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To cite this article: Marwa Hassen, Shadia M. Kadry, Nawal Zakaria & Mai H. El-Dakdoky (2018): Melatonin Improves the Therapeutic Role of Mesenchymal Stem Cells in Diabetic Rats, Toxicology Mechanisms and Methods, DOI: 10.1080/15376516.2018.1471634

To link to this article: https://doi.org/10.1080/15376516.2018.1471634

Accepted author version posted online: 01 May 2018.
Melatonin Improves the Therapeutic Role of Mesenchymal Stem Cells in Diabetic Rats

Shadia M. Kadry, Mai H. Eldakdoky, Nawal Z. Haggag, Laila A. Rashed and Marwa Tarek

Abstract

The present study was performed to investigate the role of bone marrow mesenchymal stem cells (MSCs) and/or melatonin (MT) for improvement of beta cell functions in STZ diabetic rats. Male albino rats (130-150g) were divided into six groups. Control group: received phosphate buffer saline (PBS); Melatonin group received melatonin (10 mg/kg b.wt./ day for 2 months by oral gavage); Diabetic untreated group; Diabetic group treated with melatonin; Diabetic group treated with mesenchymal stem cells (A single intravenous injection of 3×10⁶ cell in PBS) and Diabetic group co-treated with stem cells and melatonin.

The results showed significant improvement in glucose, insulin, total antioxidant and malondialdehyde level in diabetic rats treated with either MSCs alone or in combination with melatonin. The immunohistochemical analysis showed that MSCs and/or melatonin treatment reduced the rate of inflammation and apoptosis of the islet cells as well as increased the rate of pancreatic cell division. Such results were indicated by a significant improvement in the level of TNF-α, IL-10, PCNA, and caspase-3 to levels very close to the control. Co-treatment of MSCs and MT resulted in an improvement in the tissue of the pancreas and reduced number of damaged beta cells. It can be concluded that co-treatment of stem cells and melatonin has a significant role in restoring the structural and functional efficiency of beta cells in the pancreas more than stem cells alone. Such results may be due to the role of melatonin as an antioxidant in increasing the efficiency and vitality of stem cells.

Keywords: Mesenchymal stem cells, Melatonin, Diabetes, Male albino rats
Introduction

Diabetes mellitus (DM) is a common disease worldwide and is recognized as one of the causes leading to death (Godam et al. 2014). The number of people suffering from diabetes worldwide is increasing at an alarming rate, and it is predicated that the number of diabetic people will rise from 382 million to 592 million by 2035 (Cantarelli et al. 2015). As discussed by (Godam et al. 2014; Cantarelli et al. 2015) both type 1 and type 2 diabetes are characterized by a marked deficit in beta-cell mass causing insufficient insulin secretion though the different pathogenic events; autoimmunity for 1 and insulin resistance for 2.

Additionally, Diabetes is known to be a major disorder in which oxidative stress and free radical production have been implicated through several lines of evidence (Suzuki et al. 1999; Brownlee 2001). ROS have been defined as an autocatalytic mechanism that can lead to programed cell death (Jones BE et al. 2000).

Curative therapy for diabetes mellitus mainly implies replacement of missing insulin producing pancreatic β-cells, by pancreas or islet-cell transplants. The limited supply of available donor islets for transplantation, however determines that researchers must explore alternative sources of graft material or otherwise restore β cell functioning (Liu H et al. 2015). Hence, therapeutic strategy is highly desirable to regenerate insulin-producing cells and prevent the autoimmune destruction of further β-cells and immature β-cells which present in neogenic niche. An attractive possibility to treat diseases like diabetes could be represented by stem cell therapy. In the last years, the use of stem cells in clinical protocols is over and over increasing (Cantarelli et al. 2015).

Functionally stem cells can be defined as having the capacity to self-renew and the ability to generate differentiated cells. More explicitly, stem cells can generate daughter cells identical to their mother (self-renewal), as well as produce progeny with more restricted potential (differentiated cells). Besides, immunomodulatory potentials of mesenchymal stem cells (MSCs) have received great interest in regenerative and transplantation medicine (Joyce et al. 2010).
MSC differentiation is finely regulated by the action of mechanical and molecular signals from the extracellular environment (Cohen and Chen 2008). Emerging evidence suggests that melatonin may be an important regulator of precursor cell commitment and differentiation. Furthermore, radical scavenging, anti-inflammatory and anti-apoptotic properties of melatonin are expected to contribute significant homeostatic effects (Wang et al. 2013). Also, recent evidence obtained from investigations carried out in bone marrow MSC, indicates that melatonin may influence diabetes and associated metabolic disturbances not only by regulating insulin secretion, but also by providing protection against reactive oxygen species (Espino et al. 2011).

Therefore, the present study aimed to evaluate the role of bone marrow mesenchymal stem cells (MSCs) and/or melatonin for improvement of pancreatic beta cells in diabetic rat model, as well as to evaluate the role of melatonin in enhancing the performance of stem cells.

Materials and Methods

Chemicals

Streptozotocin and melatonin were purchased from Sigma Chemical Company (St. Louis, U.S.A).

Experimental Animals

Fifty two male albino rats (60 days-old, weighing 130:150g) were obtained from the animal house of the National Research Center, Cairo, Egypt. Rats were bred and maintained in an air conditioned animal house with specific pathogen–free conditions, and were subjected to a 12:12 h daylight/darkness cycle and allowed unlimited access to chow and water. All the ethical protocols for animal treatment were followed and supervised by the animal facilities, National Research Center. All animal experiments received approval from the animal care committee, National Research Center.

Induction of Diabetes

Diabetes was induced by a single intraperitoneal injection of STZ (Sigma Chemical Company (St. Louis, U.S.A), 45 mg/kg b.wt. dissolved immediately before administration in freshly prepared
0.1 mol/L citrate buffers (pH 4.5) (Asayama et al. 1994). Animals are provided with 5% glucose instead of water for the first 24 hours to counteract the hypoglycemia caused by insulin released from necrotic beta cells in the pancreas. One week after STZ injection, blood samples were withdrawn from lateral tail vein to measure the blood glucose concentration using blood glucose test meter (Glcocard II GT-1640). Rats having blood glucose ranging between 140-400 mg/dl were included in the experiment while the others were excluded.

**Preparation of BM-derived MSCs**

Bone marrow was harvested from 6-week-old male white albino rats by flushing the tibiae and femurs with Dulbecco’s modified Eagle’s medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine serum (GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia)] and suspended in complete culture medium supplemented with 1% penicillin–streptomycin (GIBCO/BRL). Cells were incubated at 37°C in 5% humidified CO2. When large colonies developed (80–90% confluence), cultures were washed twice with phosphate buffer saline (PBS, Lonza Company, Swiss) and the cells were trypsinized with 0.25% trypsin in 1 mM EDTA (GIBCO/BRL) for 5 min at 37°C. Cells were centrifuged and suspended in serum supplemented medium and incubated in 50 cm² culture flask (Falcon). MSCs were identified by their adherence to the plastic surface and their power to differentiate into osteocytes and chondrocytes (Johnstone et al. 1998; Seo et al. 2009) as well as by their morphology. CD29 (+ve), CD34 (-ve), CD90 (+ve) genes expressions were detected as markers of MSCs by flow cytometry. Dead cells were detected by using trypan blue dye, while the viable cells were counted using hemocytometer.

**Flow Cytometric Analysis**

Flow cytometric analyses were performed on a Fluorescence Activated Cell Sorter (FACS) flow cytometer (Coulter Epics Elite, Miami, FL, USA). MSC were trypsinized and washed twice with PBS. A total number of 1×10⁵ MSC were used for each run. The cells were incubated in 100 μl of
PBS with 3 μl for 20 min at room temperature. Antibody concentration was 0.1 mg mL\(^{-1}\). Cells were washed twice with PBS and finally diluted in 200 μl of PBS. The expression of surface marker was assessed by the mean fluorescence. The percentage of cells positive for CD 29, CD 90 was determined by subtracting the percentage of cells stained non-specifically with isotype control antibodies. CD 34 showed negative reaction (Haasters et al. 2009).

**Labeling of MSCs with PKH26**

PKH 26 fluorescent linker dye was used for MSCs labeling according to Sigma protocol (Saint Louis, Missouri USA). Briefly, cells were centrifuged and washed twice in serum free medium. Cells were pelleted and suspended in dye solution and then were injected intravenously into the tail vein (Morigi et al. 2008). After 10 days pancreatic sections were examined with a fluorescence microscope to detect homed labeled cells (Mokbel et al. 2011).

**Experimental Design**

Fifty two male albino rats were divided into six groups as follows:

1- **Control (6 rats)**: rats received phosphate buffer saline (150 mg/kg b.wt).

2- **Melatonin (MT) (6 rats)**: rats received melatonin by oral gavage (10 mg/kg b.wt. once a day for 8 weeks). MT was dissolved in 0.2% ethanol (0.2% ethanol vehicle has no influence according to (Gad et al. 2006).

3- **Diabetic (DM) (10 rats)**: maintained untreated (was regarded as diabetic control).

4- **DM+MT (10 rats)**: diabetic rats treated with melatonin in the same manner and dosage as mentioned before.

5- **DM+MSCs (10 rats)**: diabetic rats treated with a single i.v. injection (3×10\(^6\) cell) of mesenchymal stem cells.

6- **DM+MSCs +MT (10 rats)**: diabetic rats administered both stem cells and melatonin together in the same manner and dosages as mentioned before.

The mean body weights were recorded once a week during the experimental period.
After 2 months of MSCs administration, all rats were sacrificed under ether anesthesia at fasting state, and the pancreas tissue was weighted and fixed on formalin for histopathological and immunohistological investigation. Blood was withdrawn from the heart into tubes then serum samples were separated by centrifugation at 3000 rpm for 15 min. for biochemical examinations, serum levels of insulin, glucose, malondialdehyde (MDA), total antioxidant capacity (TAC) and interleukin 10 (IL10). Pancreatic tissues isolated and fixed in 10% formlin for the immune-histochemical studies, PCNA, caspase-3 and TNF-α. By the end of the experimentation, the relative and absolute weights were recorded for pancreatic tissues.

**Statistical Analysis**

Data were expressed as the mean ± standard error (SE). Statistical analysis was performed using the statistical package for social science (SPSS, Chicago, IL) version 22 statistical software. Data were analyzed by one-way analysis of variance followed by post hoc-least significant difference analysis; the level of significance was set at p < 0.05.

**Results**

**Characterization and Identification of MSCs:**

MSCs in culture were identified morphologically by their fibroblast and spindle shape and by their adhesiveness, fig.1 (A-C). Also, MSCs were identified by surface markers CD29 (+ve), CD90 (+ve) and CD34 (-ve) detected by flow cytometry, fig. (2).

Fig.1.docx

Fig.2.docx

**Homing of MSCs:**

PKH26 labeled stem cells were detected in the islets of Langerhans after 10 days of MSCs transplantation, strong homing of labeled cells was observed in pancreatic sections treated with mesenchymal stem cells and melatonin together, fig.3 (A&B).

Fig.3.docx
**Analysis**

As shown in tables (1-3) in the present study there are no significant changes between the control and (MT) groups. On the other hand, there are significant differences between control and diabetic group (DM) group.

As compared to diabetic group, the body weights and the absolute pancreatic weights recorded significant increase in (DM+MT) or (DM+MSCs) (P<0.01) with best results after companied treatment (DM+MT+MSCs) (P<0.001), table (1).

Table 1.docx

**Biochemical Analysis**

As shown in table (2) the insulin level decreased (p<0.001) with a parallel increase of glucose (p<0.001) in the diabetic rats (DM) as compared to control. After the treatment, reversed results were observed as compared to (DM) rats as follows: the treatment with mesenchymal stem cells (DM+MSCs) or melatonin (DM+MT) caused a significant increase (p<0.05) in serum insulin levels. Notably, the companied treatment with mesenchymal stem cells and melatonin (DM+MT+MSCs) raised insulin level to significant value (p<0.001). On the other hand, the glucose level decreased (p<0.001) in the serum of all diabetic rats after the treatment with mesenchymal stem cells, melatonin and mesenchymal stem cells and melatonin.

As compared to (DM) rats, the treatment with melatonin recorded a significant reduction (p<0.05) in MDA level. The companied treatment with mesenchymal stem cells and melatonin (DM+MT+MSCs) decreased the MDA level to a significant value (p<0.001). However, no significant change (p>0.05) was observed between the diabetic rats and those treated with mesenchymal stem cells, table (2).

In the current study, although TAC values increased non-significantly (p>0.05) in diabetic rats treated with either mesenchymal stem cells or melatonin in relation to the diabetic untreated ones,
it recorded a significant increase (p<0.01) after the companied treatment with mesenchymal stem cells and melatonin together.

Moreover, low level of serum anti-inflammatory cytokine IL-10 in diabetic model showed degrees of improvement after the treatments. In more details, the treatment with melatonin induced a significant increase (p<0.05) in IL-10, the treatment with mesenchymal stem cells recorded a significant increase (p<0.01). Additionally, the companied treatment with mesenchymal stem cells and melatonin also raised IL-10 to a significant value (p<0.001), table (2).

Table 2.docx

**Immunohistochemistry**

The results showed that total cell count which expressed PCNA immune reactivity in pancreatic islets decreased significantly for diabetic rats as compared to control. In contrast, PCNA positive cells increased (p<0.001) for treated rats [(DM+MT), (DM+MSCs) or (DM+MT+MSCs)] as compared to diabetic rats (DM). Notably, the expressions of PCNA immune reactivity increased (p>0.01) in the pancreatic islets of rats treated with mesenchymal stem cells accompanied by melatonin (DM+MT+MSCs) as compared to those treated with mesenchymal stem cells alone (DM+MSCs), table (3) and fig. (4).

Table 3.docx

Caspase-3 and TNF-α immune reactivity which showed significant increases (p<0.001) in pancreatic islets for the diabetic rats (DM) began to decrease gradually with significant difference after the treatment. Note the significant decrease of caspase-3 in the combined treatment as compared to the treatment with stem cells alone (p<0.001), table (3) and figs.(5&6).

Fig.4.docx   Fig.5.docx   Fig.6.docx

**Histopathological Studies**

Islet cells of diabetic animals showed darkly stained pyknotic cells and karyolysis and the islet cells contained vacuolated cytoplasm and slight congestion. Necrotic cells were encountered in
different regions. Pancreatic tissues also showed reduction in the value and number of islets of Langerhans and irregular shape, fig. 7(A & B). Melatonin treatment caused improvement of islet cells in different parts of tissue, while pyknotic nuclei, necrotic areas and less congestion were observed, fig. 7(C).

Pancreas sections of rats receiving a single intravenous injection for stem cells (3×10^6 cell) appeared with nearly regular shape and regenerated cells in numerous islets, fig. 7(D). Diabetic rats treated with both stem cells accompanied with melatonin for 2 month showed ameliorated structure of some cells of islets of Langerhans that contain nearly normal nuclei while other cells were abnormal with either pyknotic or karyolitic nuclei. Slightly vacuolated space was mostly still evident, fig. 7(E).

Fig.7.docx

Discussion

The present study revealed a marked depression in body weight of male albino rats given streptozotocin. However, MSCs and/or melatonin exhibit improvement in body weights of rats. The improvement due to MSCs may be attributed to their ability in self-renewing and differentiation into a variety of tissues (Aali et al. 2014). Meanwhile, the positive effect of melatonin upon body weight may be related to its possible role in ameliorating the oxidative damage induced by STZ in rats (Sekkin et al. 2015).

In the present study the treatment with melatonin or MSCs alone or together revealed significant increase in the blood insulin, while revealed significant decrease in blood glucose levels as compared to diabetic group; with best amelioration in insulin level in case of companied injection. These results may be attributed to melatonin exerts moderate beneficial effects on β-cells functions (Tajiri and Grill 1999). Also, (Sharma et al. 2015) noticed the increase in insulin after treatment with melatonin and attributed their results to the occurrence of melatonin receptors in the pancreatic islets proposes that their activation by melatonin might directly influence insulin or
glucagon production. Moreover, melatonin provides protection to the pancreatic β-cell against free radicals and oxidative stress caused by (STZ) (Anwer 2014). (Bhansali et al. 2015) attributed the mechanisms implicated in improvement in the β cell mass and/or function to fusion of MSCs with islet cells or trans-differentiation of MSCs into β-cells. (Yagi et al. 2010) showed that co-transplantation of electro fused MSCs and islet cell in rats improved blood glucose level due to bi-directional reprogramming of both β-cells and MSCs nuclei, thereby allowing the insulin gene expression. Moreover, it also resulted in increased islet cells proliferative capability and decreased apoptosis.

In the present study, treatment with melatonin or MSCs reduced MDA level and increased TAC level as compared to diabetic rats; the best result was obtained in companied injection of both melatonin and MSCs together (p< 0.01). This may be attributed to the fact that melatonin is an efficient scavenger of OH, peroxynitrite anion (ONOO⁻), O₂, nitric oxide radical (NO) and peroxy radicals. Moreover, it enhances the ability of cells to resist oxidative damage by inhibiting the pro-oxidant nitric oxide synthase (Reiter et al. 2000). It is considered as an important component of the antioxidant profile of many tissues and cells (Armagan et al. 2006). More to the point Melatonin has been shown to possess anti-inflammatory effects. Its ability to directly scavenge toxic free radicals reduces macromolecular damage in all organs. This contributes to the inflammatory response and associated tissue destruction (Reiter et al. 2000).

On the other hand, (Volarevic et al. 2010) reported that MSCs produce cytokines, chemokines, and growth factors that robustly regulate cell behavior in a paracrine fashion during the remodeling process, and their ability to act on profibrotic factors such as oxidative stress, hypoxia. Therefore, besides their ability to differentiate into many cell lines, there is secretion of a wide range of biological molecules by MSCs, such as growth factors, cytokines and chemokines. Macrophages are key inflammatory cells mediating inflammation in experimental and human diabetes. Activated macrophages elaborate a host of proinflammatory, profibrotic, and
antiangiogenic factors. These macrophage-derived products include but are not limited to TNF-α, IL-1, IL-6, reactive oxygen species (ROS) (Galkina and Ley 2006). Also TNF-α causes induction of apoptosis and necrotic cell death (Laster et al. 1988; Boyle et al. 2003).

Serum anti-inflammatory cytokine IL-10 in diabetic animals showed degrees of improvement after the treatments. In more details, the treatment with melatonin induced a significant increase (p<0.05) in IL-10, while the treatment with MSCs recorded a highly significant increase (p<0.01). Additionally, the companied treatment with MSCs and melatonin raised IL-10 to a very highly significant value (p<0.001). In contrast, the inflammatory cytokine, TNF-α immune reactivity which showed very highly significant increases (p<0.001) in pancreatic islets for the diabetic rats began to decrease gradually with significant changes after the treatment, and the highest reduction was recorded after the companied treatment.

The therapeutic potential is attributed to unique MSCs properties of specific homing to damaged tissues, inhibiting immune and inflammatory responses at the target sites, and facilitating repair the damaged tissues. MSCs regulate immune and inflammatory responses, providing therapeutic potential for treating diseases characterized by the presence of an inflammation, (Newman et al. 2009). According to (Aggarwal and Pittenger 2005), MSCs altered the cytokine secretion profile of dendritic cells (DCs), T-helper cells and NK cells to induce anti-inflammatory or tolerant phenotype, as a specific function. MSCs caused mature DC type 1 to decrease secretion of TNF alpha and mature DC type 2 to increase secretion of IL-10, additionally, MSCs caused an increase the proportion of present regulatory T cells. These properties of MSCs render these cells especially attractive for therapeutic application in several inflammatory diseases, as well as in regenerative medicine (Tögel et al. 2007).

In the present study, the proliferative capacity of the islets cells of Langerhans (PCNA) increased meanwhile the apoptotic activity (caspase-3) decreased in MSC and/or Melatonin treated diabetic rats, these results are in agree with (Arcolino et al. 2010; Gobbo et al. 2012) attributed the
significant increase of PCNA and decrease in caspase-3 to the ability of melatonin to attenuate the production of reactive oxygen species (ROS) and also regulating the expression of proteins of the apoptotic pathways and have been related to the anti-apoptotic property of this neurohormone, and can also corroborate the protective action of melatonin because PCNA is also involved in DNA damage repair and epigenomic maintenance.

(Bhansali et al. 2015) observed increase in islet cells proliferative capability and decreased apoptosis after treatment with MSCs and they attributed their result to the ability of MSCs to stimulate development of new islet cells using the transcription factor (Sox9). MSCs possess transdifferentiation and antiapoptotic ability (Tögel et al. 2007).

The therapeutic potential is attributed to unique MSCs properties of specific homing to damaged tissues, inhibiting immune and inflammatory responses at the target sites, and facilitating repair of the damaged tissues (Newman et al. 2009).

As in accordance with (Bhansali et al. 2015; Bhanudas and Gopal 2016) the result obtained from light microscope examination of pancreas sections indicated that the diabetic group resulted in severe damage in the pancreatic tissue.

The present study revealed slight improvement in histopathological alterations in the treatment of diabetic group with melatonin. These alterations were manifested as diffused vacuolar degeneration, pyknotic nuclei in addition to moderate congestion in islets of langerhans. Melatonin participation in pancreatic regeneration was attributed to its ability to scavenge free radicals as an antioxidant by stimulating messenger ribonucleic acid (mRNA) levels and activities of SOD and GPx (Tam et al. 2004; Godam et al. 2014).
The present results revealed noticeable improvement of the pancreatic tissues after treatment of diabetic group with mesenchymal stem cells. These results are in agreement with (Nugroho et al. 2016) who reported that the structure of pancreatic tissues recovered in 1 week after MSCs treatment. Recent results obtained by (Sato et al. 2005) indicated that the sections from pancreas after treatment with MSCs on day 28, showing apparently normal structure of both the pancreatic acini and the islets of Langerhans with their rounded and vesicular nuclei, such results confirm the present reported findings.

In the same concept, (Bhansali et al. 2015) postulated that treatment with MSCs in diabetic rats resulted in reorganization of islet and partial restoration of β-cell as indicated by high insulin reactivity compared with the diabetic group.

The present results also revealed that co-treatment of stem cells and melatonin was more effective than stem cell alone in modulating the biochemical alterations and histological abnormalities in the pancreatic tissue induced by diabetes. Such positive effect of melatonin may be attributed to that melatonin has strong antioxidant and protective properties due to its ability to scavenge free radicals, so it may exert its activity on stem cells so increase their vitality and their proliferation capacity.

This conclusion is in accordance with (Wang et al. 2013) who reported that oxidative stress may has a negative impact on the survival of transplanted MSCs, and it has been suggested that melatonin may be an effectively protective agent against oxidative stress-induced MSCs apoptosis. Previous studies have shown that melatonin treatment improves MSCs cell therapy for various diseases. (Liu X et al. 2013) showed that melatonin treatment improves the mobility of umbilical cord blood-derived MSCs to improve skin wound healing. (Yip et al. 2013) reported that melatonin treatment improves adipose-derived MSCs therapy for acute lung ischemia-reperfusion injury. (Farrell et al. 2014) showed that melatonin improved the survival of MSCs against
oxidative stress. Another study showed that melatonin could exert its anti-oxidant function on stem cells via its receptors (Jones DL and Wagers 2008).

Finally it can be concluded that, mesenchymal stem cell therapy has a significant role in restoring the structural and functional efficacy of beta cells in the pancreas. The results showed also that melatonin boost the role of mesenchymal stem cells in the improvement of MDA level, PCNA and caspase-3 immune-reactivates when compared to treatment of mesenchymal stem cells alone. Such result may be due to the role of melatonin as an antioxidant in increasing efficiency and vitality of stem cells.

Acknowledgments

None.

Disclosure Statement

The authors report no conflict of interest.

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40. Volarevic V, Al-Qahtani A, Arsenijevic N, Pajovic S, Lukic ML. 2010. Interleukin-1 receptor antagonist (IL-1Ra) and IL-1Ra producing mesenchymal stem cells as modulators of diabetogenesis. Autoimmunity. 43(4):255-263.


Figure 1: Rat bone marrow mesenchymal stem cell in culture. MSCs at 3 days appeared rounded in shape (A), at 7 days MSCs appeared as fibroblast and spindle shaped cells (B). MSCs at 10 days (C) MSCs reached 80–90% confluence; they were identified by their fusiform fibroblast like-structure. X200.

Figure 2: Flow cytometric characterization analyses of rat bone marrow–mesenchymal stem cells. Cells were positive for CD29 and CD90 but negative for CD34.
Figure 3: PKH26 labeled stem cells in the islets of Langerhans showing stronger red fluorescence after treatment with both MSCs and melatonin (B) than after MSCs alone (A).

Figure 4: Pancreatic section of diabetic rat showing few PCNA immune reactive cells of islets of Langerhans; (B): Pancreatic section of diabetic rats treated with melatonin showing few PCNA immune reactive cells of islets of Langerhans; (C): Pancreatic section of diabetic rats treated with MSCs revealed more expressions of PCNA in islets cells (D): Pancreatic section of diabetic rats treated with MSCs and melatonin showed a widespread of PCNA positive immune reactives in islet cells (arrows, X400).
Figure 5: (A): Pancreatic tissues of diabetic rats showing wide spread of positive immune reactives for caspase-3 in islets of Langerhans (brown color); (B): Pancreatic sections of diabetic rats treated with melatonin showing more positive immune reactives for caspase -3 in Langerhans islet cells; (C): Pancreatic section of diabetic rats treated with MSCs showing low expressions for caspase -3 in islet cells and (D): Pancreatic tissue of diabetic rats treated with MSCs and melatonin showing low expressions of caspase-3 reactivity in islet cells. X400.
Figure 6: (A): Pancreatic section of diabetic rats showing high intensities of TNF-α expression in islet cells, brown color; (B): Pancreatic section of rats treated with melatonin showing high expression of TNF-α reactivates in islet cells, (brown color). (C): Pancreatic sections of rats treated with MSCs showing moderate expression of TNF-α reactivates in islet cells. (D): Pancreatic section of rats treated with MSCs and melatonin showing low expression of TNF-α reactivity in islet cells. X400.
Figure 7: (A): diabetic pancreas showed distortion of the general pattern of islets, deeply stained pyknotic nuclei (thin arrow), karyolysis of some nuclei (arrow head), patches of necrotic areas (star) and vacuolated cytoplasm in the islet cells (thick arrow); (B): diabetic pancreas showed abnormal elongated islet with irregular margins, a reduction in the number of cells and diffused vacuolar degeneration (arrow); (C): Pancreatic tissue of diabetic rat treated with melatonin, slight improvement while still showing pyknotic nuclei (arrow) diffused vacuolar degeneration and decreased number of islet cells and (D): Pancreatic tissue of diabetic rat treated with MSCs showing amelioration of islet architecture appearing nearly with regular shape and regeneration of some cells. (H&E. X400).
Table 1- The effect of mesenchymal stem cells and/or melatonin on the body weight and pancreatic weights (absolute &relative) in induced diabetes mellitus rat models.

<table>
<thead>
<tr>
<th>groups parameters</th>
<th>Control</th>
<th>MT</th>
<th>DM</th>
<th>DM+MT</th>
<th>DM+MSCs</th>
<th>DM+MSCs +MT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain (g)</td>
<td>214.63 ±8.80</td>
<td>198.79±8.42</td>
<td>143.18 ±1.77</td>
<td>197.2 ±1.98</td>
<td>199.11 ±1.19</td>
<td>201.77 ±21.55</td>
</tr>
<tr>
<td>Absolute weight of pancreas (g)</td>
<td>0.58 ±0.06</td>
<td>0.74 ±0.08</td>
<td>0.11 ±0.07</td>
<td>0.50 ±0.01</td>
<td>0.63 ±0.13</td>
<td>0.66 ±0.03</td>
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<tr>
<td>Relative weight of pancreas</td>
<td>0.28 ±0.04</td>
<td>0.41 ±0.02</td>
<td>0.06 ±0.03</td>
<td>0.29 ±0.02</td>
<td>0.31 ±0.04</td>
<td>0.30 ±0.02</td>
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</tbody>
</table>

(MT): Melatonin group; (DM): Diabetic group; (DM+MT): Diabetic group treated with MT; (DM+MSCs): Diabetic group treated with mesenchymal stem cells and (DM+MSCs +MT): Diabetic group treated with MSCs and MT. a: Statistically significant compared to corresponding value in control (control and MT groups). b: Statistically significant compared to corresponding value in (DM) group. 1: (p<0.05), 2: (p< 0.01) & 3: (p<0.001).

Table 2- The effect of mesenchymal stem cells and/or melatonin on some serum analysis in induced diabetes mellitus rat models.

<table>
<thead>
<tr>
<th>groups parameters</th>
<th>Control</th>
<th>MT</th>
<th>DM</th>
<th>DM+MT</th>
<th>DM+MSCs</th>
<th>DM+MSCs +MT</th>
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</thead>
<tbody>
<tr>
<td>Insulin (ng/ml)</td>
<td>2.61 ±0.48</td>
<td>3.03 ±0.21</td>
<td>0.75 ±0.24</td>
<td>1.70 ±0.26</td>
<td>1.73 ±0.32</td>
<td>2.11 ±0.26</td>
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<tr>
<td>Glucose (mg/dl)</td>
<td>102.77 ±7.60</td>
<td>97.70 ±7.18</td>
<td>284.23±23.51</td>
<td>167.27 ±21.13</td>
<td>160.93 ±19.96</td>
<td>149.70±4.71</td>
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<td>MDA (m. mol/l)</td>
<td>0.08 ±0.04</td>
<td>0.13 ±0.10</td>
<td>0.51 ±0.02</td>
<td>0.25 ±0.26</td>
<td>0.43 ±0.14</td>
<td>0.06 ±0.05</td>
</tr>
<tr>
<td>TAC (m.mol/l)</td>
<td>44.17 ±5.25</td>
<td>47.10 ±4.78</td>
<td>11.93±1.42</td>
<td>21.30 ±4.77</td>
<td>24.37 ±4.07</td>
<td>32.03 ±4.42</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>179.40 ±28.57</td>
<td>205.37 ±11.55</td>
<td>73.47±17.10</td>
<td>125.17 ±5.15</td>
<td>159.80 ±31.01</td>
<td>188.37±13.99</td>
</tr>
</tbody>
</table>

(MT): Melatonin group; (DM): Diabetic group; (DM+MT): Diabetic group treated with MT; (DM+MSCs): Diabetic group treated with mesenchymal stem cells and (DM+MSCs +MT): Diabetic group treated with MSCs and MT. a: Statistically significant compared to corresponding value in control (control and MT groups). b: Statistically significant compared to corresponding value in (DM) group. c: Statistically significant compared to corresponding value in DM+MSCs. 1: (p<0.05), 2: (p< 0.01) & 3: (p<0.001).
Table 3- The effect of mesenchymal stem cells or/and melatonin on the expressions of PCNA, caspas-3 and TNF-α immune reactivity of pancreatic islets of STZ induced diabetic rats.

<table>
<thead>
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<th>DM+MSCs</th>
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(MT): Melatonin group; (DM): Diabetic group; (DM+MT): Diabetic group treated with MT; (DM+MSCs): Diabetic group treated with mesenchymal stem cells and (DM+MSCs +MT): Diabetic group treated with MSCs and MT. a: Statistically significant compared to corresponding value in control (control and MT groups). b: Statistically significant compared to corresponding value in [DM] group. c: Statistically significant compared to corresponding value in DM+MSCs. 1: (p<0.05), 2: (p< 0.01) & 3: (p<0.001).