The role of mesenchymal stem cell therapy in ameliorating diabetes-induced ovarian damage in albino rats: Histological, biochemical and immunohistochemical studies

Ahmed Y. Sedeak[a], Marwa S. Abdel-Tawab[b], Naglaa A.A. Elazeem[b], Hoda R. Reyad[b], Sherif F. Abdel-Fatah[c], Yasser Khamis[d], Ahmed A.-E. Tammam[e], Ahmed M. El-Ebiary[f], Nourhan T. Sabra[a]

Departments of [a]Anatomy and Embryology, [b]Medical Biochemistry, [c]Clinical Pharmacology, [d]Obstetrics and Gynecology, [e]Medical Physiology, Faculty of Medicine, Beni-Suef University, Beni-Suef, [f]Department of Medical Physiology, Faculty of Medicine, Fayoum University, Fayoum, Egypt

Correspondence to Marwa S. Abdel-Tawab, MD, Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Beni-Suef University, Beni-Suef 62514, Egypt. Tel: 002822517042, Mobile: 002- 01005115926; e-mail: marwa.amar@med.bsu.edu.eg

Received: 3 December 2023
Revised: 18 January 2024
Accepted: 23 January 2024
Published: 15 July 2024

Egyptian Pharmaceutical Journal 2024, 23:407–415

Background
Diabetes mellitus (DM) is a chronic disease causing a variety of complications that can damage most organs, including the gonads. Diabetes was reported to be effectively treated using mesenchymal stem cells (MSCs).
Objective
The objective of the work was to demonstrate the therapeutic role of stem cells in cases of ovarian damage due to diabetes.
Materials and methods
Eighteen adult female albino rats were divided evenly into three groups. The control group received an injection of 1 ml of saline intraperitoneally (i.p.). The streptozotocin (STZ)-treated group: that was given 60 mg/kg per body weight (i.p.) of STZ to induce DM, which was slaughtered after 4 weeks. The STZ +stem cell-treated group that got STZ then was allowed to recover for 4 weeks and then underwent an intravenous injection of one million MSC before being sacrificed. Measurements of blood glucose, serum estradiol (E2), follicular-stimulating hormone (FSH), and luteinizing hormone (LH) were performed. Histological examination with hematoxylin and eosin, Masson stain, and immunohistochemical investigation for proliferating cell nuclear antigen (PCNA) were performed on the ovarian sections.
Results and conclusion
The STZ-induced DM group showed high glucose, FSH, and LH levels and decreased the E2 levels. Also, the inflammation, degeneration, and decreased PCNA immune expression of the ovary development in the STZ-induced DM group were observed. The treatment with the MScs reversed the effect of DM on glucose, FSH, LH, and E2. Additionally, the MScs improved the pathological alterations by increasing PCNA immune expression in the ovary. It was concluded that MSCs can effectively treat diabetes-induced ovarian damage.

Keywords:
diabetes, health care, mesenchymal stem cells, ovarian damage, proliferating cell nuclear antigen

Egypt Pharmaceut J 23:407–415

1687-4315

Introduction

One of the most prevalent non-communicable metabolic chronic diseases is the diabetes mellitus (DM). Diabetes has suddenly and significantly increased globally during the past few decades. The International Diabetes Federation reported that there will be 642 million diabetic patients by 2040, up from 415 million in 2015 [1].

Most of the body's organs, such as the retina, kidney, heart, and gonads, are negatively impacted by DM in both sexes. It has negative effects on the functions of the reproductive system and decreases the rate of fertility due to ovarian damage, but the exact mechanisms are unclear [2].

Streptozotocin (STZ) is an ideal chemical agent that can be used to develop an animal model of diabetes [3]. It is a glucosamine-nitrosourea compound that has specific harmful effects on pancreatic beta cells due to the production of free radicals such as superoxide hydrogen and peroxide, leading to the induction of diabetes [4].

Diabetes and associated consequences, such as retinopathy, nephropathy, and ovarian damage, were reported to be treated with mesenchymal stem cells (MSCs) with success [5–7].

A relatively new intervention technique used to repair injured tissues is stem cell therapy. According to some

medical researchers, stem cell therapy alters the course of various human diseases. The stem cells can multiply, resulting in generations with a variety of differentiation potentials. This property, which has great promise, allows for synthesising various tissues to replace damaged and malfunctioning tissues [8].

Nitric oxide and prostaglandin estradiol (E2), two substances secreted by MSCs, improve antioxidant defences, suppress oxidation-related factors, and lessen cell necrosis [9,10]. Additionally, immunological regulation of MSCs reduces the proliferation of inflammatory cells, having an anti-inflammatory effect [11].

The primary aim of this study was to shed light on the ovarian alterations driven by diabetes while elucidating the potential therapeutic benefits of MScs through histological, biochemical, and immunohistochemical investigations.

Materials and methods

This study was conducted at Beni-Suef University's Faculty of Pharmacy's animal house between the February 15, 2023 and the August 15, 2023 in line with the standards for the handling and use of laboratory animals, the code of ethical approval was 022-375 of the institutional animal care and use committee Beni-Suef University (BSU-IACUC).

Experimental animals

The study included 18 adult female albino rats weighing 200–400 g. The female rats spent 2 weeks acclimating in the laboratory before performing the study. These rats were handled in metal cages under regular laboratory and sterile environmental circumstances. Food and water were given to the rats according to the recommended level.

The rats were divided evenly into three groups. The control group received an injection of 1 ml of saline intraperitoneally (i.p.). The STZ-treated group was given 60 mg/kg per body weight (i.p.) of STZ for the purpose of inducing DM, which was slaughtered after 4 weeks [12]. The STZ+stem cell-treated group that got STZ then was allowed to recover for 4 weeks and then underwent an intravenous injection of one million MSC before being sacrificed [13].

Chemicals

The powder solvent form of STZ was purchased from Sigma Company (St Louis, Missouri, USA). The active component of STZ, also known as N-methylnitrosocarbamoyl-D-glucosamine STZ, weighs 1 g per vial of the drug.

Diabetes mellitus induction

In the Department of Biochemistry of the Faculty of Medicine at Beni-Suef University, 60 mg/kg body weight of STZ was dissolved in sodium citrate buffer (0.1 M). It was administered intravenously within 15 min of the solution's preparation at pH 4.5. This step's objective is to trigger DM [14].

Diabetes diagnosis

Within 3 days of receiving the STZ injection, adult rats displayed polyphagia and polydipsia, which indicated that they was triggered by deteriorating the pancreatic cells of Langerhans islets [15]. An increase in blood glucose levels confirmed the diabetes diagnosis [16]. Rats with more than 200 mg/dl of blood glucose levels were considered to have DM [14].

Diabetes mellitus treatment

Preparing labelled MSCs from bone marrow was performed in Beni-Suef University's Faculty of Medicine's Biochemistry Department. Four weeks following the DM diagnosis, these stem cells were administered as a single dosage of 1 ml containing about 1×10^6 cells/rat through intravenous injection [17]. Only group III received the MSCs therapy, suspended in 1 ml of normal saline [18].

Isolating and culturing the bone marrow-derived mesenchymal stem cells

At the Medical Biochemistry Department, Faculty of Medicine, Beni-Suef University, the 5-bromo-2-deoxy-uridine labelled stem cells were performed. According to the previous study [19], these stem cells were isolated and cultured for 4 weeks. Euthanization of the rats was carried out, and all their femora and tibias were removed when they were completely sterile. They were put in Dulbecco's modified eagle medium, which was used to flush out the MSCs, and a syringe with a 23-G needle was used. The cells were carefully separated by repeatedly pipetting the bone marrow from the bones. These cells were centrifuged at 2250 rpm for 15 min after being washed twice with Dulbecco's modified eagle medium. The stem cells were grown in Dulbecco's modified eagle medium with 10% fetal bovine serum (100 U/ml penicillin G and 100 mg/ml streptomycin added at a $2.5\times10^5/cm^2$ density). The stem cells were incubated at 37°C in humidified 5% CO_2 and 95% air. Removing the nonadherent cells was done after 3 days. Every 3 days, fresh Dulbecco's modified eagle medium was added and replenished. The stem cells were

harvested after reaching 80–90% confluency over 2 weeks. They were then replanted on a six-well disc at $1.5\times10^5/cm^2$ and grown to almost confluence once more using 1 mmol EDTA, and 0.25% trypsin at 37°C for 3 min. To expand the culture, the cells were diluted by adding water at a ratio of 1 : 2 per passage. Every 3 days, the stem cells were examined under a fluorescent phase-contrast microscope (Axiocam MR R3, Carl Zeiss, Jena, Germany).

Biochemical investigations

A fine, heparinized capillary tube was used to collect blood samples for each group at the conclusion of the trial. The plasma was obtained from the centrifuged blood samples and utilized to measure glucose, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and E2. To avoid chemical damage, the rats were first given a light ether inhalation anesthetic before being killed by cervical dislocation [20]. The ovaries of rats were carefully and promptly removed.

Histological and immunohistochemical analysis

The ovaries were cut open and dissected. Then, they were treated for blocks of paraffin and sectioned at a thickness of 5 m after being fixed in 10% formalin for an overnight period – hematoxylin and eosin (H&E) [21], Masson's trichrome [22]. The immunohistochemical analysis employing proliferating cell nuclear antigen (PCNA) [23] was performed on the paraffin sections. Slices of hydrated and deparaffinized paraffin were employed. After suppressing the endogenous activity of peroxidase with 10% H_2O_2, the sections were treated with primary rabbit polyclonal antibodies against PCNA (Sigma-Aldrich). The secondary antibody (biotinylated goat anti-rabbit) was used after phosphate buffer saline for washing. The slides were incubated using the tagged avidin-biotin peroxidase, linked to the secondary antibody's biotin. After adding diaminobenzidine chromogen, which is transformed by peroxidase into a brown precipitate, the antibody binding site was visualized. The typical positive control was a piece of tonsil (it is the standard positive control as it contains PCNA molecules and can be easily detected using the stain). By omitting the injection of the main antibody, the negative control was one of the ovarian sections.

Morphometric study

The strength of the PCNA immune expression in the ovarian sections was measured morphometrically using a Leica Quin 500 computerized image analysis system (Leica Ltd, Cambridge, UK).

Statistical analysis

Statistical analysis was performed utilizing SPSS software, version 22 (SPSS Inc., Chicago, Illinois, USA). The data were expressed as a mean and SD. To compare both groups, the one-way analysis of variance test was applied. When the P values were less than 0.05, the statistical findings were regarded as significant.

Results

Mortality rate

There were no rat deaths found.

Biochemical results

As indicated in Table 1, a significantly higher mean levels of glucose, FSH, and LH and lower mean levels of E2 were found in the STZ-treated group but these changes were reversed in the STZ+stem cell-treated group as compared to the control group ($P<0.05$).

Histological results

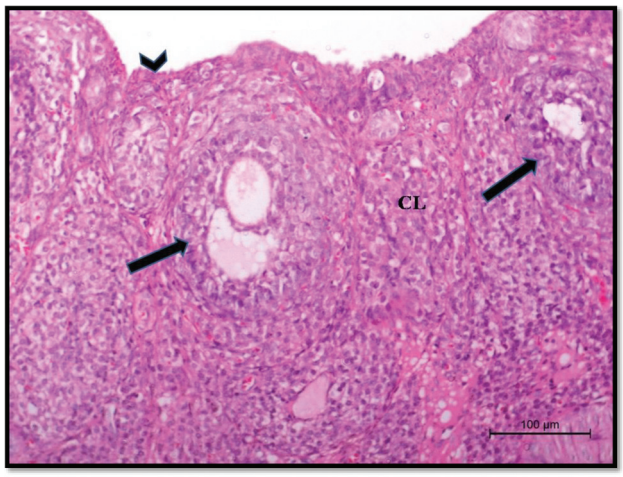
H&E staining of ovarian sections from the control group showed normal ovarian architecture formed of surface epithelium overlying a thin layer of tunica albuginea, the outer cortex, and the inner medulla. The outer cortex demonstrated different types of growing follicles in different stages of development and an intact corpus luteum, as shown in Fig. 1. Furthermore, Figs 2 and 3 showed the H&E staining of ovarian sections in the STZ-treated group, demonstrating a prominent increase in interstitial stromal cells, dilated congested blood vessels, and distorted corpus luteum with vacuolated

Table 1 Detection of the levels of blood glucose and measured hormone levels in the studied groups

The groups	Blood glucose level (mg/dl) (mean±SD)	FSH level (mIU/ml) (mean±SD)	LH level (mIU/ml) (mean±SD)	E2 level (pg/ml) (mean±SD)
The control group	91.24±7.75	0.018±0.03	0.03±0.02	31±5.62
The STZ-treated group	292.35±18.89*	0.088±0.07*	0.099±0.09*	17±9.54*
The STZ+stem cell-treated group	137.44±15.23*	0.022±0.04*	0.044±0.01*	28.2±3.12*

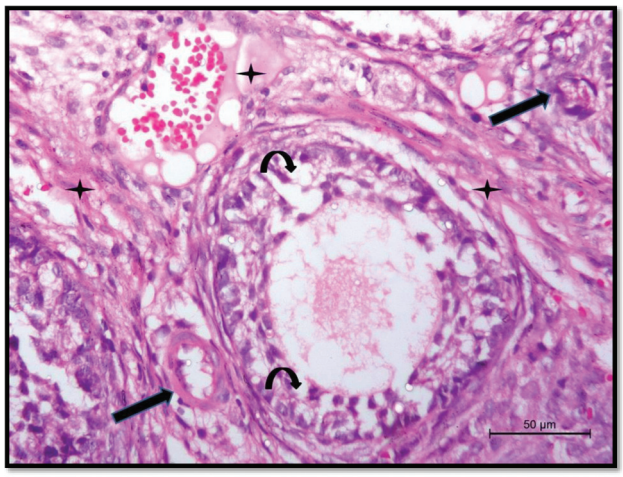
E2, estradiol; FSH, follicular-stimulating hormone; LH, luteinizing hormone; STZ, streptozotocin. *Significant differences ($P<0.05$) from the control group.

Figure 1



A photomicrograph of an ovarian section of the control group showed normal ovarian architecture formed of surface epithelium overlying a thin layer of tunica albuginea (arrowhead), outer cortex, and inner medulla. The outer cortex demonstrated different types of growing follicles in different stages of development (arrows) and the corpus luteum (CL) (H&E, ×200). H&E, hematoxylin and eosin.

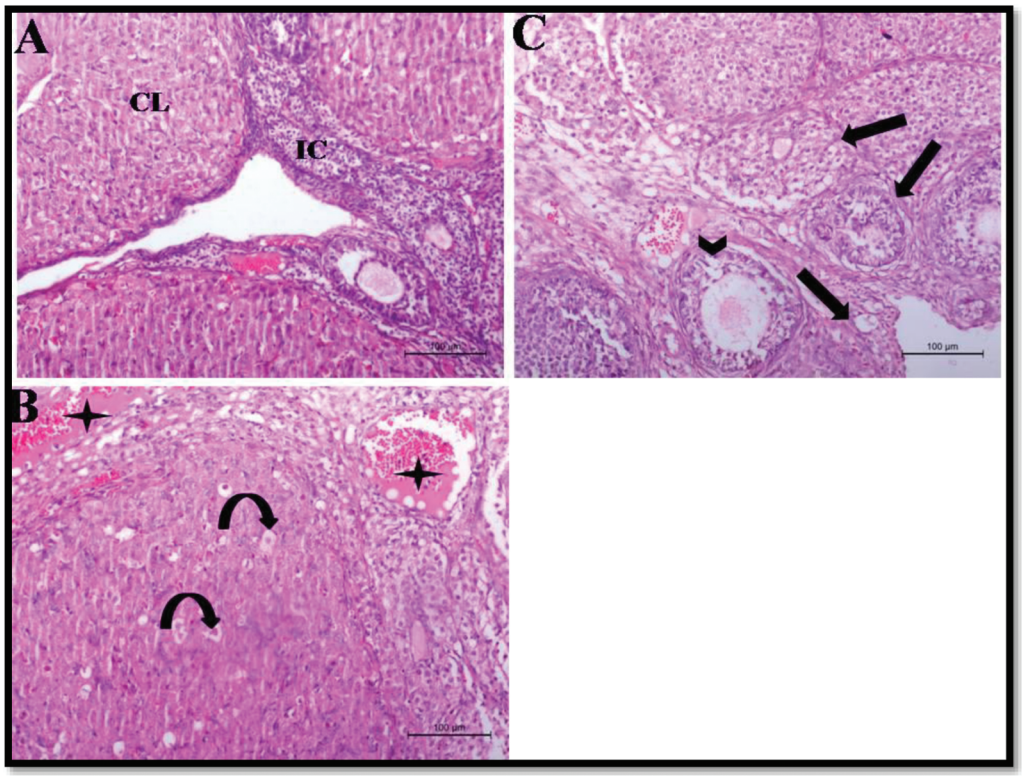
Figure 3



A photomicrograph of an ovarian section of the STZ-treated group showed distorted atretic follicles with an irregular outline and shrunken oocytes (arrows). Also, vacuolated follicular cells (curved arrows) and extravasation of blood in the ovarian stroma (stars) are demonstrated (H&E, ×400). H&E, hematoxylin and eosin; STZ, streptozotocin.

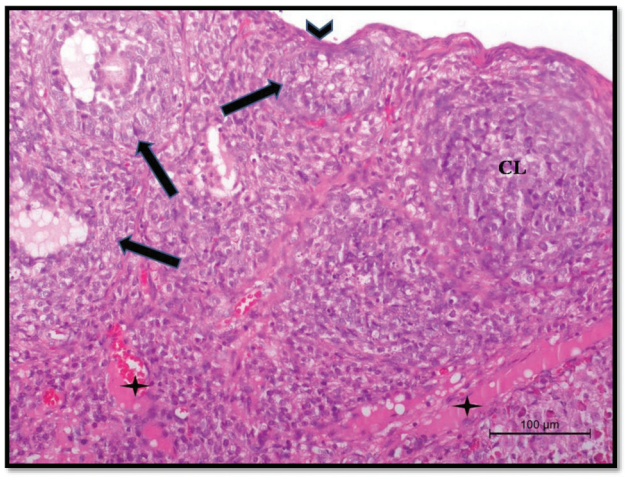
cells. Distorted atretic follicles with an irregular outline and shrunken oocytes were detected. Also, vacuolated follicular cells and extravasation of blood in the ovarian stroma were demonstrated, whereas the H&E staining of ovarian sections in the STZ+stem cell-treated group showed normal germinal epithelium, most of the

Figure 2



A photomicrograph (a–c) of an ovarian section of the STZ-treated group. (a) A prominent increase in interstitial stromal cells (IC) and distorted corpus luteum with vacuolated cells (CL). (b) An abnormal corpus luteum with vacuolated cells (curved arrows) and dilated congested blood vessels (stars). (c) Distorted atretic follicles with an irregular outline and shrunken oocytes (arrows). Also, vacuolated follicular cells are demonstrated (arrowhead) (H&E, ×200). H&E, hematoxylin and eosin; STZ, streptozotocin.

Figure 4



A photomicrograph of an ovarian cortical section of the STZ+stem cell-treated group showed germinal epithelium (arrowhead). Most ovarian follicles at different stages of development are intact (arrows). Mildly distorted corpus luteam (CL) and extravasation of blood (stars) are seen (H&E, ×200). H&E, hematoxylin and eosin; STZ, streptozotocin.

ovarian follicles at different stages of development were intact, and mildly distorted corpus luteal and extravasation of blood in the ovarian stroma were also seen in Fig. 4.

Masson's trichrome staining

In Masson's trichrome-stained ovarian sections, there was a significant deposition of collagen fibers in the stroma of the ovary in the STZ-treated group compared to the control groups. Moderate deposition of the collagen fibers was observed in the ovarian sections of the STZ+stem cell-treated group, as shown in Fig. 5.

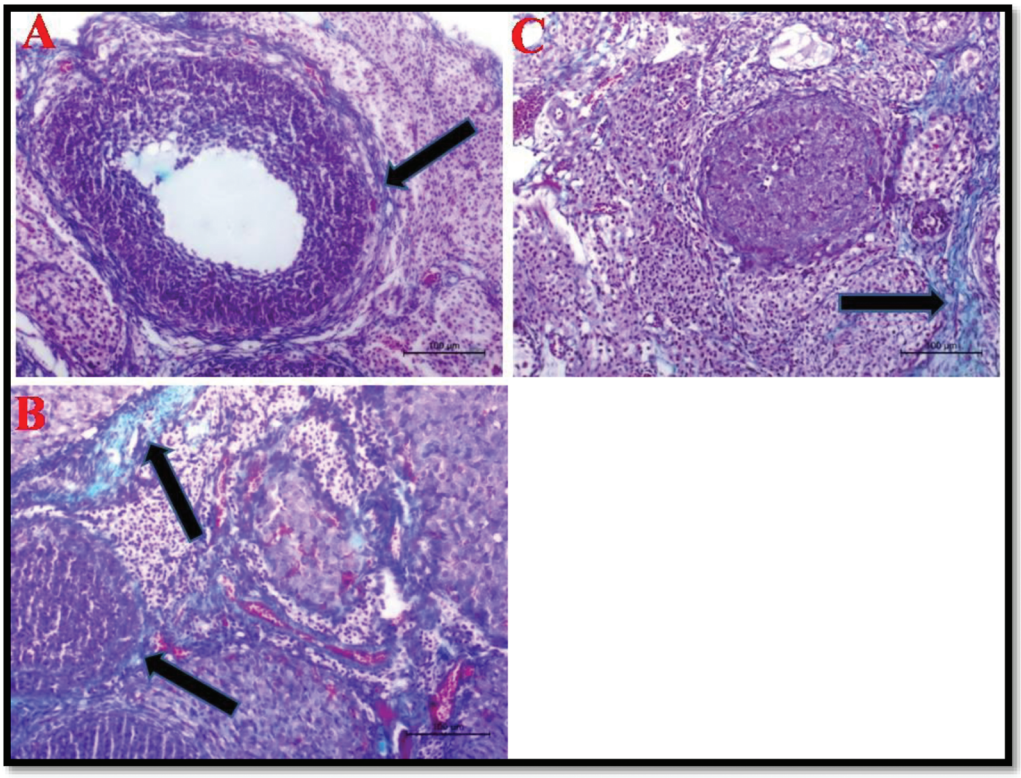
Proliferating cell nuclear antigen immunohistochemical staining

Figure 6 shows the immunohistochemical staining of the ovaries for PCNA, which demonstrated the positive e-NOS immune expression in the follicular cells and stromal cells of the ovaries. Comparatively less intense PCNA immune expression was seen in the ovarian follicles of the STZ-treated group compared to the control group. The STZ+stem cell-treated group, on the other hand, displayed more intense PCNA immune expression.

Morphometric results

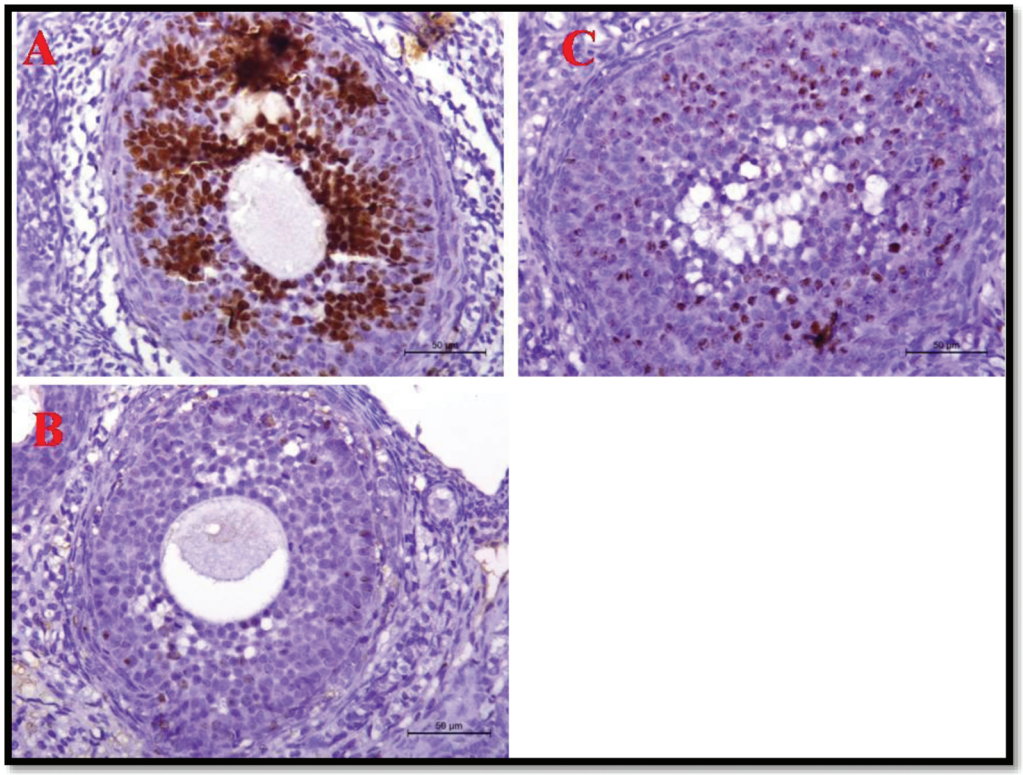
Table 2 showed that the STZ group's intensity of PCNA immune expression was significantly reduced when compared to the control group ($P<0.05$), whereas the STZ+stem cell group's intensity of

Figure 5



A photomicrograph of Masson's trichrome-stained ovarian sections of the control (a), STZ-treated (b), and STZ+stem cell-treated (c) groups showing collagen fiber deposition in the ovarian cortical stroma between follicles (arrows). (a) Minimal collagen fiber deposition. (b) Massive collagen fiber deposition. (c) Moderate deposition of the collagen fibers (Masson's trichrome, ×200). STZ, streptozotocin.

Figure 6



A photomicrograph of PCNA-immune-stained ovarian sections of control (a), STZ-treated (b), and STZ+-treated (c). (a) Brown coloration (positive PCNA immune expression) in the ovarian follicle. (b) A decreased intensity of the PCNA immune expression, while (c) shows an increase in the intensity of the PCNA immune expression (PCNA immunostaining, ×400). PCNA, proliferating cell nuclear antigen; STZ, streptozotocin.

Table 2 The morphometric results of the intensity of proliferating cell nuclear antigen immune expression in the studied groups

Groups	Intensity of PCNA immune expression (mean±SD)
The control group	67 180 856±3 346 319
The STZ-treated group	4 487 416±8 771 690*
The STZ+stem cell-treated group	56 994 580±9 921 946*

PCNA, proliferating cell nuclear antigen; STZ, streptozotocin.
*Significant differences ($P<0.05$) between the control group.

PCNA immune expression increased when compared to the STZ-treated group ($P<0.05$).

Discussion

In the present study, the levels of FSH and LH significantly increased while those of E2 significantly decreased in the diabetic group. These findings were analogous to the findings of Ahmed Sorour *et al.* [7], who declared that the level of LH was increased while levels of estrogen and progesterone were decreased in the ovaries of diabetic rats.

The current abnormal hormone results were supported by Gambineri *et al.* [24], who claimed that the major endocrinal abnormalities in insulin-resistant diabetic women include high levels of testosterone and estrogen to progesterone and, occasionally a high LH : FSH ratio leading to the polycystic ovary (PCO).

Contrary to the current findings, Soleymanzadeh *et al.* [25] found that the levels of LH and FSH and the levels of estrogen and progesterone were decreased in the diabetic rats' group. These changes were due to the decline in the level of gonadotropin-releasing hormone in cases of diabetes.

The current study showed that the levels of FSH and LH were decreased, and those of E2 were increased in the stem cell-treated group.

The same findings were observed by Ahmed Sorour *et al.* [7], who illustrated that the LH level was decreased while estrogen and progesterone levels were increased after treatment with stem cells. Also, Yan *et al.* [26] and Ding *et al.* [27] showed an improvement in the hormones after treatment with stem cells.

In agreement with the present study, Körbling and Estrov [28] suggested that these improvements to the

stem cells' anti-inflammatory properties, trophic action, homing, integration into the damaged location, and finally, transformation into tissue-specific cells.

These findings agreed with a study by Fouad *et al.* [29], who showed that stem cells improved ovarian failure in rats with cyclophosphamide-induced ovarian failure and restored their fertility. These findings may contribute to the role of stem cells in restoring the fertility of experimental rats with ovarian failure.

The current light microscopic results in the diabetic group showed a prominent increase in interstitial stromal cells, dilated congested blood vessels, and a distorted corpus luteum with vacuolated cells. Distorted atretic follicles with an irregular outline and shrunken oocytes were detected. Also, vacuolated follicular cells and extravasation of blood in the ovarian stroma were demonstrated. In the stem cell-treated group, ovarian sections showed improvement in architecture with many normal ovarian follicles. These findings agreed with the study of Ahmed Sorour *et al.* [7].

Gambineri *et al.* [24] defined PCO in all people with diabetics with different BMIs as a chronic disease with low-grade inflammation. Also, Rezvanfar *et al.* [30] reported that high blood glucose might result in oxidative stress due to increased reactive oxygen species (ROS) production, contributing to the inflammatory changes in PCO syndrome.

The previous studies claimed that the enhancement of nuclear transcription factor kappa-B; a proinflammatory mediator is included in an inflammatory condition may be involved in reproductive diseases may be caused by excessive production of ROS and defective antioxidant potential with subsequent mitochondrial malfunction.

Furthermore, Mohamed *et al.* [31] demonstrated that the environment around the ovaries can be improved by stem cells to speed up the functional healing of the damaged ovaries.

In terms of signaling pathways, Lu *et al.* [32] and Li *et al.* [33] suggested that stem cells have a role in the development of ovaries, the growth of follicles, the proliferation of granulosa cells, and the differentiation via AMPK/mTOR Hippo, PI3K/Akt, transforming growth factor beta1/Smad3 signaling pathways. Zhao *et al.* [34] demonstrated that human stem cells enhanced the PI3K pathway by elevating free levels of amino acids to restore ovarian function, improving the lipids metabolism and decreasing monosaccharides concentration.

Following stem cell transplantation, the number of follicles increased, and the number of granulosa cells that had undergone apoptosis decreased, indicating that UC MSCs may be able to enhance the environment around follicles and prevent granulosa cell apoptosis, thereby aiding in the restoration of ovarian function in the ovaries [34,35].

In the current work, the observed high increase in the distribution and density of the fibers of collagen within the ovaries of the diabetic group coincided with Dhindsa *et al.* [36], who explained that fibrosis is merely a byproduct of inflammation, which is characterized by excessive ROS production, dysregulation of apoptosis, elevated blood levels of proinflammatory mediators, and cytokines like C-reactive protein, tumor necrosis factor-alpha, transforming growth factor alpha or beta, tissue plasminogen activator, upregulation of nuclear transcription factor kappa-B, and activator protein-1 with lowering the anti-inflammatory elements' levels.

The decrease in the abundance of collagen within the ovaries of the stem cell-treated group appears to be more or less like the control, which could be explained by the previous research of Akram *et al.* [37], who attributed this to the control of inflammation and fibrosis, suggesting evidence that the paracrine impact helps the resident and wounded cells survive and recover functionally, significantly regulates the environment in the area, promotes wound healing, and decreases fibrosis.

In the current study, the PCNA immunohistochemical staining of the ovaries demonstrated decreased PCNA immune expression compared to the control group. The same findings were detected by Zhang *et al.* [38] that induced diabetes in rats by using letrozole and high-fat diets, leading to ovarian damage.

The STZ+ stem cell-treated group demonstrated increased intensity of PCNA immune expression. These findings were in accordance with Wang *et al.* [39], that induced ovarian damage after ovarian antigen injection.

A DNA polymerase accessory protein forms PCNA with a particular ring shape. The PCNA is present in multiplying eukaryotic cells and is a key coordinator in cell division, proliferation, and deoxyribonucleotide

replication [40]. The PCNA has become a widely utilized biomarker for detecting tumor cell proliferation activity [41].

In addition, the PCNA has been reported as a proliferation marker, acting as an essential regulator during ovarian follicle development in the granulosa cells [42,43]. It has been proven that PCNA is highly expressed during the growth and maturation of oocytes and follicles [44]. Furthermore, the PCNA has a critical role in different biological functions like replication and repair of DNA, chromatid cohesion, cell cycle control, and survival [45].

Conclusion

In the STZ-induced diabetic rats, the MSCs transplantation may improve the blood glucose and hormone levels (E2, FSH, and LH). Additionally, the diabetic rats' ovarian pathogenic alterations were reduced by stem cells. Thus, the stem cells opened a new field of diabetes and diabetic ovarian damage therapeutic options.

Authors' contributions

AS, NS, and MA played a vital role in the study design, data analysis, and article drafting. The biochemical work and biochemical analysis were completed by SA, AT, and AE. The analysis of the clinical data was performed by YK, NA, HR, AT, and NS. Additionally, the statistical analysis was conducted by NA, HR, YK, AE, and SA. The final review of the manuscript was completed by all authors, who also read and approved the finished product.

Acknowledgements

The authors thank the technicians and the workers of all departments performed this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Abdul Basith Khan M, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of type 2 diabetes—global burden of disease and forecasted trends. Journal of epidemiology and global health 2020;10: 107–11.

2 Agrawal IVA, Dave A, Jaiswal A. Type 2 diabetes mellitus in patients with polycystic ovary syndrome. Cureus 2023; 15:10.

3 Marino F, Salerno N, Scalise M, Salerno L, Torella A, Molinaro C, *et al.* Streptozotocin-induced type 1 and 2 diabetes mellitus mouse models show different functional, cellular and molecular patterns of diabetic cardiomyopathy. Int J Mol Sci 2023; 24:1132.

4 Capdevila J, Ducreux M, García Carbonero R, Grande E, Halfdanarson T, Pavel M, *et al.* Streptozotocin, 1982–2022: forty years from the FDA's approval to treat pancreatic neuroendocrine tumors. Neuroendocrinology 2022; 112:1155–1167.

5 Yang Z, Li K, Yan X, Dong F, Zhao C. Amelioration of diabetic retinopathy by engrafted human adipose-derived mesenchymal stem cells in streptozotocin diabetic rats. Graefe's Arch Clin Exp Ophthalmol 2010; 248:1415–1422.

6 Fang Y, Tian X, Bai S, Fan J, Hou W, Tong H, *et al.* Autologous transplantation of adipose-derived mesenchymal stem cells ameliorates streptozotocin-induced diabetic nephropathy in rats by inhibiting oxidative stress, pro-inflammatory cytokines and the p38 MAPK signaling pathway. Int J Mol Med 2012; 30:85–92.

7 Ahmed Sorour HA, Abdel Aal FS, Elgalil A, Mohamed M. Can mesenchymal stem cell transplantation and fennel ingestion alter the histology of senile diabetic rat ovary? Egypt J Histol 2019; 42:64–83.

8 Yousef HN, Sakr SM, Sabry SA. Mesenchymal stem cells ameliorate hyperglycemia in type I diabetic developing male rats. Stem Cells Int Vol 2022; 2022:7556278.

9 Pulavendran S, Vignesh J, Rose C. Differential anti-inflammatory and anti-fibrotic activity of transplanted mesenchymal vs. hematopoietic stem cells in carbon tetrachloride-induced liver injury in mice. Int Immunopharmacol 2010; 10:513–519.

10 Berardis S, Sattwika PD, Najimi M, Sokal EM. Use of mesenchymal stem cells to treat liver fibrosis: current situation and prospects. World J Gastroenterol 2015; 21:742.

11 Trzyna A, Banaś-Zbczyk A. Adipose-derived stem cells secretome and its potential application in 'stem cell-free therapy. Biomolecules 2021; 11:878.

12 Srinivasan K, Viswanad B, Asrat L, Kaul C, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res 2005; 52:313–320.

13 Ngoc PK, Phuc PV, Nhung TH, Thuy DT, Nguyet NTM. Improving the efficacy of type 1 diabetes therapy by transplantation of immunoisolated insulin-producing cells. Hum Cell 2011; 24:86–95.

14 Cesaretti MLR, Ginoza M, Ribeiro AB. Systemic hemodynamic and left ventricular function of diabetic-induced hypertensive rats. Arq Bras Endocrinol Metab 2010; 54:842–851.

15 Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis, and clinical interventions in type 1 diabetes. Nature 2010; 464:1293–1300.

16 Ghasemi A, Jeddi S. Streptozotocin as a tool for induction of rat models of diabetes: a practical guide. EXCLI J 2023; 22:274–294.

17 Kajiyama H, Hamazaki TS, Tokuhara M, Masui S, Okabayashi K, Ohnuma K, *et al.* Pdx1-transfected adipose tissue-derived stem cells differentiate into insulin-producing cells in vivo and reduce hyperglycemia in diabetic mice. Int J Dev Biol 2009; 54:699–705.

18 Carr CA, Stuckey DJ, Tatton L, Tyler DJ, Hale SJ, Sweeney D, *et al.* Bone marrow-derived stromal cells home to and remain in the infarcted rat heart but fail to improve function: an in vivo cine-MRI study. Am J Physiol Heart Circ Physiol 2008; 295:H533–H542.

19 Jiang T-S., Cai L, Ji W-Y, Hui Y-N, Wang Y-S, Hu D, *et al.* Reconstruction of the corneal epithelium with induced marrow mesenchymal stem cells in rats. Mol Vis 2010; 16:1304.

20 Liu S, Zhao C, Yang C, Li X, Huang H, Liu N, *et al.* Gambogic acid suppresses pressure overload cardiac hypertrophy in rats. Am J Cardiovasc Dis 2013; 3:227.

21 Malatesta M. Histological and histochemical methods-theory and practice. Eur J Histochem 2016; 60:1.

22 Bancroft JD, Layton C. Connective and mesenchymal tissues with their stains. Bancroft's Theory Pract Histol Tech 2012; 12:187–214.

23 Bancroft J, Suvarna K, Layton C. Bancroft's theory and practice of histological techniques E-Book. Health Sci 2018; 126:138.

24 Gambineri A, Patton L, Altieri P, Pagotto U, Pizzi C, Manzoli L, *et al.* Polycystic ovary syndrome is a risk factor for type 2 diabetes: results from a long-term perspective study. Diabetes 2012; 61:2369–2374.

25 Soleymanzadeh F, Mahmoodi M, Shahidi S. Effect of vitex agnus-castus ethanolic extract on sex hormones in streptozotocin-induced diabetic rats. J Family Reprod Health 2020; 14:102.

26 Yan L, Wu Y, Li L, Wu J, Zhao F, Gao Z, *et al.* Clinical analysis of human umbilical cord mesenchymal stem cell allotransplantation in patients with premature ovarian insufficiency. Cell Prolif 2020; 53:e12938.

27 Ding L, Yan G, Wang B, Xu L, Gu Y, Ru T, *et al.* Transplantation of UC-MSCs on collagen scaffold activates follicles in dormant ovaries of POF

patients with long history of infertility. Sci China Life Sci 2018; 61:1554–1565.

28 Körbling M, Estrov Z. Adult stem cells for tissue repair—a new therapeutic concept? New Engl J Med 2003; 349:570–582.

29 Fouad H, Sabry D, Elsetohy K, Fathy N. Therapeutic efficacy of amniotic membrane stem cells and adipose tissue stem cells in rats with chemically induced ovarian failure. J Adv Res 2016; 7:233–241.

30 Rezvanfar M, Rezvanfar M, Ahmadi A, Saadi HS, Baeeri M, Abdollahi M. Mechanistic links between oxidative/nitrosative stress and tumor necrosis factor alpha in letrozole-induced murine polycystic ovary: biochemical and pathological evidence for beneficial effect of pioglitazone. Hum Exp Toxicol 2012; 31:887–897.

31 Mohamed SA, Shalaby S, Brakta S, Elam L, Elsharoud A, Al-Hendy A. Umbilical cord blood mesenchymal stem cells as an infertility treatment for chemotherapy induced premature ovarian insufficiency. Biomedicines 2019; 7:7.

32 Lu X, Bao H, Cui L, Zhu W, Zhang L, Xu Z, *et al.* hUMSC transplantation restores ovarian function in POI rats by inhibiting autophagy of theca-interstitial cells via the AMPK/mTOR signaling pathway. Stem Cell Res Ther 2020; 11:1–13.

33 Li Z, Zhang M, Zheng J, Tian Y, Zhang H, Tan Y, *et al.* Human umbilical cord mesenchymal stem cell-derived exosomes improve ovarian function and proliferation of premature ovarian insufficiency by regulating the hippo signaling pathway. Front Endocrinol 2021; 12:711902.

34 Zhao Y, Ma J, Yi P, Wu J, Zhao F, Tu W, *et al.* Human umbilical cord mesenchymal stem cells restore the ovarian metabolome and rescue premature ovarian insufficiency in mice. Stem Cell Res Ther 2020; 11:1–15.

35 Cui L, Bao H, Liu Z, Man X, Liu H, Hou Y, *et al.* hUMSCs regulate the differentiation of ovarian stromal cells via TGF-β1/Smad3 signaling pathway to inhibit ovarian fibrosis to repair ovarian function in POI rats. Stem Cell Res Ther 2020; 11:1–12.

36 Dhindsa G, Bhatia R, Dhindsa M, Bhatia V. Insulin resistance, insulin sensitization and inflammation in polycystic ovarian syndrome. J Postgrad Med 2004; 50:140.

37 Akram KM, Samad S, Spiteri MA, Forsyth NR. Mesenchymal stem cells promote alveolar epithelial cell wound repair in vitro through distinct migratory and paracrine mechanisms. Respir Res 2013; 14:1–16.

38 Zhang S, Tu H, Yao J, Le J, Jiang Z, Tang Q, *et al.* Combined use of Diane-35 and metformin improves the ovulation in the PCOS rat model possibly via regulating glycolysis pathway. Reprod Biol Endocrinol 2020; 18:1–11.

39 Wang Z, Wei Q, Wang H, Han L, Dai H, Qian X, *et al.* Mesenchymal stem cell therapy using human umbilical cord in a rat model of autoimmune-induced premature ovarian failure. Stem Cells Int 2020; 2020:3249495.

40 Essers J, Theil AF, Baldeyron C, van Cappellen WA, Houtsmuller AB, Kanaar R, *et al.* nuclear dynamics of PCNA in DNA replication and repair. Mol Cell Biol 2005; 25:9350–9359.

41 Cardano M, Tribioli C, Prosperi E. Targeting proliferating cell nuclear antigen (PCNA) as an effective strategy to inhibit tumor cell proliferation. Curr Cancer Drug Targets 2020; 20:240–252.

42 Tom⊠nek M, Chronowska E. Immunohistochemical localization of proliferating cell nuclear antigen (PCNA) in the pig ovary. Folia Histochem Cytobiol 2006; 44:269–274.

43 Zhang H, Jiang X, Zhang Y, Xu B, Hua J, Ma T, *et al.* microRNA 376a regulates follicle assembly by targeting PCNA in fetal and neonatal mouse ovaries. Reproduction 2014; 148:43–54.

44 Xu B, Hua J, Zhang Y, Jiang X, Zhang H, Ma T, *et al.* Proliferating cell nuclear antigen (PCNA) regulates primordial follicle assembly by promoting apoptosis of oocytes in fetal and neonatal mouse ovaries. PLoS ONE 2011; 6:e16046.

45 Moldovan G-L., Pfander B, Jentsch S. PCNA, the maestro of the replication fork. Cell 2007; 129:665–679.