

Repeated Intravenous Dosing of Human Umbilical Cord Mesenchymal Stem Cells Improves Glycemic Control and Organ Protection in a Type 2 Diabetes Rat Model

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Background: Type-2 diabetes mellitus (T2DM) is a chronic disorder marked by insulin resistance, beta (β)-cell dysfunction, and persistent hyperglycemia, often leading to diabetic kidney disease. There is growing recognition that inflammation exacerbates diabetes. Human umbilical cord mesenchymal stem cells (hUC-MSCs) have shown promising immunomodulatory effects in the treatment of T2DM; however, the optimal dosing remains inconclusive.

Objective: This study evaluates the effectiveness of early, late, and repeated doses of Cytopeutics[®] hUC-MSCs in a T2DM rat model.

Methods: Male Sprague Dawley (SD) rats were allocated into five groups: normal control group and diabetic control group receiving saline, and three diabetic treatment groups receiving hUC-MSCs at different time points—early, late, and repeated. The male rats were fed a high-fat diet (HFD), followed by diabetes induction via a single intraperitoneal injection of streptozotocin (STZ) at 35 mg/kg body weight (BW). Each treatment group received 3.5×10^6 cells/rat intravenously. At the end of the study, blood and tissue were collected before termination for glycemic control (hemoglobin A1C (HbA1c), fasting serum glucose (FSG), and fasting urine glucose (FUG) levels), pro-inflammatory factor high sensitivity C Reactive Protein (hs-CRP), and histopathological analyses.

Results: The diabetic control group exhibited significantly poorer glycemic control along with extensive pancreatic and kidney damage compared to the normal control group. The early dose group showed limited glycemic control, but reduced hs-CRP sustainably compared to the diabetic control group. The late dose group demonstrated glycemic control partially, and preserved pancreatic β -cells and kidney from damage but did not reduce hs-CRP. The repeated dose group reduced HbA1c, FSG, FUG and hs-CRP up to the end of the study and is also associated with fewer pancreatic β -cells and renal tissue damage.

Conclusion: Repeated hUC-MSCs administration demonstrated better glycemic control, modulated inflammatory responses, protected β -cells and kidneys from damage in a T2DM rat model.

Keywords: hUC-MSCs, T2DM, HbA1c, hs-CRP, insulin resistance

Introduction

Type-2 diabetes mellitus (T2DM), a condition marked by insulin resistance and hyperglycemia, contributes to both microvascular complications and macrovascular complications.¹ Low-grade systemic (LGS) inflammation plays a key role in T2DM pathogenesis,² with high-sensitivity C-reactive protein (hs-CRP) emerging as a pro-inflammatory marker associated with hyperglycemia in diabetes.^{3,4}

Mesenchymal stem cells (MSCs) have shown promise in treating T2DM due to their immunomodulatory and reparative properties, as demonstrated in both preclinical and clinical studies.^{5–7} Among the various MSC sources, human umbilical

cord mesenchymal stem cells (hUC-MSCs) are often preferred because their collection and isolation are non-invasive, they possess a higher proliferative capacity, and they exhibit lower immunogenicity, making them suitable for allogeneic use.^{8,9}

By secreting anti-inflammatory cytokines, chemokines, and trophic factors, hUC-MSCs regulate immune cell responses, suppress chronic low-grade inflammation, and enhance insulin sensitivity, thereby helping to preserve pancreatic islet function and improve T2DM-related markers.^{10–13} Evidence from our preclinical and clinical studies further supports this immunomodulatory mechanism, demonstrating dose-dependent suppression of pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF- α) and Interleukin (IL)-6 in animal models and sustained increases in anti-inflammatory markers (IL-1Ra, IL-10) in human trials.^{6,14}

The dose, timing, and frequency of MSC administration appear to influence therapeutic outcomes in T2DM treatment.⁷ In an early- and late-stage beta (β)-cell dedifferentiation study of T2DM rat model, the multiple infusions of hUC-MSCs showed better improvement in glucose homeostasis in the early treatment group compared to the late treatment group.¹⁰ Similarly, another study reported that while a single dose of bone marrow (BM)-MSCs transiently reduced hyperglycemia, multiple early-phase doses of BM-MSCs reversed hyperglycemia and sustained normoglycemia for at least 9 weeks, whereas late-phase treatment was ineffective.¹⁵

This study evaluates the efficacy of Cytopeutics® hUC-MSCs administered as early, late, and repeated doses in a T2DM rat model using Sprague Dawley (SD) rats induced by high-fat diet (HFD) and low-dose streptozotocin (STZ), which closely mimics key features of human T2DM.^{16,17} The association between hyperglycemia and systemic inflammation in diabetes was also investigated. This research could offer valuable insights into the most effective dosing regimen for hUC-MSCs treatment in T2DM, highlighting the MSCs infusion strategy may be critical for clinical translation in humans.

Materials and Methods

Isolation of hUC-MSCs

The use of hUC-MSCs in this study was conducted under a Clinical Trial Exemption (CTX) approval granted by the National Pharmaceutical Regulatory Agency (NPRA), Ministry of Health Malaysia (CTX-230204). This authorization allows Cytopeutics to obtain human umbilical cords and manufacture hUC-MSCs for clinical investigational use in compliance with national regulatory requirements. Human umbilical cord samples were obtained from full-term, healthy infants after delivery, with written consent from both parents, in accordance with the national Standard for Cord Blood Banking and Transplantation and the Association for the Advancement of Blood & Biotherapies (AABB) standards. Donor eligibility included screening for genetic mutations, infections, cancers, and other hereditary diseases across three generations (newborns, parents, and grandparents). Following delivery and placental detachment, segments of 15–40 cm were aseptically excised proximal to the placenta using sterile instruments, disinfected, and placed in sterile containers containing sodium chloride irrigation solution. Samples were sealed within biohazard bags, transported at 4–37°C, and processed within 48 h at a certified Good Manufacturing Practice (GMP) contract laboratory of Cytopeutics, Cyberjaya, Malaysia, in compliance with the Malaysia Guidelines for Stem Cell Research and Therapy.⁶ Upon arrival, cords were assigned unique identifiers and processed in a cleanroom Grade B (Class 100). Tissue was minced and digested with Collagenase Type I, and the suspension was centrifuged. Cell expansion was done in established growth medium maintained at 37°C, 5% CO₂, and 95% air incubator. Fresh growth medium was added after removing non-adherent cells, and the adherent cells were allowed to grow until they reached confluence over the following three days. Further expansion was carried out by culturing the cells in additional flasks until the required number was attained. The desired cell number (30×10^6 cells/vial) was added to cryopreserved vials. For the preparation of the cells, the vials were thawed, aspirated, and suspended in normal saline before centrifugation. The cell pellets were resuspended and prepared for cell counting and treatment once the supernatant was carefully removed and replaced with normal saline. The consistency and quality of the stem cells processed for use in this research were validated, tested, and confirmed negative for mycoplasma, murine pathogens, endotoxins, and other contamination agents.¹⁸ The final cell number per animal prepared for each infusion was 3.5×10^6 cells/rat in 0.2 mL (infusion volume).

Animal and Ethics

Male SD rats, aged 5–6 weeks old, weighing 200–250g, were quarantined and acclimatized for 1 week prior to experiment initiation. Animals were housed group-wise, and autoclaved corncocks were used as the bedding material. The animals were housed under controlled conditions with $22 \pm 3^\circ\text{C}$, $50 \pm 20\%$ relative humidity, a 12-hour light/dark cycle, and 15 to 20 fresh air changes per hour (ACPH). Animals were fed ad libitum a certified irradiated laboratory rodent diet (Altromin Spezialfutter, Germany, Diet 1324), a high-fat diet for diabetic animals (60% kCal from fat, D12492i, Research Diets, Inc., USA), and offered reverse osmosis filtered drinking water in polycarbonate bottles. Ethical approval was granted by the Institutional Animal Ethics Committee (SYNGENE/IAEC/1379/05-2022). All animal procedures were performed and documented in compliance with the Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines.

Study Design

The primary outcome of this study would be the changes in glycemic control, as evidenced by hemoglobin A1c (HbA1c), fasting serum glucose (FSG), and fasting urine glucose (FUG), as well as systemic inflammation, as evidenced by hs-CRP. The secondary outcomes were the histological changes in the pancreas and kidney. The sample size was estimated based on the research equation method approach.¹⁹ The sample size calculation is provided in the [Supplementary Materials](#). Based on this calculation, 70 rats were required, with an additional 20% allowance for anticipated mortality, giving a target of 83 animals. On day -29, male SD rats (n=83) were grouped into a normal control group (n=12) and an HFD group (n=71). On day -28 onward, the normal control group received normal chow diet, while the HFD group continued on a high-fat diet throughout the study. On day -7, the animals in the HFD group were fasted overnight (~12 hrs) before the administration of STZ at 35 mg/kg body weight (BW) intraperitoneally (i.p.), while the normal control group received saline with the same route of administration. Following STZ administration, the HFD rats were given a 10% sucrose solution for the next 48 hrs to prevent the occurrence of hypoglycemia. Seven HFD rats died post-STZ administration, reducing the HFD group to 64 animals.

On day -2, FSG was measured after an overnight fast. Diabetic rats were identified based on FSG levels ≥ 100 mg/dL; animals below this threshold (n=16) were excluded. Three rats from the normal control group were also excluded as the blood glucose levels were notably higher than normal levels. After these exclusions, 57 rats remained for randomization and analysis, which is lower than the initial target because the additional dropouts for failure to meet the diabetic threshold had not been anticipated. On day -1, the remaining 48 diabetic rats were stratified based on the FSG levels and randomly assigned to treatment groups using a computer-generated random-number list prepared by a technician not involved in subsequent procedures: diabetic control (n=15), early dose (n=15), late dose (n=10), and repeated dose (n=8). Both the diabetic control and normal control (n=9) groups received normal saline. Treatment with hUC-MSCs was administered as follows: early dose group on days 0 and 14, late dose group on days 28 and 42, and repeated dose group on days 0, 14, 28, and 42. Each rat was considered an experimental unit, as treatment and measurements were assigned and collected on an individual basis. Investigators responsible for assessments were blinded to group allocation until all data were collected.

Body weight was recorded at specific time points to monitor any treatment-related changes in the animals. All animals were monitored daily for clinical signs of distress throughout the study, with no specific humane endpoint established. To reduce animal stress, urine and blood collection were scheduled on different days from the treatment administration. Blood was collected via tail nipping, and urine was collected using clean metabolic cages, with animals fasted overnight (~12 hours) beforehand. The samples were examined using Dimension Xpand Plus (Siemens, Germany) to measure FSG and FUG levels for short-term glycemic control measurement.

On day 29, selected animals from the normal control (n=5), diabetic (n=6), and early dose (n=6) groups were humanely euthanized by exsanguination under deep isoflurane anesthesia. Similarly, on day 56, the remaining animals in the normal control (n=4), diabetic (n=6), early dose (n=6), late dose (n=8), and repeated dose (n=7) groups were sacrificed following the same euthanization method. Prior to termination on days 29 and 56, blood was collected from the respective groups via retro-orbital plexus (ROP) under mild isoflurane anesthesia to measure HbA1c and hs-CRP levels. Rodent HbA1c generally indicates glycemic control commensurate with the life span of their red blood cell (RBC) which

is approximately 53–60 days.²⁰ In view with their shorter RBC lifespan compared to humans, therefore the changes in rat RBC HbA1c can be appreciated in 3 to 4 weeks as demonstrated by previous groups.^{21,22}

While previous T2DM studies have primarily focused on TNF- α and IL-6 as key inflammatory markers,^{3,23} we selected hs-CRP because it is a clinically relevant biomarker that reflects the combined effects of multiple cytokines and is strongly associated with T2DM. Unlike individual cytokines, hs-CRP reflects the cumulative downstream effect of multiple pro-inflammatory pathways and thus provides a robust indicator of systemic inflammation. Importantly, hs-CRP has been strongly linked to insulin resistance, diabetes progression, and cardiovascular risk, and is widely recognized as a predisposing marker for metabolic and vascular complications.²⁴ Its use as a proven clinical marker strengthens the clinical relevance of our findings.⁵ Following termination, the pancreas and kidney were harvested and weighted prior to histological assessment. A flow chart summarizing the study design is shown in Figure 1.

Dose Selection and Justification

According to previous studies, the dosages of hUC-MSCs used in the diabetic animal models typically range from 1×10^6 cells/rat to 5×10^6 cells/rat, with 1×10^6 cells/rat and 3×10^6 cells/rat being the most commonly used.⁷ Therefore, a dose of 3.5×10^6 cells/rat of hUC-MSCs was selected, as it falls within this commonly used range in T2DM animal models. Previous findings demonstrated that a single intravenous infusion of up to 40×10^6 cells/kg BW and repeated doses of 2.0×10^6 cells/kg BW were safe in healthy mice without any reaction, rejection, morbidity, or mortality.¹⁴ By using standard body surface area conversion methods, the corresponding dosage was converted to human equivalent dose (HED) of 1.5×10^6 cells/kg BW,²⁵ which lies within the range of intravenous hUC-MSCs doses already tested in early-phase clinical trials for metabolic and inflammatory disorders ($0.8\text{--}3 \times 10^6$ cells/kg).^{5,26} This calculation demonstrates that the experimental dose is biologically relevant and clinically translatable.

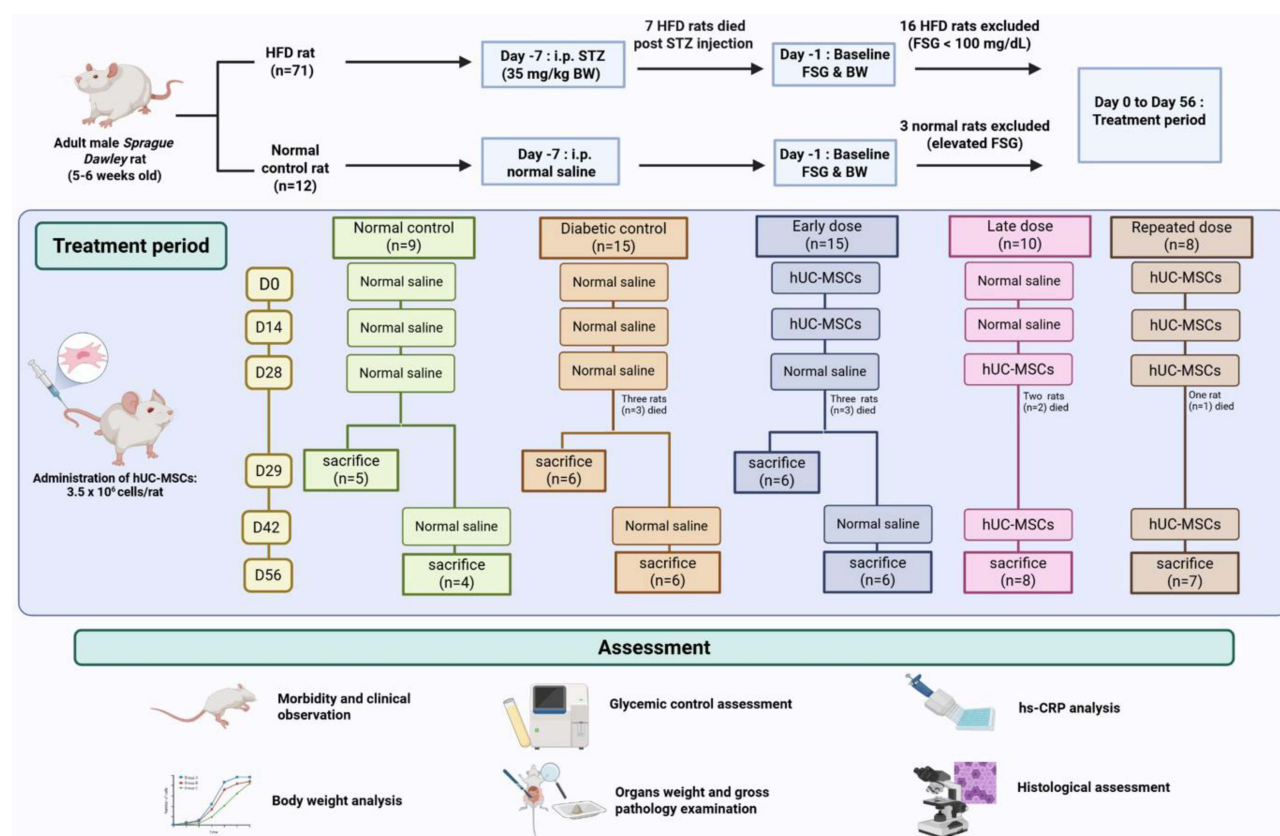


Figure 1 The flow chart of the study shows the study design and the assessment conducted with proposed animal numbers from day –28 to day 56. The diabetic rats were fed with HFD, and the normal rats were given a normal chow diet throughout the study. i.p.: intraperitoneal injection. Created in BioRender. Rahim, E. (2025) <https://BioRender.com/a32kzje>. **Abbreviations:** BW, Body weight; HFD, High-fat diet; FSG, Fasting serum glucose; STZ, Streptozotocin.

Histopathological Analysis

Following termination, the pancreas and kidney were harvested and preserved in buffered formalin, embedded in paraffin, sliced into sections, and stained with hematoxylin and eosin (H&E) for tissue morphological assessment. The slides were examined under the light microscope and independently reviewed by an in-house veterinary pathologist who was blinded to group allocation. The organs were scored with semi-qualitative severity scoring. The pancreatic sections were scored for islet morphological changes such as islet depletion and degeneration, as the STZ administration causes islet atrophy through β -cell loss, depleting the central core of islet tissue.²⁷ While the severity in the kidney tissues was evaluated based on any tubular changes, including swelling of tubules, tubular vacuolation, and mineralization, which is profound in early diabetic nephropathy.^{28,29} The scores ranged from 0-none, 1-minimal, 2-mild, 3-moderate, and 4-severe.

Further specific staining was also performed on both the pancreas and the kidney. Kidney sections were additionally stained with Periodic Acid-Schiff (PAS) to evaluate glycogen deposition. The pancreas tissues were subjected to immunostaining of anti-insulin and anti-glucagon. The pancreas sections deparaffinization was performed using xylene, then rehydrated, and subjected to heat-induced antigen retrieval in boiled water. The sections were then blocked with peroxide blocking solution (Cat# HK111-50K, BioGenex) for 5 minutes. Subsequently, the tissues were subjected to protein blocking for 5 minutes before being incubated for one hour with primary antibody containing anti-insulin antibody (ab63820) in 1:750 dilution and anti-glucagon antibody (ab10988) in 1:100 dilution in a humidified chamber. The slides were later incubated with secondary antibody (goat anti-rabbit IgG polymer detection kit MP-7451-50) for 30 minutes, followed by goat anti-mouse IgG H&L-alkaline phosphatase (ab205719 - 1:500) for one hour. The slides were then washed in phosphate-buffered saline (PBS) prior to counterstaining with 3,3'-diaminobenzidine (DAB), then counterstained with Mayer's hematoxylin before being placed in xylene, concentration gradient alcohol to dehydrate, and the images were examined by a light microscope.

For immunohistochemical (IHC) analysis of the pancreas, 5 representative islets were selected per animal, and their respective areas (μm^2) were measured. Positively stained α -cells and β -cells within each islet were counted. Mean α - and β -cell expression was defined as the percentage of α - or β -cells relative to the total number of islet cells (%). Total α - and β -cell expression was calculated as the total number of α - and β -cells across the five islets, normalized to islet area (cells/ mm^2).

Statistical Analyses

The results were analyzed based on the 29-day and 56-day experiments. A 29-day analysis was performed on the animal terminated on day 29, while 56-day analysis was performed on the animals terminated on day 56. Statistical analyses were performed using Statistical Packages for the Social Sciences (SPSS), and graphs were generated with GraphPad Prism version 10.3.0 (GraphPad Software, San Diego, CA). The normality of the data was examined by using Shapiro–Wilk test and Kolmogorov–Smirnov test. Data are presented as mean $\pm$ SEM. Parametric data included 29-day and 56-day body weight, FSG, FUG, HbA1c, hs-CRP, pancreas weight, total α - and β -cells, mean α - and β -cells, kidney weight, and kidney mineralization. Non-parametric data included islet damage score and kidney vacuolation. Between-group differences at each time point were analyzed using one-way ANOVA for parametric data or the Kruskal–Wallis test for non-parametric data. For post-hoc analysis, Tukey HSD was used when homogeneity was met; otherwise, Dunnett's test was applied. The Dunn's test was used for pairwise comparison in the Kruskal–Wallis test. A simple Pearson correlation analysis was conducted to observe the correlation between the glucose marker, HbA1c, with the histopathological and inflammation markers. A multiple linear regression was conducted to determine the independent predictors of nephropathy, and the 95% confidence interval (CI) of the regression coefficient was reported. A p-value < 0.05 was considered statistically significant.

Results

Clinical Signs and Mortality in Diabetic Rats

During the 4 weeks preceding the treatment period, 7 rats in the HFD group died following STZ administration on day -7 , with clinical signs observed before death, such as weakness and lethargy. The animals in the HFD group died at day -4 ($n=2$), day -3 ($n=1$), and day -2 ($n=4$), indicating the after-effect of STZ-induced β -cell damage (Figure 1).

During the 8-week treatment, 9 additional rats died in the diabetic control (n=3 out of 15), early dose (n=3 out of 15), late dose (n=2 out of 10), and repeated dose (n=1 out of 8) groups (Figure 1). The animals in both the diabetes control and the hUC-MSCs-treated groups exhibited similar clinical signs of lethargy and weakness several days prior to death, accompanied by persistent hyperglycemia. There was no increase in mortality in any of the hUC-MSCs-treated groups compared to the normal control group. Furthermore, there is no significant difference in body weight between the diabetic control and the hUC-MSCs-treated groups up to the end of experiment.

Effect of hUC-MSCs on Glycemic Control in Diabetic Rats

To explore the effect of hUC-MSCs on glycemic control in diabetic rats, HbA1c, FSG, and FUG levels were measured for the 29-day and 56-day experiments (Figures 2 and 3a).

Changes in HbA1c Levels

As expected, there was a significant increase in HbA1c levels in the diabetic control group compared to the normal control group on both days 29 ($7.53 \pm 0.16\%$ vs $3.80 \pm 0.00\%$; $p < 0.001$) and 56 ($7.63 \pm 0.31\%$ vs $3.85 \pm 0.05\%$; $p = 0.006$), indicating poor glycemic control in the diabetic control group. However, only the repeated dose group showed significantly lower HbA1c levels than the diabetic control group at the end of the study on day 56 ($5.93 \pm 0.37\%$ vs $7.63 \pm 0.31\%$; $p = 0.010$) (Figure 3a and Table S2).

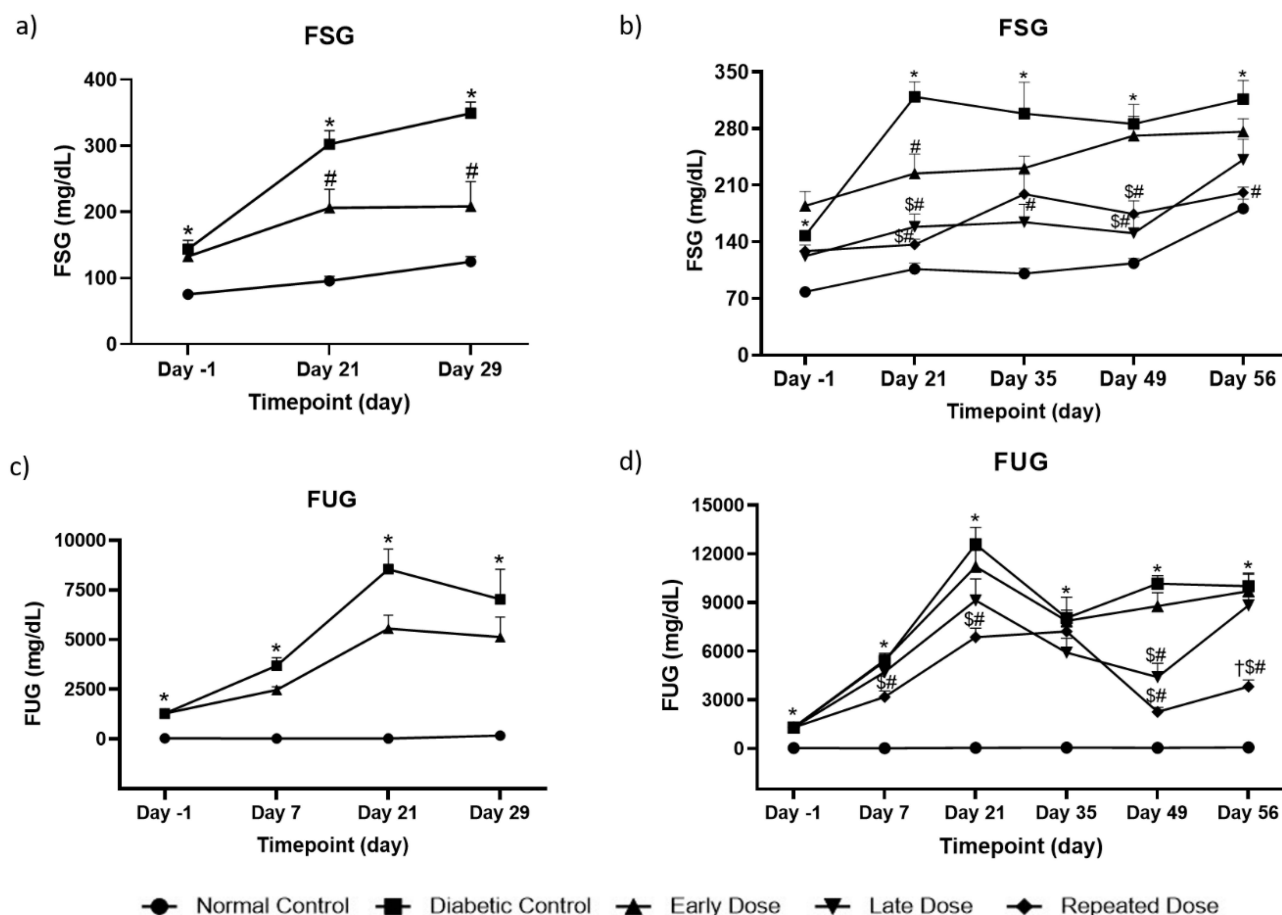


Figure 2 Figures show the changes of (a) FSG 29-day experiment, (b) FSG 56-day experiment, (c) FUG 29-day experiment, and (d) FUG 56-day experiment. * $p < 0.05$ compared to normal control, # $p < 0.05$ compared to diabetic control, \$ $p < 0.05$ compared to early dose, and † $p < 0.05$ compared to late dose. Data presented as mean $\pm$ SEM. One-way ANOVA analysis with post hoc Tukey's test for multiple comparisons between groups was performed for both 29-day and 56-day of FSG and FUG. 29-day experiment: normal control, n=5, diabetic control, n=6, early dose, n=6, 56-day experiment: normal control, n=4, diabetic control, n=6, early dose, n=6, late dose, n=8, repeated dose, n=7. **Abbreviations:** FSG, Fasting serum glucose; FUG, Fasting urine glucose; mg/dL, milligram per deciliter.

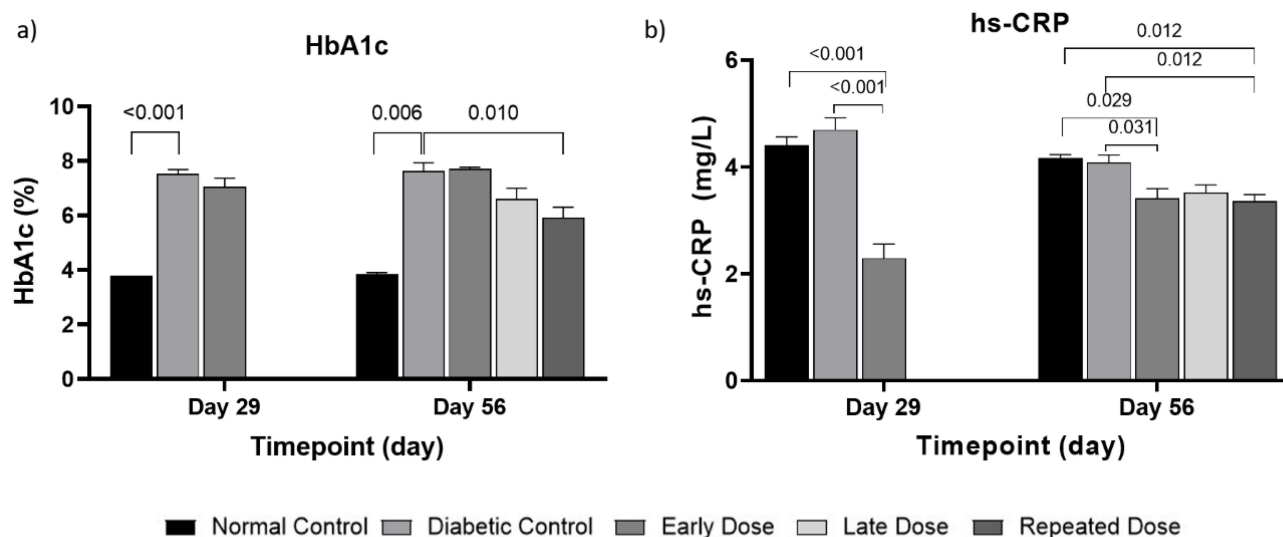


Figure 3 The figures show the effect of hUC-MSCs at different dose phases on (a) HbA1c and (b) hs-CRP levels in 29-day and 56-day experiments. Data presented as mean $\pm$ SEM. One-way ANOVA analysis with post hoc Tukey's test for multiple comparisons was performed for both 29-day and 56-day of HbA1c and hs-CRP. 29-day experiment: normal control, n=5, diabetic control, n=6, early dose, n=6, 56-day experiment: normal control, n=4, diabetic control, n=6, early dose, n=6, late dose, n=8, repeated dose, n=7. **Abbreviations:** HbA1c, Hemoglobin A1c; hs-CRP, high sensitive C-reactive protein.

Changes in FSG Levels

In the 29-day experiment, the early dose group showed significantly lower FSG levels compared to the diabetic control group on both day 21 (206.10 ± 27.96 mg/dL vs 302.50 ± 20.41 mg/dL; $p = 0.015$) and day 29 (208.50 ± 37.38 mg/dL vs 349.10 ± 17.10 mg/dL; $p = 0.004$) (Figure 2a).

In the 56-day experiment, significant reductions in FSG were observed earlier and across more groups. On day 21, significant lower FSG levels in the early dose (224.37 ± 24.07 mg/dL; $p = 0.004$), late dose (158.42 ± 15.68 mg/dL; $p < 0.001$), and repeated dose (136.60 ± 6.64 mg/dL; $p < 0.001$) groups were observed compared to the diabetic control (319.15 ± 18.59 mg/dL). At the same time point, the late dose and repeated dose groups also showed significantly lower FSG levels than the early dose group ($p = 0.046$ and $p = 0.006$, respectively) (Figure 2b).

By day 49, FSG levels in the late dose (150.72 ± 20.00 mg/dL; $p = 0.002$) and repeated dose (174.27 ± 16.32 mg/dL; $p = 0.018$) groups were significantly lower compared to the early dose (271.10 ± 24.10 mg/dL). At the same time point, these groups also showed significantly lower FSG levels than the diabetic control (285.81 ± 23.99 mg/dL; $p < 0.001$ and $p = 0.005$, respectively). However, on day 56, only the repeated dose group showed significantly lower FSG levels compared to the diabetic control group (200.78 ± 6.85 mg/dL vs 316.30 ± 22.92 mg/dL; $p = 0.002$) (Figure 2b).

Changes in FUG Levels

In the 29-day experiment, no significant difference in FUG levels between diabetic control and early dose group in all time points (Figure 2c). In 56-day experiment, both the late dose and repeated dose groups also showed significantly lower levels compared to the diabetic control group on day 49 (4396.63 ± 854.60 mg/dL vs $10,162.17 \pm 496.00$ mg/dL; $p = 0.001$ and 2256.57 ± 273.20 mg/dL vs $10,162.17 \pm 496.00$ mg/dL; $p < 0.001$, respectively). Compared to the early dose group, the late dose group showed significantly lower FUG levels on day 49 (4396.63 ± 854.60 mg/dL vs 8770.50 ± 827.10 mg/dL; $p = 0.029$). The repeated dose group demonstrated significantly lower FUG levels than the early dose group on day 7 (3176.90 ± 355.80 mg/dL vs 5451.80 ± 336.70 mg/dL; $p = 0.006$), day 21 (6859.10 ± 560.40 mg/dL vs $11,208.00 \pm 1000.00$ mg/dL; $p = 0.042$), day 49 (2256.60 ± 273.20 mg/dL vs 8770.50 ± 827.10 mg/dL; $p = 0.002$), and day 56 (3821.70 ± 396.30 mg/dL vs 9696.00 ± 1035.60 mg/dL; $p = 0.011$). Additionally, on day 56, the repeated dose group also exhibited significantly lower FUG levels compared to the late dose group (3821.70 ± 396.30 mg/dL vs 8824.00 ± 565.10 mg/dL; $p < 0.001$) (Figure 2d).

Overall, the early dose group showed some glycemic control early, but this was not sustainable. The late dose group showed partial reduction, while the repeated dose group demonstrated better and more sustained reduction in glucose levels across all 3 glycemic parameters measured.

Effect of hUC-MSCs on Inflammatory Marker Hs-CRP Levels in Diabetic Rats

We further assessed the effects of hUC-MSCs on hs-CRP levels in diabetic rats on days 29 and 56 (Figure 3b and Table S2). Both normal control and diabetic control groups showed higher hs-CRP levels (> 4 mg/L) on both days 29 and 56.

Following administration of hUC-MSCs, there was a significant reduction in hs-CRP levels in the early dose group compared to the diabetic control group on days 29 (2.29 ± 0.27 mg/L vs 4.70 ± 0.22 mg/L; $p < 0.001$) and 56 (3.41 ± 0.18 mg/L vs 4.08 ± 0.14 mg/L; $p = 0.031$). Similarly, the repeated dose group showed significantly lower hs-CRP levels than the diabetic control group on day 56 (3.35 ± 0.13 mg/L vs 4.08 ± 0.14 mg/L; $p = 0.012$). Interestingly, the early dose group also showed a significant reduction in hs-CRP levels compared to the normal control group on days 29 (2.29 ± 0.27 mg/L vs 4.41 ± 0.15 mg/L; $p < 0.001$) and 56 (3.41 ± 0.18 mg/L vs 4.17 ± 0.06 mg/L; $p = 0.029$), while the repeated dose group demonstrated significant reduction on day 56 (3.35 ± 0.13 mg/L vs 4.17 ± 0.06 mg/L; $p = 0.012$).

Effect of hUC-MSCs on Pancreatic Islet Cells in Diabetic Rats

Histological (H&E staining) and immunohistochemical (IHC staining) analyses were performed to evaluate islet morphology and the expression of insulin and glucagon (Figures 4 and 5). The pancreatic histological scores were tabulated in Table 1. Based on the gross pathology, the weight of the pancreas is only significantly higher in the diabetic control group compared to the normal control group.

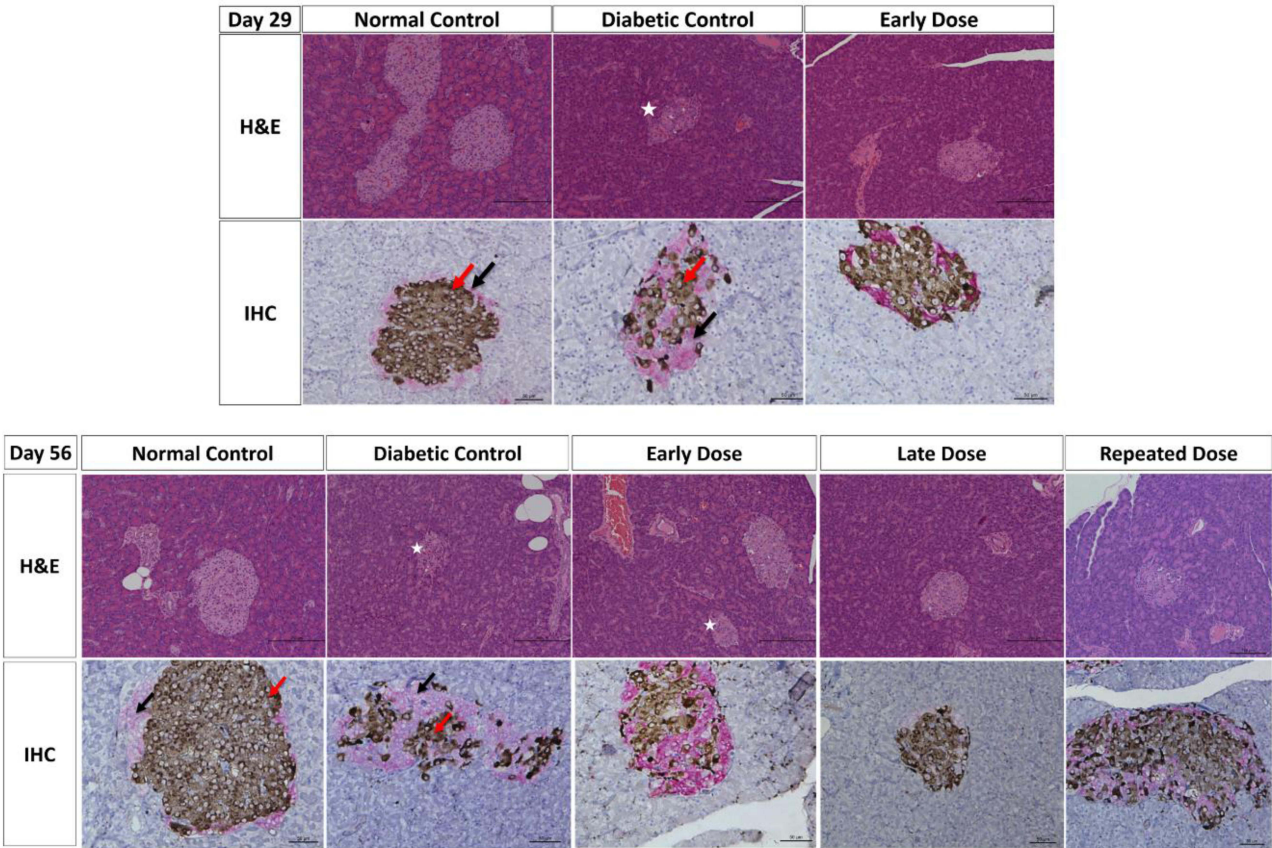


Figure 4 Representative histological images of the pancreas showing their islets in all groups at days 29 and 56. Black arrow indicates glucagon expression, the red arrow indicates insulin expression, and the star symbol indicates islet degeneration and size depletion H&E: hematoxylin and eosin staining and IHC: immunohistochemistry staining. (For H&E, scale bar = 100–200 µm; for IHC, scale bar = 50 µm).

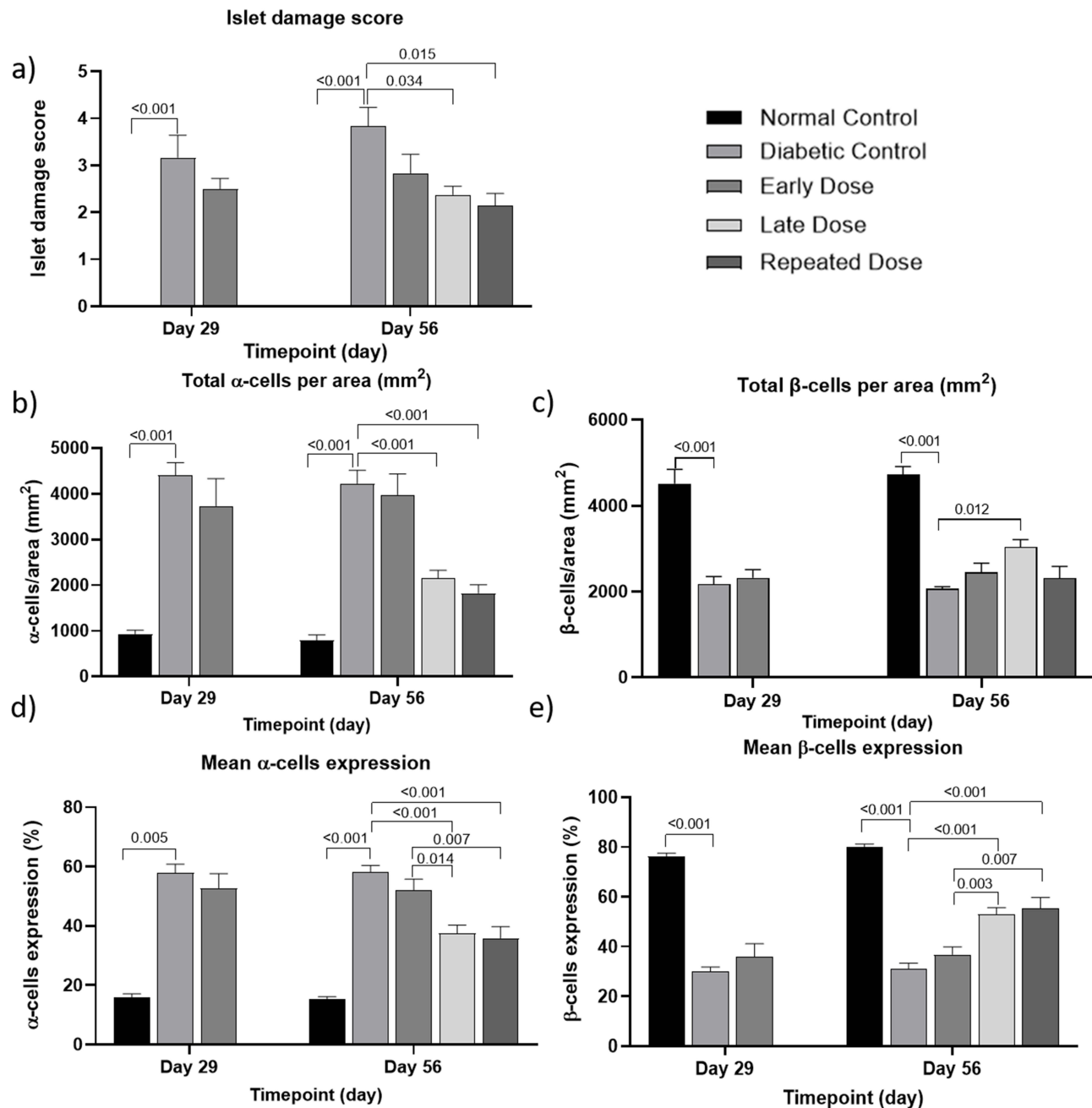


Figure 5 Graphs showing (a) islet damage scores, (b) total expression of α cells, (c) total expression of β cells, (d) mean expression of α cells, and (e) mean expression of β cells. Data are presented as mean $\pm$ SEM. One-way ANOVA analysis with post hoc Tukey's test for multiple comparisons was performed for both 29-day and 56-day of pancreas histology scores. Kruskal–Wallis test followed by Dunn's test for multiple comparisons was performed for islet damage score on both 29-day and 56-day experiment. 29-day experiment: normal control, $n=5$, diabetic control, $n=6$, early dose, $n=6$, 56-day experiment: normal control, $n=4$, diabetic control, $n=6$, early dose, $n=6$, late dose, $n=8$, repeated dose, $n=7$.

Abbreviations: g, gram; mm², millimetersquare; pmol/L, picomole per litre.

The normal control group exhibited normal islet structure, with a well-defined shape and balanced insulin and glucagon expressions. In contrast, the diabetic control group exhibited significantly higher islet damage scores, increased total α -cell counts, elevated mean α -cell expression, reduced total β -cell counts and mean β -cell expression as compared to normal control group on days 29 and 56 (all p -values < 0.01), indicating extensive islet injury and dysregulated glucagon/insulin balance in the diabetic control group.

Table 1 The Pancreas Histological Scores Between the Groups at Day 29 and Day 56

Parameter		Normal Control	Diabetic Control	Early Dose	Late Dose	Repeated Dose	P Value
Islet damage score	Day 29	0.00 ± 0.00	3.17 ± 0.48*	2.50 ± 0.22	—	—	0.002 ^b
	Day 56	0.00 ± 0.00	3.83 ± 0.40*	2.83 ± 0.40	2.38 ± 0.18 [#]	2.14 ± 0.26 [#]	0.001 ^b
Total α-cells/area (mm ²)	Day 29	934 ± 86.00	4411 ± 271.00*	3735 ± 601.00	—	—	< 0.001 ^a
	Day 56	794 ± 120.30	4229 ± 289.60*	3981 ± 455.52	2151 ± 177.04 [#]	1826 ± 182.48 [#]	< 0.001 ^a
Total β-cells/area (mm ²)	Day 29	4515 ± 330	2173 ± 177*	2320 ± 189	—	—	< 0.001 ^a
	Day 56	4730 ± 179	2065 ± 49*	2444 ± 218	3039 ± 172 [#]	2311 ± 274	< 0.001 ^a
Mean α-cells (%)	Day 29	15.84 ± 1.30	59.49 ± 3.18*	54.76 ± 4.90	—	—	< 0.001 ^a
	Day 56	15.38 ± 0.80	58.32 ± 2.07*	52.18 ± 3.57	37.51 ± 2.77 ^{#†}	35.91 ± 3.84 ^{#†}	< 0.001 ^a
Mean β-cells (%)	Day 29	76.08 ± 1.46	29.26 ± 1.93*	35.81 ± 5.29	—	—	< 0.001 ^a
	Day 56	79.89 ± 1.31	30.94 ± 2.34*	36.82 ± 2.99	53.07 ± 2.60 ^{#†}	55.26 ± 4.49 ^{#†}	< 0.001 ^a

Notes: For post hoc analysis, * ($p < 0.05$) compared to normal control, [#] ($p < 0.05$) compared to diabetic control, and [†] ($p < 0.05$) compared to early dose. ^aOne-way ANOVA analysis with post hoc Tukey's test for multiple comparisons was performed for both 29-day and 56-day of total and mean α and β cells. ^bKruskal–Wallis test followed by Dunn's test for multiple comparison was performed for islet damage score on both 29-day and 56-day experiment.

Early administration of hUC-MSCs did not significantly reduce islet damage scores or glucagon expression, nor did it significantly increase insulin expression compared to the diabetic control group on either day 29 or 56. Whereas on day 56, both the late dose and repeated dose groups demonstrated a significant reduction in islet damage scores ($p = 0.034$ and $p = 0.015$, respectively), total α-cell counts, mean α-cell expression, and significantly higher mean β-cell expression compared to the diabetic control group. Notably, the late dose group showed a significant increase in total β-cell counts compared to the diabetic control ($p = 0.012$). All p -values < 0.001 unless otherwise indicated.

On day 56, both groups also demonstrated significantly lower mean α-cell expression than the early dose group ($p = 0.014$ and $p = 0.007$, respectively) and significantly higher mean β-cell expression ($p = 0.003$ and $p = 0.007$, respectively). Overall, these findings suggest that late and repeated doses of hUC-MSCs may protect pancreatic islet cells from damage and regulate the insulin and glucagon expressions in diabetic rats (Figure 5).

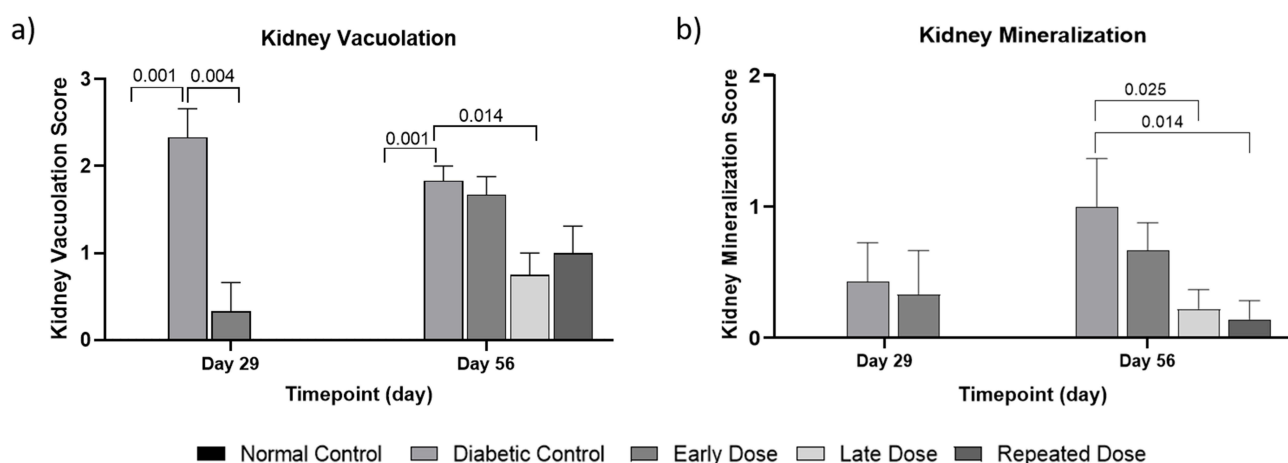


Figure 6 Graphs show the effect of hUC-MSCs at different dose phases on (a) kidney vacuolation tubule and (b) kidney mineralization tubule in the 29- and 56-day experiment. Data presented as mean ± SEM. One-way ANOVA analysis with post hoc Tukey's test for multiple comparisons was performed for both 29-day and 56-day kidney mineralization scores. Kruskal–Wallis test followed by Dunn's test for multiple comparisons was performed for both 29-day and 56-day kidney vacuolation scores. 29-day experiment: normal control, $n = 5$, diabetic control, $n = 6$, early dose, $n = 6$, 56-day experiment: normal control, $n = 4$, diabetic control, $n = 6$, early dose, $n = 6$, late dose, $n = 8$, repeated dose, $n = 7$.

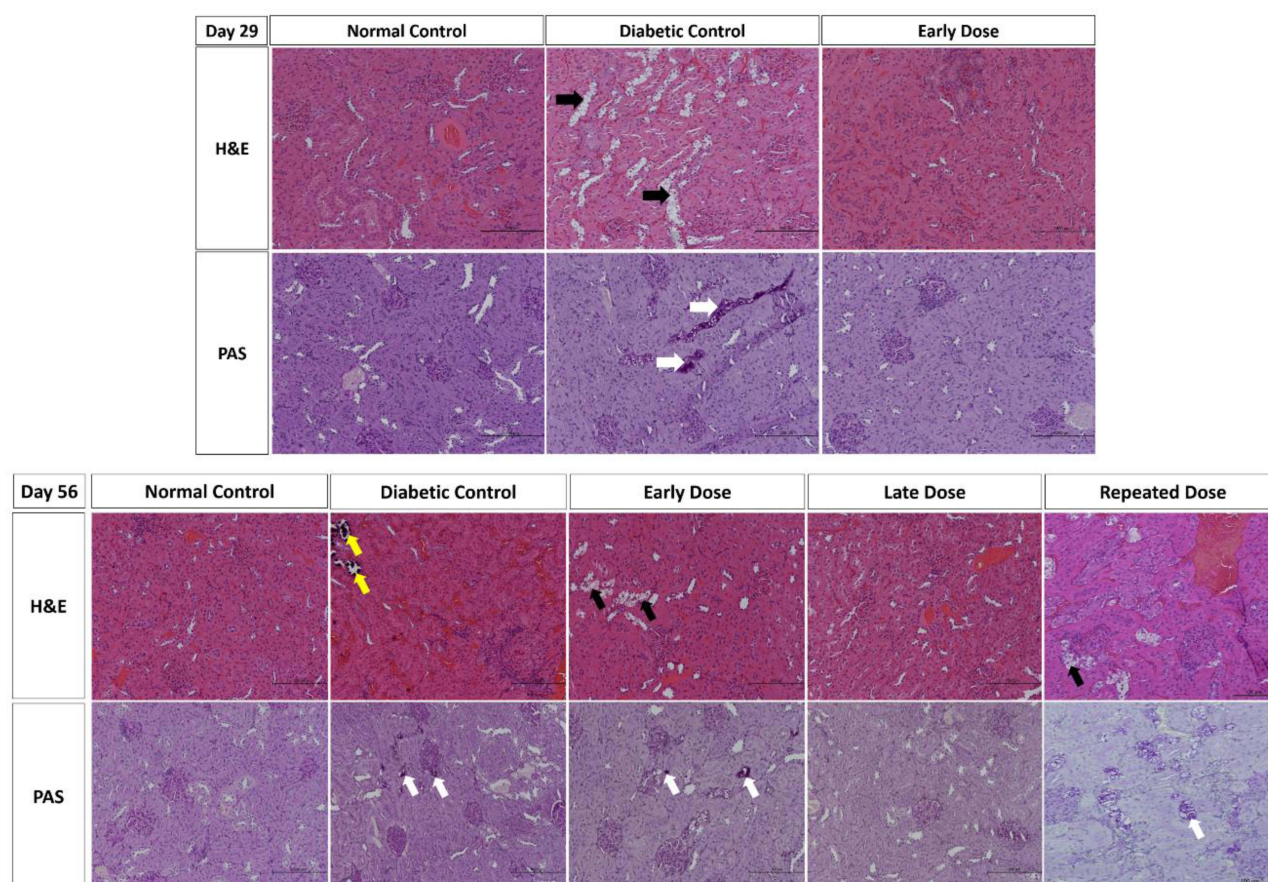


Figure 7 Histological images of the kidney section at days 29 and 56 show tubule vacuolation and mineralization in the diabetic control group. The black arrow shows vacuolation tubule, the yellow arrow shows mineralization tubule, and the white arrow shows glycogen deposition. Less vacuolation and mineralization were observed in hUC-MSCs treated diabetic groups. (For H&E and PAS staining, scale bar = 100–200 μ m).

Abbreviations: H&E, hematoxylin and eosin staining; PAS, Periodic Acid-Schiff staining.

Effect of hUC-MSCs on Renal Damage in Diabetic Rats

To evaluate the effect of hUC-MSCs on renal damage in diabetic rats, H&E and PAS stainings were performed. Based on the gross pathology, no significant changes were observed in the kidney weight across all groups. Histological analysis revealed prominent tubular injury, including vacuolation (black arrows), glycogen accumulation (white arrows), and mineral deposits (yellow arrows) (Figures 6 and 7). The renal histological scores were tabulated in Table 2.

No glomerular abnormalities were noted, indicating that damage was primarily confined to the tubules. H&E staining showed significant tubular vacuolation in the diabetic control group compared to the normal control group on both days 29 ($p = 0.001$) and 56 ($p = 0.001$). Albeit not statistically significant, mineralization scores were higher in the diabetic

Table 2 The Kidney Histological Scores Between the Groups at Day 29 and Day 56

Parameter		Normal Control	Diabetic Control	Early Dose	Late Dose	Repeated Dose	P Value
Kidney vacuolation	Day 29	0.00 $\pm$ 0.00	2.33 $\pm$ 0.33*	0.33 $\pm$ 0.33 [#]	–	–	0.002 ^b
	Day 56	0.00 $\pm$ 0.00	1.83 $\pm$ 0.17*	1.67 $\pm$ 0.21	0.75 $\pm$ 0.25 [#]	1.00 $\pm$ 0.31	0.003 ^b
Kidney mineralization	Day 29	0.00 $\pm$ 0.00	0.50 $\pm$ 0.34	0.33 $\pm$ 0.33	–	–	0.400 ^a
	Day 56	0.00 $\pm$ 0.00	1.00 $\pm$ 0.37	0.67 $\pm$ 0.21	0.25 $\pm$ 0.16 [#]	0.14 $\pm$ 0.14 [#]	0.049 ^a

Notes: For post hoc analysis, * ($p < 0.05$) compared to normal control group and [#] ($p < 0.05$) compared to diabetic control group. ^aOne-way ANOVA analysis with post hoc Tukey's test for multiple comparisons was performed for both 29-day and 56-day kidney mineralization scores. ^bKruskal–Wallis test followed by Dunn's test for multiple comparison was performed for both 29-day and 56-day kidney vacuolation scores.

control group compared to the normal control group. There was no significant increase in kidney weight between the diabetic control and normal control groups. Kidney tubular vacuolation was significantly less seen in the early dose group on day 29 ($p = 0.004$) and in the late dose group on day 56 ($p = 0.014$) compared to the diabetic control group. Mineralization scores were significantly lower in both the late dose ($p = 0.025$) and repeated dose ($p = 0.014$) groups on day 56 compared to the diabetic control group. Overall, these findings suggest that hUC-MSCs have therapeutic potential in ameliorating kidney tubular injury in diabetic rats.

Correlation Analysis

Using Pearson correlation analysis, we found that glucose marker HbA1c was significantly associated with several histopathological and cellular parameters (Table S1). Specifically, HbA1c showed positive correlations with kidney vacuolation ($r = 0.56$, $p < 0.001$), kidney mineralization ($r = 0.48$, $p < 0.001$), islet damage score ($r = 0.71$, $p < 0.001$), total α -cells counts ($r = 0.68$, $p < 0.001$) and mean α -cells expression ($r = 0.73$, $p < 0.001$). In contrast, HbA1c was negatively correlated with total β -cell counts ($r = -0.55$, $p < 0.001$) and mean β -cell expression ($r = -0.75$, $p < 0.001$). A trend towards a positive correlation was also observed between HbA1c and hs-CRP levels ($r = 0.601$, $p = 0.051$), indicating a possible association, albeit not statistically significant. However, hs-CRP levels were significantly correlated with kidney vacuolation ($r = 0.71$, $p = 0.01$), indicating a strong positive correlation. Furthermore, multiple regression analysis identified both hs-CRP (coefficient, b : 0.46; 95% CI: 0.16, 0.75) and HbA1c (coefficient, b : 0.42; 95% CI: 0.24, 0.61) as independent predictors of nephropathy, accounting 45% variation in kidney vacuolation (adjusted $R^2 = 0.45$).

Discussion

This study aimed to evaluate the therapeutic effects of intravenous administration of hUC-MSCs on glycemic control, inflammation, pancreatic islet preservation, and renal protection in a T2DM rat model. Our preclinical study comparing early, late, and repeated dosing reflects the clinical situation, whether treatment soon after diagnosis offers better long-term control than delayed intervention. Early hUC-MSCs administration during this period is aimed at preserving pancreatic islet and kidney function, rather than to reverse advanced damage. The current findings may support an early-intention-to-treat strategy, which could improve long-term outcomes when translating hUC-MSCs therapy to patient care.

In this study, the model was established using a combination of HFD and low-dose STZ, which induced insulin resistance and β -cell damage, thereby effectively replicating human T2DM.^{16,30} At baseline (day -1), rats developed early-stage T2DM, with FSG levels ranging from prediabetic (100–126 mg/dL) to diabetic thresholds (>126 mg/dL).^{16,31,32} While previous studies focused on T2DM models with advanced complications, the current study aimed to provide evidence on the effects of hUC-MSCs during the early stage of the disease.

Among the three dosing regimens of hUC-MSCs tested (early, late, and repeated), the repeated dose of hUC-MSCs demonstrated the most substantial therapeutic effects in glycemic control. This group demonstrated better glycemic control markers as shown by lower FSG, FUG, and HbA1c levels compared to both the diabetic control and other dosing groups. These findings were consistent with a previous study reported on the efficacy of multiple infusions of hUC-MSCs in ameliorating hyperglycemia at the early stage of T2DM.¹⁰ In contrast, the early dose group showed transient improvements in glycemic parameters, while late dose group was unable to sustain glycemic control, as indicated by increased FUG levels from day 49 to day 56 and no significant reduction in HbA1c levels.

In terms of systemic inflammation, the repeated dose group significantly reduced hs-CRP levels to even lower than normal controls, indicating a potent anti-inflammatory immunomodulatory effect. Interestingly, the early dose group also decreased hs-CRP on days 29 and 56, despite limited glycemic improvement. Conversely, the late dose group did not reduce systemic inflammation. These findings are consistent with previous study reported that early administration of hUC-MSCs in diabetic nephropathy mice resulted in a significant reduction of circulating IL-1 β and TNF- α , whereas later administration failed to suppress these pro-inflammatory cytokines.³³

Pancreatic islet preservation was another prominent beneficial outcome associated with repeated hUC-MSCs administration. Histological and immunofluorescence analyses demonstrated preserved islet architecture and restoration of the insulin-to-glucagon ratio, indicating a preservation of β -cell function and regulation of α -cell hyperactivity. This was aligned with previous studies that demonstrated pancreatic islet recovery after repeated intravenous administrations of

BM-MSCs initiated early.^{15,34} This structural integrity was not maintained in the early dose group, where islet damage was still evident. Renal protection was also most apparent in the repeated dose group, with reduced vacuolar degeneration observed in renal tissues.

Multivariate regression analysis identified both HbA1c and hs-CRP as independent predictors of renal injury, collectively accounting for 45% of the variance in kidney vacuolation. A trend toward a positive correlation between HbA1c and hs-CRP ($p = 0.051$) suggests a potential interaction between metabolic and inflammatory factors in diabetic nephropathy. These results are in line with prior studies linking hyperglycemia and systemic inflammation to diabetic kidney damage.^{35,36} In the present study, HbA1c was also significantly associated with renal and islet damage, as well as the imbalance in insulin and glucagon expression within the islets.

Hsiao et al (2025) reported similar findings to the current study regarding the immunomodulatory and renoprotective effects of hUC-MSCs in a type 1 diabetes mellitus (T1DM) model. The study reported that the multiple infusions of hUC-MSCs (3×10^7 cells/kg) significantly reduced pro-inflammatory cytokines including IL-1 β and TNF- α compared to diabetic kidney disease (DKD) control group. The renoprotective effects, including reduced glomerulosclerosis and inflammation, were most prominent with the most frequent hUC-MSCs infusions.³⁷ While both studies highlight the benefits of hUC-MSCs in renal protection, the current study extends these findings by also investigating the effects of hUC-MSCs on glycemic control and pancreatic β -cell preservation in a T2DM model. In contrast, Hsiao et al focused solely on renal outcomes in T1DM-induced DKD. Despite differences in disease models and study focus, both studies support the therapeutic potential of repeated high-dose hUC-MSCs infusions, but the current research shows more comprehensive benefits in T2DM rats, including both renal protection and better glycemic control.

The observed therapeutic effects are likely mediated by their immunomodulatory and paracrine properties of hUC-MSCs. Previous studies have shown that hUC-MSCs reduce hs-CRP levels in conditions such as lung infection, cardiovascular diseases,³⁸ and diabetic retinopathy.³⁹ Similarly, BM-MSCs have shown the ability to lower hs-CRP both in T1DM and T2DM animal models.⁴⁰ In the current study, hUC-MSCs effectively reduced the systemic hs-CRP levels, suggesting effective modulation of inflammatory responses. Additionally, hUC-MSCs have also been reported to suppress pro-inflammatory M1 macrophages and promote their polarization to anti-inflammatory M2 macrophages, thereby helping to reduce islet inflammation.^{41–43} In line with previous reports, the current findings suggest that hUC-MSCs home to inflamed tissues and secrete paracrine factors that support tissue regeneration and attenuate damage, particularly in pancreatic and renal structures.^{18,44,45}

Taken together, these results highlight the importance of initiating treatment during the early stage of diabetes to bring blood sugars and inflammation under control, but also underscore the need for repeated administration to achieve therapeutic benefit, especially in preserving pancreatic beta islet and renal tissue. These results also highlight the need to understand the mechanism of action of hUC-MSCs following dosing regimen to support clinical translation as previously reported.⁷

While the results of this study highlight the therapeutic potential of hUC-MSCs at the onset of T2DM, several limitations should be acknowledged. The relatively small sample sizes per group, with some groups having fewer than 10 rats, may reduce the robustness of the findings. The relatively short duration of the experiment limited the assessment of long-term diabetic complications such as cardiomyopathy, nephropathy, neuropathy, and retinopathy, which typically manifest after extended periods in diabetic models.^{46–48} In addition, the use of only male rats limits evaluation of potential sex-specific differences. Male rats are more susceptible to diet-induced insulin resistance and glucose intolerance, providing a more consistent disease phenotype.⁴⁹ In contrast, female rats were not used in this study as hormonal fluctuations may introduce variability and reduce model consistency and reproducibility.^{50,51} However, to improve translational relevance, future studies should include both sexes. Considering the rat-to-human age conversion ratio,⁵² the two-month diabetes duration used in this study corresponds to approximately six human years. Looking ahead, larger, sex-balanced studies with extended follow-up, detailed mechanistic analyses, and formal dose-finding experiments are still needed to establish the translational relevance of hUC-MSCs therapy before advancing to human trials. Nevertheless, our findings provide preclinical evidence supporting the therapeutic potential of hUC-MSCs in managing and mitigating diabetic complications in a rat model.

Conclusion

This study highlights the therapeutic potential of hUC-MSCs in managing type 2 diabetic rat model and demonstrates the repeated systemic administration at 3.5×10^6 cells/rat was well tolerated. Repeated dosing when initiated early is better than early dose or late dose in reducing glucose levels and inflammation, preserving pancreatic β -cell, and conferring renal protection.

Data Sharing Statement

The data analyzed to support the findings of this study are available from the corresponding author upon request.

Ethics Statement

Animal ethics approval was obtained from the Institutional Animal Ethics Committee (SYNGENE/IAEC/1379/05-2022), Syngene International, Bangalore, India, reported according to ARRIVE and International Committee of Medical Journal Editors (ICMJE) guidelines.

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Author Contributions

Sze-Piaw Chin: Conceptualization, Methodology, Validation, Writing-Original Draft, Visualization, Project Administration. Erlena Nor Asmira Abd Rahim: Formal Analysis, Investigation-Data collection, Data Curation, Writing-Original Draft, Visualization. Natasha Najwa Nor Arfuzir: Methodology, Formal Analysis, Data Curation, Writing-Original Draft, Visualization. Kong-Yong Then: Validation, Writing-Review & Editing, Funding Acquisition. Soon-Keng Cheong: Conceptualization, Writing-Review & Editing, Supervision. All authors gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

S.P.C. advises Cytopeutics Sdn Bhd. on regulatory, clinical, and research activities; reports personal fees from Cytopeutics during the conduct of the study and outside the submitted work; and has a patent Treatment of diabetes issued to Assigned to company. S.P.C., S.K.C. and K.Y.T. sits on Cytopeutics medical advisory board. The authors have no other conflicts of interest to declare.

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