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RESEARCH ARTICLE

Umbilical Cord Blood Mesenchymal Stem Cells Mitigated Diabetic Hepato-Renal Insufficiency in Alloxan-Induced Type 1 Diabetes in Dogs: Biochemical and Histopathological Approach

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ABSTRACT

Canine diabetes mellitus (DM) is a common metabolic endocrine condition, characterized by persistent hyperglycemia and insulin insufficiency. DM has severe irreversible pathological disorders including retinopathy, neuropathy, hepatopathy, and nephropathy. The search for alternative approaches for restoring pancreatic endocrine function is therefore of paramount clinical interest. Umbilical cord blood mesenchymal stem cells (UCB-MSCs) were isolated and identified as one of Mesenchymal stem cells (MSCs) which have a regenerative role by enhancing resident stem cells and soluble factors that stimulate the internal repairing process. Nine mongrel dogs were randomly allocated into three equal groups, 3 dogs each: control, diabetic, and diabetic-UCB-MSCs treated group. The isolated cells displayed surface markers of MSCs cluster of differentiations (CD) CD90, CD105, and CD73. Moreover, the findings showed that UCB-MSCs transplantation in diabetic dogs induced a remarkable decrease in fasting blood glucose (FBG) level, AST, ALT, ALP, γ-GT, total protein (TP), albumin, blood urea nitrogen (BUN), and serum creatinine than the diabetic group. Additionally, UCB-MSCs administration markedly improves hepatic and renal oxidative status besides improving the histopathological changes with appearing multiple regenerative signs. Therefore, MSCs provide a promising therapeutic strategy for DM-associated disorders in dogs.

Keywords: Diabetes mellitus, Dogs, UCB-MSCs, and Oxidative stress.

Introduction

DM is a widespread long-lasting metabolic disease that is associated with several complications and causes high mortality among diabetic population. It is characterized by an abnormality in protein, fat, and carbohydrates metabolism [1-3].

Additionally, Because of metabolic acidosis, osmotic diuresis, dehydration, and, in the event of extreme hyperosmolarity, coma, cats and dogs with diabetic ketoacidosis may have markedly high blood glucose concentrations, azotemia, and decreased total CO2. Glucose will be detected through urinalysis. Additionally, it might demonstrate the presence of casts, germs,

ketones, and/or protein. A urine culture should always be carried out in glucosuric animals since urinary tract infections are frequently present and cannot be ruled out by the absence of an active urine sediment [4,5].

The main clinical manifestations of diabetes mellitus (DM) are polyuria, polydipsia, polyphagia, and weight loss [6-8]. Diabetic complications are two types as microvascular and macrovascular. The microvascular one includes retinopathy, nephropathy, and neuropathy. The primary macrovascular complications are cerebrovascular disease manifested or presented as strokes, accelerated cardiovascular disease, myocardial infarction and liver dysfunction [9-11].

Persistent hyperglycemia is the main cause of diabetic complications which lead to free radicles production as superoxide anion, hydroxyl radicals and glucose autoxidation [12,13].

DM is related to some of hepatic troubles that damages hepatic tissue such as unusual glycogen accumulation, cirrhosis, fibrosis, hepatic carcinomas, chronically high levels of hepatic enzymes, acute liver diseases, and hyperglycemia [14]. Furthermore, liver damage caused by free radicals production leads to hepatic inflammatory response, hepatocyte apoptosis, and fibrogenesis [15,16]. Diabetic nephropathy (DN) is a recurrent consequence of DM that is responsible for finally occurring renal failure. There are numerous mechanisms sharing in the complex pathophysiology of renal disorders in diabetic animals; angiopathy of glomerular capillaries in the kidney glomeruli which results from persistent elevated blood glucose level, followed by oxidative stress release, apoptosis, inflammation, cirrhosis and endoplasmic reticulum (ER) stress [17]. DN causes kidney injury or renal dysfunction that is indicated by proteinuria and noticeable rise in the levels of serum creatinine and urea [18,19].

Renal oxidative stress brought on by hyperglycemia resulting from lowering mitochondrial membrane potential, which consequently result in an increase reactive nitrogen and oxygen species (RNS/ROS).This altered macro- and microvascular structure resulted in DNA destruction, precipitation of extracellular matrix protein overexpression, mesangial growth, atrophy of glomeruli and glomerular fibrosis [20]. Moreover, Oxidative stress can also cause the release of proinflammatory cytokines and chemokines, which can lead to inflammation and additional kidney damage [21].

DN is associated with the destruction of podocytes, which leads to glomerulosclerosis, cellular hypertrophy, and podocytopenia [22].

Podocytes have a restricted regeneration capacity, in contrast to additional forms of cells. As a result, when podocytes are destroyed, the glomerular filtration barrier becomes leaky, which exaggerates podocyte damage and causes proteinuria [23].

Mesenchymal stem cells (MSCs) have the potential to be used as a cell-based therapy to treat several illnesses, including diabetes, liver damage, and neurodegenerative disorders [24]. MSCs are isolated from various tissue types, such as umbilical cord (UC-MSCs), bone marrow (BM-MSCs) and adipose tissue (AD-MSCs) [25,26]. MSCs considered one of the most promising valid regenerative tools as displayed an immunomodulatory, anti-inflammatory,

and antiapoptotic activity. MSCs can also privilege the immune system since it does not express the human leukocyte derived antigen (HLA-DR) thus, their transplant rejection is scanty or absent [27,28].

Furthermore, MSCs possess antioxidant and neoagiogenic activity via secreting several growth and trophic factors as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin like growth factor 1(IGF-1), heme oxygenase 1 (HO-1), and indoleamine 2,3 dioxygenase (IDO) that involved in the activation of the internal repairing mechanism via a paracrine activity [29-31]. Interestingly, MSCs can significantly improve the renal function, inhibit inflammation and fibrosis, and arrest the progress of early DN. These outcomes lay the base for the therapeutic use of UCB-MSCs as an innovative DN therapy approach [32]. Furthermore, it was reported that UC-MSC infusion enhanced liver function [33]. Among those cells is UCB-MSCs that exert higher regenerative potency and plasticity because they express to what extent many embryonic transcription factors such as OCT4, NanoG, and REX-2 that make them a potential candidate for our study.

Thus, the aim of this study was to explore UCB-MSCs' effects in alleviating hepatic and renal complication of DM, and to the best of our knowledge it's the first record for the transplantation of the UCB-MSCs in diabetic dogs to alleviate the hepato-renal complication of T1DM in dogs.

Materials and methods

Animals

In this investigation, nine adult mongrel male dogs were purchased from the animal house faculty of Veterinary Medicine Zagazig University. The dogs were clinically healthy by physical examination, normal full serum biochemical analysis, and urinalysis. At the beginning of the experiment, the dogs' ages were 1.67 ± 0.4 years old with average body weight was between 19-24 kg. All dogs were housed in a separate metal cage in the same environmental, hygienic and nutritional circumstances through the whole experimental period and fed a home-made diet containing chicken, bone, bread and rice twice a day along with free access to water. The environmental conditions for the animal house were 24 ^{o}C, 65% relative humidity, and 12/12 h light/dark cycle. Before the start of the experiment, each dog received a protective dose (1ml/ 50 Kg body wt S/C) of anthelmintic medication (Dectomax ®, Zoetis, USA) before starting the experiment to ensure that no dog has any external or internal parasites. Internal parasite infestation was excluded by performing fecal examination according to method described by Mosallanejad *et al.* [34] and Abdullaziz *et al.* [35]. All dogs were kept for a period of 2 weeks for acclimatization.

Experimental design

Nine mongrel adult dogs were randomly allocated into three equal groups 3 dogs each:

Control group: dogs received a single I/V dose of phosphate buffer saline (PBS) at the time of diabetes induction and another two doses during the transplantation of the UCB-MSCs. Diabetic group: dogs received a single I/V dose of alloxan 50 mg/kg body weight and PBS at the time of the MSCs transplantation [36], and diabetic-UCB-MSCs treated groups: one month post diabetes induction dogs were received two I/V doses of UCB-MSCs 5 x 10^6 cell/kg in the cephalic vein with 2 weeks

interval. Three blood samples were collected after one week, 4 weeks, and nine weeks for all experimental animals, then serum was separated and stored at -20 ^{o}C until performing the biochemical analysis. After that, the dogs were euthanized and tissue samples of the liver and kidney were collected in divided into two parts, first part was collected on 10% neutral formalin buffer solution for histopathological examination and the second part was preserved in -80 ^{o}C for the oxidant/antioxidant activity.

Induction of diabetes mellitus by alloxan-monohydrate (ALX)

All experimental dogs were fasted over the whole night before receiving ALX , with unrestricted access to water. Induction of type 1 diabetes using the technique previously outlined by Watanabe *et al.* [36]. Diabetic dogs injected with a single intravenous dose of 50mg/kg body weight, using 5 % ALX (sigma-Aldrich), dissolved in physiological saline, to all diabetic group under fasting conditions. The preparation of the drug and the injection were done rapidly to avoid the effect of denaturation because ALX is highly reactive and unstable. To avoid hypoglycemic effect of ALX post administration, all the experimental dogs received 10% glucose IV [37]. Three days post administration of ALX, glucometer was employed to measure the fasting blood glucose level which displayed a FBG exceeding 250 mg/dL considered diabetic and was enrolled in the study.

Blood and tissue samples

After 6 h of fasting, 5 ml of blood sample was collected from the cephalic vein of each dog without anticoagulant that was left for proper coagulation then the samples were centrifuged at 3000 rpm for 15 min. Serum samples were separated and stored at -20 °C until measuring the biochemical parameters. Liver and kidney samples were collected and divided into 2 parts first part were collected on 10% neural formalin buffer and second part was wrapped in aluminum foil at stored at -80 °C for the measuring of the oxidant/antioxidant activity in the tissue homogenate.

All dogs were evaluated and monitored for nine weeks after accommodation period and starting the experiment by through clinical examination, fasting blood glucose measurement. Histopathological examination and oxidative stress measurement were applied for the three groups. At the end of the study, dogs were euthanized using pentobarbital 100 mg/kg IV [38].

Ethical statement

The sample of cord blood was collected from the Zagazig University hospitals. All the samples were collected under aseptic conditions in 50 ml capacity sterile falcon tubes containing heparin and DMEM media (Lonza, Belgium) with 1% penicillin-streptomycin-amphotericin from the donor with already written consent. All methods were performed in accordance with ARRIVE guidelines (https://arriveguidelines.org). All experimental procedures were approved by Zagazig University institutional animal care and use committee (ZUIACUC/2/F/24/2024).

Isolation and Expansion of UCB-MSCs

Isolation of UCB-MSCs

The cord blood was diluted 1:2 with PBS, the diluted CB was loaded on the Ficoll-Paque solution (Lonza Bioscience, Ficoll-Paque TM Plus). Following 30 min of room temperature density gradient centrifugation at 400g, MNC were

extracted from the interphase and twice washed with phosphate-buffered saline (PBS, Lonza Bioscience). Automated cell analyzers (XE-2100, Sysmex; Hmx Hemocytometer, Beckman Coulter) were used to count the number of cells. The assay for colony-forming unit fibroblasts (CFU-F) was carried out as follows. MNC were cultivated in culture in 100-mm-diameter BD Falcon culture dishes at a density of 1-2 x 106/cm2. For three days, the cells were allowed to attach. The non-adherent cell population was then removed, and fresh culture DMEM supplemented with 10% (V/V) foetal bovine serum (FBS), 50 IUL-1 penicillin, 2 mML-glutamine, and 50 µg mL-1 streptomycin (Lonza Bioscience) 100 U streptomycin was added in place of the old culture DMEM. To decrease the adherence of monocytes in the plates, dexamethasone (107 M) (Sigma–Aldrich) was added to the primary culture medium for a week. Non-adherent cells were then eliminated with a medium change, and the remaining cells were then fed culture medium without dexamethasone on a weekly basis. Every week, the medium was changed twice, and cell counts were used to create proliferation curves. Adherent cells measuring between 60 and 70 percent confluence were collected using 0.25% trypsin and 0.5 mM EDTA (Lonza Bioscience) after the culture reached 80% confluence [39,40].

Quantification of UCB-MSCs surface markers

Thirty milligrams of the cell pellet were used for the extraction of the total RNA using QIAzol (QIAGEN, Germany) and then converted to cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, United States) [41]. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was carried out in a Rotor-Gene Q 2 plex real-time PCR machine (QIAGEN, Germany). A total reaction volume of 20 µL (10 µL TOPreal^{TM} qPCR 2X PreMIX (Enzynomics, Korea), 1 µL of the forward and reverse primers (Thermo Fisher, USA), 8 µL of RNAse free water) with a cyclic condition of initial denaturation at 95 C for 10min, 40 cycle of denaturation at 95 for 15 sec, annealing 60 for 30 sec, and extension at 72 for 25 sec followed melting curve analysis according to the method previously described by [42]. By applying the $^{2-\Delta\Delta}$CT method the fold change of the target genes was performed. The normalization of the target gene expression was done by using the most stably expressed housekeeping gene GAPDH according to the result of the expression stability of different housekeeping which was analyzed by GeNorm online tool https://genorm.cmgg.be/ for assaying the stability of the housekeeping expression across the different experimental groups and the most stable expressed housekeeping that displayed a non-significant change across the different study groups was the one that used in the data normalization of the target gene [43].

Table 1: Primers sequences used in RT-qPCR assay

Target Gene	Primer (′5 ------ ′3)	Size	Accession no.
CD105	F:CTGACCTGTCTGGTTGCACA R:TCAGAGGCTTCACTGGGCT	198	NM_000118.4
CD90	F:AAGACCCCAGTCCAGATCC R:GACTGTTAGCAGGAGAGCGA	78	NM_006288.5
CD73	F:TGACACACGGCATTAGCTGT R:CTGGAGAGGGACAAGTGCAG	139	NM_001204813.2

Gapdh	F:CCATGGGGAAGGTGAAGGTC R: CTTCCCGTTCTCAGCCATGT	146	NM_001357943.2

Biochemical sampling and analysis

Measurement of fasting blood glucose (FBG) each week according to the method that applied by [40]. Analysis of serum liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), total protein (TP) and albumin were determined by using commercial kits supplied by (Spinreact, Spain) according to the supplier instructions. Serum urea and creatinine measurement was carried out by using commercial kits (Spinreact, Spain) according to the manufacturer guidelines. The oxidant/antioxidant activity was measured in the renal and hepatic tissue homogenate that was previously prepared by collecting 1g of tissue on 9 mL of phosphate buffered saline which homogenized using tissue homogenizer (Invitrogen, USA). Tissue homogenate was centrifuged at 3000 rpm for 20 min, then the supernatant was collected for assaying the following oxidant/antioxidant parameters. The lipid peroxidation marker (MDA) and total antioxidant capacity (TAC) were estimated in the kidney and liver tissues using sandwich ELISA kits (MyBioSource, USA) following the manufacture instruction [44].

Histopathological examination

The kidney and liver specimens; three samples per each were removed and preserved in a 10% neutral buffered formalin solution. They were then gradually dehydrated using alcohol (70–100%), cleaned in xylene and embedded in paraffin Hematoxylin and eosin (HE) dyes were used to generate five-micron-thick paraffin slices, which were subsequently inspected under a microscope [45].

Statistical analysis

GraphPad Prism 10.2.2. was used for applying statistical analysis by using the one-way analysis of variance (ANOVA). The post-hock analysis was applied by Tukey test. The comparison of the data indicated a significant effect when $p < 0.05$. The values are the mean of three dogs for each group ± standard error of the mean (SEM). Checking the data normality was done before performing any statistical analysis using Shapiro-Wilcox test for better selection of a suitable statistical package either parametric or non-parametric analysis.

Results

Identification of the UCB-MSCs

To Identify the UCB-MSCs the expression of the mesenchymal stem cells surface markers was assayed in comparison to the mononuclear cell layers of the human blood (MNC). The results illustrated that the UCB-MSCs displayed a significant upregulation ($p < 0.001$, 0.0001, and 0.0001) in the expression levels of CD105, CD90, and CD73 respectively than the MNC (Figure 1).

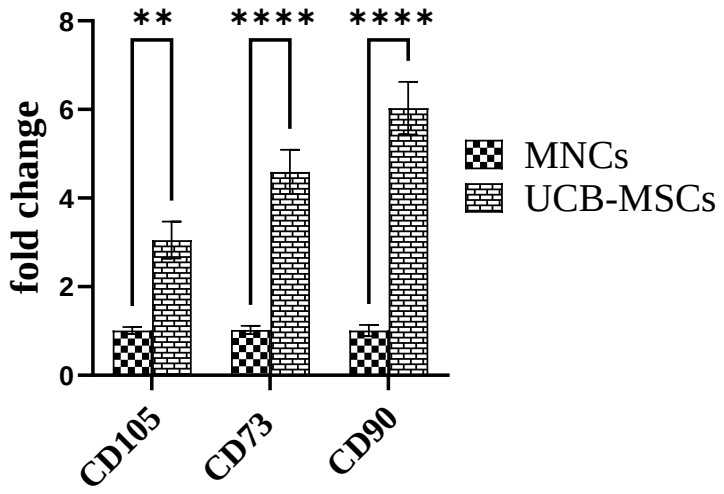


Figure 1: Relative expression of the UCB-MSCs surface markers (CD105, CD90, and CD73) in comparison to hMNCs. Values are the means of 5 values per group ± SEM. *****p*<0.01 and *******p*<0.0001.

Effect of UCB-MSCs transplantation on of FBG level on type 1 diabetes

According to the results of the current study, fasting blood glucose (FBG) level of diabetic dogs increased significantly ($p < 0.001$) one week after diabetes induction than the control group and reached its highest level (470 mg/dl) at 7th week. While the treated dogs with UCB-MSCs showed a significant ($p < 0.001$) decrease in FBG level from the first week (318.5 mg/dl) post UCB-MSCs administration and reached its lowest level (71.89 mg/dL) at 9th week in comparison with the control group than the diabetic group (Figure 2). Furthermore, UCB-MSCs transplantation brought the FBG to the normal physiological tone of the control group from the 4th week and remained with a non-significant fluctuation throughout the experimental period (Figure 2).

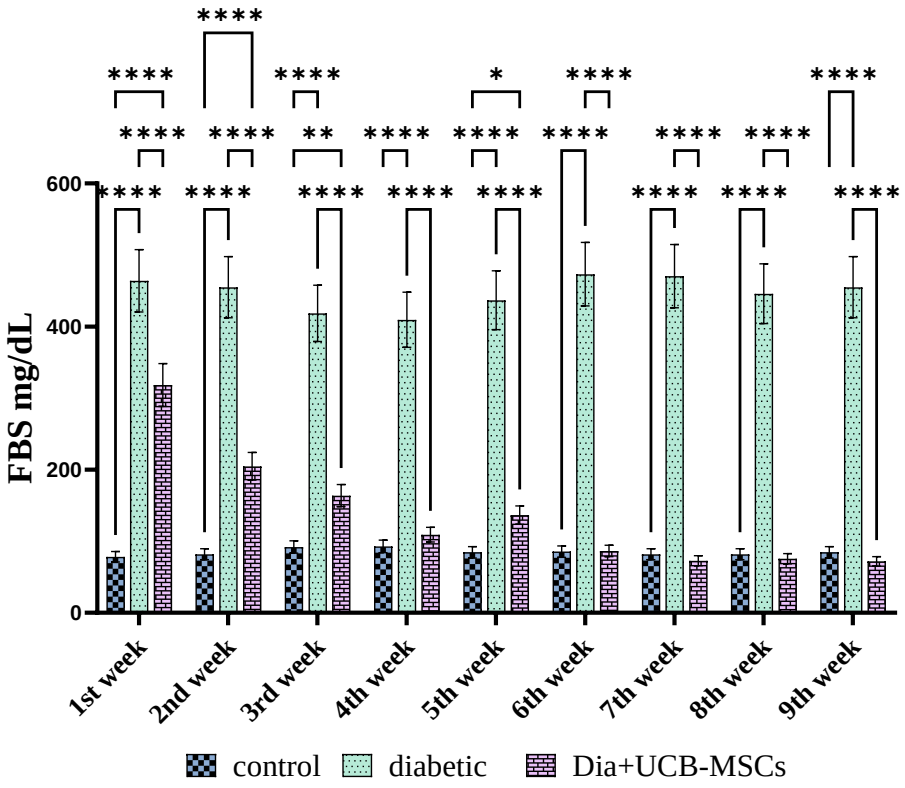


Figure 2: Effect of UCB-MSCs transplantation on the mean value of FBG (mg/dl) on type 1 diabetes during nine weeks post transplantation, represent the mean ± SEM of 3 dogs per group. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

Effect of UCB-MSCs transplantation on the level of liver function; AST, ALT, ALP, GGT, TP and albumin on type 1 diabetes

The current study showed that diabetic dogs displayed a significant (p<0.001) rise in liver enzymes levels ALT, AST, ALP, GGT (U/l), while revealed a significant (p<0.001) decline in serum total protein and albumin (U/L) in comparison to the control groups. Conversely, the group treated with UCB-MSCs demonstrated a significant (p<0.001) reduction in serum liver enzymes ALT, AST, ALP, and GGT (U/l) and a significant (p<0.001) elevation in serum total protein and albumin. Interestingly UCB-MSCs brought out the level of hepatic enzymes (ALT, AST, ALP and GGT) and serum albumin towards the normal physiological tone near the control group value at the conclusion of the trial (Table 2).

Table 2: Effect of UCB-MSCs transplantation on the mean value of liver enzymes in type 1 diabetes during nine weeks post transplantation.

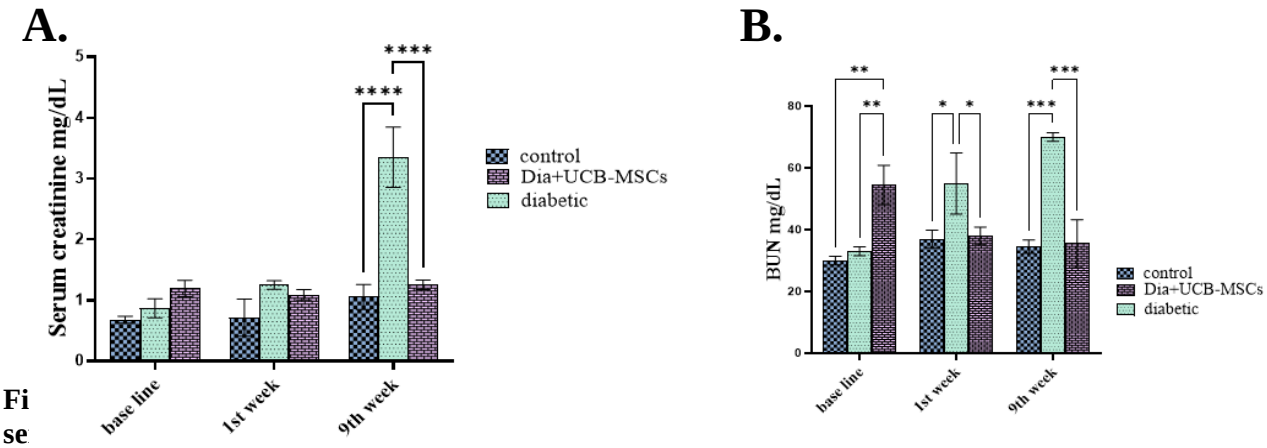
Parameter	Weeks	Control	Diabetic	Dia+UCBMSCs
AST (U/l)	Baseline	20 ± 1^{Ac}	24.5 ± 1.5^{Cb}	30 ± 1^{Aa}
	1st week	21.5 ± 5.5^{Ab}	46 ± 3^{Ba}	39.5 ± 0.5^{Aa}
	9th week	21 ± 3^{Ab}	73 ± 2^{Aa}	27.5 ± 7.5^{Ab}
ALT (U/l)	Baseline	22.5 ± 1.5^{Aa}	25.5 ± 0.5^{Ca}	28 ± 3^{Aa}
	1st week	20.5 ± 1.5^{Ab}	39.5 ± 1.5^{Ba}	27 ± 3^{Ab}
	9th week	21 ± 1^{Aa}	64.5 ± 4.5^{Ac}	29.5 ± 0.5^{Ab}
ALP (U/l)	Baseline	200 ± 5^{Ac}	277.5 ± 12.5^{Ca}	245 ± 26^{Ab}
	1st week	217.5 ± 7.5^{Ab}	317.5 ± 2.5^{Ba}	293.5 ± 6.5^{Aa}
	9th week	198.5 ± 1.5^{Ac}	395 ± 15^{Aa}	279.5 ± 0.5^{Ab}
GGT (U/l)	Baseline	14.5 ± 1.5^{Ac}	32 ± 3^{Ab}	56 ± 2^{Aa}
	1st week	16 ± 4^{Ac}	54.5 ± 2.5^{Aa}	34.5 ± 2.5^{Ab}
	9th week	22 ± 3^{Ac}	70 ± 1^{Aa}	40.5 ± 0.5^{Ab}
TP (g/dl)	Baseline	6.015 ± 0.395^{Aa}	5.02 ± 0.1^{Ab}	4.3 ± 0.3^{Bb}
	1st week	5.95 ± 0.15^{Aa}	4.5 ± 0.3^{Ab}	5.55 ± 0.25^{Aa}
	9th week	6.95 ± 0.15^{Aa}	3.4 ± 0.3^{Bb}	5.985 ± 0.015^{Aa}
Albumin (mg/dl)	Baseline	4.395 ± 0.225^{Aa}	4.065 ± 0.175^{Aa}	3.655 ± 0.045^{Aa}
	1st week	4.45 ± 0.35^{Aa}	2.95 ± 0.15^{Bc}	3.55 ± 0.15^{Ab}
	9th week	4.45 ± 0.25^{Aa}	2.3 ± 0.3^{Bc}	3.735 ± 0.065^{Ab}

Small superscript letter refers to row effect and capital superscript letter refer to column comparison. Values are the mean of 3 dogs per group ± SEM. Value carry different superscript indicate significant change ($p < 0.05$). AST :aspartate transaminase, ALT :alanine aminotransferase, ALP :alkaline phosphatase, GGT :gamma glutamyl transferase, and TP: total protein.

Effect of UCB-MSCs transplantation on kidney function; serum creatinine and BUN level on type 1 diabetes

Serum creatinine level was significantly (p <0.001) higher in diabetic group than in the control dogs after nine weeks. While it is significantly (p < 0.0001) lower in UCB-MSC treated dogs than the control dogs after nine weeks.

Fascinatingly, transplantation of the UCB-MSCs in the diabetic dogs brought the serum creatinine level towards the normal physiological tone (Fig. 3A). After nine weeks BUN was significantly (p < 0.001) high in diabetic dogs in contrast to the control group, while the UCB-MSC treated group significant (p < 0.001) decrease than the control group (Figure 3B).



Fi se transplantation, represent the mean ± SEM of 3 dogs per group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

Effect of UCB-MSCs transplantation on the oxidative stress in type 1 diabetes

The diabetic group showed a significant (p<0.001) decrease in the mean of hepatic TAC (ng/mg) than the control group. While dogs treated with UCB-MSCs showed a significantly (p< 0.01) increase than the diabetic group (Figure 4 A).The diabetic group showed a significant (p<0.01) increase in the mean of hepatic MDA (Nmol/mg) than the control group. However, dogs treated with UCB-MSCs showed a significantly (p< 0.05) decrease in the level of hepatic MDA value than the diabetic group (Figure 4 B).

Also, the findings demonstrated that in contrast to the control group, the mean of renal TAC (ng/mg) was significantly (p<0.01) lower in the diabetic group. On the other hand, dogs received UCB-MSC treatment displayed a significant (p<0.01) increase in the mean of renal TAC (ng/mg) when compared to the diabetic group at the end of the study (Figure 4 C). Additionally, the diabetic group showed a significant (p<0.001) increase in the mean of renal MDA (Nmol/mg) compared with the control group, however dogs treated with UCB-MSCs showed a significant (p<0.01) reduction in the mean of renal MDA (Nmol/mg) than the diabetic group (Figure 4 D).

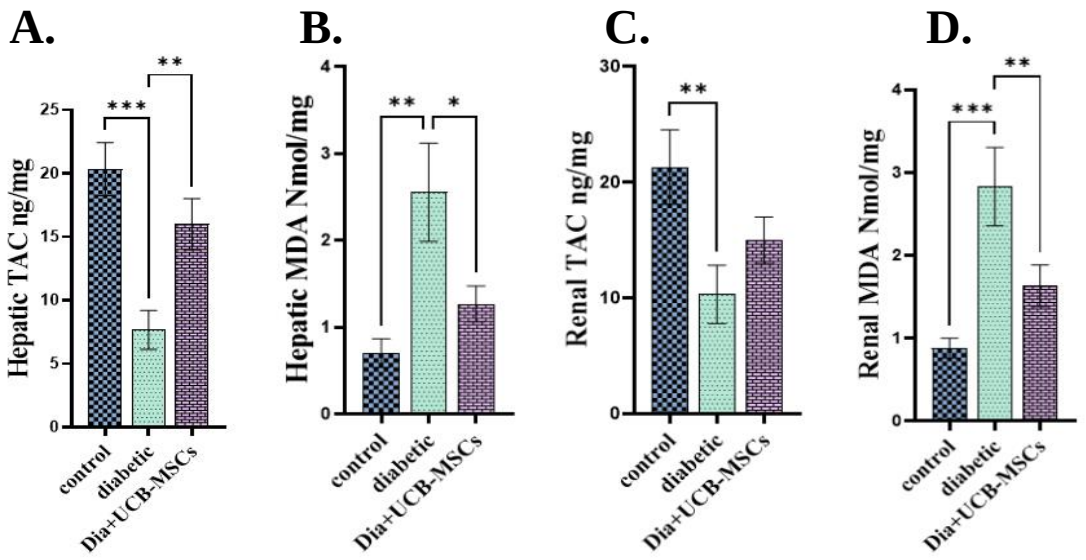


Figure 4: Effect of UCB-MSCs transplantation on the mean value of oxidative stress including hepatic and renal TAC and hepatic and renal MDA, on type 1 diabetes during nine weeks post transplantation, represent the mean ± SEM of 3 dogs per group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

Effect of UCB-MSCs transplantation on the histopathological examinations of kidney and liver on type 1 diabetes

Control group: The kidney's renal tubule and glomeruli displayed normal histological structures (Figure 5A). The liver's hepatocyte, hepatic sinusoidal, and portal region histological structures were all normal (Figure 6A).

Diabetic group: The kidney showed different shapes of necrotic renal tubules. Some of necrotic renal tubules represented by more eosinophilic cytoplasm and disappear of nucleus with disassociation of the cells on the basement membrane, other showed vacuolated epithelial cells with presences of intertubular homogenous eosinophilic proteinaceous substance and mononuclear cell infiltration with hemorrhage and brown pigment of hemosiderin were also detected, few renal tubules showed basophilic substances of calcification. The glomeruli showed atrophy of glomeruli tufts replaced by homogenous eosinophilic proteinaceous substance (Figure 5 B and C.) while the liver showed congestion of hepatic sinusoids and focal area of necrosis represented by pyknotic nuclei, the portal area showed proliferation of collagen fiber and mononuclear cells infiltration mixed with homogenous eosinophilic proteinaceous substance (Figure 6 B). Some hepatocytes showed faint eosinophilic vacuoles inside its cytoplasm and proliferation of Kupffer cells (Figure 6 C). The liver showed multiple areas of necrosis, one area replaced by mononuclear cells (Figure 6 D) and other area replaced by homogenous eosinophilic proteinaceous substance and mononuclear cells infiltration.

Treated group: The kidney restores normal histological structure of normal renal tubules with presences of mild congestion of glomeruli tufts (Figure 5 D). The liver restores hepatic cells' normal histological structure with central vein dilatation (Figure 6 E and F).

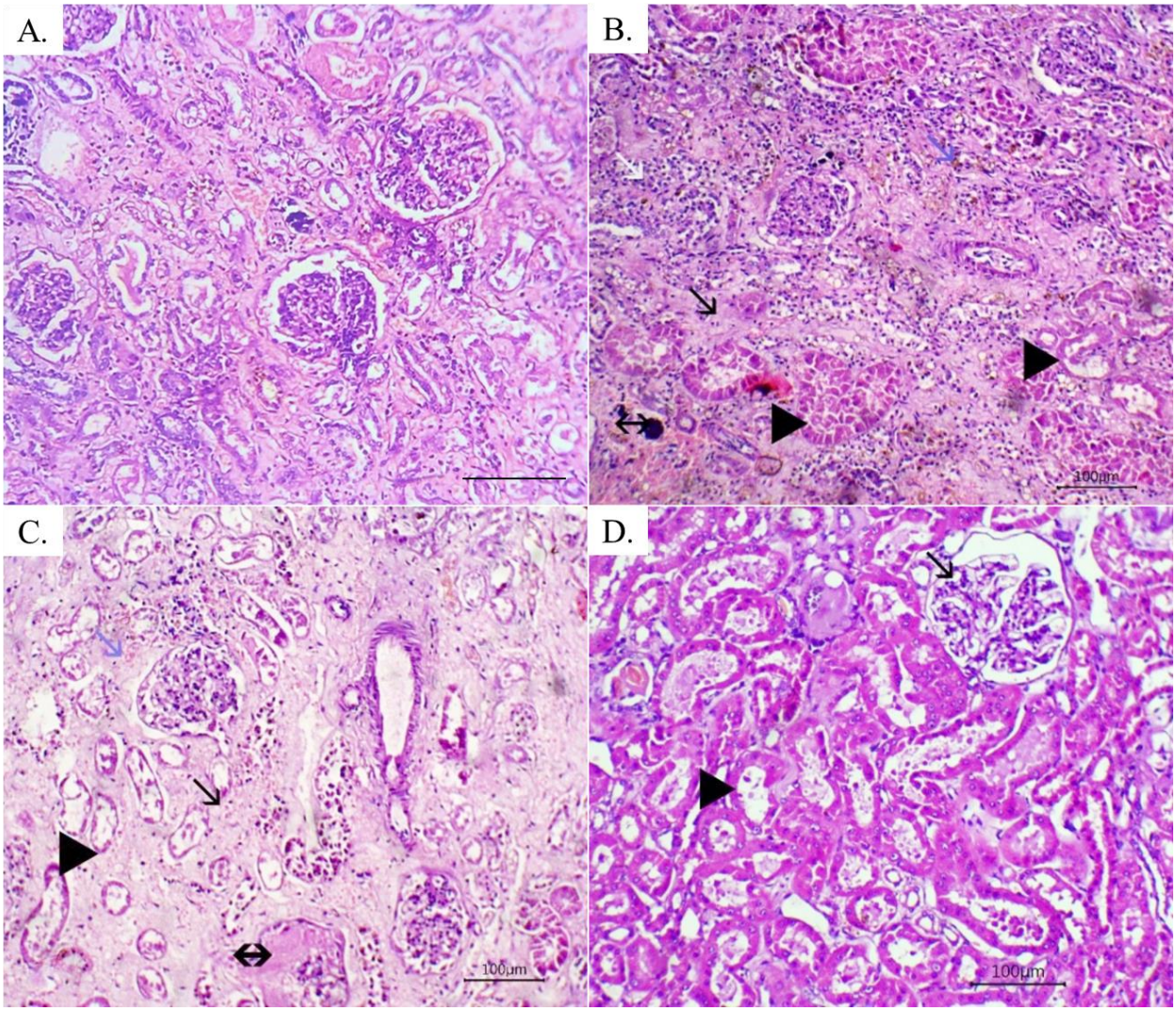


Figure 5: photomicrographs of H&E-stained sections in the renal cortex. Control group (A), Diabetic group (B&C) and Treated group (D). The diabetic group, the kidney (B&C): B-necrotic renal tubules (arrowhead), proteinaceous substance (black arrow), mononuclear cells (white arrow) hemosiderin pigment (blue arrow), calcified tubules (arrow with 2 head). C-necrotic renal tubules (arrowhead), proteinaceous substance (black arrow), hemorrhage (blue arrow), eosinophilic proteinaceous substance in the glomeruli (arrow with 2 head). Treated group (D), the kidney-normal renal tubules (arrowhead), congestion of glomeruli tufts (black arrow), bare = 100 µm.

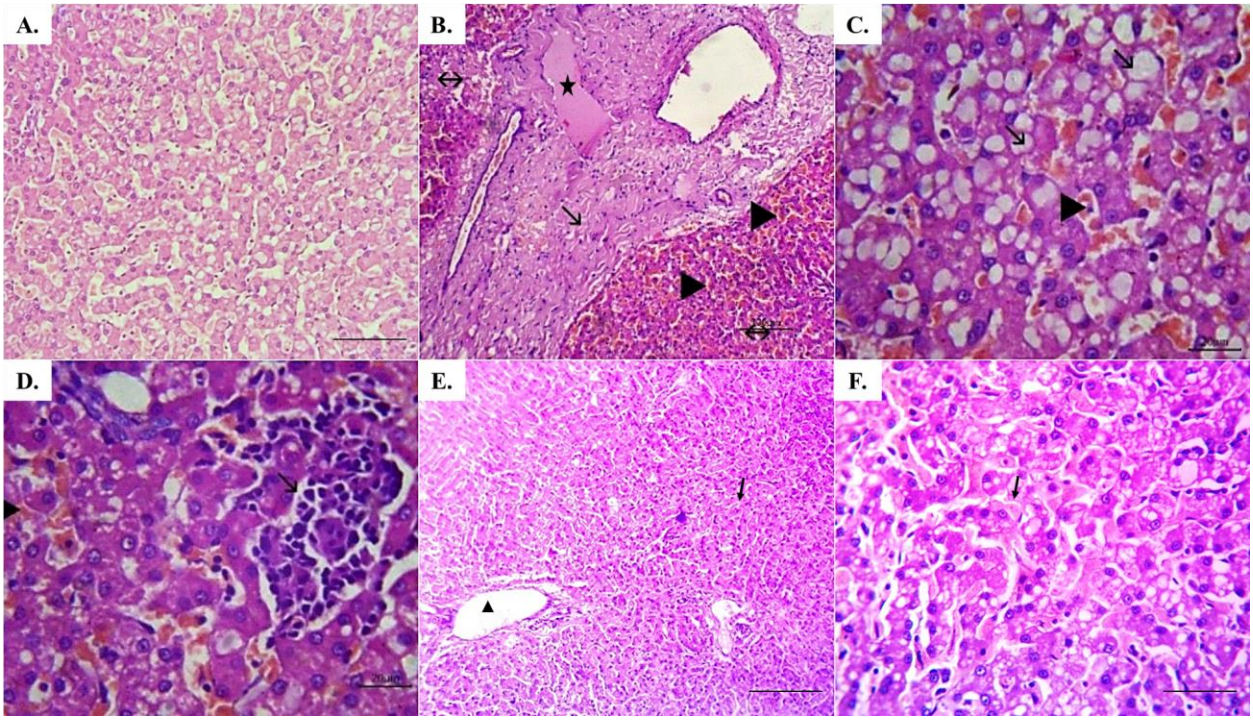


Figure 6: Photomicrograph of H&E-stained liver of control group (A), diabetic group (B-D), and treated group (E&F). B-the portal area showing collagen fiber (black arrow), proteinaceous substance (star), congestion of hepatic sinusoids (arrow head), necrotic area (arrow with 2 head), bar = 100 µm. D: congestion of hepatic sinusoids (arrow head), necrotic area (arrow with 2 head), eosinophilic vacuoles (arrow) , bar = 20 µm. C- congestion of hepatic sinusoids (arrow head) necrotic area replaced by mononuclear cells (black arrow), bar = 20 µm. D: congestion of hepatic sinusoids (arrow head) necrotic area replaced by mononuclear cells (white arrow), proteinaceous substance (black arrow), eosinophilic vacuoles (arrow with 2head), bar = 20 µm. Liver in E and F showed normal hepatocytes (black arrow), E- dilation of central vein (arrowhead), bar =100 µm.

Discussion

DM is defined as a widespread endocrine disease which disturbs a cell's ability to absorb glucose because of an absolute or relative insulin deficiency [46]**.** Currently, diabetes mellitus (DM) affects roughly 1 in 500 dogs and 1 in 250 house cats. It mostly affects dogs aged 5 to 12, with uncommon cases occurring in puppies under 3 years old [47]. DM brought on by either a partial or complete insulin deficiency and characterized by high glucose levels, glycosuria and weight loss [6,7].

There are few therapeutic options for DM, such as insulin therapy, dietary change, and managing concurrent disorders. However, none of these approaches may result in tight control of blood glucose levels [48]. Consequently, innovative approaches, such as stem cell therapy, have been employed recently to restore pancreatic endocrine function [49].

MSCs can control immunological disturbances, resulting in the death of β-cells, additionally MSCs exhibit regenerative and immunomodulatory characteristics. Thus, stem-cell transplantation can be used to treat dogs

with insulin-dependent diabetic mellitus (IDDM) [50].

DM-related persistent and chronic hyperglycemia is linked to severe complications, prolonged damage and various organs disappointment including the liver, kidneys, heart, nerves, eyes, and blood capillaries. Additional symptoms of chronic hyperglycemia include diminished growth and greater susceptibility to certain illnesses. Comorbid disorders such neuropathy, nephropathy, vasculopathy, and retinopathy are the main contributors to diabetes morbidity and mortality [51,52].

According to the current study, the serum level of blood glucose in diabetic dogs was significantly higher than that of the control dogs [53-55]. Hyperglycemia can be attributed to destructive influence of alloxan monohydrate upon beta cells in the pancreas via generating oxygen free radicals [56].

However, our data also displayed significant reduction in the serum level of blood glucose in UCB-MSCs treated group. This might return to effect of MSCs which induce pancreatic beta cells regeneration via secreting growth factors, restoring its normal execratory function and anti-inflammatory effect through signaling mechanisms such as, JAK/STAT, Wnt/β-catenin, PI3K/AKT/mTOR, NF-κB, and notch signaling pathways .Moreover, MSCs can activate polarization of macrophage to the M2 phenotype [57,58].

Regarding liver enzymes, AST, ALT, ALP and GGT, dogs with type 1 diabetes revealed higher significance in comparison with the control group at the end of the experiment. Our findings align with previous findings [54-55,59-60]. The elevated levels of hepatic enzymes indicate hepatocyte damage resulting from the inflammatory process in hyperglycemic settings [61]. Additionally, our results revealed a remarkable decrease in albumin levels total protein, these outcomes match with those that were reported by Num-Adom *et al.* [62]. Hypoproteinemia may be attributed to kidney damage caused by diabetic hyperglycemia [63]. While dogs treated with UCB-MSC revealed a marked improvement and reduction in the hepatic enzymes' levels, this might return to the ability of UCB-MSCs to control apoptosis, prevent or even delay fibrosis of liver and other related disorders, and release exosomes that can lower pro-inflammatory factor levels and NLRP3 inflammasome expression, all of which have an anti-inflammatory effect [64].

The data concerning BUN and serum creatinine levels revealed that diabetic dogs showed a significant increase in BUN and serum creatinine levels in comparison with control dogs at the end of the experiment, indicating the presence of renal injury. These findings were in agreement with previous studies [53,59,65-67] that declared that diabetic hyperglycemia induces an elevation in serum creatinine and BUN levels.

The impact of hyperglycemia on tubular cells and podocyte function and vitality can account for the aforementioned outcomes as described by Hamza *et al*. [18] who discovered that podocyte apoptosis thought to be the primary controller of solute transport through the nephron tubulointerstitial part, additionally, podocytes damage were linked to renal cirrhosis and proteinuria [68]. While UCB-MSC treated group showed a considerable decrease in serum creatinine, BUN, and reduced proteinuria, these results were ascribed to the UCB-MSCs regenerative ability throughout enhancing resident stem cells and soluble

factor, which consequently stimulate the internal repairing process [69]. This regenerative function of UCB-MSCs causes a reduction in inflammatory cytokine generation and activates kidney' anti-apoptotic proteins expression [32].

Concerning oxidative stress biomarkers, in the diabetic group, the total antioxidant capacity (TAC) of liver and kidneys were reduced significantly, while significant increase in hepatic and renal lipid peroxidation marker (MDA) levels. These results can be a consequence of decreasing the defense systems' antioxidant capacity and/or oxidation of glucose [70,71]. Meanwhile, UCB-MSCs treated group showed extremely reduced MDA levels with increased the levels of various antioxidants' mRNA expression, including TAC [70,72].

Histopathological examination of liver the diabetic group showed congestion of hepatic sinusoids and focal area of necrosis represented by pyknotic nuclei, the portal area revealed proliferation of collagen fiber and mononuclear cells infiltration mixed with homogenous eosinophilic proteinaceous substance .While kidney showed different shapes of necrotic renal tubules compared with control group. Some of necrotic renal tubules represented by more eosinophilic cytoplasm and disappearance of nucleus with disassociation of the cells on the basement membrane, other showed vacuolated epithelial cells with presences of intertubular homogenous eosinophilic proteinaceous substance and mononuclear cell infiltration with hemorrhage and brown pigment of hemosiderin were also detected, few renal tubules showed basophilic substances of calcification. The glomeruli showed atrophy of glomerular tufts replaced by homogenous eosinophilic proteinaceous substance. These findings agreed with those of previous studies [36,53].

Fortunately, dogs received UCB-MSCs transplantation revealed restoring the normal histological structure of liver with dilation of the central vein. Also, renal tissue restored the normal histological structure of normal kidney's tubules with the presence of mild congestion of glomerular tufts; these results agreed with those of Khamis *et al*. [73]. This improvement in the histopathological examination is related to the mechanism in which MSCs release cytokines and growth factors that stimulate immunosuppressive, proliferative, anti-inflammatory, and anti-apoptotic effects [74]. Additionally, found that MSCs might be able to reach the damaged kidney and liver, assist in restoring renal function and regenerate the tubular epithelium without linking forces with local tubular cells. Finally, it was suggested that MSCs need to provide either endocrine or paracrine elements that explain the advantageous effects on the renal regeneration after damage [75].

Conclusion

Intravenous UCB- MScs injections may help reverse the kidney and liver damage caused by type 1 diabetes. These outcomes highlighted the possible application of UCB-MSCs as a unique therapeutic strategy for diabetic nephropathy and hepatopathy.

Conclusion

The authors declare no conflict of interest.

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الملخص العربي

الخلايا الجزعيه الميزنكيميه المشتقه من دم الحبل السري تخفف من حده الاعتلال الكلوي والكبدي المحدث من الداء السكري النوع الأول ف الكلاب باستخدام الالوكسان : دراسه علي مستوي التغير البيو كيميائي و الباثيولوجي.

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2 قسم الفارماكولوجي ,كليه الطب البيطري , جامعه الزقازيق 44511 , الشرقيه , مصر .

3 قسم الباثولوجيا والباثولوجيا الاكلينيكيه ,كليه الطب البيطري , جامعه الزقازيق 44511 , الشرقيه , مصر .

يعتبر مرض السكرى أحد أشهر امراض الغدد الصماء الشائعة بين فصيلة الكلاب ويتميز المرض بارتفاع مستمر في مستويات الجلوكوز في الدم بالتزامن مع نقص الأنسولين. فيما يتعلق بالعواقب الوخيمة المرتبطة بمرض السكري والتى تؤثر على العديد من الأعضاء الأساسية مسببة اضطرابات مرضية حادة وغير قابلة للعلاج تشمل هذه الاضطرابات اعتلال الشبكية والاعتلال العصبي واعتلال الكبد واعتلال الكلية. ولذلك فإن البحث عن طرق بديلة لاستعادة وظيفة الغدد الصماء البنكرياسية ذات أهمية سريرية قصوى. قد تساعد الخلايا الجذعية الوسيطة المستخرجة من نخاع العظام فى حل هذه المشكلة حيث تتميز هذه الخلايا الجذعية بان لها دور تجديدي من خلال تعزيز الخلايا الجذعية المقيمة والعوامل القابلة للذوبان التي تحفز عملية الإصلاح الداخلي. أجريت هذه الدراسة على تسعة كلاب تم تقسيمها إلى ثلاث مجموعات ثلاثة كلاب داخل كل مجموعة : المجموعة الضابطة ومجموعة تم اصابتها بمرض السكري ومجموعة تم علاجها باستخدام الخلايا الجذعية. وأظهرت النتائج أن الخلايا المعزولة أظهرت دلالات سطحية للخلايا الجذعية ممثلة فى CD90 وCD105 وCD73. علاوة على ذلك، أظهرت النتائج أن زرع الخلايا الجذعية في الكلاب المصابة بداء السكري أدى إلى انخفاض ملحوظ في مستوى جلوكوز الدم الصائم، الاسبرتيت أمينوترانسفيريز والالانين أمينوترانسفيريز ، انزيم الفوسفاتاز قلوي ، إنزيم ناقلة الببتيد جاما جلوتاميل ، البروتين الكلى، الألبومين، اليوريا والكرياتينين في الدم مقارنة بمجموعة مرضى السكري. بالإضافة إلى ذلك، أظهرت الكلاب المعالجة بـالخلايا الجذعية ارتفاعًا ملحوظًا فى قيم قدرة مضادات الأكسدة الكلية مع انخفاضً في مستوي المالونالدهيد بالاضافة الى استعادة التركيب النسيجي الطبيعي للكبد والكلى. لذلك، ونظرًا لنتائجها المشجعة، توفر الخلايا الجذعية الوسيطة استراتيجية علاجية واعدة للاضطرابات المرتبطة بمرض السكرى.