Original article

Effects of Insulin and Resveratrol versus Mesenchymal Stem Cells (MSCs) on the Pancreas of Diabetic Rats (Type-I) (Light and Electron Microscope Study)

Samia M. Manawy , Essam M.Eid, Ali M. Ali, Fatma M. Ahmed, Nehal F.Shaheen

Abstract:

Anatomy and Embryology Department, Faculty of Medicine Benha University, Egypt.

Corresponding to:
Dr. Fatma M. Ahmed.
Anatomy and Embryology Department, Faculty of Medicine Benha University, Egypt.
Email:
fatma.gaballah@fmed.bu.edu.eg

Background: transplantation of mesenchymal stem cells (MSCs) provides an effective strategy to protect pancreatic tissue injury in diabetes type 1. Many factors act as obstacles to their best regenerative capacity. Aim of the work: To evaluate the role of Adipose-derived mesenchymal stem cell (AD-MSCs) in the treatment of type 1 DM and to compare their efficacy alone and with a combination with resveratrol and long-acting insulin in the pancreas of albino rats with streptozotocin induced T1DM. **Methods:** 10 out of the 50 healthy adult male albino rats served as donors for stem cells and the other 40 rats were subdivided into two main groups: Group I (control group (16 rats), Group II (STZ-diabetic group, (24) rats which were injected with a single intraperitoneal injection of streptozotocin 50 mg/kg BW. This group was subdivided into 4 groups. GII a: untreated rats, GII b: received 1×10^6 of cultured rat ADMSCs once intravenously into the tail vein, GII c which was given MSCs plus resveratrol in combination, GII d which was given MSCs & resveratrol and insulin glargine. Pancreatic samples were prepared for immune histochemical study, light and electron microscopic examination, and morphometric studies. **Results:** The AD-MSCs-treated group revealed moderate improvement in histological structure and fasting blood glucose level. AD-MSCs, resveratrol and insulin-treated group demonstrated higher improvement than that of the MSCs treated group alone or dual combination of AD-MSCs plus resveratrol. **Conclusion:** Better synergistic protective effect on damaged pancreatic tissues can be observed in STZ induced DM rats treated with AD- MSC coupling with resveratrol and insulin treatment.
Key words: AD-MSCs, type1 DM, Resveratrol, Insulin

Introduction

Diabetes mellitus (DM) is a major public health problem worldwide. It affects around 415 million people and is set to rise to 642 million by the year 2040 [1].

Type1 DM is an autoimmune, metabolic disease characterized by selective destruction of pancreatic β-cells leading to a loss of endogenous insulin production and secretion with lifelong insulin treatment [2]. Recently regenerative therapy via transplantation of MSC has emerged as an effective strategy to protect against tissue and organ injury [3]. Hyperglycemic state induces MSCs apoptosis, as impairing the balance between reactive oxygen substances and antioxidant mechanisms leading to decrease their therapeutic effects by decreasing their homing and differentiation [4]. Resveratrol is a natural agent possesses great therapeutic potential for protecting against injures in multiple tissues as a result of its antioxidative and anti-inflammatory properties [4].

Material and methods:

This study is an experimental research approved by the Research Ethics Committee of faculty of medicine, Benha University (MD 3-11-2020).

Animals

This study was conducted on 40 adult male albino rats weighing between 120 and 200 gm, taken from and housed at the laboratory animal house unit of Kasr Al-Ainy Faculty of Medicine, Cairo University; the rat housed in plastic cages at 22± 3°C and 14 h: 10 h light: dark, rats were fed a standard diet and water for a period of 4 weeks (from 10April 2021 to 8 May 2021), after an accommodation period of one week. Strict cleaning and maintenance measures were applied to keep the animals in a healthy state. All ethical rules for animal treatment were followed.

Chemicals

Streptozotocin (STZ): It was obtained from Sigma Chemical Company (Sigma-Aldrich, St. Louis, MO) in the form of 1 gm white to yellow powder vial.

Insulin glargine:(Lantus®): It is extended-action biosynthetic human insulin It was obtained from Sanofi–Aventis Pharma, Bad Soden, Germany, in the form of 10 ml vial containing 100 units/ ml.

Adipose tissue-derived mesenchymal stem cells (AD-MSCs):

The AD-MSCs were prepared in the stem cell unit, faculty of medicine, Cairo University and were used at a dose of AD-MSCs (1×106 cells/ ml) suspended in 1ml phosphate buffer saline (PBS) and were injected once intravenously through the tail vein.

Experimental Design

10 out of the 50 albino rats used in this study served as donors for stem cells and the other 40 rats were divided into two major groups:

1) **Group I** (16 rats); the control group subdivided into 4rat for each:

- Negative control: did not receive any medications.
- Positive control A: received a single intraperitoneal injection of 1 ml of sodium citrate buffer (0.1 mM/ L, PH 4.5)
- Positive control B: received saline solution orally.
- Positive control C was injected with 1ml PBS,

2) **Group II: STZ-diabetic group (24 rats)** were injected with a single intraperitoneal injection of STZ 50 mg/kg BW dissolved in 0.1 mM/L sodium citrate buffer, pH 4.5. This group further subdivided into 4 groups (6 rats for each).

GIIa: untreated diabetic that didn't receive any medications.

GIIb: received $1x10^6$ of cultured rat AD-MSCs, suspended in 1ml PBS; the cells were injected once intravenously through the tail vein[5].

GIIc: treated with resveratrol (5-10 mg/kg BW) dissolved in saline solution orally once
daily)and AD-MSCs in combination [6].

GIId: treated with resveratrol (5-10 mg/kg BW dissolved in saline solution orally once
daily), insulin glargine (2-6U/rat/day)[2], and AD-MSCs.

At the end of the experiment blood samples from all rats were taken for measuring of the blood glucose level then rats were anesthetized by light ether inhalation and sacrificed by cervical dislocation. The abdomen was opened by midline incision and pancreas was removed and rapidly dissected out and divided into two parts: one fixed in 10 % formalin solution for light microscopic examination and the other was fixed in 2.5% glutaraldehyde for electron microscopic examination.

Histopathological study:
Pancreatic specimens subjected for light microscopic study, were fixed in 10% formalin saline for 24h and 5 μm paraffin sections were cut and stained with H&E for histological study and Masson's trichrome stain for collagen fibers [7]. Slide visualization and image photographing were performed in the Anatomy Department, Faculty of Medicine, Benha University, Egypt. For this, Nikon Eclipse 80i upright microscope (Nikon Corporation, Japan) with a fitted digital camera, Toup CamTM Xcam full HD camera (ToupTek Europe, Ultramacro Ltd., UK) was used.

Transmission electron microscopic study: -
The pancreatic specimens were collected and ultrathin sections were performed [8].It was done and photographed in Tanta EM Unit, Faculty of Medicine, Tanta University using JOEL (JEM-100 SX, Akishima, Tokyo, Japan).

Immunohistochemical study: -
Immunohistochemistry of paraffin-embedded pancreatic sections was performed caspase 3 antibodies by using

the avidin– biotin peroxidase complex technique [9].The slides were studied by light microscopy in Anatomy Department, Benha faculty of Medicine, Benha University.

Biochemical study [10]:-
Before scarification of rats fasting, blood glucose level was measured after a fasting period of 6-8 hours. Blood drop was taken from the distal end of the tail and analyzed immediately via a blood glucose monitoring device by the glucose oxidase method using a Glucometer (Accu-Check Active, Roche Diagnostics, Mannheim, Germany*)*

Morphometric study [11]:were performed to measure.

1.The pancreatic islet size.

2.Area percent of collagen fibers in Masson's trichrome stained sections.

3.Area percent of caspas-3 immunoreactivity.at a magnification of × 400.

Statistical analysis
The values obtained from morphometric study and level of blood glucose level were expressed as mean ± SD for each group. Statistical comparison among different groups was evaluated using a one-way analysis of variance (ANOVA) and post hoc Least Significant Difference (LSD) test. Calculations were done with SPSS software, version 20 (IBM SPSS Statistics for Windows, Armonk, NY, USA). Statistical significance was defined as *p*-value less than 0.05[12].

Results
I) Histological analysis
Hematoxylin and Eosin results:
Group I: The sections appeared as closely packed lobules of pancreatic acini surrounding well-packed endocrine islets of Langerhans. The acini were formed of pyramidal cells with basal nuclei and apical acidophilic cytoplasm. The islets consisted of clusters of large beta cells centrally and smaller alpha cells peripherally, the cells were separated by blood capillaries. Most of the nuclei of

islets and acini were regular and rounded (Fig.1a).

Group IIa: showed loss of the normal architecture of islets of Langerhans with distortion of its outline and the cells appeared degenerated and necrotic with pyknotic & fragmented nuclei. There were lytic vacuoles giving them a foamy and rarefaction appearance. (Fig.1b).

Group IIb: The islets showed improvement in the architecture with intact boundaries except for the presence of a few vacuoles between the cells of islets. Beta cells appeared nearly normal, pale polygonal cells, Alpha cells appeared with dense nuclei and acidophilic cytoplasm at the periphery. scanty cells appeared with pyknotic nuclei and vacuolated cytoplasm. Pancreatic acini appeared with normal architecture (Fig.1c).

Group IIc: showed marked improvement in the morphological appearance of pancreatic cells when compared with the previous groups. The islets appeared with regular outlines and no vacuolation. The islet cells were arranged in well-packed cords. Beta cells appeared pale cells with prominent nucleoli at the center and Alpha cells appeared with smaller dark basophilic nuclei at the periphery. Pancreatic acini showed normal architecture**. (Fig.1d).

Group IId: The pancreatic sections restored the normal architecture as the same finding in the previous group (GIIc) with more increase in size and cellular density. (Fig.1e).

Masson's trichrome results:

The diabetic untreated group showed excess, blue-stained collagen fibers inside the islets of Langerhans and within thin septa around the pancreatic acini (Figs.2b). The control, GIIc and GIId groups exhibited thin blue collagen fibers inside the islets of Langerhans and within thin septa around the pancreatic acini (Figs.2 a, d, & e).

The AD-MSCs treated group exhibited mild blue collagen fibers deposition (Figs.2c).

II)Immunohistochemical study:

Caspase 3 Immune histo-chemical:

The control group showed negative brownish cytoplasmic coloration *(*Fig.4a), While GIIa showed a strong positive brownish cytoplasmic immune reaction in the islet and the acinar cells. (Fig.4b).

The AD-MSCs group showed mild immunoreactivity of caspase 3 in the islet and the acinar cells (Fig.4c), while a minimal immune reactivity appeared in groups (GIIc) and (GIId). (Fig.4 d & e).

III)Transmission electron microscopic result (TEM):

Group I: The Beta cells appeared with a rounded regular euchromatic nucleus with a prominent nucleolus. Its cytoplasm contains numerous secretory granules with condensed cores with angular sides and large surrounding halos, mitochondria appeared regular. Normal Golgi apparatus. Alpha cells exhibited characteristic homogeneous electron-dense granules with no or very narrow surrounding hallo. (Fig.4a&b).

Acinar cells appeared intact with euchromatic basal nuclei, and numerous electron-dense large secretory granules, zymogen granules, of variable sizes in the apical part. Normal mitochondria, and rough endoplasmic reticulum (rER) were also evident in the acinar cells. (Fig.4c).

Group IIa: The β cells appeared distorted with irregular pyknotic nuclei and rarified vacuolated cytoplasm. There was a decrease in the secretory granules with a fusion of some, and degenerated swollen mitochondria was obvious. (Figs. 5d&e).

The acinar cells appeared with irregular contour of the nucleus, damaged swollen mitochondria, dilated cistern of GA, and decreased secretory granules some of them were empty. (Fig.4f).

Group IIb: The β cells appeared with a nearly normal nucleus. The Nuclei appeared intact with prominent nucleolus except one nucleus appeared pyknotic. The secretory granules were numerous and intact except a few granules appeared empty. Scanty vacuoles and dilated

cisterns of rER could be seen, others with regular appearance (Figs.5 g&h).

The acinar cells appeared intact with normal nuclei. The secretory granules appeared normal except some granules appeared dissolved with faint density. Normal mitochondria and rER could be seen (Fig.4 i).

Group IIc: The pancreatic section showed the β cell with a normal nucleus. The cytoplasm had numerous intact secretory granules, normal mitochondria, and normal Golgi apparatus (Figs. 5a&b). The acinar cells appeared with nearly normal nuclei with prominent nucleolus. Its cytoplasm exhibited many normal secretory granules, normal mitochondria, and rough endoplasmic reticulum (Fig.5c).

Group IId: The pancreatic section showed beta cells restoring their normal appearance with numerous characteristic secretory granules, regular nuclei, normal mitochondria, and normal Golgi apparatus. (Figs. 6d&e). The acinar cells restored their normal appearance like control group. (Fig.5f).

IV)Morphometric and statistical results

- **Mean values of the diameter of the islet of Langerhans by pixels' table 1 and histogram (Fig.1f)**

Group IIa: showed a significant decrease (P< 0.05) when compared with all other groups.

Group IIb: group showed a significant decrease when compared with the control, GIIc, and Group IId groups. Also, there was a significant increase when compared with GIIa (P< 0.05).

Group IIc showed a significant decrease when compared with the control and GIId group. Also, there was a significant increase when compared with GIIa, and GIIb (P< 0.05).

Group IId group had non-significant change compared with the control group. Also, there was a significant increase (P< 0.05) when compared with GIIa, GIIb, and GIIc.

- **Mean area percentage of collagen fibers deposition table 1 and histogram (Fig.2f)**

Group IIa: showed a significant increase (P< 0.05) when compared with the control and other treated groups.

Group IIb: Exhibited a significant increase (P< 0.05) when compared with the control, GIIc, and GIId groups. Also, there was a significant decrease when compared with control and GIIa, GIIb and GIIc groups.

Group IIc: Had a significant increase when compared with the control and GIId groups. Also, there was a significant decrease when compared with GIIa & GIIb (P< 0.05)

Group IId: Has non-significant change compared with the control group. Also, there was a significant decrease (P< 0.05) when compared with GIIa, GIIb, and GIIc groups.

- **Mean area percentage of caspase 3 table 1 and histogram (Fig.1f)**

Group IIa: showed a significant increase (P< 0.05) when compared with the control and other treated groups. Group IIb: showed a significant increase (P< 0.05) when compared with the control, GIIc, and GIId groups. Also, there was a significant decrease (P< 0.05) when compared with GIIa. Group IIc: showed a significant increase (P< 0.05) when compared with the control and GIId groups. also, there was a significant (P< 0.05) decrease when compared with GIIa & GIIb groups. Group IId: Had non-significant change compared with the GI. Also, there was a significant decrease (P< 0.05) when compared with GIIa, GIIb, and GIIc groups.

Mean values of FBG mg/dl Table 1:

Group IIa: showed a significant increase (P< 0.05) when compared with the control group.

GIIb: showed a significant increase (P< 0.05) when compared with the control, GIIc, and GIId groups. GIIc group, showed a significant increase (P< 0.05) when compared with the control and GIId groups. Also, there was a significant (P< 0.05) decrease when compared with GIIa

& GIIb. GIId had non-significant change compared with the control group. Also, there was a significant decrease (P< 0.05) when compared with GIIa, GIIb, and GIIc groups.

Table (1) showing the Mean and ± SD of fasting blood glucose (mg/dl), the area percentage of caspase 3, collagen fiber deposition, and ean of the diameter of the islet for all groups with comparison between all groups by Post Hoc LSD test.

	Group I		Group II diabetic		
	Control	**untreated Group**	**AD-MSCs Group**	**AD-MSCs +res**	**AD-MSCs +res.+ insulin**
FBG (mg /dl)	98.17±3.7	551.17± 11.1 a, c, d, e	16.05 ± 0.9 a,b,d,e	129.7 ±7.3 a,b,c,f	100.7 ± 6.1 a, b, c, d
Area % of caspase 3	1.2 ± 1	44.6 ± 3.3 a, c, d, e	14.7 ± 1.7 a, b, d, e	10.27± 1.4 a, b, c, e	8 ± 0.95 b, c, d
Area% of collagen fibers	4.8 ±1.5	48.4 ± 7.2 a, c, d, e	21.4 ± 1.9 a, b, d, e	16.05 ± 0.9 a, b, c, e	27.85±0.45 b, c, d
Mean of the diameter of the islet	817.44± 80.1	392.4 ± 43.1 a, c, d, e	617.5 ± 66.5 a, b, d, e	698.9 ±70.3 a, b, c, e	698.9 ±70.3 b, c, d

Data expressed as mean ± SD, *: significance ≤ 0.05.
a: Significance with GI   b: Significance with GIIa
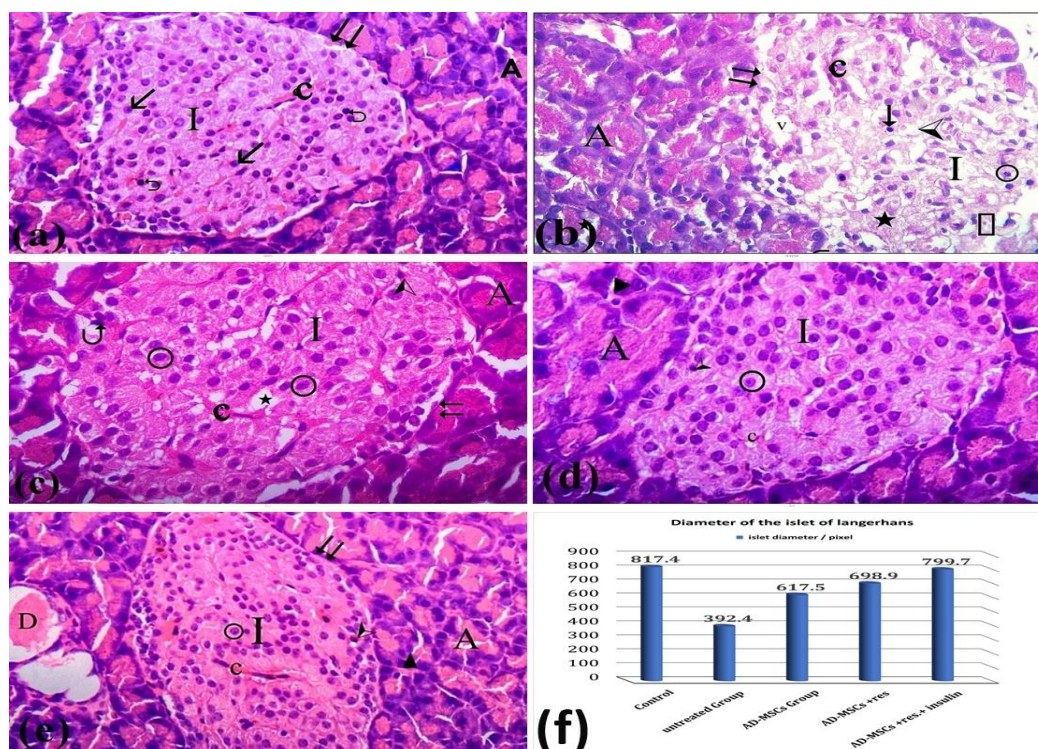c: Significance with GIIb   d: Significance with GIIC
 e: Significance with GIID.

Fig. (1): A photomicrograph of a pancreatic section of the **control group Fig.1 (a)** showing: normal pancreatic tissue architecture. The acini (A) appear intact with basal basophilia and apical acidophilia and the islet of Langerhans (I) is buried in it with a regular outline (double arrow). The islet cells are arranged in a cord- like manner separated by blood capillaries (c). The β-cells (arrows) lie in the core of the islet and are bigger than Alpha cells (curved arrows) that lie at the periphery. (H&E X400)

Group IIa untreated diabetic group Fig.1 (b) showing; marked loss of normal architecture of islets (I) with irregular outline (double arrow). The islet cells show marked degenerative changes in the form of vacuolated rarefied cytoplasm (star) and karyorrhectic (fragmented) nuclei (circle), dark pyknotic nuclei (arrow), complete loss of cells (rectangular), and swollen cell (head arrow). blood capillary (c) Some pancreatic acini (A) appear distorted with vacuolation (star). (H&E X400)

AD-MSCs Group Fig.1 (c) showing the islet of Langerhans (I) with intact boundary (double arrow) except the presence of few lytic vacuoles between the cells of the islets of Langerhans (star). Intact Beta cells appear pale vesicular cells (circle) and dark nuclear alpha cells appear at the periphery (head arrow). There are multiple capillaries between cells (c) Scanty cells appear with pyknotic nuclei (curved arrow). Pancreatic acini (A) appear with normal architecture. (H&E X400)

AD-MSCs+ res. Group Fig.1 (d) showing the islets of Langerhans (I) with normal architecture and regular outline (double arrow) no vacuolation appears. Beta cells appear pale vesicular cells with prominent nucleoli (circle). Alpha cells appear with smaller dark basophilic nucleus at periphery (head arrow). Some blood capillaries appear between cells (c). Pancreatic acini (A) appear with normal architecture basally located basophilic nucleus with prominent nucleolus (triangle) with apical acidophilic secretion. (H&E X400)

AD-MSCs+ res.+ insulin Group Fig.1 (e) showing the islets of Langerhans (I) with regular outline (double arrow) and no vacuolation. Islets cells arranged in well packed cords with normal rounded Beta cells (circle) and smaller in size with dark basophilic Alpha cells at the periphery (head arrow). Some blood capillaries appear between cells (c). Pancreatic acini (A) appear intact with basally basophilic nucleus with prominent nucleolus (triangle) and apical acidophilic secretion. blood vessels (BV). Note increasing the size and cellularity of islets. (H&E X400).Fig.1 (f): Histogram showing mean values of the diameter of the islet of Langerhans among the study groups.
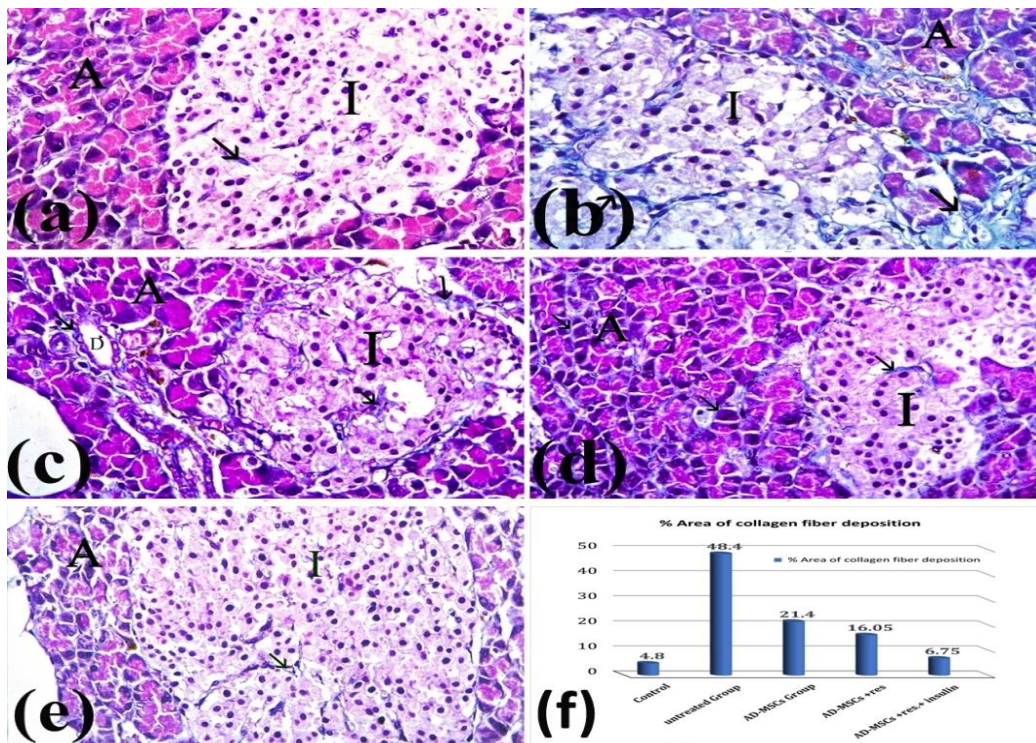
Fig. (2): Photomicrograph of pancreatic sections of group 1 (a) showing thin blue-stained collagen fibers (arrow) inside the islets of the pancreas (I) and around the acini (A). Group IIa, (b) showing marked excess collagen fibers inside the islets (I) and between the acini (A). Group IIb, (c) showing excess, collagen inside the islets (I) and between acini (A). Group IIc, (d) showing mild deposition of collagen fibers inside the islets (I), duct (D), and between the acini (A). Group IIb (e) showing minimal, collagen fibers inside the islets (I) and between acini (A) (Masson's Trichrome X 400). Fig.2 (f): Histogram showing mean % area of collagen deposition among the study groups.
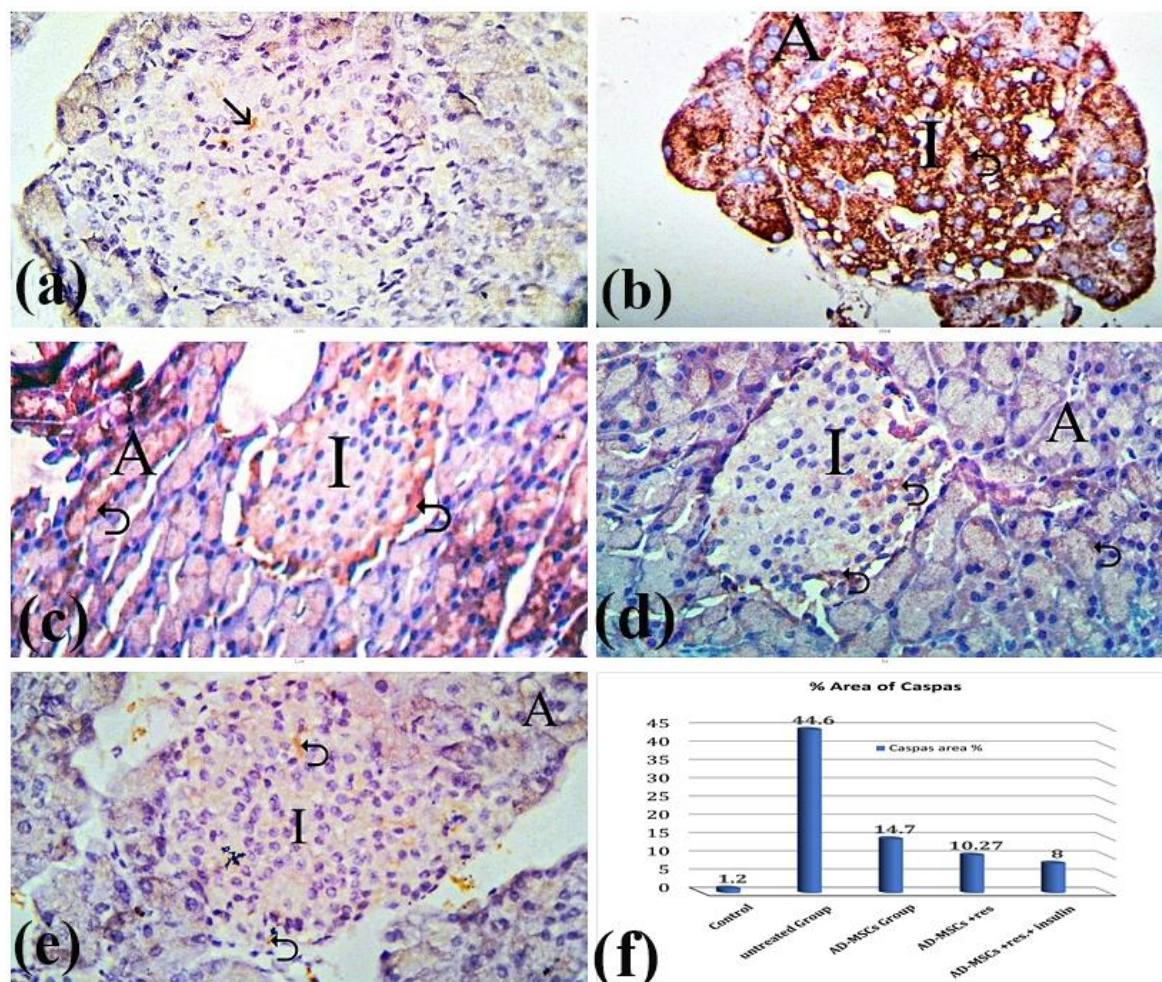

Fig. (3): A photomicrograph of a pancreatic section of group1 (a): showing negative, trace, brownish cytoplasmic immune-reaction for caspase-3 (arrow) in the islet's cells and acinar cells. Group IIa (b): Showing strong positive cytoplasmic immune reaction of caspase-3 (arrow) in the islet's cells (I) and the acinar cells (A). Group IIb (c): Showing mild cytoplasmic immunoreaction (arrow) of caspase-3 in islets cells (I) and acinar cells (A). Group IIC (d) and Group IIb (e): showing minimal scanty cytoplasmic immunoreaction (arrow) in islets cells (I) and acinar cells(A). Caspase 3 X400). Fig.4 (f): Histogram showing Mean percentage area of Caspase 3 immunoreactivity among the study groups.
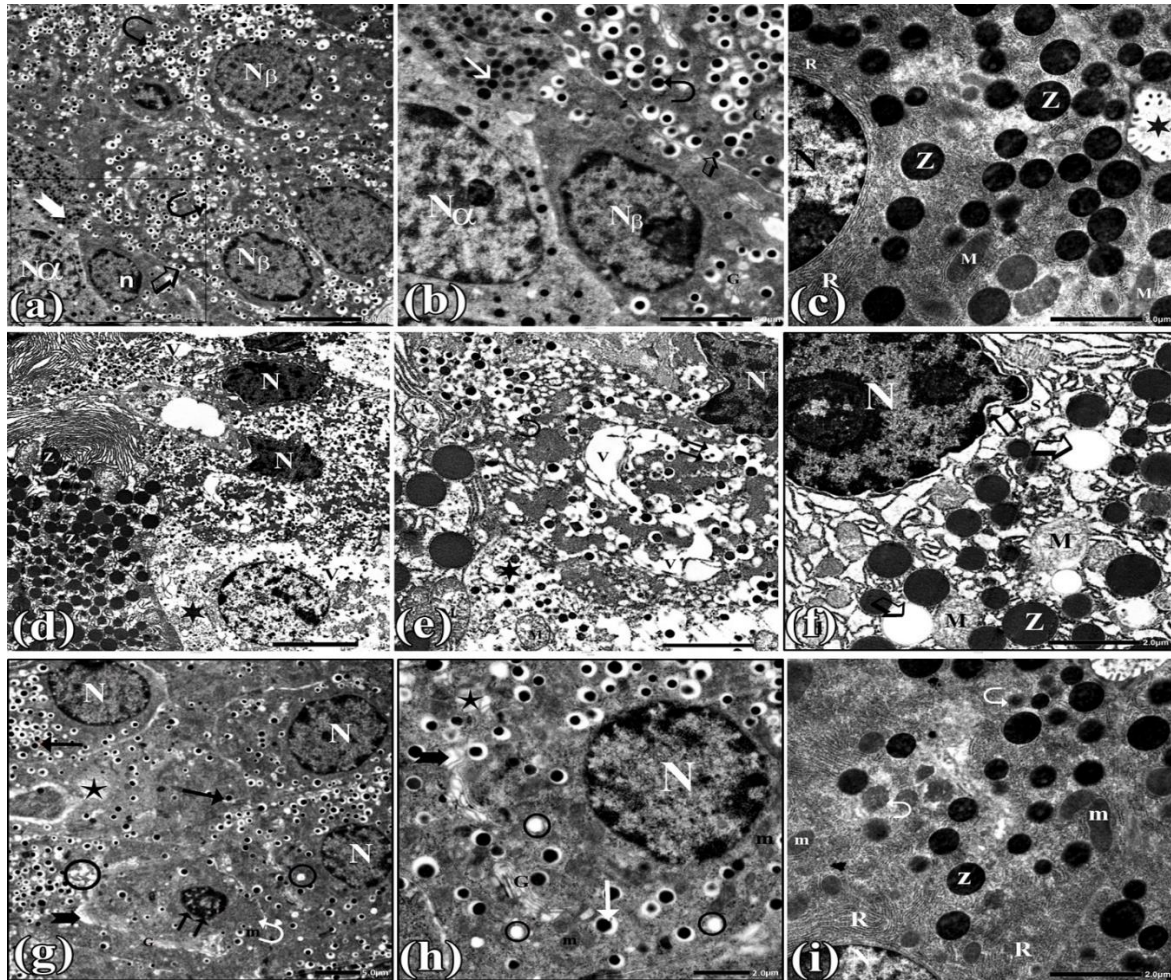
Fig. (4): Transmission electron micrographs of the rat pancreatic tissue. Control group (Fig.4 a, b, c). β cells in (fig.4a& b) appears with a rounded regular nucleus (Nβ) with a prominent nucleolus (n), numerous secretory granules with condensed core and large surrounding halos (curved arrow), normal Golgi apparatus (G). Alpha cell appears with regular nucleus (Nα) and homogeneous electron-dense granules with no surrounding hallo (tailed arrow) notice, the junction between cells (arrow). Acinar cells (Fig.4 c) appear intact with euchromatic nucleus (N), and numerous electron-dense large zymogen granules (z), of variable sizes, intact mitochondria (m), and rough endoplasmic reticulum (R). Untreated Diabetic group (Fig.4 d, e, f): The β cells appear (Fig.4d & e) distorted with irregular pyknotic nuclei (N), rarified (star) and vacuolated (V) cytoplasm, and degenerated swollen mitochondria is obvious (M) The acinar cells (Fig.4 f) appear with irregular contour of the nucleus (N), damaged swollen mitochondria (M), dilated spaces (S), and secretory granules some of them are empty (arrow). AD-MSCs group (Fig.4 g, h, i): The β cells (Fig.4 g & h) appear with a nearly normal nucleus (N) except one nucleus appears pyknotic (double arrow). The cytoplasm shows multiple normal secretory granules (arrow) except few granules are empty (circle), intact mitochondria (m), intact Golgi apparatus (G) with few swollen cisterns (bifid arrow), and rarified areas (star). (Fig.4 i): A part of acinar cell with part of its nucleus appears (N). The cytoplasm contains many intact zymogen granules (Z) few of them have faint density (curved arrow), normal mitochondria with regular cristae (m), and rough endoplasmic reticulum (R). (TEM x 8000 -TEM X 20000)
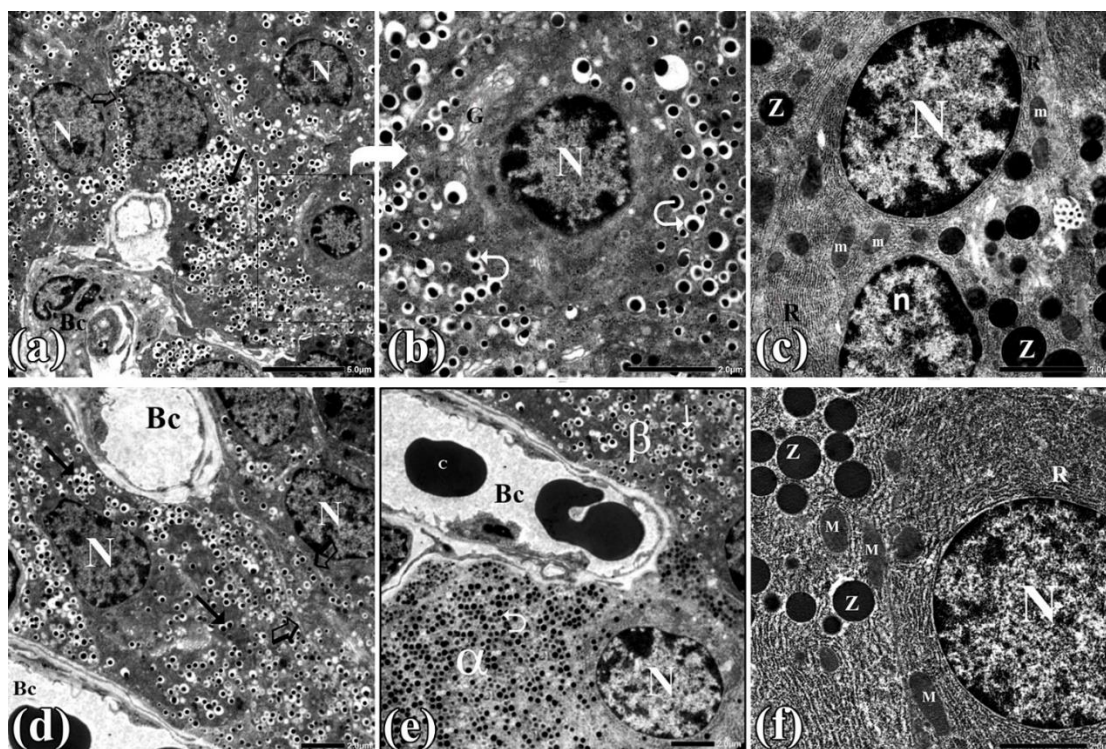
Fig. (5): Transmission electron micrographs of the rat pancreatic tissue MSCs +Res. group (Fig.5 a, b, c): The β cell (a&b) has a normal nucleus (N). The cytoplasm has numerous intact secretory granules (arrow), normal Golgi apparatus (G). The acinar cells(c) appear with nearly normal nuclei (N) with prominent nucleolus(n). Its cytoplasm exhibits intact secretory granules (Z), normal mitochondria (m), and rough endoplasmic reticulum (R). MSCs +Res. +insulin group (Fig.5 d, e, f): β cells restoring their normal appearance with numerous characteristic secretory granules (dense arrow) and regular nuclei (N). Notice, blood capillary (Bc) with blood cell (c) and cell junction (Fig.5 d & e). The acinar cells restoring their normal appearance like control group. (Fig. 5f). (TEM X8000 -X 20000).

Discussion

Numerous therapeutic approaches have been taken for the replacement of β-cells; stem cell transplantation is one of the most promising alternatives. Stem cells have shown the potential efficacy to treat T1DM by reconstitution and preservation of islet β-cell function. [13].

In the present study, H&E-stained sections of GIIa showed extensive destruction of Langerhans islets with ill-defined boundaries, reduction of their size, and focal lytic areas could be seen. The cells appeared degenerated with pyknotic nuclei. Also, pancreatic acini appeared distorted with vacuolation. These results were in agreement with those of [14, 15].

These results were confirmed by a TEM examination that showed degeneration in both islets and acini. The cells of islets appeared with irregular pyknotic nuclei, rarified cytoplasm, obvious lytic vacuolation, decreased shrunken secretory granules, swollen cisternae of GA, and damaged swollen mitochondria. These findings were agreed by [16] who reported pathological changes in both endocrine and exocrine parts of the diabetic group.

Also, [17] reported an obvious degenerative and apoptotic changes in beta cells. They clarified that it was because of lipid peroxidation. They proved that by a significant increase in pancreatic malondialdehyde MDA, a lipid peroxidation indicator.

In the current study, the sections of GIIb pancreatic tissues by light and TEM showed regeneration of islets cells in comparison with GIIa, the islet of Langerhans restored normal architecture with intact boundary except scanty lytic

vacuoles between the cells. Beta cells appeared pale with prominent nucleoli. Scanty cells appeared with a pyknotic nuclei and vacuolated cytoplasm. Pancreatic acini appeared normal. These results were in consistent with [18] who explained this improvement by MSCs by their immunomodulatory properties and differentiation of MSC into insulin-secreting cells. Also, the results agreed by [19], who showed that MSCs administration reduce the hyperglycemia and are accompanied by increased islet-neogenesis. and [20] who explained this improvement by the ability of AMSCs to differentiate into insulin-producing cells & numerous transcription factors like insulin gene enhancer protein (Isl-1) and Pax-6.

The Masson's trichrome stains results were in consistent with [21,22] who reported minimal collagen fibers between pancreatic lobules in the control group and a marked increase in collagen deposition in the diabetic group while, resveratrol-preconditioned stem cells treated rats showed minimal deposition.

This study demonstrated that using resveratrol in combination with AD-MSCs increases its efficacy rather than using it alone as appeared histologically by H&E. The islets restored their normal architecture, size, cellularity, and there weren't degenerated cells. The results were confirmed by ultrastructure and biochemical studies. These results were in consistent with [23] who found that AD-MSCs precondition with resveratrol increases the survival marker expression, leading to enhanced AD-MSCs viability. They verified its viability by measuring the survival proteins e.g. phosphorylated serine/threonine protein kinase (p-Akt) expression they found that hyperglycemia reduced p-Akt expression in ADSC. By contrast, the p-Akt expression was increased despite hyperglycemia, if AD-MSC was preconditioned with resveratrol. [22] clarified this as resveratrol improved the homing of transplanted cells and augmented their regenerative, and anti-

fibrotic activities by decreasing free radicals that are responsible in part for cellular and DNA damage. They approved these results by measuring the oxidative stress parameters and the expression of apoptosis and fibrosis markers.

In this study, using a long acting insulin analog in combination with both AD-MSCs and resveratrol showed the best improvement histologically and functionally. This amelioration was verified by restoring the normal histological structure of the islets of Langerhans with an increase in the cellularity and the size of islets, preserved ultrastructure of β cells, and restoring normal blood glucose level. We clarified that hyperglycemia may affect the apoptotic pathway resulting in an increasing apoptotic process with decreasing viability of AD-MSCs, so decreasing the hyperglycemia by insulin treatment in addition using resveratrol, enhance viability and the regenerative power of MSCs. rather than using MSCs alone or with resveratrol only.

[24,25] revealed that STZ-induced hyperglycemia could initiate pancreatic β-cells apoptosis in diabetic rats, through elevating apoptotic factors like annexin, and caspases-3,8,9 associated with decreased anti-apoptotic Bcl-2 expression. Also, those results were supported by the results of [18,26, 27] who showed that controlling the blood glucose level by insulin treatment can promote beta-cell regeneration and decrease the apoptosis.

The immunohistochemical results were in agreement with [27, 28] who published that resveratrol coupling with ADSC showed a protective effect on pancreatic damage through the increased sirtuin 1 expression and suppression of caspase-3. Also, they reported a significant suppression of survival proteins and increased apoptotic proteins such as cytochrome C, Caspase3&9 can be observed in the diabetic group by contrast, significant upregulation of survival proteins and decreased apoptotic proteins can be found

in (ADMSC) and (res+ ADMSC) groups when compared to the diabetic group.

These results have been supported by morphometric and statistical results. GIIb showed a significant increase in Mean of the diameter of the islet when compared with GIIa. These results were consistent with [18,29, 30] and there was a significant decrease in FBG level, area % of caspase 3, and collagen fibers as compared with GIIa this agreed by [5, 31, 32] who explained that pancreatic fibrosis may be caused by pancreatic stellate cells that have been activated by hyperglycemia, resulting in fibrotic changes.

Group IIc had a significant increase in the mean diameter of islets and a significant decrease change in FBG level, collagen fibers, and caspase 3 immune reactivity as compared with GIIa and GIIb. These results were agreed by [22, 23, 28].

Group IId has non-significant change in FBG level, collagen fibers deposition, and caspase area percentage as compared with the control group and increased significance changes as compared to GIIa, GIIb, and GIIc

Conclusions:

From this study, we concluded that transplantation of AD-MSCs showed a moderate ameliorative effect on all histopathological features and FBG level in STZ diabetic rats.

Considering the efficacy of AD-MSCs there was a best synergistic effect of triple combination (MSC +res. +insulin) than dual combination (AD-MSCs +resveratrol).

Conflict of interest

None of the contributor's declared any conflict of interest

Reference:

1. Al-Lawati Ja. Diabetes Mellitus: A Local and Global Public Health Emergency! Oman Med J. 2017;32(3):177–9.
2. Luippold G, Bedenik J, Voigt A, Grempler R. Short- And Longterm Glycemic Control of Streptozotocin-Induced Diabetic Rats Using Different Insulin Preparations. Plos One. 2016;11(6):E0156346.
3. Han Y, Li X, Zhang Y, Han Y, Chang F, Ding J. Mesenchymal Stem Cells For Regenerative Medicine. Cells. 2019;8(8):886.
4. Hu C, Li L. The Application of Resveratrol to Mesenchymal Stromal Cell-Based Regenerative Medicine. Stem Cell Res Ther. 2019;10(1):307.
5. El Aasar He, Rashed L, Sadik Ae, Amer R, Emam H. The Role of the Adipose Tissue-Derived Mesenchymal Stem Cells Enriched With Melatonin In Pancreatic Cellular Regeneration. Folia Morphol. 2022;81(4):931–41.
6. Das S, Behera Jp, Rojaramani Y, Mohanty Rr. Effects of Resveratrol On Oxidative Stress In High Fat Diet /Streptozocin Induced Diabetic Wistar Albino Rats. Int J Basic Clin Pharmacol. 2019;8(3):482–7.
7. Suvarna Ks, Layton C, Bancroft Jd. Bancroft's Theory And Practice Of Histological Techniques E-Book. Elsevier Health Sciences; 2018. 573.
8. Hayat Ma. Principles and Techniques of Electron Microscopy: Biological Applications. 4th edition 45-61 (Cambridge University Press; 2000).
9. Strzalka W, Ziemienowicz A. Proliferating Cell Nuclear Antigen (Pcna): A Key Factor In Dna Replication And Cell Cycle Regulation. Ann Bot. 2011 ;107(7):1127–40.
10. Elzawahry E, Salem M, Bakry S, Rashed L, Hussein As. Therapeutic Roleof Mesenchymal Stem Cells and Vitamin D On Some Biochemical and Haematological Parameters in streptozotocin-Induced Diabetic Male Albino Rats. Al-Azhar Bull Sci. 2018;29(Issue 1-C):1–14.
11. Shawky Lm, Morsi Aa, El Bana E, Hanafy Sm. The Biological Impacts of Sitagliptin On the Pancreas of a Rat Model of Type 2 Diabetes Mellitus: Drug Interactions With Metformin. Biology. 2019;9(1):6.
12. Mchugh Ml. Multiple Comparison Analysis Testing In Anova. Biochem Medica. 2011;21(3):203–9.
13. Wan Xx, Zhang Dy, Khan Ma, Zheng Sy, Hu Xm, Zhang Q, Et Al. Stem Cell Transplantation In The Treatment Of Type 1 Diabetes Mellitus: From Insulin Replacement To Beta-Cell Replacement. Front Endocrinol. 2022; 13:859638.
14. El Mongy Nf, El- Arab Ali El-Arab S, Kamal Ramadan B, Yousef El-Saeed M. Effects Of Adipose Tissue Mesenchymal Stem Cell Therapy On Diabetic Rats. Al-Azhar Med J. 2017;46(1):193–210.
15. El-Sawah Sg, Althobaiti F, Rashwan Hm, Aldhahrani A, Abdel-Dayem Ma, Fayad E, Et

Al. Anti-Inflammatory and Antioxidant Potential Capacities of Ad-Mscs and Bm-Mscs In Suppressing Pancreatic B-Cells Auto-Immunity And Apoptosis In Rats With T1dm Induced Model. Biocell. 2022;46(3):745.

16. Yousef Hn, Sakr Sm, Sabry Sa. Mesenchymal Stem Cells Ameliorate Hyperglycemia In Type I Diabetic Developing Male Rats. Stem Cells Int. 2022 ;2022: 1-15.

17. Yousry Mm, Farag Ea. Interactive Effects of Endothelial Colony-Forming Cells with Adipose Mesenchymal Stem Cells On the Pancreas Of Type 2 Diabetic Rat Model: A Histological Study. Egypt J Histol . 2023 ;46(2):866–85.

18. Morsy Shereen M, M. Ameen A, M. Atwa M, I. Mohamed M. Role of Adipose Derived Mesenchymal Stem Cells in Improving Streptozotocin-Induced Diabetes Mellitus In Rats. Al-Azhar Med J. 2015;44(4):279–93.

19. Bhansali S, Kumar V, Saikia Un, Medhi B, Jha V, Bhansali A, Et Al. Effect of Mesenchymal Stem Cells Transplantation On Glycaemic Profile & Their Localization In Streptozotocin Induced Diabetic Wistar Rats. Indian J Med Res. 2015 ;142(1):63–71.

20. Yousry Mm, Farag Ea. Interactive Effects of Endothelial Colony-Forming Cells with Adipose Mesenchymal Stem Cells On the Pancreas Of Type 2 Diabetic Rat Model. A Histological Study. Egypt J Histol. 2022 ;*46*(2), 866–885.

21. Domouky Am, Hegab as, Al-Shahat A, Raafat N. Mesenchymal Stem Cells and Differentiated Insulin Producing Cells Are New Horizons for Pancreatic Regeneration In Type I Diabetes Mellitus. Int J Biochem Cell Biol. 2017; 87:77–85.

22. Khalil Sg, Younis Nn, Shaheen Ma, Hammad Sk, Elswefy Se. Evaluation of in Vivo and Ex Vivo Pre-Treated Bone Marrow-Derived Mesenchymal Stem Cells with Resveratrol In Streptozotocin-Induced Type 1 Diabetic Rats. J Pharm Pharmacol. 2023;75(9):1186–97.

23. Chen Ts, Kuo Ch, Day Ch, Pan Lf, Chen Rj, Chen Bc, Et Al. Resveratrol Increases Stem Cell Function In The Treatment Of Damaged Pancreas. J Cell Physiol. 2019 ;234(11):20443–52.

24. Muruganathan U, Srinivasan S, Vinothkumar V. Antidiabetogenic Efficiency of Menthol, Improves Glucose Homeostasis and Attenuates Pancreatic B-Cell Apoptosis In Streptozotocin–Nicotinamide Induced Experimental Rats Through Ameliorating Glucose Metabolic Enzymes. Biomed Pharmacother. 2017; 92:229–39.

25. Aminzadeh A, Maroof Nt, Mehrabani M, Juybari Kb, Sharifi Am. Investigating The Alterations of Oxidative Stress Status, Antioxidant Defense Mechanisms, Map Kinase and Mitochondrial Apoptotic Pathway in Adipose-Derived Mesenchymal Stem Cells From Stz Diabetic Rats. Cell J Yakhteh. 2020 ;22(Suppl 1):38.

26. Grossman Ej, Lee Dd, Tao J, Wilson Ra, Park Sy, Bell Gi, Et Al. Glycemic Control Promotes Pancreatic Beta-Cell Regeneration in Streptozotocin-Induced Diabetic Mice. Plos One. 2010 ;5(1): E8749.

27. Cheng Y, Shen J, Ren W, Hao H, Xie Z, Liu J, Et Al. Mild Hyperglycemia Triggered Islet Function Recovery in Streptozotocin-Induced Insulin-Deficient Diabetic Rats. J Diabetes Investig . 2017 ;8(1):44–55.

28. Chen Ts, Lai Pf, Kuo Ch, Day Ch, Chen Rj, Ho Tj, Et Al. Resveratrol Enhances Therapeutic Effect On Pancreatic Regeneration in Diabetes Mellitus Rats Receiving Autologous Transplantation of Adipose-Derived Stem Cells. Chin J Physiol. 2020;63(3):122–7.

29. Aali E, Mirzamohammadi S, Ghaznavi H, Madjd Z, Larijani B, Rayegan S, Et Al. A Comparative Study of Mesenchymal Stem Cell Transplantation with Its Paracrine Effect On Control Of Hyperglycemia In Type 1 Diabetic Rats. J Diabetes Metab Disord. 2014 ;13(1):76.

30. Lei Lt, Chen Jb, Zhao Yl, Yang Sp, He L. Resveratrol Attenuates Senescence of Adipose-Derived Mesenchymal Stem Cells and Restores Their Paracrine Effects On Promoting Insulin Secretion Of Ins-1 Cells Through Pim-1. Eur Rev Med Pharmacol Sci. 2016;20(6):1203–13.

31. Kadry Sm, El-Dakdoky Mh, Haggag Nz, Rashed La, Hassen Mt. Melatonin Improves the Therapeutic Role Of Mesenchymal Stem Cells In Diabetic Rats. Toxicol Mech Methods. 2018 ;28(7):529–38.

32. El-Haroun H, Salama Rm. Comparative Study On the Therapeutic Effects of Bone Marrow Mesenchymal Stem Cells Versus Platelet Rich Plasma On the Pancreas of Adult Male Albino Rats With Streptozotocin-Induced Type 1 Diabetes Mellitus. Folia Morphol. 2022;81(1):65–81.