Journal of Molecular Histology (2026) 57:233
https://doi.org/10.1007/s10735-026-10896-y

ORIGINAL PAPER

Cell-free MSC secretome therapy ameliorates diabetic testicular injury via antioxidant mechanisms

Serbay Ozkan[1] · Basak Isildar[2] · Aslı Erdogan Oner[1] · Ismail Unal[3] · Ebru Emekli-Alturfan[4] · Meltem Kurus[1] · Meral Koyuturk[5]

Received: 16 May 2026 / Accepted: 4 July 2026 / Published online: 14 July 2026

Abstract

Diabetes mellitus is a metabolic disorder characterized by persistent hyperglycaemia that may impair male reproductive function. Mesenchymal stem/stromal cell-derived conditioned medium (MSC-CM) has emerged as a promising cell-free therapeutic approach for diabetes-associated complications. This study investigated the effects of CM obtained from MSCs cultured under normoxic (N-CM) or deferoxamine (DFX)-induced hypoxia-mimetic conditions (DFX-CM) on diabetes-related testicular dysfunction in streptozotocin (STZ)-induced diabetic rats. Diabetes was induced in Sprague Dawley rats using a single intraperitoneal injection of STZ (55 mg/kg). Diabetic rats received intraperitoneal administration of N-CM or DFX-CM for 3 weeks. Serum reproductive hormones were analysed by ELISA. Histological and histomorphometric evaluations were performed using haematoxylin and eosin staining, while ultrastructural alterations were examined by transmission electron microscopy (TEM). Oxidative stress markers and antioxidant enzyme activities were also assessed in testicular tissue. Diabetes reduced serum follicle-stimulating hormone (FSH), gonadotropin-releasing hormone, and luteinizing hormone levels, although only FSH showed a significant decrease. Histological analyses revealed mild impairment of spermatogenesis in diabetic rats without significant morphometric alterations. TEM demonstrated marked ultrastructural abnormalities in diabetic testes, including Sertoli cell degeneration and disrupted spermatogenic cell morphology. Both CM treatments attenuated these alterations, with improved ultrastructural organization observed particularly in Sertoli cells. Diabetes also induced oxidative stress, evidenced by reduced catalase and superoxide dismutase activities and elevated malondialdehyde and H_2O_2 levels. N-CM treatment showed the strongest antioxidant effects. These findings suggest that MSC-CM, particularly N-CM, may alleviate diabetes-induced testicular damage by restoring oxidative balance and preserving seminiferous tubule ultrastructure.

Keywords Diabetes · Reproductive dysfunction · Mesenchymal stem/stromal cells · Conditioned media · Oxidative stress

✉ Serbay Ozkan
ozkanserbay@gmail.com; serbay.ozkan@istanbul.edu.tr

Basak Isildar
basakisildar@gmail.com

Aslı Erdogan Oner
asli.erdogan@ikc.edu.tr

Ismail Unal
ismail.unal@medipol.edu.tr

Ebru Emekli-Alturfan
eiemekli@marmara.edu.tr

Meltem Kurus
meltem.kurus@ikcu.edu.tr

Meral Koyuturk
koyuturk@iuc.edu.tr

1 Faculty of Medicine, Histology and Embryology Department, İzmir Katip Çelebi University, Izmir, Turkey

2 Faculty of Medicine, Histology and Embryology Department, Balıkesir University, Balıkesir, Turkey

3 Faculty of Medicine, Department of Medical Biochemistry, Istanbul Medipol University, Istanbul, Turkey

4 Faculty of Dentistry, Department of Basic Medical Sciences, Marmara University, Istanbul, Turkey

5 Cerrahpaşa Faculty of Medicine, Department of Histology and Embryology, İstanbul University-Cerrahpaşa, Istanbul, Turkey

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from insufficient insulin production due to pancreatic β-cell dysfunction or destruction (Khatri et al. 2021). It is associated with a wide range of systemic complications, including cardiovascular disease, nephropathy, neuropathy, and reproductive dysfunction (Atkinson et al. 2014). Although exogenous insulin therapy remains the primary treatment strategy, it primarily controls glycaemia and does not adequately prevent the progression of long-term complications (He et al. 2021). Among these complications, reproductive dysfunction has gained increasing attention. Hyperglycaemia has been suggested to disrupt the hypothalamic–pituitary–gonadal (HPG) axis, leading to hormonal imbalances, including altered testosterone levels (Olojede et al. 2022). Preclinical studies have demonstrated that diabetes is associated with reduced sperm count and motility, as well as structural alterations in testicular tissue (Olojede et al. 2022). These findings highlight the need for novel therapeutic strategies targeting both endocrine regulation and tissue-level damage. In recent years, mesenchymal stem/stromal cells (MSCs) have emerged as a promising therapeutic approach for diabetes and its complications (Isildar et al. 2023). Their beneficial effects are generally attributed to paracrine mechanisms, including the secretion of bioactive factors with angiogenic, anti-apoptotic, anti-inflammatory, immunomodulatory, and antioxidant properties (Wu and Mahato 2014; Endlich et al. 2017; Xv et al. 2017; Isildar et al. 2024; Ozkan et al. 2024; Sun et al. 2025). Accordingly, MSC-derived conditioned medium (MSC-CM), which contains a complex mixture of soluble factors and extracellular vesicles, has been increasingly investigated as a cell-free therapeutic alternative. Compared with direct cell transplantation, MSC-CM offers advantages such as improved safety, reduced immunogenicity, and greater reproducibility, while maintaining comparable therapeutic efficacy in many experimental settings (Nagaishi et al. 2016, 2017; Kerans et al. 2018).

The therapeutic potential of MSC-derived cell-free products has been demonstrated across a variety of disease models and organ systems, supporting their use as an alternative to cell-based therapies. For example, CM derived from adipose tissue MSCs improved cardiovascular function and attenuated tissue injury in an insecticide-induced cardiac damage model, as evidenced by enhanced mean arterial pressure, normalization of electrocardiographic parameters, reduced oxidative stress, and suppression of pro-inflammatory cytokines, including TNF-α and IL-6 (Zangiabadi et al. 2025). Similarly, extracellular vesicles derived from umbilical cord MSCs ameliorated ischemic injury in proximal tubular epithelial cells by promoting morphological recovery, restoring ATP production capacity, and enhancing antioxidant metabolite accumulation (Faria et al. 2023). Beyond cardiovascular and renal applications, MSC secretome has also shown regenerative effects in cutaneous wound healing. Secretome obtained from Wharton's jelly-derived MSCs stimulated human dermal fibroblast proliferation, accelerated scratch-wound closure, promoted extracellular matrix remodelling and type I procollagen secretion, and enhanced anti-inflammatory and antioxidant responses (Lee et al. 2026). Collectively, these findings indicate that MSC-derived secretome exerts broad cytoprotective, anti-inflammatory, and antioxidant effects across different tissues, highlighting its potential as a versatile therapeutic strategy for diseases characterized by oxidative stress and tissue degeneration.

The therapeutic potential of MSCs and their secretome can be enhanced through preconditioning strategies. Approaches such as hypoxic culture, three-dimensional systems, or exposure to hypoxia-mimetic agents have been shown to modulate the secretory profile of MSCs, thereby improving their regenerative capacity (Oses et al. 2017a; Isildar et al. 2022, 2024; Ozkan et al. 2022). Among these, deferoxamine (DFX), an iron-chelating hypoxia mimetic, has been widely used to induce hypoxia-like responses in MSCs (Oses et al. 2017a; Ozkan et al. 2022; Isildar et al. 2024). In our previous studies, DFX preconditioning significantly enhanced the protein content of MSC-CM and increased the levels of key growth factors, including vascular endothelial growth factor, leading to improved pancreatic β-cell recovery and immunomodulatory effects (Isildar et al. 2022, 2024). When the role of oxidative stress as an underlying mechanism for diabetes-induced tissue damage, including in the testes, strategies that enhance the antioxidant capacity of MSC-derived products may provide additional therapeutic benefit (Caturano et al. 2023). In this context, hypoxia-mimetic preconditioning has been shown to improve the antioxidant potential of MSCs (Chang et al. 2019; Isildar et al. 2025). Therefore, in the present study, it was aimed to investigate diabetes-associated reproductive dysfunction with a focus on alterations in the HPG axis and their relationship with oxidative stress in testicular tissue. Furthermore, the therapeutic efficacy of CM derived from DFX-preconditioned MSCs was evaluated in a rat model of diabetes by assessing its effects on oxidative stress and testicular histopathological and morphometric alterations.

Material & method

Culture of hUC-MSCs and collection of CM

In this study, our established human umbilical cord-derived mesenchymal stem/stromal cell (hUC-MSC) lines, which had been previously isolated and characterized in our earlier study, were used for the collection of CM (Ozkan et al. 2022). hUC-MSCs were expanded until they reached 70–80% confluence, after which the culture medium was removed and replaced with serum-free medium. The cultures were then divided into two experimental conditions: (i) normoxic control CM (N-CM) and (ii) hypoxia-mimetic preconditioned CM (DFX-CM), in which 150 µM deferoxamine (DFX) was added to the culture medium. The hypoxia-mimetic effect of this DFX concentration has been previously confirmed in hUC-MSCs by increased hypoxia-inducible factor-1 alpha (HIF-1α) expression under identical experimental conditions (Ozkan et al. 2022). After 48 h of incubation, the CM were collected and centrifuged to remove cellular debris. Subsequently, the supernatants were filtered to eliminate residual DFX and concentrated using centrifugal filtration units with a 3 kDa molecular weight cut-off membrane (Ozkan et al. 2022).

Animal models and treatment groups

Ethical approval for the animal experiments was obtained from the Experimental Animal Ethics Committee of Bezmiâlem Vakıf University (Date: 10.05.2023; Approval No: E.106223). A total of 20 male Sprague–Dawley rats, aged 10–12 weeks and weighing 350–400 g, were included in this study. Prior to the experimental procedures, the animals were acclimatized to the housing environment for one week. Rats were maintained under standard laboratory conditions, including a 12 h light/dark cycle, ambient temperature of 20 ± 2 °C, relative humidity of 45–55%, and ad libitum access to food and water. Type 1 diabetes mellitus was induced in 16 rats by a single intraperitoneal injection of streptozotocin [STZ (Sigma S0130); 55 mg/kg] dissolved in citrate buffer, while the remaining control animals received citrate buffer only and were designated as the negative control group. Blood glucose levels were measured from the tail vein by using a glucometer (Contour Plus, Bayer) on day 3 following STZ administration, and animals with glucose levels exceeding 250 mg/dL were considered diabetic and included in the study (Isildar et al. 2024). Four weeks after STZ injection, diabetic rats were randomly divided into three groups: diabetic untreated group (D, n = 4), diabetic rats treated with normoxic CM (D + N-CM, n = 6), and diabetic rats treated with DFX-preconditioned CM (D + DFX-CM, n = 6). The treatment groups received intraperitoneal injections of either 1 ml of N-CM or DFX-CM; four doses per week for the next 3 weeks. One week after the final administration, the animals were anesthetized with ketamine/xylazine and sacrificed. Blood samples were collected for hormonal analyses, and testicular tissues were harvested for biochemical and histological evaluations. Body weight and blood glucose levels were monitored weekly throughout the experimental period.

ELISA analyses of serum hormone levels

At the end of the experimental period (week 8), blood samples were collected via cardiac puncture and centrifuged at 800 × g for 10 min. The serum supernatants were separated and stored for further analyses. Serum testosterone (Cloud-Clone, CEA458Ge), luteinizing hormone (LH; Cloud-Clone, CEA441Ra), follicle-stimulating hormone (FSH; Cloud-Clone, CEA830Ra), and gonadotropin-releasing hormone (GnRH; Cloud-Clone, CEA843Ra) levels were measured using commercially available ELISA kits according to the manufacturers' instructions. Optical density (OD) values were determined using a microplate reader, and hormone concentrations were calculated from the corresponding standard curves.

Light microscopic analyses

Right testis obtained from each rat was fixed in Davidson's fixative for light microscopic examination, while the left one was cut into 1 mm^3 pieces and fixed in glutaraldehyde for electron microscopic analysis. For light microscopy, tissues were dehydrated through a graded alcohol series, embedded in paraffin, and sectioned at 5 µm thickness (Bancroft et al. 2013). Following haematoxylin and eosin (H&E) staining, sections were examined with an Olympus BX43 light microscope and photographed with an Olympus SC100 camera and processed with imaging software (Olympus Labsens, version 1.3). Ten seminiferous tubules from each rat were randomly selected and evaluated at 40× magnification. Spermatogenesis was assessed using the Johnsen scoring system (Table 1) in which a score of 10 represented seminiferous tubules containing complete spermatogenesis and perfect tubules, whereas a score of 1 indicated complete tubular degeneration and no cell (Meydanli et al. 2018). In addition, seminiferous tubule diameter and germinal epithelium thickness were measured using Fiji software, a distribution of ImageJ (Schindelin et al. 2012). For each animal, measurements obtained from the ten randomly selected seminiferous tubules were averaged to generate a single representative value, and these mean values were used for statistical analysis and data presentation.

Table 1 Johnsen's tubular biopsy scores (JTBS)

Score	DESCRIPTION
1	No cells in tubular sections
2	No germ cells present
3	Only spermatogonia present
4	Only a few spermatocytes present
5	No spermatozoa, no spermatids but several or many spermatocytes present
6	Only a few spermatids present
7	No spermatozoa but many spermatids present
8	Only a few spermatozoa present
9	Many spermatozoa present but disorganized spermatogenesis
10	Complete spermatogenesis and perfect tubules

Transmission electron microscopic evaluation

For ultrastructural analysis, testicular tissue samples were fixed in glutaraldehyde followed by osmium tetroxide, dehydrated through an increasing alcohol series, and embedded in epoxy resin. Ultrathin sections were prepared and stained with uranyl acetate and lead citrate (Bancroft et al. 2013). The sections were then examined using a transmission electron microscope (TEM, JEOL, JEM-1011). Ultrastructural evaluation focused on the morphology of Sertoli and Leydig cells, stages of spermatogenesis, mitochondrial integrity, endoplasmic reticulum organization, cytoplasmic lipid accumulation, and vacuole formation (Meydanli et al. 2018).

Oxidative stress markers in testicular tissues

To evaluate oxidative stress status in testicular tissues, levels of malondialdehyde (MDA; Elabscience, Cat. No: E-BC-K025-S), superoxide dismutase (SOD; Elabscience, Cat. No: E-BC-K019-S), catalase (CAT; Elabscience, Cat. No: E-BC-F006), and hydrogen peroxide (H_2O_2; Elabscience, Cat. No: E-BC-K102-S) were measured using commercially available assay kits. Testicular tissues stored at −80 °C were homogenized and lysed prior to analysis. All assays were performed according to the manufacturer's protocols. Optical density values were measured using a spectrophotometer, and the concentrations or activities of oxidative stress parameters were calculated accordingly.

Statistical analysis

Statistical analyses were performed using GraphPad Prism (Version 10.5.1 for Windows/Mac, GraphPad Software, San Diego, California USA). Data sets were first assessed for normality. For normally distributed data, one-way ANOVA followed by Tukey's multiple comparisons test was used to compare groups. For data sets that did not satisfy the normality assumption, the non-parametric Kruskal–Wallis test was applied. A P value <0.05 was considered statistically significant. Results are presented as mean ± standard error of the mean (SEM).

Results

ELISA analyses of serum hormone levels

To investigate diabetes-associated alterations in circulating reproductive hormones and to evaluate the potential therapeutic effects of the two different CM treatments, serum testosterone, FSH, GnRH, and LH levels were measured by ELISA. Serum testosterone levels remained comparable among all experimental groups, with no statistically significant differences observed. In contrast, diabetic animals exhibited reduced serum concentrations of FSH, GnRH, and LH compared with the control group. However, this reduction reached statistical significance only for FSH levels ($p<0.01$). Treatment with both N-CM and DFX-CM resulted in increased FSH concentrations compared with the untreated diabetic group; however, these improvements did not show statistical significance (Fig. 1).

Histological and morphometric evaluations of seminiferous tubules

Johnsen's tubular biopsy scoring performed on H&E-stained sections revealed mean scores of 8.88 ± 0.08 in the control group, 7.82 ± 0.13 in the diabetic group, 8.10 ± 0.15 in the D + N-CM group, and 8.00 ± 0.17 in the D + DFX-CM group (Fig. 2a-e). Histological examination of the control testes demonstrated normal seminiferous tubule architecture with an intact germinal epithelium and abundant mature spermatozoa within the tubular lumen (Fig. 2a). In contrast, testes from the diabetic group exhibited a marked reduction in mature spermatozoa within the seminiferous tubule lumina, while the tubular architecture was largely preserved with only mild disorganization of the germinal epithelium. (Fig. 2b). The D + N-CM and D + DFX-CM groups showed a modest increase in the presence of more mature spermatozoa within the tubular lumen (Fig. 2c and d). Consistent with these histological observations, Johnsen's scores tended to be higher in the treatment groups than in the untreated diabetic group, indicating a trend toward improved spermatogenesis. Nevertheless, no statistically significant differences were observed between the control and experimental groups or between the treatment groups and the diabetic group ($P>0.05$) (Fig. 2e). In addition to Johnsen scoring, seminiferous tubule diameter and seminiferous epithelial height were evaluated in testicular sections.

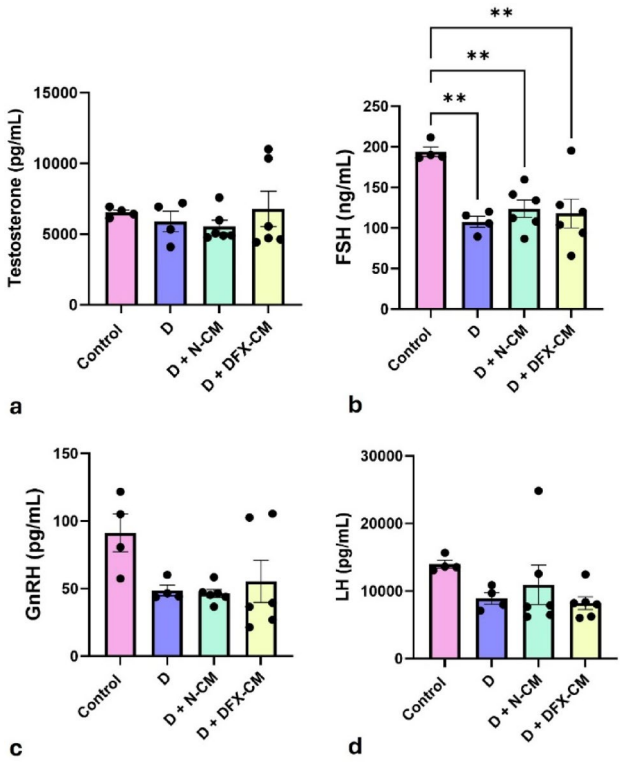

Fig. 1 Graphical representation of ELISA analysis for serum testosterone (**a**), follicle-stimulating hormone (FSH) (**b**), gonadotrophin-releasing hormone (GnRH) (**c**) and luteinizing hormone (LH) (**d**). $^{**}p<0.01$ ($n=4$ for control and D and $n=6$ for D+N-CM and D+DFX-CM groups; mean ± SD; one-way ANOVA with Tukey's post hoc test)

No statistically significant differences were observed among the groups for either parameter (Fig. 2f-g).

Ultrastructural analysis of seminiferous tubules

TEM examination of the seminiferous tubules in the control group demonstrated normal ultrastructural architecture. Sertoli cells exhibited irregularly contoured nuclei. Spermatogonia were oval-shaped and located adjacent to the basement membrane. Spermatocytes showed varying degrees of chromatin condensation within their nuclei and were closely associated with the cytoplasmic processes of Sertoli cells. Spermatids at different stages of maturation were observed embedded within the Sertoli cell cytoplasm. No ultrastructural abnormalities were detected in any of the germ cell populations, indicating preserved spermatogenic organization and cellular integrity. In contrast, the diabetic group exhibited marked degenerative alterations, including an increased number of lysosomes in Sertoli cells, spermatogonia with dispersed heterochromatin, primary spermatocytes with disrupted nuclear membranes, and widened intercellular spaces within the seminiferous epithelium. These diabetes-induced ultrastructural abnormalities were relatively reduced in both treatment groups compared with the untreated diabetic group. Furthermore, substantial restoration was observed in the ultrastructure of Sertoli cells as well as in the spermatogenic cells of the seminiferous epithelium following CM treatments (Fig. 3).

Analysis of oxidative stress markers in testicular tissue

To assess diabetes-induced oxidative damage in the testicular tissue and the potential protective effects of CM treatments, antioxidant enzyme activities, including SOD and CAT, as well as levels of oxidative stress markers H_2O_2 and MDA, were analysed. CAT activity was significantly reduced in all diabetic groups compared with the control group, indicating impaired antioxidant defence under diabetic conditions. Treatment with N-CM significantly improved CAT activity compared with both the untreated diabetic group and the DFX-CM-treated group ($p<0.01$ and $p<0.05$, respectively). Similarly, SOD activity was significantly higher in the N-CM-treated group than in both the untreated diabetic group and the DFX-CM-treated group ($p<0.0001$ and $p<0.05$, respectively), suggesting enhanced antioxidant capacity following N-CM administration. MDA, lipid peroxidation marker (Gaweł et al. 2004), was significantly elevated in the untreated diabetic group compared with controls ($p<0.0001$). Notably, both N-CM and DFX-CM treatments significantly reduced MDA levels, restoring them to values comparable with the control group. In contrast, H_2O_2 levels were significantly increased in all diabetic groups; untreated diabetic, D+N-CM, and D+DFX-CM groups, compared with the control group ($p<0.01$, $p<0.05$, and $p<0.01$, respectively) (Fig. 4). Overall, these findings indicate that diabetes-associated hyperglycaemia induces marked oxidative stress in the testes. CM treatments, particularly N-CM, partially ameliorated this oxidative imbalance by enhancing antioxidant enzyme activity and reducing lipid peroxidation.

Discussion

Diabetes mellitus can impair reproductive function through prolonged hyperglycaemia, highlighting the need for effective therapeutic approaches (He et al. 2021). Given the regenerative and cytoprotective potential of MSC-derived CM, particularly following hypoxic preconditioning (Ozkan et al. 2022; Isildar et al. 2024), this study investigated whether normoxic- or DFX-induced hypoxia-conditioned MSC-CM could alleviate diabetes-related reproductive dysfunction in a single-dose STZ-induced rat model. In the present study, diabetes induced reproductive endocrine

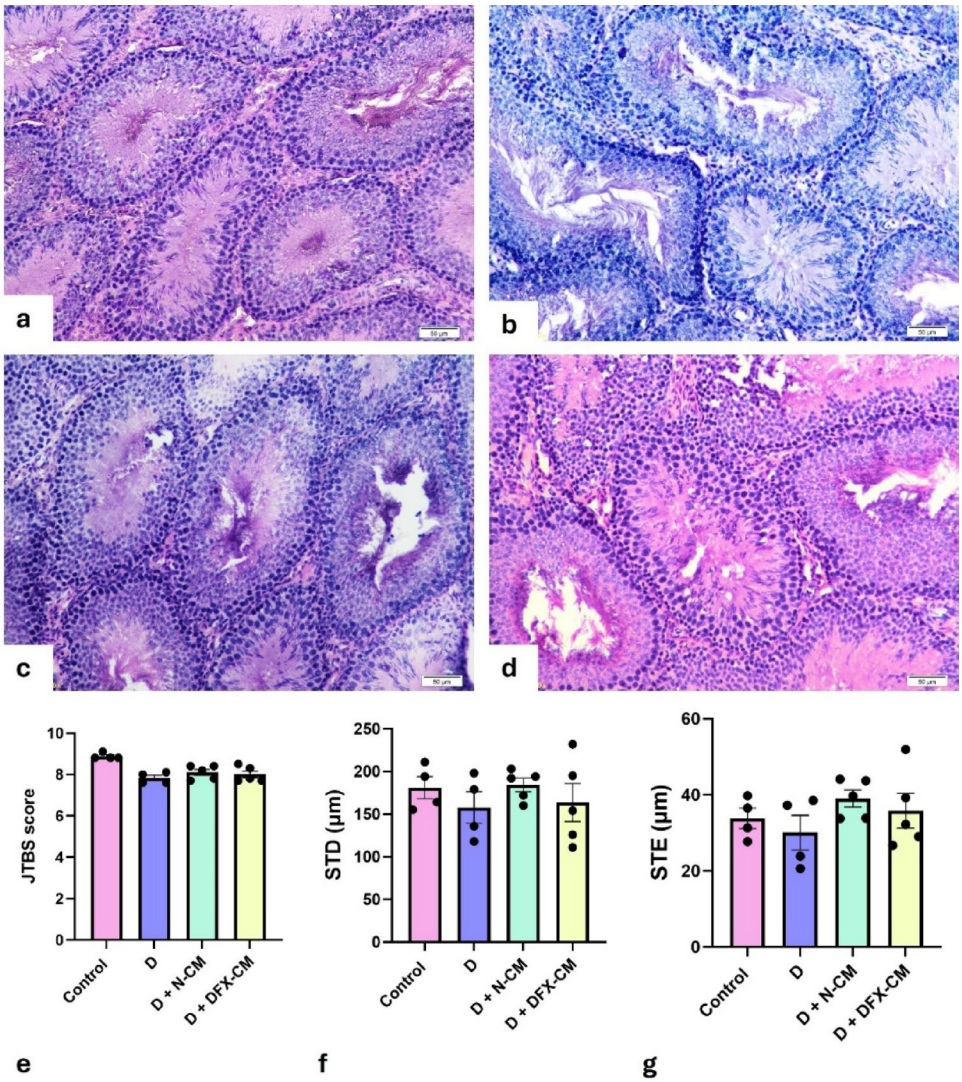

Fig. 2 Representative figures of H&E staining for control (**a**), diabetes (**b**), D + N-CM (**c**) and D + DFX-CM (**d**) groups. Graphical expression of semiquantitative Johnsen's tubular biopsy score (JTBS) analysis (**e**). Graphical expressions of histomorphometric analysis for the diameter of the seminiferous tubule (STD) (**f**) and the thickness of the seminiferous tubule epithelium (STE) (**g**). ($n = 4$ for control and D and $n = 5$ for D + N-CM and D + DFX-CM groups; mean ± SD; one-way ANOVA with Tukey's post hoc test)

dysregulation, characterized by reduced serum GnRH, FSH, and LH levels, although these hormonal alterations were not accompanied by significant histopathological or morphometric changes in the testes. Nevertheless, ultrastructural examination revealed marked cellular alterations within the seminiferous epithelium, together with impaired antioxidant defence and increased oxidative stress, indicating that diabetes-related testicular injury occurs primarily at the subcellular level. Importantly, treatment with both MSC-CM formulations ameliorated these ultrastructural abnormalities and restored redox balance, with N-CM demonstrating more pronounced antioxidant and protective effects than DFX-CM.

Serum reproductive hormone levels, including testosterone, FSH, GnRH, and LH, were evaluated. Testosterone levels were not significantly altered under diabetic conditions. Physiologically, testosterone production and regulation are controlled by the hypothalamic–pituitary–gonadal (HPG) axis. In this pathway, hypothalamic GnRH stimulates the anterior pituitary to release FSH and LH (Mbiydzenyuy and Qulu 2024). FSH acts primarily on Sertoli cells to promote androgen-binding protein (ABP) production and support spermatogenesis, whereas LH stimulates Leydig cells to synthesize testosterone (Mbiydzenyuy and Qulu 2024). In the present study, serum GnRH, FSH, and LH levels were reduced in all diabetic groups, although statistical significance was reached only for FSH. Given the unchanged serum testosterone levels observed in the present study, assessment of intratesticular testosterone concentrations, particularly within the seminiferous tubules, together with local ABP expression, may provide a more comprehensive evaluation of diabetes-associated alterations in androgen activity. ABP plays a crucial role in maintaining high local concentrations of testosterone and dihydrotestosterone within the seminiferous tubules (Ma et al. 2015). Therefore, the absence of intratesticular testosterone and ABP analyses represents a limitation of this study. These findings suggest that hyperglycaemia may disrupt the HPG axis, consistent

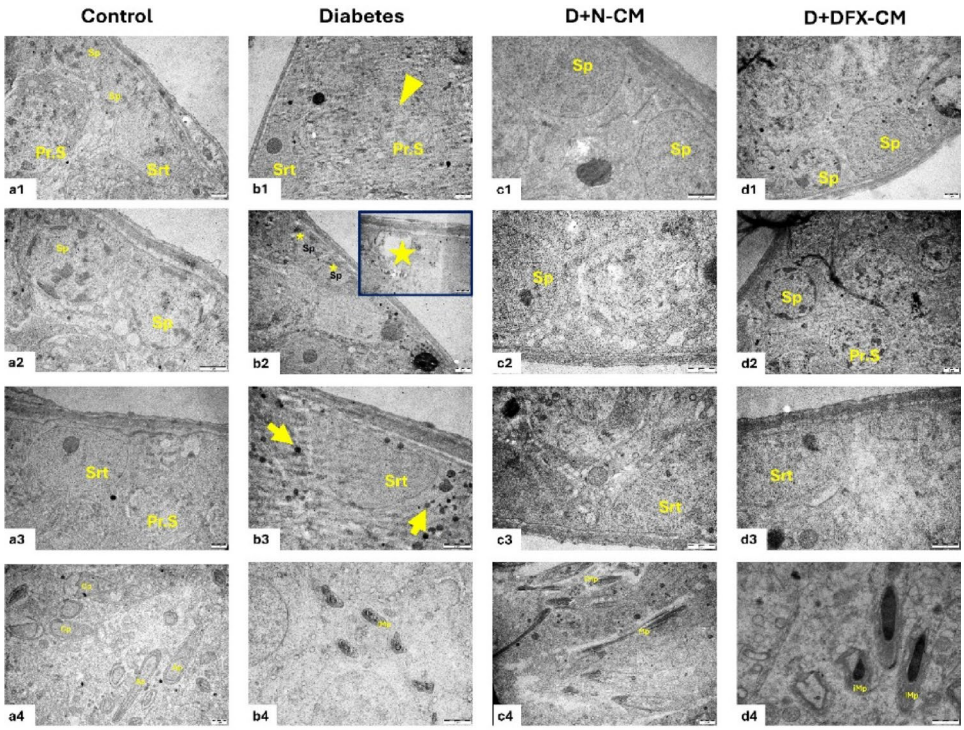

Fig. 3 Representative transmission electron microscopy (TEM) images of seminiferous tubules from the control (**a**), diabetes (**b**), D + N-CM (**c**), and D + DFX-CM (**d**) groups. Images 1–2 show spermatogenic cells, including spermatogonia (Sp), identified by their round nuclei located adjacent to the basement membrane of the seminiferous tubule; primary spermatocytes (Pr.S), characterized by larger nuclei with prominent chromatin condensation and a more adluminal position within the seminiferous epithelium; and Sertoli cells (Srt), recognized by their irregularly shaped euchromatic nuclei with prominent nucleoli and cytoplasm extending between adjacent spermatogenic series cells. Image 3 shows Sertoli cells, and image 4 presents spermatids at different developmental stages: cap phase (Cp), acrosomal phase (Ap), initial maturation phase (iMp), and maturation phase (Mp). Arrow: lysosome; star: intercellular space; asterisk: dispersed heterochromatin; arrowhead: disintegrated nuclear membrane

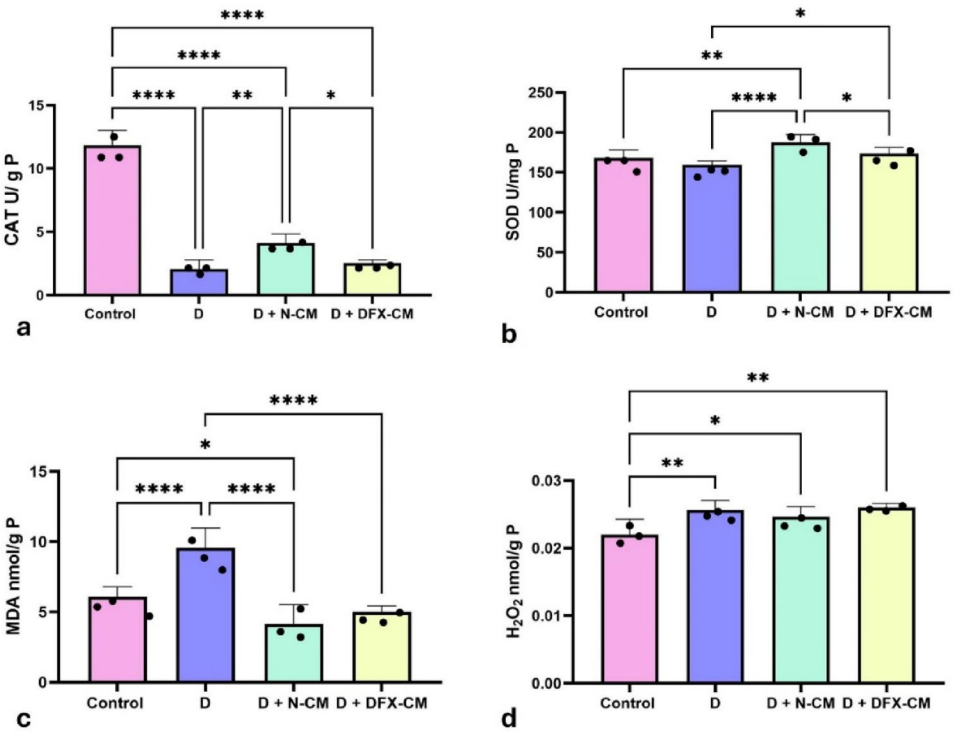

Fig. 4 Graphical representation of CAT (**a**), SOD (**b**), MDA (**c**), and H_2O_2 (**d**) levels in testicular tissues. Data are presented as mean ± SD. Statistical significance was defined as ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$. ($n = 3$ per group; mean ± SD; one-way ANOVA with Tukey's post hoc test)

with previous reports (Barsiah et al. 2019). Furthermore, this apparent endocrine dysregulation, which was not reflected in circulating testosterone levels, may indicate the presence of compensatory mechanisms preserving systemic androgen homeostasis despite upstream hormonal disturbances. Regarding the CM treatment groups, both CM formulations produced modest increases in FSH levels. In addition, slight elevations in GnRH were observed in the DFX-CM-treated group, while LH levels were marginally increased in the N-CM-treated group. Although these changes did not reach statistical significance, they may indicate a partial restorative effect of CM treatment on hyperglycaemia-induced reproductive endocrine dysfunction. Further studies with larger sample sizes and direct evaluation of the testicular hormonal microenvironment are required to confirm these preliminary observations.

Next, we evaluated whether hyperglycaemia-associated disruption of the HPG axis contributed to impaired spermatogenesis and deterioration of sperm parameters. In this context, our histopathological analyses revealed that the spermatogenic process was adversely affected in the diabetic groups, accompanied by a reduction in spermatozoa numbers. However, these changes did not reach statistical significance when compared with the control group. Similarly, histomorphometric assessments, including measurements of seminiferous tubule diameter and epithelial thickness, showed no significant differences among the experimental groups. These findings suggest that diabetes-associated disruption of the HPG axis may exert its effects on the testes initially at the cellular and subcellular levels, before overt structural alterations become detectable by routine light microscopic examination. The absence of marked differences in serum testosterone levels among the groups further supports this interpretation. To further characterize these subtle changes, testicular tissues were examined by TEM. While the control group exhibited normal cellular ultrastructure, the diabetic group demonstrated several degenerative alterations consistent with previous reports, including increased lysosomal content in Sertoli cells, spermatogonia enriched with heterochromatin, and primary spermatocytes with disrupted nuclear membrane integrity (Meydanli et al. 2018; Öztaş et al. 2019). In addition, widening of the intercellular spaces within the seminiferous epithelium was observed. These ultrastructural abnormalities are indicative of early cellular stress, impaired Sertoli cell support, and disruption of germ cell homeostasis under hyperglycaemic conditions. Notably, both treatment groups exhibited fewer ultrastructural abnormalities compared with the untreated diabetic group. Improvements were particularly evident in Sertoli cell morphology and in the organization of spermatogenic cells within the seminiferous epithelium. Collectively, the histopathological, morphometric, and ultrastructural findings indicate that the deleterious effects of hyperglycaemia on testicular tissue are more pronounced at the subcellular level than at the gross histological level, and that MSC-CM may exert protective and restorative effects against diabetes-induced testicular damage.

Sertoli cells regulate the proliferation of spermatogonial cells, coordinate meiotic progression, and facilitate the final stages of spermiogenesis. Consequently, any functional impairment in Sertoli cells may substantially compromise seminiferous epithelial integrity and germ cell development, even before overt histological damage becomes apparent (Olojede et al. 2022). The ultrastructural abnormalities identified in the present study, especially the increased lysosomal content and disruption of cellular organization, may therefore reflect early Sertoli cell dysfunction induced by the diabetic microenvironment. One potential mechanism underlying these changes is dysregulated glucose metabolism, which may impair cellular bioenergetics and promote excessive generation of ROS, leading to oxidative stress. This mechanism may explain the subcellular pathological alterations detected by TEM, as oxidative damage is known to affect membrane integrity, organelle function, and chromatin organization within testicular cells. In support of this, our findings demonstrated that diabetic conditions were associated with impaired oxidative homeostasis, as evidenced by reductions in antioxidant defence parameters including, CAT and SOD enzymes and elevations in oxidative stress markers like MDA and H_2O_2 relative to the control group. One of the therapeutic actions of MSC-CM is attributed to their antioxidant properties (Gao et al. 2014; Oses et al. 2017b). In that regard, treatment with CM, particularly N-CM, significantly increased SOD and CAT levels compared with the untreated diabetic group. In parallel, MDA levels were significantly reduced in both CM-treated groups, approaching those observed in the control group. These data indicate that MSC-CM can effectively restore redox balance in hyperglycaemia-exposed testicular tissue. Importantly, the biochemical improvement in oxidative stress status corresponded with the ultrastructural recovery observed in Sertoli cells and spermatogenic cells. This association suggests that oxidative stress is a key mediator of diabetes-induced testicular injury and that the protective effects of MSC-CM may be largely attributable to enhancement of endogenous antioxidant defences.

To our knowledge, this is among the first studies to demonstrate that hyperglycaemia disrupts cellular stress management within testicular tissue and that MSC-CM, particularly N-CM, can mitigate these alterations and contribute to structural recovery at the ultrastructural level. Notably, in the current study, N-CM exhibited more pronounced protective effects than DFX-CM. This difference may be attributable to changes in the secretory profile and metabolic activity

of MSCs induced by DFX-mediated hypoxia mimetic preconditioning. Although hypoxia is widely used to enhance the release of regenerative paracrine factors, and we previously demonstrated that 24-hour preconditioning reduced the total oxidant status of DFX-CM (Isildar et al. 2025), it may simultaneously impair other antioxidant properties of MSCs, potentially diminishing the therapeutic efficacy of their secretome in pathological conditions where oxidative stress plays a central role. In this regard, the lack of a comprehensive analysis of the molecular composition of the two CM represents another limitation of the present study. Therefore, under the current experimental conditions, the greater efficacy of N-CM may reflect a more favourable balance of cytoprotective and antioxidant mediators capable of counteracting hyperglycaemia-induced oxidative injury in testicular tissue.

Conclusion

In conclusion, this study demonstrated that diabetes induces disruption of the HPG axis and causes ultrastructural damage within the seminiferous tubules, potentially mediated by impaired antioxidant defence mechanisms and increased oxidative stress. Administration of MSC-CM, particularly N-CM, partially restored redox balance and improved diabetes-associated structural alterations in testicular tissue. Although further studies are required to clarify the specific paracrine factors responsible for these effects, the present study provides proof-of-concept evidence that MSC-CM represents a promising therapeutic strategy for diabetes-related testicular dysfunction.

Author contributions S.O., B.I, and M.K. contributed to the study conception and design. Material preparation, data collection and analysis were performed by S.O., B.I., I.U., and A.E.O. The first draft of the manuscript was written by S.O and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by Izmir Katip Celebi University, Scientific Research and Project Coordinatorship (2024-GAP-TIPF-0008).

Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of Interests The authors have no competing interests to declare that are relevant to the content of this article.The authors declare no competing interests.

Ethical approval All animal experiments were conducted with the approval of the Experimental Animal Ethics Committee of Bezmiâlem Vakıf University (approval no. E.106223; approved on 10 May 2023).

References

Atkinson MA, Eisenbarth GS, Michels AW (2014) Type 1 diabetes. Lancet

Bancroft J, Layton C, Suvarna S (2013) Bancroft's theory and practice of histological techniques content strategist. Michael Houston Content Development Specialists

Barsiah S, Behnam-Rassouli M, Shahabipour F et al (2019) Evaluation of testis hormonal and histopathological alterations in type I and type II diabetic rats. J Cell Biochem. https://doi.org/10.1002/jcb.28936

Burrack AL, Martinov T, Fife BT (2017) T cell-mediated beta cell destruction: Autoimmunity and alloimmunity in the context of type 1 diabetes. Front Endocrinol (Lausanne).

Caturano A, D'Angelo M, Mormone A et al (2023) Oxidative Stress in Type 2 Diabetes: Impacts from Pathogenesis to Lifestyle Modifications. Curr Issues Mol Biol 45:6651–6666. https://doi.org/10.3390/cimb45080420

Chang H-H, Hsu S-P, Chien C-T (2019) Intrarenal Transplantation of Hypoxic Preconditioned Mesenchymal Stem Cells Improves Glomerulonephritis through Anti-Oxidation, Anti-ER Stress, Anti-Inflammation, Anti-Apoptosis, and Anti-Autophagy. Antioxid (Basel Switzerland) 9. https://doi.org/10.3390/antiox9010002

Endlich K, Kliewe F, Endlich N (2017) Stressed podocytes—mechanical forces, sensors, signaling and response. Pflugers Arch Eur J Physiol

Faria J, Calcat-I-Cervera S, Skovronova R et al (2023) Mesenchymal stromal cells secretome restores bioenergetic and redox homeostasis in human proximal tubule cells after ischemic injury. Stem Cell Res Ther 14:353. https://doi.org/10.1186/s13287-023-03563-6

Gao X, Song L, Shen K et al (2014) Bone marrow mesenchymal stem cells promote the repair of islets from diabetic mice through paracrine actions. Mol Cell Endocrinol. https://doi.org/10.1016/j.mce.2014.03.004

Gaweł S, Wardas M, Niedworok E, Wardas P (2004) [Malondialdehyde (MDA) as a lipid peroxidation marker]. Wiad Lek 57:453–455

He Z, Yin G, Li QQ et al (2021) Diabetes mellitus causes male reproductive dysfunction: a review of the evidence and mechanisms. Vivo 35:2503–2511. https://doi.org/10.21873/invivo.12531

Isildar B, Ozkan S, Ercin M et al (2022) 2D and 3D cultured human umbilical cord-derived mesenchymal stem cell-conditioned medium has a dual effect in type 1 diabetes model in rats: immunomodulation and beta-cell regeneration. Inflamm Regen 42:55. https://doi.org/10.1186/s41232-022-00241-7

Isildar B, Ozkan S, Koyuturk M (2023) Therapeutic potential of mesenchymal stem cell-derived conditioned medium for diabetes mellitus and related complications. https://doi.org/10.1002/adtp.202300216. Adv Ther n/a:2300216

Isildar B, Ozkan S, Sahin H et al (2024) Preconditioning of human umbilical cord mesenchymal stem cells with deferoxamine potentiates the capacity of the secretome released from the cells and promotes immunomodulation and beta cell regeneration in a rat model of type 1 diabetes. Int Immunopharmacol 129:111662. https://doi.org/10.1016/j.intimp.2024.111662

Isildar B, Ozkan S, Neccar D, Koyuturk M (2025) Preconditioning and post-preconditioning states of mesenchymal stem cells with deferoxamine: a comprehensive analysis. Eur J Pharmacol 996:177574. https://doi.org/10.1016/j.ejphar.2025.177574

Kerans F, Lungaro L, Azfer A, Salter D (2018) The Potential of intrinsically magnetic mesenchymal stem cells for tissue engineering. Int J Mol Sci 19:3159. https://doi.org/10.3390/ijms19103159

Khatri R, Petry SF, Linn T (2021) Intrapancreatic MSC transplantation facilitates pancreatic islet regeneration. Stem Cell Res Ther. https://doi.org/10.1186/s13287-021-02173-4

Lee NE, Hwang JI, Bang CY et al (2026) Wharton's jelly mesenchymal stem cell-secretome enhances skin rejuvenation via ApoA4 and SERPINH1. Regen Ther 31:101071. https://doi.org/10.1016/j.reth.2026.101071

Ma Y, Yang H-Z, Xu L-M et al (2015) Testosterone regulates the autophagic clearance of androgen binding protein in rat Sertoli cells. Sci Rep 5:8894. https://doi.org/10.1038/srep08894

Mbiydzenyuy NE, Qulu L-A (2024) Stress, hypothalamic-pituitary-adrenal axis, hypothalamic-pituitary-gonadal axis, and aggression. Metab Brain Dis 39:1613–1636. https://doi.org/10.1007/s11011-024-01393-w

Meydanli EG, Gumusel A, Ozkan S et al (2018) Effects of resveratrol on high-fructose-induced testis injury in rats. Ultrastruct Pathol 42:65–73. https://doi.org/10.1080/01913123.2017.1397075

Nagaishi K, Mizue Y, Chikenji T et al (2016) Mesenchymal stem cell therapy ameliorates diabetic nephropathy via the paracrine effect of renal trophic factors including exosomes. Sci Rep 6:34842. https://doi.org/10.1038/srep34842

Nagaishi K, Mizue Y, Chikenji T et al (2017) Umbilical cord extracts improve diabetic abnormalities in bone marrow-derived mesenchymal stem cells and increase their therapeutic effects on diabetic nephropathy. Sci Rep 7:8484. https://doi.org/10.1038/s41598-017-08921-y

Olojede SO, Lawal SK, Faborode OS et al (2022) Testicular ultrastructure and hormonal changes following administration of tenofovir disoproxil fumarate-loaded silver nanoparticle in type-2 diabetic rats. Sci Rep. https://doi.org/10.1038/s41598-022-13321-y

Oses C, Olivares B, Ezquer M et al (2017a) Preconditioning of adipose tissue-derived mesenchymal stem cells with deferoxamine increases the production of pro-angiogenic, neuroprotective and anti-inflammatory factors: Potential application in the treatment of diabetic neuropathy. PLoS ONE 12:e0178011. https://doi.org/10.1371/journal.pone.0178011

Oses C, Olivares B, Ezquer M et al (2017b) Preconditioning of adipose tissue-derived mesenchymal stem cells with deferoxamine increases the production of pro-angiogenic, neuroprotective and anti-inflammatory factors: Potential application in the treatment of diabetic neuropathy. PLoS ONE. https://doi.org/10.1371/journal.pone.0178011

Ozkan S, Isildar B, Ercin M et al (2022) Therapeutic potential of conditioned medium obtained from deferoxamine preconditioned umbilical cord mesenchymal stem cells on diabetic nephropathy model. Stem Cell Res Ther 13:438. https://doi.org/10.1186/s13287-022-03121-6

Ozkan S, Isildar B, Sahin H et al (2024) Comparative analysis of effects of conditioned mediums obtained from 2D or 3D cultured mesenchymal stem cells on kidney functions of diabetic rats: Early intervention could potentiate transdifferentiation of parietal epithelial cell into podocyte precurso. Life Sci 343:122543. https://doi.org/10.1016/j.lfs.2024.122543

Öztaş E, Yılmaz TE, Güzel E et al (2019) Gliclazide alone or in combination with atorvastatin ameliorated reproductive damage in streptozotocin-induced type 2 diabetic male rats. Saudi Pharm J. https://doi.org/10.1016/j.jsps.2019.01.003

Schindelin J, Arganda-Carreras I, Frise E et al (2012) Fiji: an open-source platform for biological-image analysis. Nat Methods 9:676–682. https://doi.org/10.1038/nmeth.2019

Sun J, Jia Y, Chen S et al (2025) Adipose mesenchymal stem cell-derived extracellular vesicles alleviate renal fibrosis by reducing epithelial-mesenchymal transition via the FOXS1/Wnt/β-catenin signaling pathway. Int Immunopharmacol 146:113880. https://doi.org/10.1016/j.intimp.2024.113880

Wu H, Mahato RI (2014) Mesenchymal stem cell-based therapy for type 1 diabetes. Discov Med

Xv J, Ming Q, Wang X et al (2017) Mesenchymal stem cells moderate immune response of type 1 diabetes. Cell Tissue Res 368:239–248. https://doi.org/10.1007/s00441-016-2499-2

Zangiabadi I, Askaripour M, Rajizadeh MA et al (2025) Conditioned medium from human adipose-derived mesenchymal stem cells attenuates cardiac injury induced by Movento in male rats: role of oxidative stress and inflammation. BMC Pharmacol Toxicol 26:13. https://doi.org/10.1186/s40360-025-00847-w