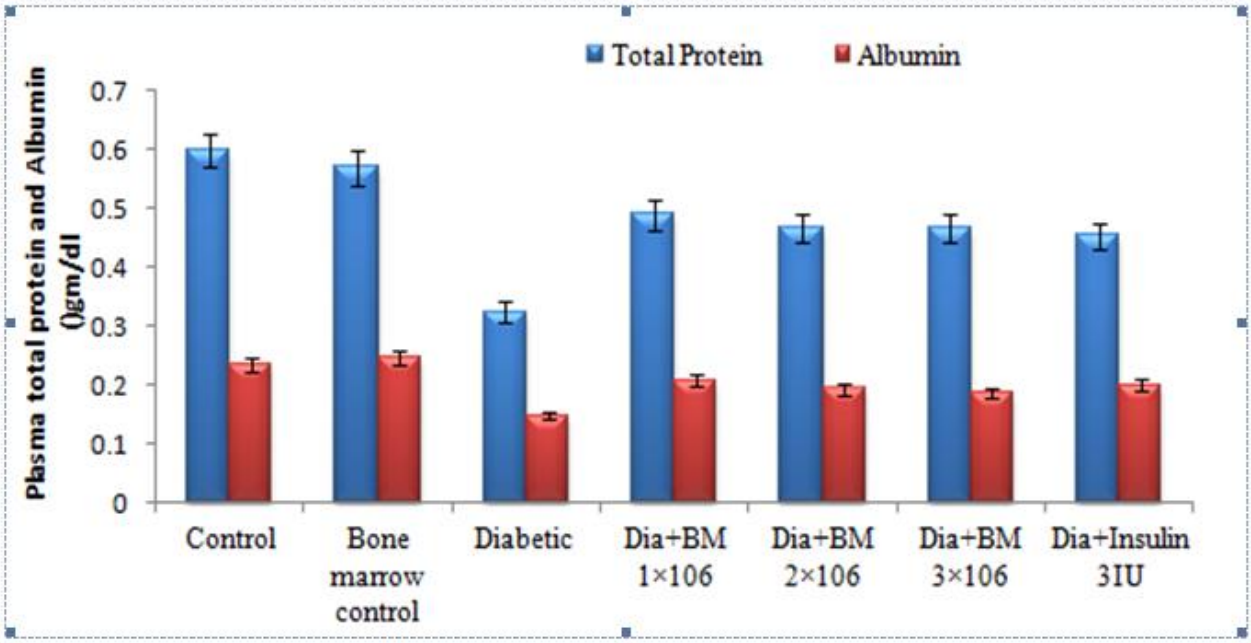


Fig-3.1: **Effect of Bone marrow stem cells on Plasma total protein and Albumin levels in normal and STZ--induced diabetic male Wistar rats**

Each value is mean ± S.D. 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 (DMRT).

Histological Studies

Hematoxylin and eosin stained sections

Hematoxylin and eosin stained sections of pancreas, kidney and liver of untreated rats revealed that each islet of Langerhans is formed of numerous compactly arranged cells occurring as dense cords (Fig4A-4D). 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 psychotic 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 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 (5A-5D) [7]. In diabetic rats both the capsule and glomeruli was found to be disrupted, whereas such 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 were found distributed with hepatocytes, whereas the in diabetic rats the cell were found get disrupted (6A-6D). Upon BMSC treatment the hepatocytes were found to be as in the normal. Insulin treated diabetic rat's also possessed normal liver histology.

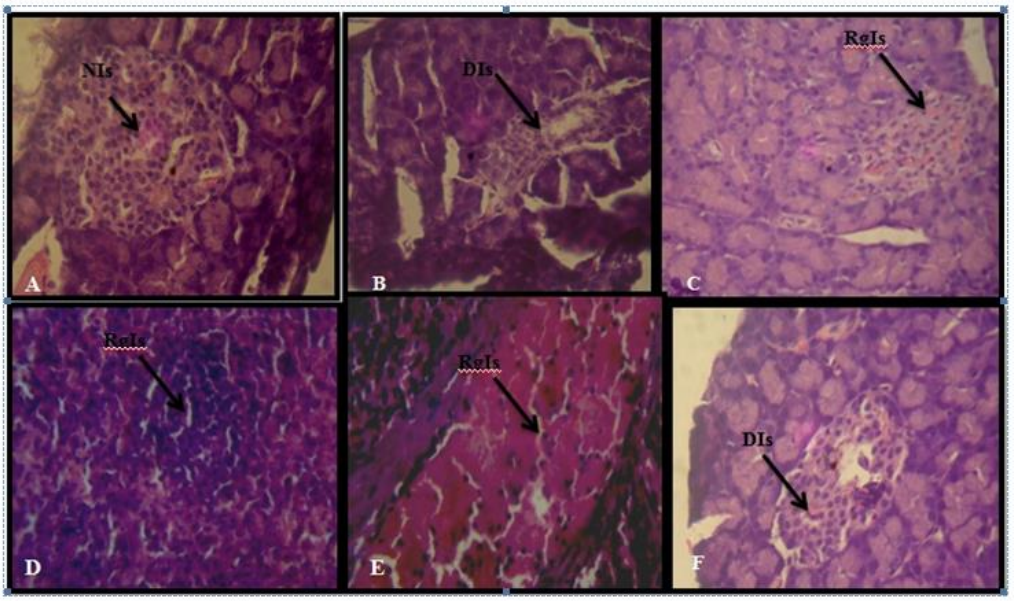


Fig-4: Effect of BMSCs on pancreas of the STZ-induced male albino Wistar rats

A: Photograph of Islet of normal rat. (200x)(I-Islets of Langerhans)
B: Islets of Langerhans of diabetic control. Cellular boundary has been disrupted in islet cells (200x)
C: Islets of BMSCs (1×10^6) treated diabetic rats. (200x) (I-Islets of Langerhans)
D: Islets of BMSCs (2×10^6) treated diabetic rats. (200x) (I-Islets of Langerhans)
E: Islets of BMSCs (3×10^6) treated diabetic rats. (200x) (I-Islets of Langerhans)
F: Islets of insulin-treated diabetic rats. (200x)

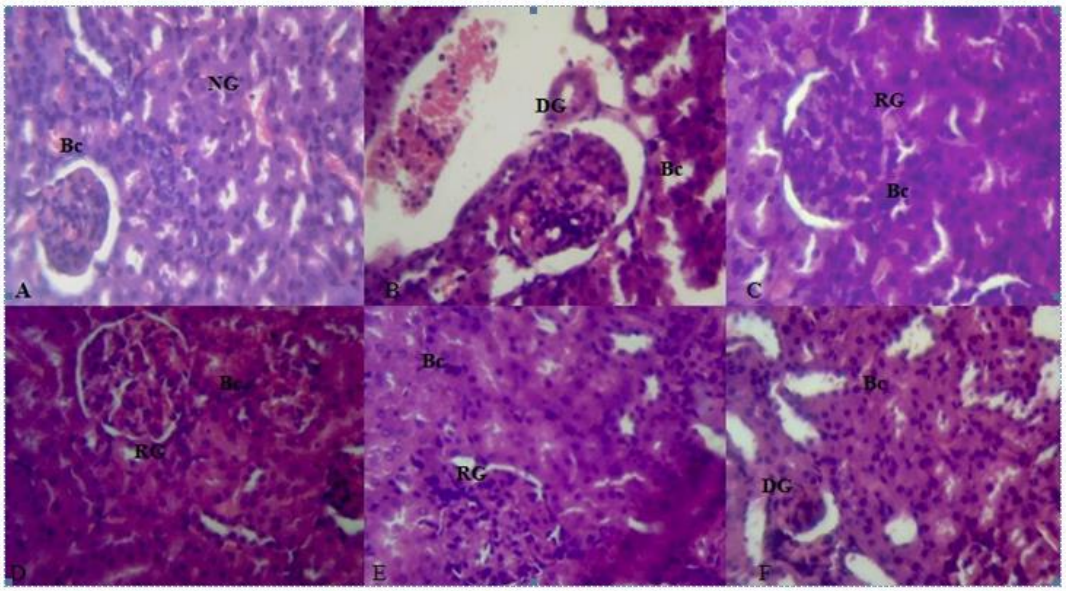


Fig-5: Effect of BMSCs on kidney of the STZ-induced male albino Wistar rats

A: Photograph of Kidney of normal rat. (200x)(Bc- Bowman's capsule; G-Glomeruli)
B: Paraffin section of kidney of diabetic control. Destruction of the capsule (200x)
C: Kidney section of BMSCs (1×10^6) treated diabetic rats. (200x) (Bc- Bowman;s capsule; G-Glomeruli)
D: Kidney section of BMSCs (2×10^6) treated diabetic rats. (200x) (Bc- Bowman;s capsule; G-Glomeruli)
E: Kidney section of BMSCs (3×10^6) treated diabetic rats. (200x) (Bc- Bowman;s capsule; G-Glomeruli)
F: Paraffin section of insulin-treated diabetic rat kidney. (200x) (Bc- Bowman;s capsule; G-Glomeruli)

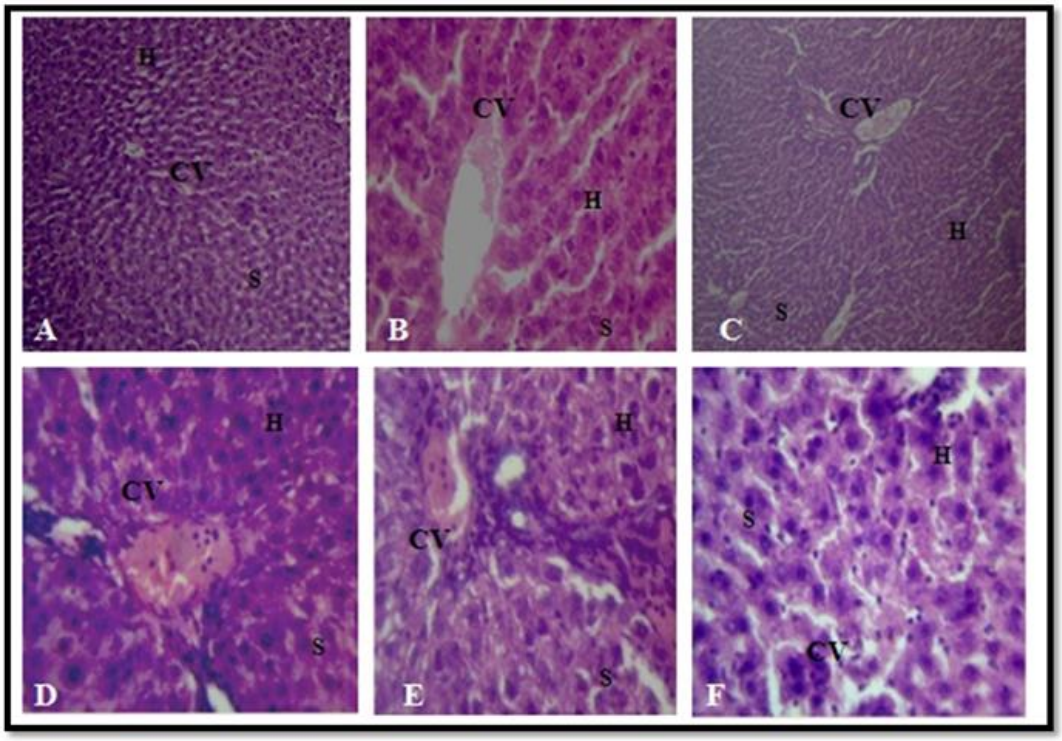


Fig-6: Effect of BMSCs on liver of the STZ-induced male albino Wistar rats

A: Photomicrograph of liver of normal Wistar rat. (200x)
B: Paraffin section of liver of diabetic control. Destruction of liver cells (200x) (H-hepatocytes)
C: Liver section of BMSCs (1×10^6) treated diabetic rats. (200x) (H-hepatocytes)
D: Liver section of BMSCs (2×10^6) treated diabetic rats. (200x) (H-hepatocytes)
E: Liver section of BMSCs (3×10^6,) treated diabetic rats. (200x) (H-hepatocytes)

F: Paraffin section of insulin-treated diabetic rat liver. (200x) (H-hepatocytes)

Antioxidant analysis

Effect of BMSCs on lipid peroxidation

Effect of BMSC on lipid peroxidation in STZ-induced rats determined is depicted in figure 8 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.

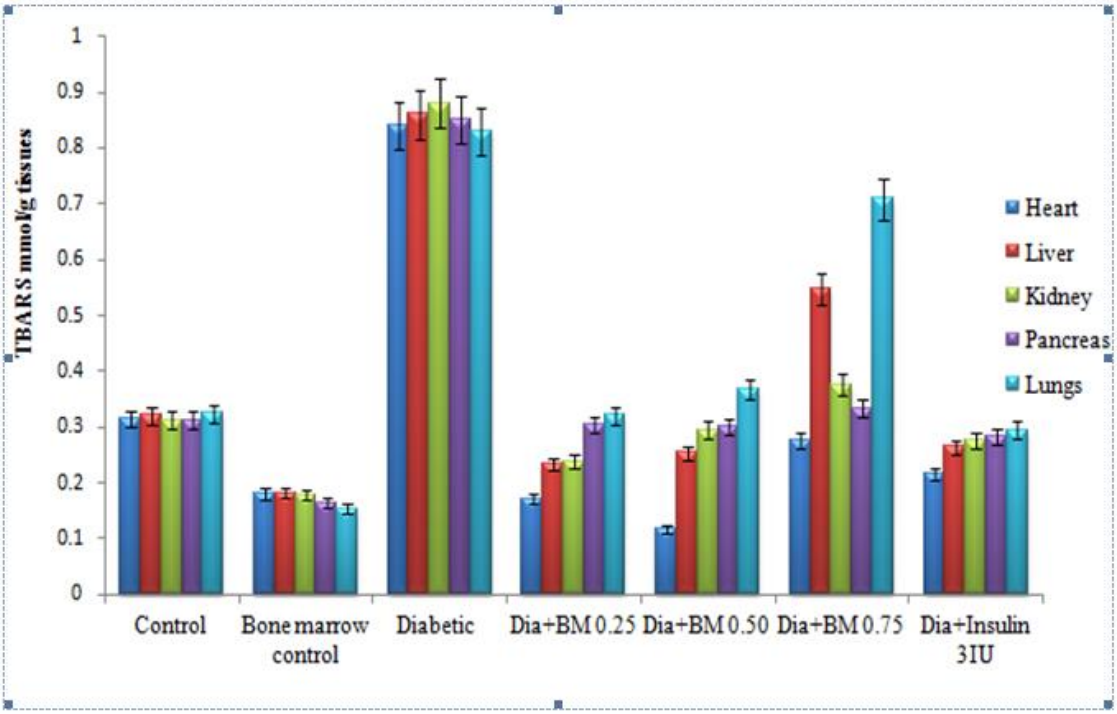


Fig-7: **Effect of Bone marrow stem cells on Tissue Lipid peroxidation in normal and streptozotocin induced diabetic male Westar rats**

Each value is mean ± S.D. 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 (DMRT).

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 figures 8, 9 &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.

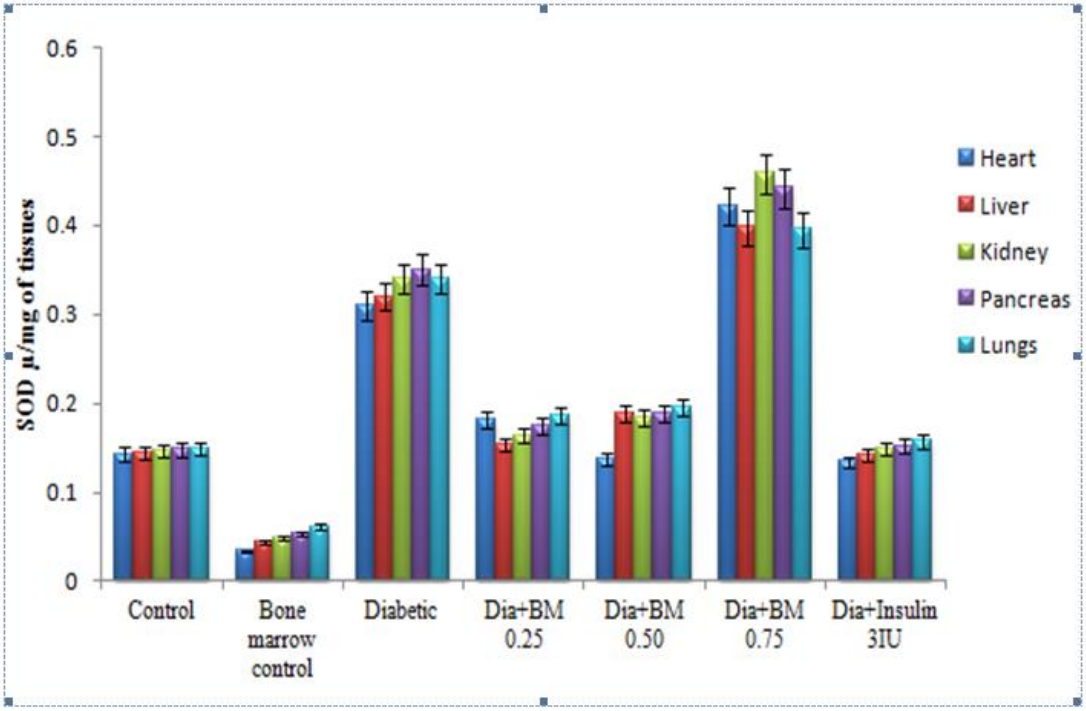


Fig-8: **Effect of Bone marrow stem cells on tissue Superoxide dismutase in normal and streptozotocin induced diabetic male Westar rats**

Each value is mean ± S.D. 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 (DMRT).

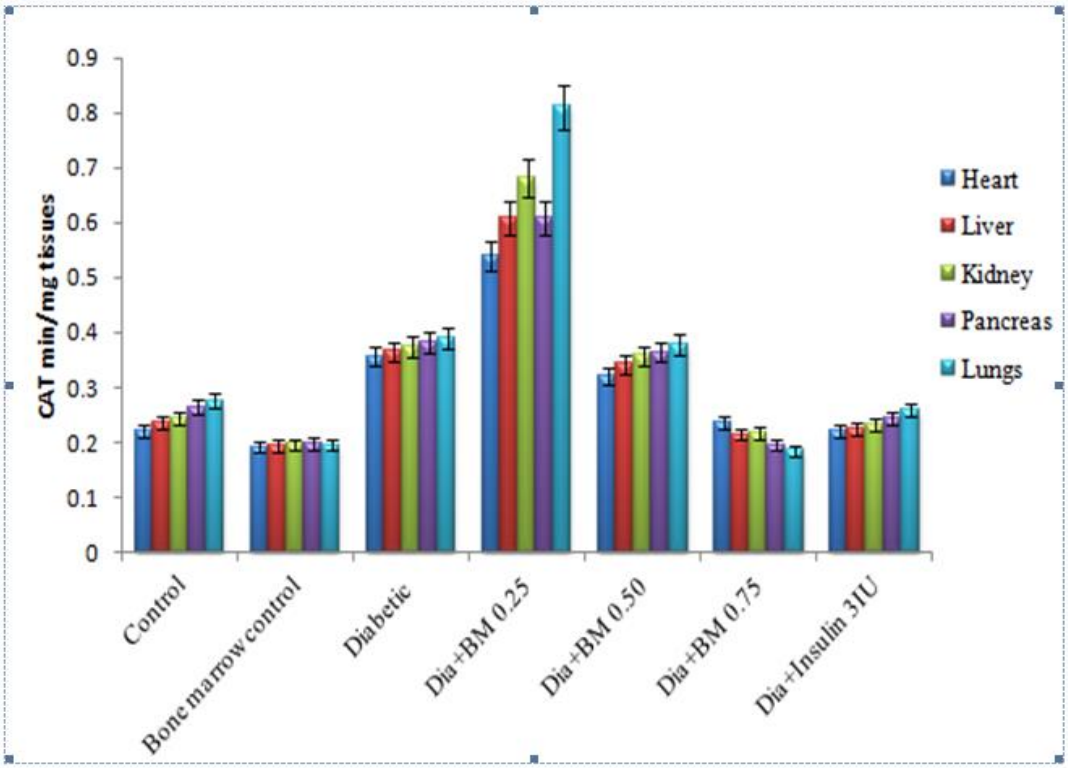


Fig-9: **Effect of Bone marrow stem cells on tissue Catalase in normal and streptozotocin induced diabetic male Wistar rats**

Each value is mean ± S.D. 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 (DMRT).

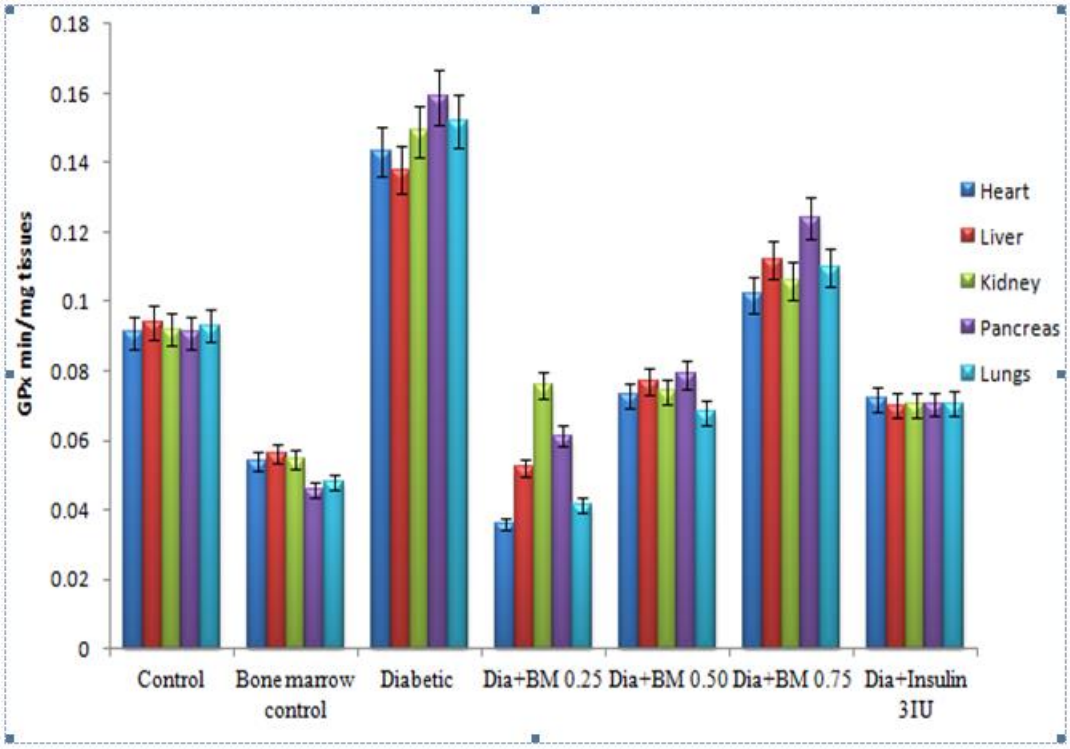


Fig-10: **Effect of Bone marrow stem cells on tissue Glutathione Peroxidase in normal and streptozotocin induced diabetic male Wistar rats**

Each value is mean ± S.D. 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 (DMRT).

Effect of BMSCs on reduced glutathione

The non-enzymatic antioxidant GSH levels in liver, pancreas, kidney, lungs and heart tissues are depicted in figure-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.

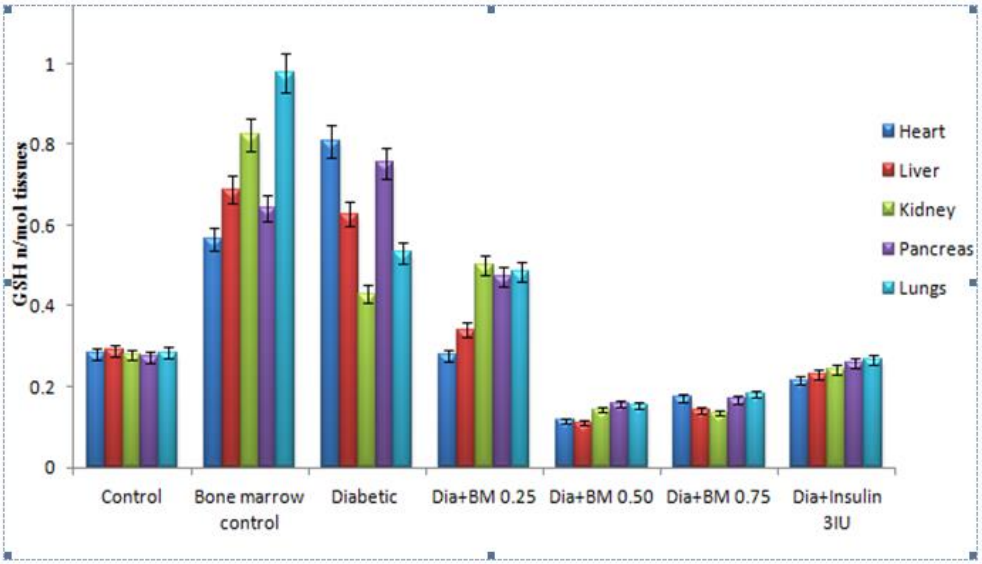


Fig-11 Effect of Bone marrow stem cells on tissue reduced glutathione in normal and streptozotocin induced diabetic male Wistar rats

Each value is mean ± S.D. 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 (DMRT**).**

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 [5]. Diabetes mellitus 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. Diabetes mellitus may be suspected or recognized clinically by 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 mesenchymal stem cells -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 tissue, like adipose tissue, dental pulp, placenta, Wharton's jelly and umbilical cord. MSCs are hypo-immunogenic, allowing allogeneic transplant without histocompatibility or recipient conditioning being required [3] 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 bone marrow stem cells 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 glycogenosis. In Diabetes mellitus the normal capacity of the liver to synthesize glycogen is impaired. Liver plays a very important role in buffering the postprandial 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 propertyand 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. Endogenous antioxidant enzymes namely Glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) level decreases in diabetic rat [13]. GPx plays a pivotal role in H_2O_2 catabolism. CAT is a heme protein which catalyses the reduction of hydrogen peroxides and protects the tissues from hydroxyl radical [5]. Hence the present study confirms the β-cell regenerative efficacy of BMSCs in diabetic effects by several parameters such as 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 are giving better result for the treatment of type 1 diabetes mellitus.

- Diabetes mellitus is a serious and complex chronic condition and the metabolic disorder is characterized byhyperglycemia and disturbance of carbohydrates, proteins, and fat metabolism, secondary to an absolute or relative lack of hormone insulin [2]. T1DM is undoubtedly a terrible, lifelong disease, involving substantial short-term and long-term complications such as Diabetic polyneuropathy (DPN), atherosclerosis, and myocardial infarction [4].
- 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 role in diabetes and some antioxidant also have a role in reduction of diabetes and some related problems[3].
- In Diabetes mellitus the normal capacity of the liver to synthesize glycogen is impaired. Liver plays a very important role in buffering the postprandial hyperglycemia and is involved in the synthesis of glycogen from glucose [9]. The free radicals produce various byproducts plays a crucial role to regulate the progression of liver disease [5].
- Now a day's stem cell therapy is used as treatment for diabetes. Mesenchymal stem cells (MSCs) have been indicated as a novel emerging regenerative therapy for diabetic neuropathy because of their multipotency. In Bone marrow stem (BMSCs) cell have the ability to prevent DPN in STZ-induced diabetic rat [7].

- 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 [8].
- BMSCs were found to normalize 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 [11].
- The BMSCs were also found to have hepato and renal protective effects on the diabetic animals [10]. BMSCs showed potential antioxidant effects by normalizing TBARS, GSH, SOD, CAT and GPx levels in the diabetic groups.

CONCLUSION

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, 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.

CONFLICT OF INTERESTS

The authors have no conflict of interests to declare regarding the publication of this paper.

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