Original Article

Role of Human Umbilical Cord Blood derived Stem Cells versus their Conditioned Medium on the Regeneration of Pancreatic Beta Cells in a Rat Model of Diabetes Mellitus

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ABSTRACT

Introduction: An efficient alternate source of mesenchymal stem cells (MSCs) was obtained from umbilical cord blood (UCB). Therefore, this work was designed to assess the role of human UCB-derived MSCs versus their conditioned medium (CM) on the regeneration of beta cells of the pancreas in a rat model of diabetes.

Materials and Methods: Forty adult male Wistar rats were divided equally into 4 groups; Group I (control), Group II (diabetic), each rat was injected with a single dose of 35 mg/kg of streptozotocin (STZ) intraperitoneally. After being diabetics, they were left without treatment, Group III (UCB-MSCs treated), injected with 1x106 cells /ml of UCB-MSCs once into tail vein after confirmation of being diabetic, Group IV (CM treated), injected intramuscularly with 0.5 ml of CM once per week after being diabetic. After 2 weeks and 4 weeks from being diabetic, the pancreas specimens from all groups were processed for H&E stain and immunohistochemically for anti-insulin and anti-caspase-3 antibodies.

Results: Diabetic group showed distortion of the architecture of islets of Langerhans and resulted in decrease size of islets and appearance of many empty spaces within them. There was significant reduction in body weight, serum insulin, serum C-peptide level and, insulin immunohistochemical stained positive cells. Moreover, significant rise in blood glucose and in caspase-3 immunohistochemical stained positive cells was found. UCB-MSCs and CM treated subgroups showed an obvious histological and biochemical improvement when compared to diabetic group.

Conclusion: Transplantation of UCB-MSCs was more efficient in the regeneration of pancreatic beta cells than injection of conditioned medium in the rat model of diabetes. However, CM still could prevent progress of degeneration of beta cells of islets of Langerhans as indicated by its anti-inflammatory and anti-apoptotic effect.

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Key Words: Conditioned medium, diabetes, mesenchymal stem cells, streptozotocin, umbilical cord blood.

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INTRODUCTION

In the population of young adolescents, diabetes mellitus (DM) type I, referred to as insulin-dependent diabetes, has become a relatively common chronic health problem[1]. Long-term problems from this illness may lead to high rates of morbidity and mortality, which may lower quality of life. Maintaining appropriate blood glucose levels is a daily struggle for patients with type I diabetes[2]. There has been a rise in the number of individuals admitted to hospitals because of the major side effects as diabetic ketoacidosis and severe hypoglycemia. The hallmark of diabetes type I is a complete deficiency of insulin due to an autoimmune degeneration of pancreatic beta cells mediated by T cells[3].

Mesenchymal stem cells (MSCs) are multipotent adult stem cells and capable of self-renewal and altering the immune system. Because they can secrete growth factors that have a beneficial effect on injured tissues, it has been discovered that they can regenerate tissues[4]. Freshly donated umbilical cord blood (UCB) can be used to separate MSCs, which offers benefits over other tissues like bone marrow and adipose tissues since it can be obtained readily and doesn't require invasive procedures[5]. Numerous investigations, both clinical and experimental, showed that UCB-MSCs-transplantation lowers blood glucose levels and improves hyperglycemia. It was discovered that they generate growth factors that function through a paracrine mechanism to enhance the microenvironment of the pancreas and help the regeneration and proliferation of beta cells of islets of Langerhans[6].

Nonetheless, research on growth factors generated by stem cells has demonstrated that these substances, in the absence of stem cells, can repair tissues under a variety of damaging circumstances[7]. Conditioned medium (CM) was named such because stem cells produce these growth factors in culture medium known as secretome, microvesicles, or exosome[8].

When compared to stem cell utilization, the CM has many advantages because it is easier to make, package, freeze-dry, and transport. Additionally, because it is cell-

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free, it is not necessary to ensure compatibility between the donor and recipient to lessen the potential problems related to rejection[9]. Consequently, there is a good chance that stem cell-derived conditioned media will be developed into therapeutics for regenerative medicine[10].

AIM OF THE WORK

The aim of this work was to assess the effects of mesenchymal stem cells derived from human umbilical cord blood and their conditioned media on beta cells of the pancreas in a STZ-induced diabetes mellitus rat model.

MATERIALS AND METHODS

Animals

This experiment was conducted on 40 adult male Wistar rats of an average weight 150 - 200 grams. All animals were housed in Ain Shams Faculty of Medical Ain Shams Research Institution (MASRI) with water and food ad libitum. They were kept in wire mesh cages at normal room temperature. All animal procedures were approved by the Animal Ethical Committee of Ain Shams University and were conducted according to the guidelines outlined in the Guide for the Care and Use of Laboratory Animals, under approval number: FMASU R56/2024.

Experimental design

The animals were divided randomly into 4 groups, 10 animals each as follows:

Group I (Control group): each rat was injected intraperitoneally once with 0.1 mol/L citrate buffered solution (pH 4.4).

Group II (Diabetic group): each rat was injected with a single intraperitoneal injection of 35 mg/kg body weight of streptozotocin (STZ) (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 0.1 mol/L citrate buffered solution (pH 4.4). When blood glucose levels were assessed three days after STZ injection and were more than 250 mg/dL, the rats were classified as diabetic and enrolled in the study[11].

Group III (UCB-MSCs treated group): each rat was injected with STZ as in Group II. After confirmation to be diabetic, each rat was injected once with 1× 106 cells/ml of UC-MSCs through tail vein[12].

Group IV (CM treated group): each rat was injected with STZ as in Group II. After confirmation of being diabetic, each rat was given an intramuscular injection of 0.5 ml of CM as a single dose once a week till the end of the experiment[13].

Each group was further subdivided equally into two subgroups (n=5 each), subgroup A; rats were sacrificed 2 weeks after confirmation of being diabetic and subgroup B; rats were sacrificed 4 weeks after confirmation of being diabetic.

Umbilical cord blood collection

Human umbilical cord blood was collected after maternal informed consent in Gynecology and Obstetrics Department in Ain Shams University Hospital. Clinical data, such as pregnancy specifics and cord blood characteristics, were gathered from every donor. Venipuncture was used to obtain cord blood (volume: >20 ml) from full-term deliveries as soon as the cord was clamped and before the placenta was delivered. The blood was kept in a sterile bag with 30 milliliters of citrate phosphate dextrose within the collection bag as an anticoagulant[14]. The sterile bag containing the cord blood was delivered right away to the stem cell research lab in the Histology Department of the Ain Shams University Faculty of Medicine for processing.

Isolation, expansion, and characterization of UCB-MSCs

The cord blood was diluted at a 1:1 volume ratio with phosphate-buffered saline (PBS). Then, the blood-PBS mix was added upon Ficoll-Histopaque surface placed in sterile test tube carefully and very slowly to avoid breaking through the Ficoll layer.

The tube was centrifuged at 400G for 20 minutes. After centrifugation four layers were appeared from bottom to top, RBCs (red pellet), Ficoll (white layer), MNCs (buffy coat), plasma (yellow layer). Micropipette was used to aspirate the MNCs layer carefully without taking up too much plasma or Ficoll. Then, the MNCs layer underwent a washing process utilizing PBS followed by centrifugation for 10 minutes. The cell pellets were resuspended in complete medium (Dulbecco's modified Eagle's medium with 10% fetal bovine serum, 1% penicillin and streptomycin). The cells were seeded in appropriate tissue culture vessels and incubated in a humidified incubator with 5% CO2 at 37° C. Nonadherent cells were eliminated by changing the media three days after the first plating. After 14 days, the remaining adhering cells were checked for confluency appearance and fed twice a week. Trypsin/EDTA was utilized to extract MSCs at 90% confluence, which were then cultivated at a density of 4000 cells/cm2. The culture medium was replaced twice a week. Trypsinization and subculture was repeated, and cells of the third passage were harvested after reaching confluence and were suspended in PBS to be used in this study[14].

Using flowcytometry, the third passaged UCB-MSCs were identified by their surface markers. Anti-rat CD45 and CD73 monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC) were incubated with the cells (Thermo Fisher Scientific, USA). After being washed and preserved in 1% formaldehyde, the cells were examined using a Beckman Coulter NAVIOS.EX FACSCalibur Flow Cytometer. Cell Quest was used to examine the data.

Conditioned medium preparation

The UCB-MSCs, after 3 passages, were cultured with 1 ml of DMEM devoid of serum for a duration of 48 hours. Subsequently, the conditioned medium (CM) was collected in sterile tubes and centrifuged at 1800 rpm for 10 minutes to eliminate any cellular remnants or detached cells. The

conditioned medium was filtered through a 0.22 micrometer syringe filter to remove any potential contaminants. It was then stored in -80 °C until it was required[15].

Body weight measurement

A body weight flow chart was established to track any changes in the animals' weight throughout the investigation. At the start of the experiment, two and four weeks following the induction of diabetes, the body weight of each rat in each subgroup was measured.

Biochemical assays and enzyme-linked immunosorbent assay (ELISA)

At the time of scarification, blood samples were obtained from rats in each subgroup to measure the level of serum C-peptide, serum insulin, and glucose.

A drop of tail capillary blood was used to assess the blood glucose concentration using the glucose oxidase assay on a glucometer (Accu-Chek Active, Roche, Germany). Serum insulin concentration levels were determined utilizing a rat insulin ELISA kit (DRG, USA) and the enzyme immunoassay technique[16].

Rat insulin enzyme-linked immunosorbent assay kit (C-Peptide ELISA Kit, Sigma-Aldrich, St. Louis, MO) was used to quantify serum C-peptide.

Histological study

After anesthesia by ether inhalation, rats were sacrificed by decapitation at the end of the week 2 and week 4 of the experiment. The tail of pancreatic specimens was then collected, fixed for 24 hours in Bouin's solution, and processed for paraffin block. The blocks were sliced serially of 5 µm thick and stained with H&E.

Immunohistochemical study

By using immunohistochemistry according with the manufacturer's instructions, the amount of insulin present in the islet β-cells and the rate of apoptosis in the pancreatic islets were identified. Briefly, the pancreatic sections were deparaffinized, rehydrated, and treated with 3% H_2O_2 for 15 minutes to inhibit endogenous peroxidase activity. The sections were treated with 2% bovine serum albumin (BSA) and antigen retrieval for one hour. The sections were then treated with polyclonal anti-caspase-3 antibody (1:100; Sigma Aldrich; catalogue no. C9598) and polyclonal anti-insulin antibody (Dako, Stockport, UK; catalogue no. A0564) at a dilution of 1:200. After washing, the sections were incubated for half an hour with a secondary goat anti-rabbit peroxidase-conjugated antibody. Using 0.2% 3,3′-diaminobenzidine (DAB) stain, the antigen-antibody binding site was visible as dark brown color. To counterstain the nuclei, the sections were stained with hematoxylin. The primary antibody step was skipped to obtain the negative controls[17].

Morphometric study

The Leica Q win V.3 image analyzer program was utilized to quantify the surface area of islets of Langerhans in the sections stained with H&E and the area percentage of protein expression in immunohistochemical stained sections (surface area percentage of insulin positive cells and caspase 3 positive cells). This was carried out using an objective lens of 40X (five high-power fields, 40X/section) on five distinct non-overlapping stained slices of the pancreatic islets of Langerhans from each group's rats. Every rat's reading was considered as a single variable. The gathered data was then statistically examined.

Statistical analysis

The morphometric measurements were analyzed by IBM's Statistics for Windows SPSS version 16 software and were represented as mean ± standard deviation (SD). Tukey's test was used for multiple comparisons after a one-way analysis of variance (ANOVA). *P values* less than 0.05 indicated statistical significance in the results.

RESULTS

UCB-MSCs culture and identification

After 12 days in primary culture, the cultured UCB-MSCs achieved 85% confluence. Following repeated passaging, the MSCs developed a uniform morphology and resembled fibroblasts. Moreover, flow cytometry was used to identify MSCs at passage 3. Of these, 99.9% of MSCs were positive for the mesenchymal marker CD73, while less than 12.4% expressed the hematological marker CD45 (common leukocyte antigen) (Figure 1).

Physical parameter analysis

Diabetic rats of subgroups IIA&IIB exhibited a significant reduction in body weight as compared to the control group. Comparing the mean body weight of the UCB-MSC treated subgroups IIIA and IIIB to that of the diabetic subgroups revealed a significant rise ($P < 0.05$). Furthermore, the mean body weight of the CM-treated subgroups (IVA&IVB) was significantly greater ($P < 0.05$) than that of the diabetic subgroups. Additionally, compared to subgroup IVA, subgroup IIIA displayed a non-significant difference ($P>0.05$). Subgroup IIIB, however, showed a significantly higher mean body weight than subgroup IVB ($P < 0.05$) (Table 1).

Biochemical parameters analysis

When comparing the blood glucose levels of the diabetic subgroups to the control group, there was a significant increase ($P<0.05$). Rats in the four treated subgroups had a marked and significant ($P<0.05$) decrease in blood glucose levels following CM or UCB-MSC delivery when compared to the diabetic subgroups. Additionally, as shown in Table 1, there was no significant difference ($P>0.05$) between the CM and UCB-MSC treated subgroups. The blood insulin and C-peptide levels in the diabetic rats were significantly ($P<0.05$) lower as compared to the control group. UCB-MSCs or CM treatment led to a statistically significant ($P<0.05$) rise in their levels as compared to

the diabetic subgroups IIA&IIB. However, compared to CM treated subgroups (IVA&IVB), UCB-MSCs treated subgroups (IIIA&IIIB) demonstrated a significant rise in blood insulin and C peptide (P<0.05) (Table1).

Histological results

Pancreatic histological analysis

In the present study, the rats of control group in both subgroups A and B showed nearly similar results. H&E-stained sections of the pancreas revealed the normal architecture of pancreas. The predominant exocrine component of pancreas consisted of closely packed secretory acini. Islets of Langerhans appeared as pale oval areas surrounded by a delicate capsule inside pancreatic lobules. They appeared formed of irregular branching and anastomosing cords of cells separated by blood capillaries (Figure 2a).

Two weeks following the onset of diabetes (subgroup IIA), the pancreas displayed disarray in its endocrine structure as indicated by a noticeable decrease in the number of Langerhans cells, accompanied by the emergence of vacant areas within the islet. Some cells having deeply acidophilic cytoplasm with small darkly stained nuclei (Figure 2b). The alterations in the structural morphology of the islets were worsened after four weeks from induction of diabetes (subgroup IIB). The islets exhibited reduction in their sizes and decrease in the number of cells within it leaving many vacant areas. Many cells have small darkly stained nuclei. Some cells showed deeply acidophilic cytoplasm with eccentric flattened nuclei (Figure 2c). However, when UC-MSCs were administered (subgroup IIIA), the islet of Langerhans revealed the presence of large number of normal sized and shaped cells, however, few cells still showed small and darkly stained nuclei. Mononuclear inflammatory cellular infiltration could be seen (Figure 2d). Four weeks after stem cell therapy (subgroup IIIB) showed nearly restoration of normal morphological structure of the islets as number of islets cells were apparently increased with marked reduction in the empty spaces. The cells were pale stained with vesicular nuclei (Figure 2e). Treatment with CM (subgroup IVA) still showing focal structural changes of islet of Langerhans. Some empty spaces existed within the islet with small darkly stained nuclei in some cells. Capillary congestion and mononuclear inflammatory cellular infiltration were also observed (Figure 2f). Four weeks after CM treatment (subgroup IVB) revealed improvement in morphology with apparent rise in the size and number of islet cells. Few cells showed small and darkly stained nuclei (Figure 2g).

A significant (P<0.05) decrease in the mean surface area of the islets was seen in the diabetic group (subgroups IIA and IIB) in comparison to the control group. Conversely, the mean surface area of islets exhibited a significant (P<0.05) rise in UCB-MSCs and CM treated subgroups when compared to diabetic subgroups. On the other hand, compared to subgroup IVB treated with CM, stem cell-treated subgroup IIIB demonstrated a statistically significant rise in the mean surface area of islets (P<0.05) (Table 2, Figure 3).

Immunohistochemical analysis for anti-insulin-stained pancreatic sections

Nearly all the beta cells of islets of Langerhans showed strong cytoplasmic staining for insulin antibody in the control group (Figure 4a). A moderate decrease in islet cell positive immune reaction was observed in diabetic subgroup IIA (Figure 4b). Subgroup IIB had significantly reduced immunoreactivity, primarily in the outer region of the islets (Figure 4c). After receiving stem cell treatment for two weeks (subgroup IIIA), numerous islet cells displayed a mild immune response (Figure 4d). Most of the islet cells showed intensely positive immune reaction for insulin in sections of subgroup IIIB (Figure 4e). Some cells in the islets of Langerhans exhibited moderately positive immune reaction in subgroup IVA (CM treated) (Figure 4f). Numerous cells of the islets exhibited strong positive reaction in subgroup IVB (Figure 4g).

The mean area percentage of insulin immune positive cells in group II exhibited a significant reduction ($P < 0.05$) in comparison to the control group. In contrast, groups III and IV demonstrated a significant rise ($P < 0.05$) in the mean area percentage of insulin immunopositivity following the transplantation of UCB-MSCs and the injection of their conditioned medium after a duration of 4 weeks in comparison to the diabetic group. Nevertheless, it is worth mentioning that the subgroup IIIB, which was treated with stem cells, exhibited a remarkable rise in the mean area percentage of insulin positive cells as opposed to the subgroup IVB, which was treated with CM ($P < 0.05$) (Figure 5, Table 2).

Immunohistochemical analysis for caspase 3-stained pancreatic sections

Caspase-3 immunohistochemical staining exhibited a lack of reactivity in most cells within the control group (Figure 6a). In contrast, the diabetic subgroups displayed a strong positive immune response to caspase-3 in the cytoplasm and nuclei of a considerable number of cells within the islet of Langerhans, in comparison to the control group (Figures 6b,c). The subgroups treated with stem cells and conditioned media also demonstrated a mild to moderate positive immune response towards caspase-3 (Figures 6d-g).

Quantitatively, subgroups IIA and IIB showed a significant rise ($P < 0.05$) in the proportion of caspase-3 immune-positive cells as compared to the control group. Subgroups IIIA and IIIB, which were treated with UCB-MSCs, as well as subgroups IVA and IVB, which received CM treatment, demonstrated a significant decrease ($P < 0.05$) in the average proportion of caspase-3 immune-positive cells as compared to subgroups IIA and IIB. However, subgroup IIIA exhibited a substantial reduction in the mean proportion of caspase-3 compared to subgroup IVA ($P < 0.05$). Furthermore, subgroup IIIB displayed an insignificant difference ($P > 0.05$) when compared to subgroup IVB (Figure 7,Table 2).

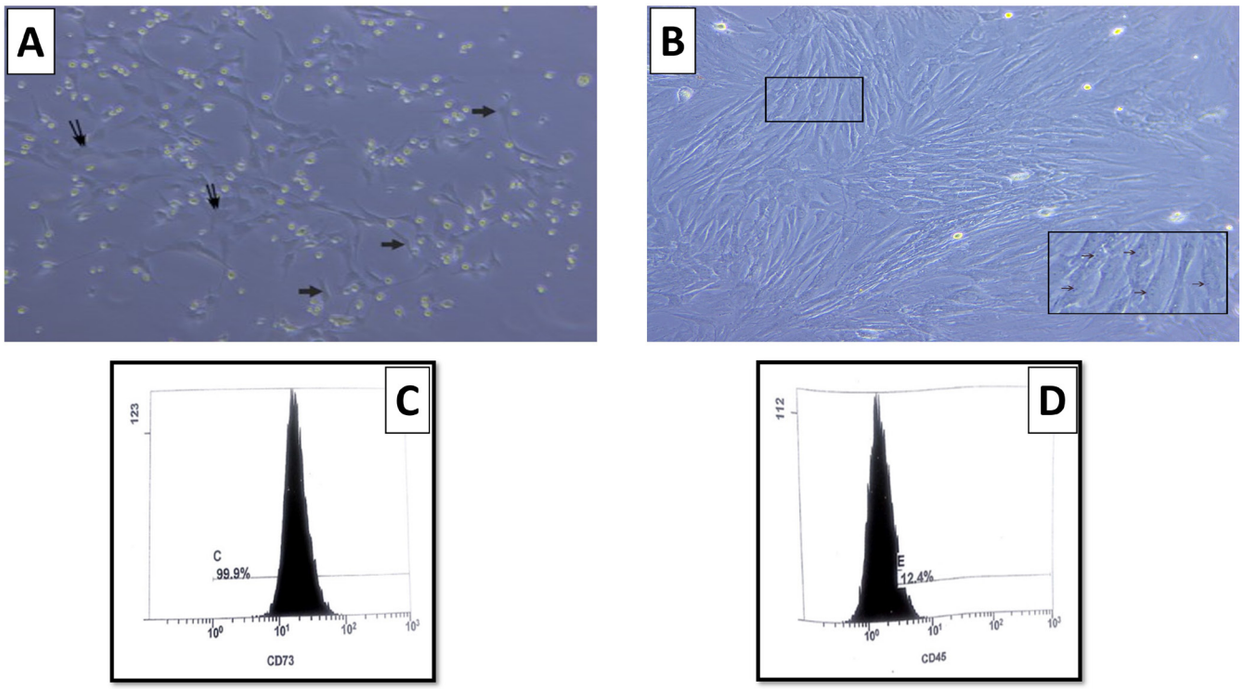

Fig.1: Morphology and characterization of UC-MSCs. (A) on day 12 after initial seeding, UC-MSCs exhibiting spindle-shaped cells with vesicular nuclei and granular cytoplasm (↑) reaching about 80% confluency. (B) Passage 3 reveals UC-MSCs consisting of a uniform population of spindle-shaped cells with cytoplasmic processes, granular cytoplasm, and vesicular nuclei. Notably, numerous nuclei exhibit more than one nucleolus (inset ↑). (Phase contrast microscopy x100). (C&D) Flowcytometric analysis of the UC-MSCs at passage 3 showing more than 99% of the cells express CD73, whereas they are negative for CD45.

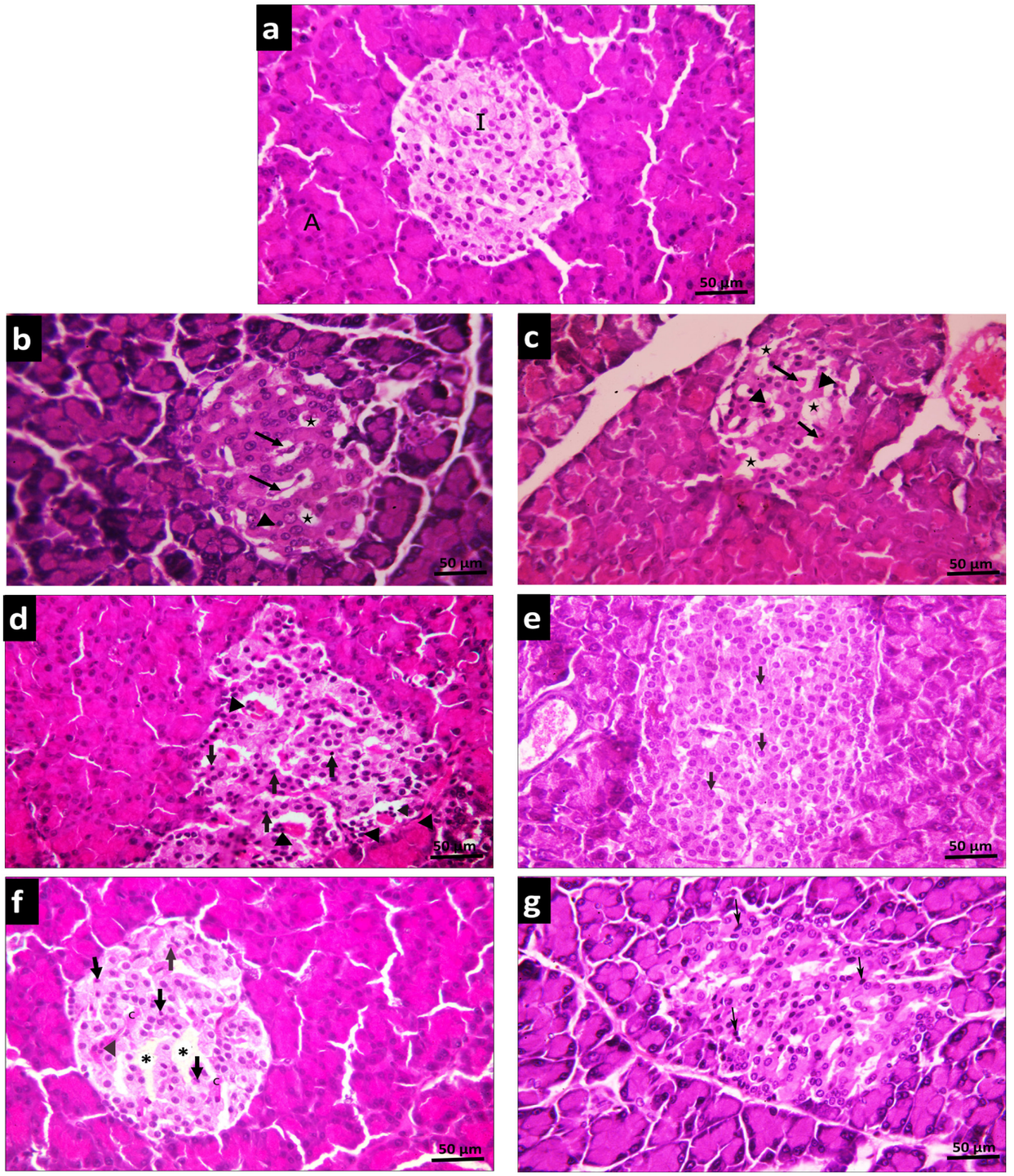

Fig. 2: Histological examination of Langerhans islets of pancreas (H&E, x400). (a) Control group I showing an islet formed of pale stained cells (I). The islet cells are variable in size having vesicular nuclei and surrounded with pancreatic acini (A). (b) Subgroup IIA showing some cells having deeply acidophilic cytoplasm (▲) with small and darkly stained nuclei. Many areas of the islet show empty spaces (*). Notice some cells appear shrunken, irregular, and vacuolated within the islet (↑). (c) Subgroup IIB showing apparent decrease in number of cells of the islet of Langerhans with some areas of empty spaces (*) within the islet. Many cells have small and darkly stained nuclei (▲). Notice some cells have deeply acidophilic cytoplasm with eccentric flattened nuclei (↑). (d) Subgroup IIIA showing apparent increase in the number of cells of the islet of Langerhans. Few cells have small and darkly stained nuclei (↑). Mononuclear inflammatory cells infiltration (▲) could be seen. (e) Subgroup IIIB showing nearly regular outline of an islet. Notice an apparent increase in the cellularity within the islet and marked reduction in empty spaces. Most of cells possess vesicular nuclei (↑). (f) Subgroup IVA showing an islet of Langerhans with small and darkly stained nuclei in some cells (↑) associated with capillary congestion (c) and mononuclear inflammatory cellular infiltrations (▲). Some empty spaces are seen within the islet (*). (g) Subgroup IVB showing apparent increase in the cellularity within the islet of Langerhans. Few cells show small and darkly stained nuclei (↑).

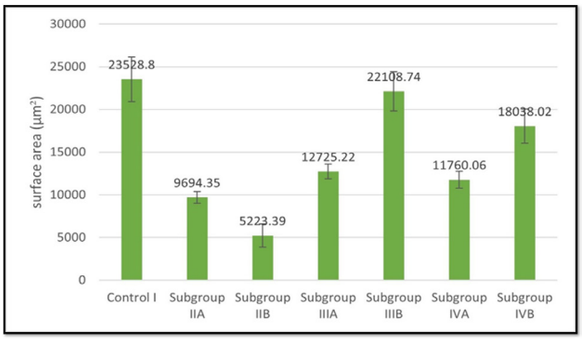

Fig. 3: Histogram showing the mean surface area (µm²) of islets among various study subgroups.

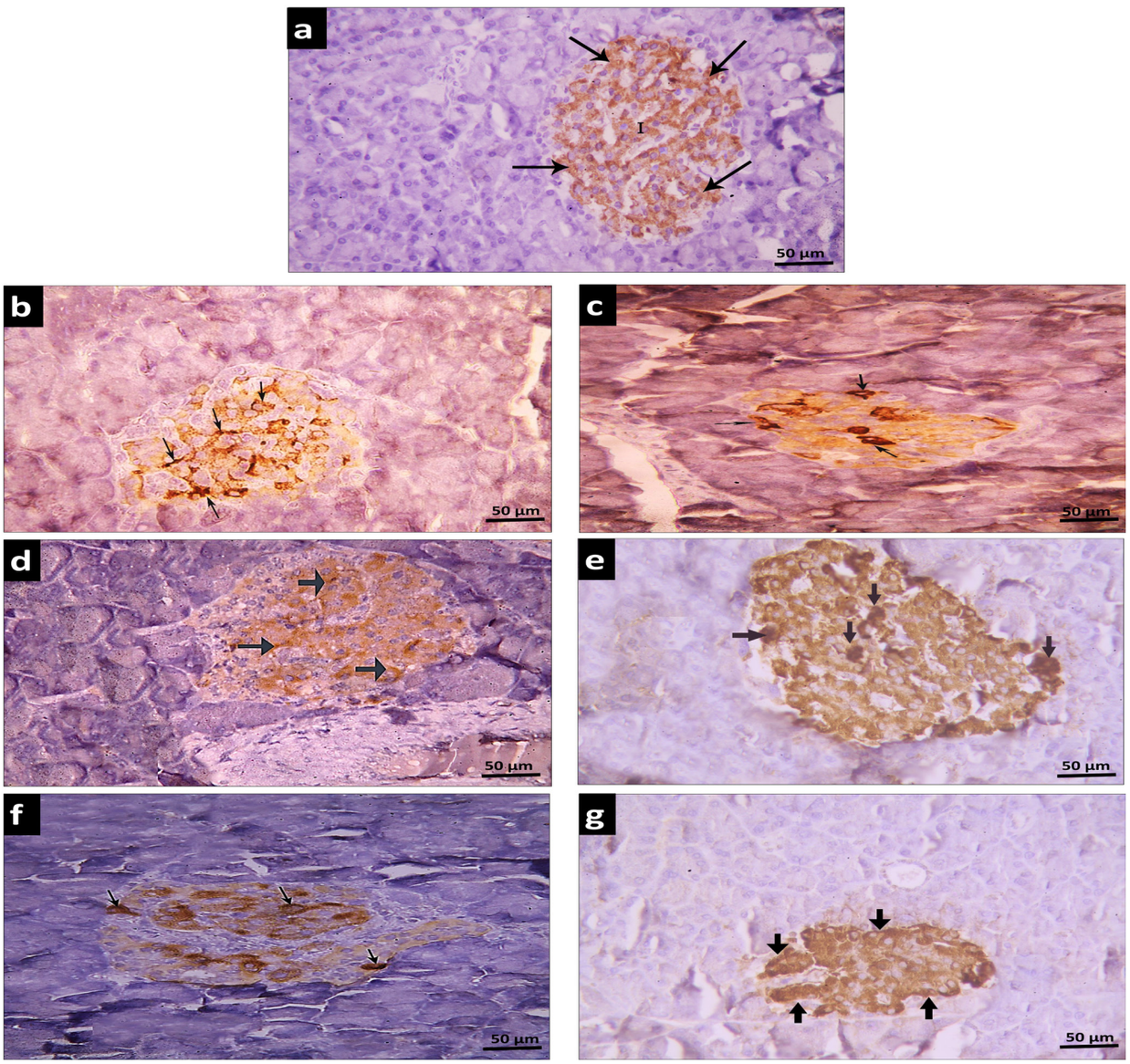

Fig. 4: Immunohistochemical detection of anti-insulin antibody in beta cells of pancreas. (a) Group I (control group) is demonstrating a Langerhans islet (I) that exhibits a high degree of cytoplasmic immunoreactivity (↑) in the majority of its cells. (b) Subgroup IIA showing moderate positive immunoreactivity in some cells of the islet of Langerhans (↑). (c) Subgroup IIB showing weak brownish reaction in the cytoplasm of most of beta cells. Notice only few cells exhibit positive reaction (↑). (d) Subgroup IIIA showing moderate brownish reaction in the cytoplasm of most of the beta cells (↑). (e) Subgroup IIIB showing intense positive reaction in the cytoplasm of most of beta cells (↑) of the islet. (f) Subgroup IVA showing moderate positive reaction in the cytoplasm of some beta cells (↑) of the islet. (g) Subgroup IVB exhibiting intense positive reaction in the cytoplasm of most of beta cells (↑) of the islet. (Anti-insulin Immunostaining x400)

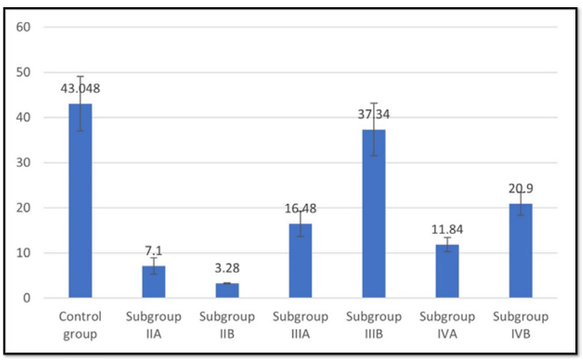

Fig. 5: Histogram showing the mean area percentage of insulin immune-positive cells across various study subgroups.

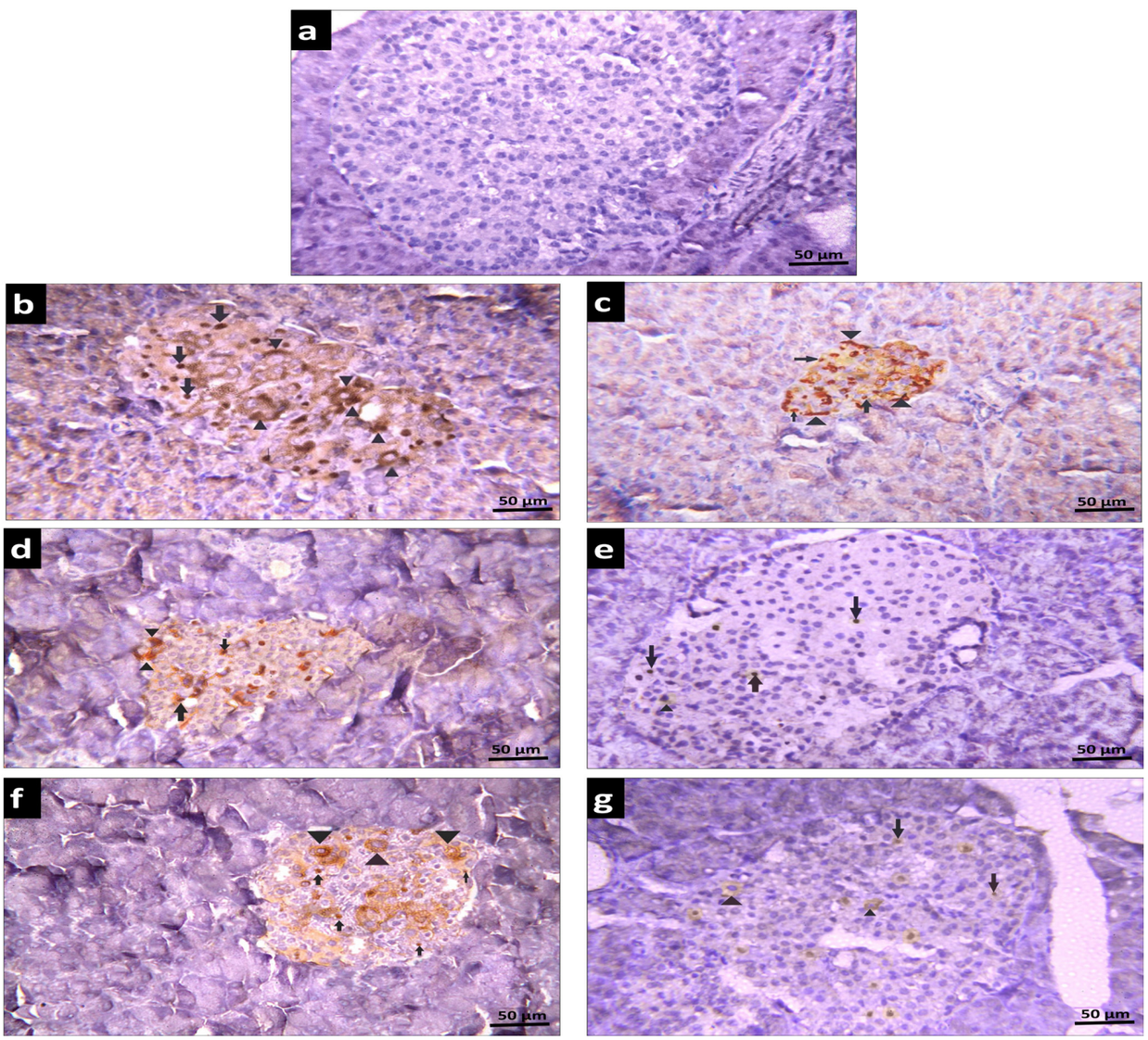

Fig. 6: Immunohistochemical detection of caspase 3. (a) control group I reveals negative reaction in most cells of the islet of Langerhans. (b) Subgroup IIA exhibits intense positive immune reaction in cytoplasm of many cells of the islet (▲). Notice nuclear expression of the other cells (↑). (c) Subgroup IIB showing intense positive immunoreactivity in cytoplasm of many cells of the islet (▲) and immune expression in the nuclei of other cells (↑). (d) Subgroup IIIA showing moderate positive immunoreactivity in cytoplasm of some cells of the islet (▲) and nuclear expression of the other cells (↑). (e) Subgroup IIIB showing few cells with mild positive nuclear reaction (↑) and cytoplasmic reaction (▲) for caspase-3 in the islet of Langerhans. (f) Subgroup IVA showing moderate positive reaction in some cells of the islet of Langerhans. Some cells exhibit positive immunoreactivity in the cytoplasm (▲), other cells show nuclear expression (↑). (g) Subgroup IVB showing an islet with few cells having mild positive nuclear reaction (↑) and cytoplasmic reaction (▲) for caspase 3. (Caspase-3 immunohistochemical staining x400)

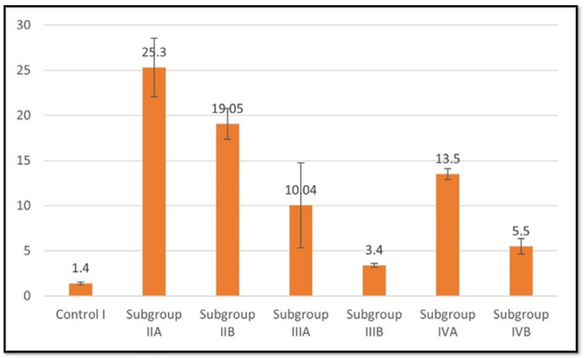

Fig. 7: Histogram showing the mean area percentage of caspase-3 immune-positive cells among various study subgroups.

Table 1: Illustrating the significance difference between mean ± standard deviation of the body weight (gram), level of blood glucose (mg/dL), serum insulin level (µU/mL), and C-peptide level (ng/ml) across various study subgroups.

Groups	Mean of body weight (grams)± SD	Mean level of blood glucose (mg/dl) ± SD	Insulin level (µU/mL) ±SD	C-peptide level (ng/ml) ±SD
Control group I	185.2 ± 10.3	120 ± 6.4	49.88±2.25	0.278 ±0.063
Subgroup IIA	169.811.8±[a]	352± 60.5[a]	17.74±1.08[a]	0.113±0.007[a]
Subgroup IIB	154.88.07±[ab]	558± 34.97[ab]	9.84±0.47[ab]	0.086±0.001[ab]
Subgroup IIIA	192.6± 11.3b[ab]	192± 18[ab]	27.5±1.31[ab]	0.175±0.007[ab]
Subgroup IIIB	235± 7.9[ac]	136± 5.2[c]	42.34±3.07[ac]	0.254±0.003[ac]
Subgroup IVA	185± 14.5[b]	217± 23.16[ab]	22.5±1.59[abd]	0.156±0.006[abd]
Subgroup IVB	212 ± 7.8[ace]	160± 8.1[ac]	34.5±1.59[ace]	0.21 ±0.015[ace]

Values are expressed as means ±SD. Significance calculated by (LSD= least significant difference) at $P<0.05$ [a] Significant change from control group I, [b] significant change from subgroup IIA, [c] significant change from subgroup IIB, [d] significant change from subgroup IIIA, [e] significant change from subgroup IIIB

Table 2: Illustrating the significance difference between mean ± standard deviation of mean surface area of islets of Langerhans (µm^2), mean area % of insulin immunopositively cells, and mean area % of caspase-3 immune positive cells across various study subgroups.

Groups	Mean surface area of islets (µm^2) ±SD	Mean area % of insulin immunopositively cells ±SD	Mean area % of caspase-3 immune positive cells (±SD)
Control group I	23528.8 ± 2600.75	43.048 ± 6.04	1.4 ± 0.15
Subgroup IIA	9694.35 ± 678.78[a]	7.1± 1.8[a]	25.3 ± 3.23[a]
Subgroup IIB	5223.39 ± 1368.04[ab]	3.28 ± 0.14[ab]	19.05 ± 1.71[ab]
Subgroup IIIA	12725.22 ± 862.88[ab]	16.48± 2.81[ab]	10.04 ± 4.78[ab]
Subgroup IIIB	22108.74 ± 2296.5[c]	37.34± 5.8[ac]	3.4 ± 0.22[c]
Subgroup IVA	11760.06 ± 1007.34[ab]	11.841.57±[ab]	13.5 ± 0.6[abd]
Subgroup IVB	18038.02 ± 1991.99[ace]	20.92.52±[ace]	5.5 ± 0.87[ac]

Values are expressed as means ±SD. Significance calculated by (LSD= least significant difference) at $P<0.05$ [a] Significant change from control group I, [b] significant change from subgroup IIA, [c] significant change from subgroup IIB, [d] significant change from subgroup IIIA, [e] significant change from subgroup IIIB

DISCUSSION

The present study was designed to compare the efficacy of mesenchymal stem cells derived from human umbilical cord blood and their conditioned medium on the beta cells of islets of Langerhans of the pancreas in an experimentally induced rat model of diabetes mellitus. In the current study, diabetes mellitus type I was induced by administrating a single intraperitoneal injection of 1ml of streptozotocin 35 mg/kg body weight. Streptozotocin (STZ) was chosen in this experiment as it was previously proved by many authors to induce diabetes mellitus in experimental animal both biochemically and histologically[18].

In the current study, diabetic subgroup IIA revealed severe injury in the Langerhans islets including decrease the number of cells with appearance of some empty spaces which were assumed that were previously occupied by islet cells. More noticeable structural changes were observed in subgroup IIB, many cells showed features of apoptosis in the form of small deeply stained nuclei (pyknotic nuclei), deeply acidophilic cytoplasm in other cells indicating acidophilic degeneration. Capillary congestion inside islets and mononuclear inflammatory cellular infiltration were also noticed. A significant reduction (P<0.05) in surface area of islets of Langerhans in both subgroups was observed. In agreement with these findings, previous studies demonstrated that the administration of STZ through parenteral means, at elevated dosages, results in the targeted damage of beta cells that secreting insulin. This leads to the manifestation of type 1 diabetes in adult animals[19-20]. They attributed the observed empty spaces within the islets to the activity of phagocytic cells responsible for cleaning. In addition, there was observed deterioration of islets and degeneration of acini accompanied by vascularization and capillary congestion, along with the infiltration of inflammatory cells, mainly T-lymphocytes. The pathogenesis in type 1 diabetes mellitus is associated with T-cell infiltration of the pancreatic islets and is characterized by a progressive T-cell-mediated destruction of the insulin-secreting beta-cells[21]. These changes most likely led to a comparative decrease in the mass and quantity of islets. Furthermore, the presence of pyknotic nuclei, vacuoles, and remnants of degenerated beta cells with deeply acidophilic cytoplasm were also identified[22].

When comparing subgroups IIA and IIB to the control group, immunohistochemical staining for anti-insulin sections showed a significant reduction in the mean area percentage of insulin immune-positive cells. The reduction in insulin positive response resulted from both loss of insulin production and direct damage to beta cells. Other researchers have also provided an explanation for this, mentioning that STZ interferes with the cellular metabolic oxidative systems[23].

The mean area percentage of caspase-3 positive cells in the diabetes subgroups increased significantly as compared to the control group. The apparent drop in the cell count and the size of the islets were thought to be the causes of the outcome. According to reports, the final stage of beta cell death in diabetes type I is apoptosis. The death receptor signal, mitochondria, and endoplasmic reticulum stress are the three main mechanisms that contribute to beta cell apoptosis and boost caspase-3 activity[24].

In addition, subgroups IIA and IIB showed significant elevation in the level of blood glucose associated with significant reduction in body weight. Laboratory pancreatic serum insulin level and C-peptide level showed significant reduction in subgroup IIA and subgroup IIB in comparison to control group. It was stated that serum insulin level is an important diagnostic marker for diabetes mellitus. Some researchers recorded that the decrease in insulin level and C-peptide level was due to significant loss of beta cells[25]. Moreover, other investigators stated that in mice model of diabetes induced by STZ administration caused significant reduction in body weight and significant increase in glucose level. They attributed that prolonged exposure to hyperglycemia caused oxidative stress which resulted in glycosylation and oxidation of proteins involved in the pathogenesis of diabetes[26].

After intravenous UCB-MSC delivery in subgroups IIIA&IIIB of the current study, the pancreatic architecture was almost identical to that of the control group. There was a notable rise in the area percentage of insulin immune-positive cells to subgroups IIA and IIB. In the meantime, a significant reduction in caspase-3 positive cells was noticed. Furthermore, a significant rise in the body weight, serum insulin and C-peptide levels and a significant drop in the mean blood glucose level in subgroups IIIA and IIIB were observed as compared to the diabetic group. Similar findings were reported in few earlier investigations[27-28].

Two theories were proposed to explain how mesenchymal stem cells were able to preserve the pancreatic islets of Langerhans. It was believed that the first one, which expressed trophic and immunomodulatory substances, included paracrine pathways. Even if the stem cells were unable to settle in the wounded tissues, the trophic factors could regenerate them through the growth factors, anti-apoptotic, immunomodulatory, and angiogenic factors[29]. The increase in pancreatic islet cellular proliferation shown by an increased positive reaction in immunohistochemical staining for anti-insulin and the significantly higher mean area percentage of insulin-positive cells in the UCB-MSC treated group (subgroups IIIA and IIIB) compared to the diabetic group (subgroup IIA and IIB) in the current study might support this assumption.

The second theory suggests that mesenchymal stem cells preserve the pancreas by differentiating into islets of Langerhans beta cells. It was discovered that the mesenchymal stem cells endured and developed into beta cells, resulting in an expansion in the quantity and dimensions of Langerhans cells[30].

The unique benefits of UCB-MSCs, including their painless collecting process, ease of availability, high

differentiation efficiency, rapid self-renewal, and decreased risk of graft versus host illness, made them the favored stem cell type in the current study. A unique combination of prenatal and postnatal MSC properties, no ethical issues with biomaterial acquisition, strong proliferative and differentiation potential, lack of tumorigenicity, karyotype stability, and strong immunomodulatory activity have been added to the benefits of using human umbilical cord blood as a source of MSCs[31]. Prior research demonstrated that these cells did not result in tumor growth or an immunological rejection reaction when grafted into normal rat tissues[32-33].

In the current study, administration of the conditioned medium (CM) intramuscularly was based on cytokines and growth factors present within it that will influence the local cells at the wounded location to trigger the tissue healing process. The CM resulted into improvement in the structure of the islets of Langerhans. This improvement appeared as an apparent increase the cellularity within the islet where most of cells showed vesicular nuclei and significant rise (P<0.05) in surface area of the islets. A significant rise in the area percentage of insulin immunopositively cells was observed in comparison to both subgroup IIA and IIB. Significant decrease in caspase-3 positive cells was also detected. Same results were reported by other researchers[34-35].

Furthermore, the mean value of blood glucose level in CM treated group (subgroups IVA and IVB) was significantly decreased as compared to the diabetic group (subgroups IIA and IIB). Moreover, body weight showed significant increase in these subgroups. In the present study serum insulin level and C-peptide level in subgroups IVA and IVB were significantly increased compared to diabetic subgroups.

According to some reports, MSCs generate and secrete various chemokines, cytokines, and growth factors which affect neighboring cells' behavior. These substances that are secreted increase angiogenesis, decrease fibrosis and apoptosis, improve neuronal survival and differentiation, drive remodeling of the extracellular matrix, limit inflammation locally, and modulate immunological responses[36].

Additionally, under hypoxic conditions, the UC-MSC exosomes in the conditioned medium dramatically enhanced the sustainability of the islet beta cells and prevented their apoptosis. UC-MSC exosomes increased the antiapoptotic protein and downregulated the apoptotic proteins that cleaved caspase-3. It was determined that UC-MSC exosomes may help diabetic patients by enhancing the survival and function of encapsulated islets[35]. Likewise, the C2C12 cell line's insulin resistance was shown to be improved by the administration of CM. Treatment with CM derived from umbilical cord blood-MSCs enhanced the insulin-signaling pathway and the translocation of glucose transporter type 4 (GLUT4). Also, the contents and functions of the mitochondria were enhanced[37].

CONCLUSION

It was concluded that transplantation of umbilical cord derived mesenchymal stem cells and their conditioned medium could be utilized in the treatment of STZ-induced diabetes. Because UCB-MSCs preserve pancreatic tissues better than CM, their application may be more efficacious.

RECOMMENDATION

UCB-MSCs are known to have β-cell regeneration and immunoregulatory capabilities, but further investigations are needed before clinical applications may be tried. Therefore, it is advised to employ CM as a novel cell-free therapy method with wide applicability for the treatment of illness and injury that has positive results and no serious safety concerns.

CONFLICT OF INTERESTS

There are no conflicts of interest..

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الملخص العربى

دور الخلايا الجذعية الوسيطة المشتقة من دم الحبل السري البشرى مقابل الوسط المُكَيف لها في تجديد خلايا البنكرياس بيتا في نموذج لمرض السكرى في الجرذان

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الخلفية: تم الحصول على مصدر بديل فعال للخلايا الجذعية الوسيطة من دم الحبل السري. لذلك، صممت هذه الدراسة لتقييم دور الخلايا الجذعية الوسيطة المشتقة من دم الحبل السري البشرى مقابل الوسط المكيف لها على تجديد خلايا البنكرياس بيتا في نموذج لمرض السكري فى الجرذان.

المواد والطرق: تم تقسيم أربعين من ذكور جرذان وستر البالغة بالتساوي إلى أربع مجموعات. المجموعة الأولى (الضابطة)، المجموعة الثانية (مرضى السكري)، تم حقن كل جرذ داخل الغشاء البروتوني بجرعة واحدة مقدارها ٣٥ مل/كجم من الستربتوزوتوسين. بعد التأكد من مرض السكري، تركت الجرذان دون علاج، المجموعة الثالثة (المعالجة بالخلايا الجذعية)، حقنت ١x١٠٦ خلية / مل من الخلايا الجذعية مرة واحدة في الوريد الذيل بعد التأكد من كونهم مرضى السكري، المجموعة الرابعة (المعالجة بالوسط المكيف)، حقنت في العضل ٠,٥ مل من الوسط المكيف مرة واحدة في الأسبوع بعد الإصابة بمرض السكري حتى نهاية التجربة. تم ذبح الجرذان بعد أسبوعين وأربعة أسابيع من الاصابة بمرض السكري، تمت معالجة عينات البنكرياس من جميع المجموعات للصبغ بصبغة الهيماتوكسلين والإيوسين وللتفاعل المناعي النسيجي الكيميائي للأجسام المضادة للأنسولين ومضادات الكاسباز -٣.

النتائج: أظهرت مجموعة السكري تشويها في بنية جزر لانجرهانز ونتج عنها انخفاض حجم الجزر وظهور العديد من المساحات الفارغة داخلها. كان هناك انخفاض كبير في وزن الجسم، والأنسولين في الدم ومستوى الببتيد C ، وفي الخلايا الإيجابية بالأنسولين المناعي. علاوة على ذلك، حدث ارتفاع كبير في نسبة الجلوكوز في الدم وفي الخلايا الإيجابية بالمواد الكيميائية المناعية الكاسباز -٣. أظهرت المجموعات الفرعية المعالجة بالخلايا الجذعية الوسيطة المشتقة من دم الحبل السري والوسط المكيف تحسنا نسيجيا وكيميائيا حيويا واضحا عند مقارنتها بمجموعة مرضى السكري.

الاستنتاج: كان زرع الخلايا الجذعية الوسيطة المشتقة من دم الحبل السري البشرى أكثر فعالية في تجديد خلايا بيتا البنكرياس من حقن الوسط المكيف لها في مرض السكري من النوع الأول الناجم عن الستربتوزوتوسين. ومع ذلك، يمكن أن يمنع الوسط المكيف تقدم التدمير في خلايا بيتا في جزر لانجرهانز كما يتضح من تأثيره المضاد للالتهابات والمضاد لموت الخلايا المبرمج.