The potential role of melatonin on bone marrow mesenchymal stem cells therapy in pancreatic tissue of streptozotocin-induced diabetic rats.

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Abstract

The present study was established to investigate the role of bone marrow mesenchymal stem cells (MSCs) and melatonin (MT), alone or in combination with each other for improvement of beta cell functions in STZ-induced diabetic rat model. Also, to evaluate the role of melatonin in increasing the efficacy of MSCs. Fifty two male albino rats (130-150g) were divided into six groups. Control: received phosphate buffer saline PBS; Melatonin group received melatonin (10 mg/kg b.wt. day for 2 months by oral intubation); Diabetic untreated group; Diabetic group treated with melatonin; Diabetic group treated with mesenchymal stem cells (MSCs) (A single intravenous injection of 3×10^6 cell in PBS)and Diabetic group treated with both stem cells and melatonin. Diabetes was induced by a single i.p. injection of 45mg/kg b.wt. of streptozotocin. The results showed that treatment of diabetic rats with either MSCs alone or in combination with melatonin resulted in an increase in body weight and pancreas weight. The biochemical analysis showed significant improvement in glucose, insulin, total antioxidant and malondialdehyde level in diabetic rats treated with either MSCs alone or in combination with melatonin. On the other hand, the results of imumuno-histochemical analysis showed that MSCs and/or melatonin treatment reduced the rate of inflammation and apoptosis of the Islet cells of pancreases 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. The microscopic examination of Langerhans islands showed that treatment with MSCs either alone or in combination with melatonin resulted in an improvement in the tissue of the pancreas and reduced number of damaged beta cells. It can be concluded that stem cell therapy has a significant role in restoring the structural and functional efficiency of beta cells in the pancreas. The results also showed that co- treatment of stem cells and melatonin was more effective than stem cell alone. Such result may be due to the role of melatonin as an antioxidant in increasing the efficiency and vitality of stem cells.

Keywords: Diabetic rats; Inflammatory markers; Melatonin; Mesenchymal stem cells; Oxidative stress; Pancreas.

Introdution

Diabetes mellitus (DM) is a common disease worldwide and is recognized as one of the causes leading to death (**Godam *et al.,* 2015**). 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 **Meier *et al.* (2006)** and **Cantarelli *et al.* (2015**), both type 1 and type 2 diabetes is characterized by a marked deficit in beta-cell mass causing insufficient insulin secretion regarded of 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**

and Brownlee, 2001). ROS have been defined as an autocatalytic mechanism that can lead to programed cell death **(Jones *et al.,* 2000).**

Curative therapy for diabetes mellitus mainly implies replacement of missing insulin-producing pancreatic β-cells, with 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 (**Urbán *et al.,* 2009 and Liu *et al.,* 2015).** Hence, a therapeutic strategy aimed to regenerate insulin-producing cells and prevent the autoimmune destruction of remnant and neogenetic β-cells are highly desirable. 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 **(Rayan *et al.,* 2005).**

MSC differentiation is finely regulated by the action of mechanical and molecular signals from the extracellular environment. 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 on 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 **(Javier *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

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

2- 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 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.

3-Induction of diabetes:-

The animal model of diabetes was induced by a single intraperitoneal injection of STZ (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**). These 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.

4- Preparation of bone marrow BM-derived MSC:-

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) and 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 CO_2. 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 cm^2 culture flask (Falcon). MSCs were identified by their adherence to the plastic surface and their power to differentiate into osteocytes and chondrocytes (**Seo *et al.,* 2009 and Johnston *et al.,* 1998**) 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 cytometry

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 1×10^5 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 one of tail veins (**Marina *et al.,* 2008).** After 10 days pancreatic sections were examined with a fluorescence microscope to detect homed labeled cells (**Mokbel *et al.,* 2011).**

Fifty two male albino rats were divided into 6 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., daily for 2 months).

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 intravenous (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 month of MSCs administration, all rats were sacrificed under ether anthesia at fasting state, and the pancreas was weighted and fixed in 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 (IL-10)). By the end of the experimentation, the relative and absolute weights were recorded for pancreatic tissues.

Results

Characterization and identification of MSCs:

MSCs in culture were identified morphologically by their fusiform shape and their adhesiveness. Fig.1 (A-C). Also, MSCs were identified by surface markers (CD29 (+ve), CD90 (+ve) and CD34 (-ve)) and detected by flow cytometry. Fig. (2).
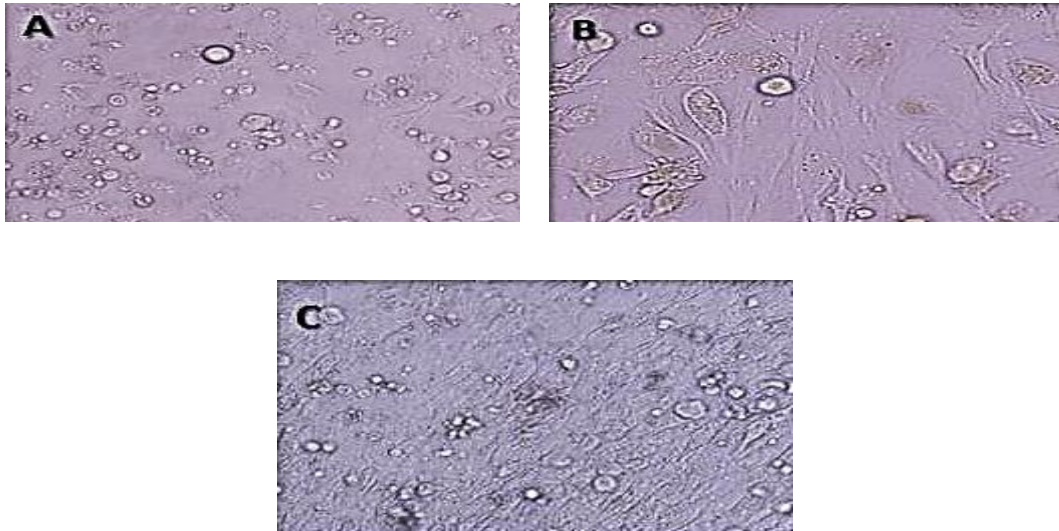
Fig.(1): Rat bone marrow mesenchymal stem cell in culture. MSCs at 3days appeared rounded in shape (A), at 7days MSCs appeared as fibroblast and spindle shaped cells (B). MSCs at 10days (C) MSCs reached 80–90% confluence, they were identified by their fusiform fibroblast like-structure.X200
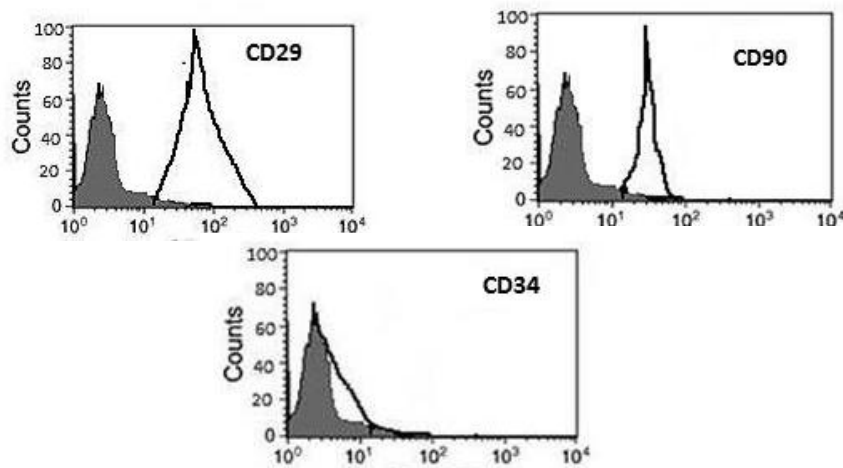

Fig.(2). Flow cytometric characterization analyses of rat bone marrow–mesenchymal stem cells. Cells were positive for CD29 and CD90 but negative for CD34.

Homing of MSCs:

PKH26 labeled stem cells were detected in in the islets of Langerhans after 10 days of MSCs transplantation, strong homing of labeled cells was observed in pancreatic sections treated with mesechymal stem cells and melatonin together.Fig.3 (A&B).
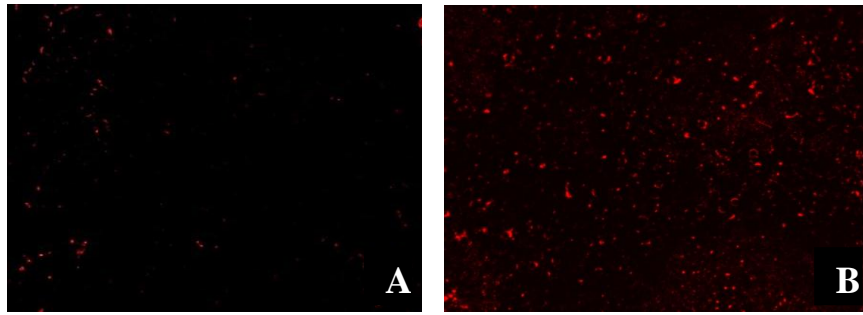
Fig.(3): PKH26 labeled stem cells in the islets of Langerhans showing stronger red flourescence after treatment with both MSCs and melatonin (B) than after MSCs alone (A).

Biochemical analysis:

In the present study there are no significant changes between the control and MT groups. On the other hand, there are statistical changes between control and diabetic model (MD group).

As compared to MD rats, the body weights and the absolute pancreatic weights recorded statistical increase in DM+MT or DM+MSCs, with best results in companied injection (DM+MT+MSCs). Fig.(4).
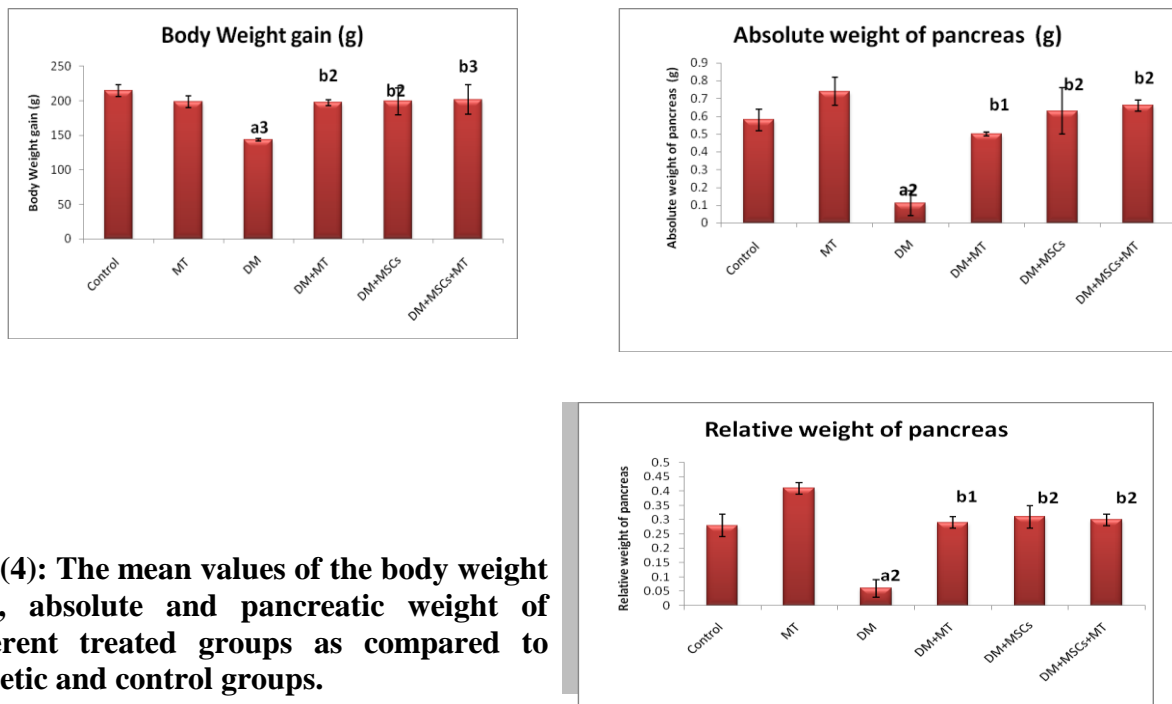
Fig. (4): The mean values of the body weight gain, absolute and pancreatic weight of different treated groups as compared to diabetic and control groups.

a: Statistically significant compared to corresponding value in control (control and melatonin (MT) groups). b: Statistically significant compared to corresponding value in DM group. 1: ($p<0.05$), 2: ($p< 0.01$) & 3: ($p<0.001$).

As shown in fig.(5), the insulin level decreased ($p<0.001$) with a parallel increase of glucose level ($p<0.001$) in the diabetic rats (DM) as compared to control. After the treatment, reversed parallel 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 a highly significant value ($p<0.001$). On the other hand, an important observation was that, the glucose level decreased very highly significantly 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. As more important result, the companied treatment with mesenchymal stem cells and melatonin (DM+MT+MSCs) decreased the MDA level to a highly significant value ($p<0.001$). However, no significant change was observed between the diabetic rats and those treated with mesenchymal stem cells. fig.(6).

In the current study, although TAC values increased non-significantly in diabetic rats treated with either mesenchymal stem cells or melatonin in relation to the diabetic untreated ones, it recorded a highly 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, while the treatment with mesenchymal stem cells recorded a highly significant increase ($p<0.01$). Additionally, the companied treatment with mesenchymal stem cells and melatonin raised IL-10 to a very highly significant value ($p<0.001$). fig.(6).
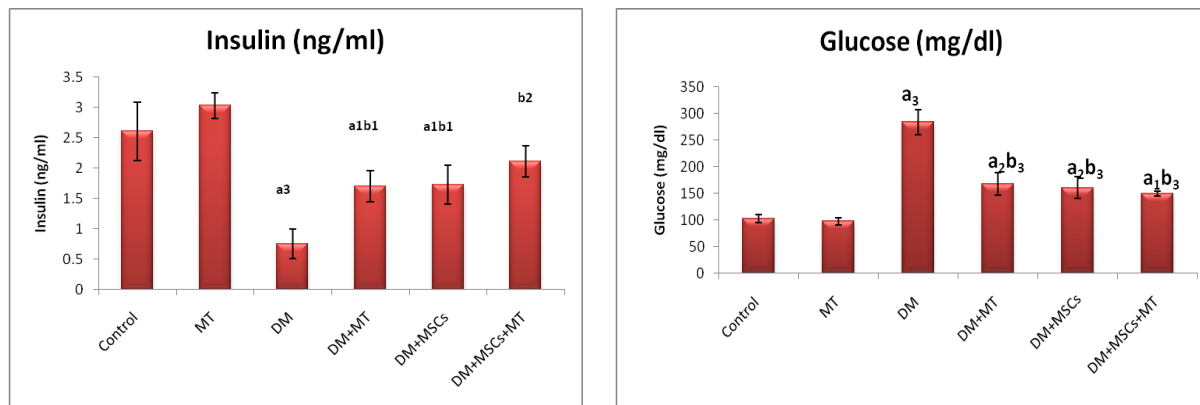

Fig. (5): The mean values of serum insulin and glucose of different treated groups as compared to diabetic and control groups.
a: Statistically significant compared to corresponding value in control (control and melatonin (MT) groups). b: Statistically significant compared to corresponding value in DM group.
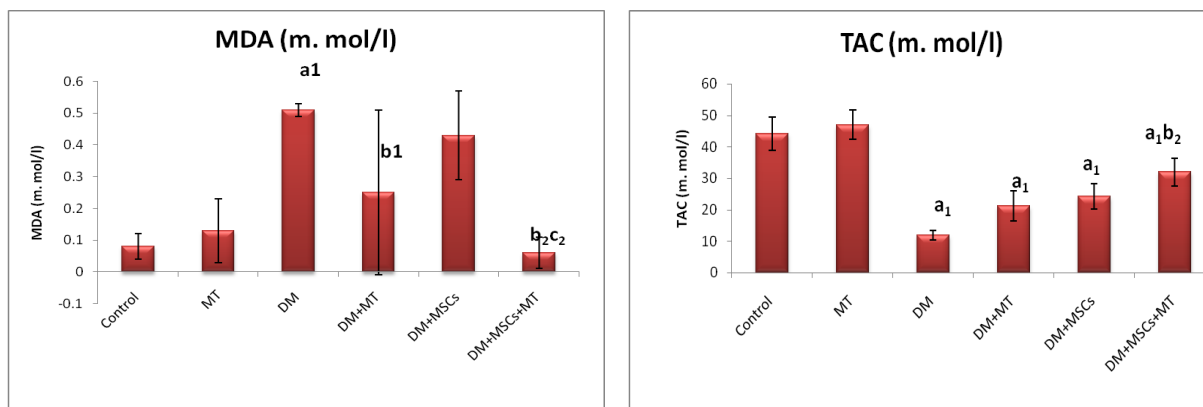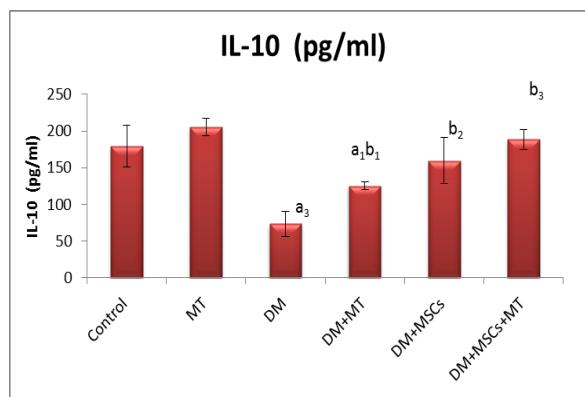*1: ($p<0.05$), 2: ($p<0.01$) & 3: ($p<0.001$).*

Fig. (6): The mean values of serum MDA, TAC and IL-10 of different treated groups as compared to diabetic and control groups.

a: Statistically significant compared to corresponding value in control (control and melatonin (MT) groups). b: Statistically significant compared to corresponding value in DM group. 1: (p<0.05), 2: (p< 0.01) & 3: (p<0.001).

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) (Figs.7 & 8).

Caspase-3 and TNF-α immune reactivity which showed very highly significant increases (p<0.001) in pancreatic islets for the diabetic rats (DM) began to decrease gradually with statistical changes after the treatment, and the most observed reduction was recorded after the accompanied treatment (DM+MT+MSCs). Note the statistical decrease of caspase-3 in the accompanied treatment as compared to the treatment with stem cells alone (p<0.001) (Figs.7, 9 & 10).
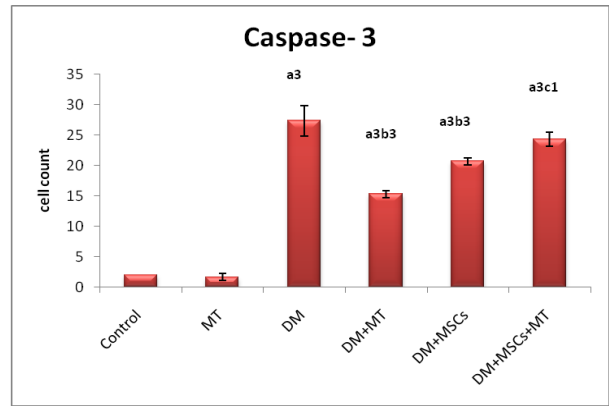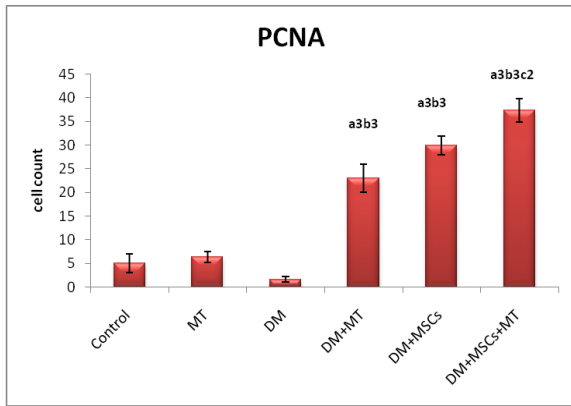
Fig. (7): The mean counts of positive cells in immunostained pancreatic islands of different treated rats as compared to diabetic and controls.

a:Statistically significant compared to corresponding value in control (control and melatonin (MT)groups). b:Statistically significant compared to corresponding value in DM group. 2: (p< 0.01), 3: (p<0.001).
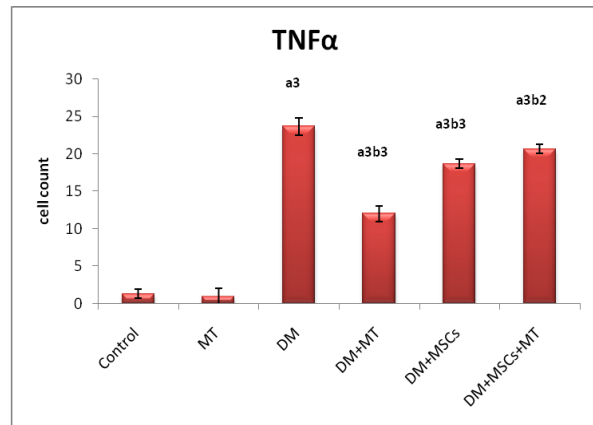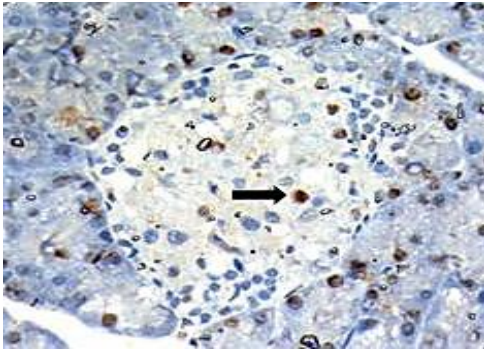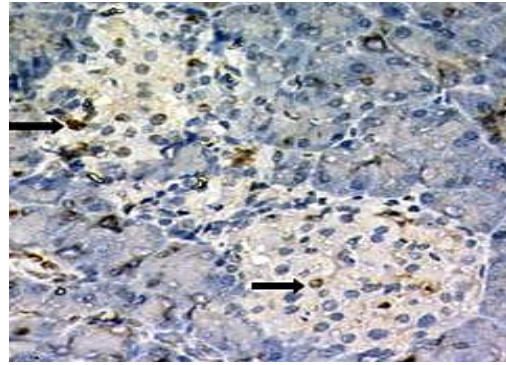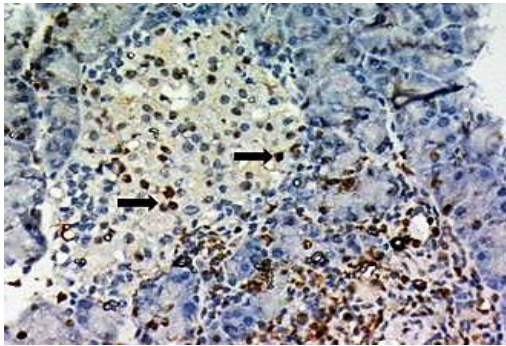
Fig.8 (A-D): Immuno-stained pancreatic sections of different treated rats for PCNA. (x400).
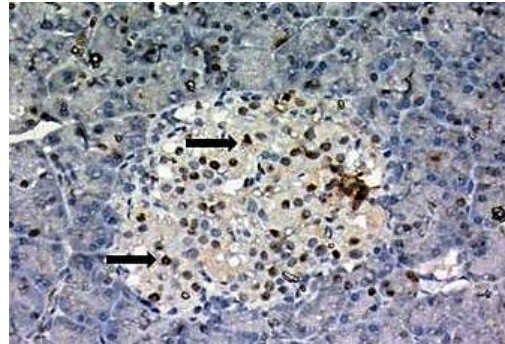

(A): Pancreatic section of diabetic rat showing few PCNA immune reactive cells of islets of Langerhans (arrow).

(B): Pancreatic section of diabetic rats treated with melatonin showing few PCNA immune reactive cells of islets of Langerhans (arrows).
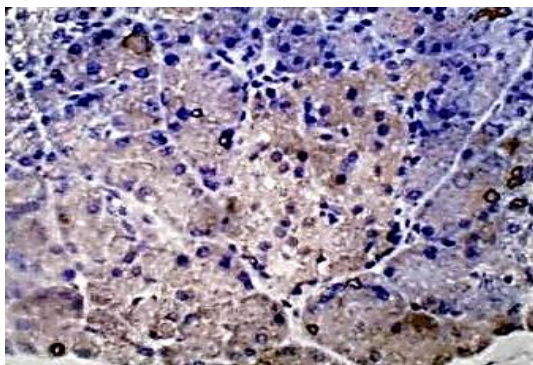

(C): Pancreatic section of diabetic rats treated with MSCs revealed more expressions of PCNA cells of islets of Langerhans (arrows).
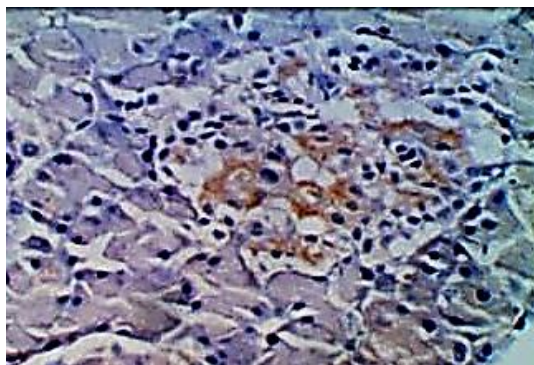

(D): Pancreatic section of diabetic rats treated with MSCs and melatonin showed a widespread of PCNA positive immune reactivates in islet cells (arrows).
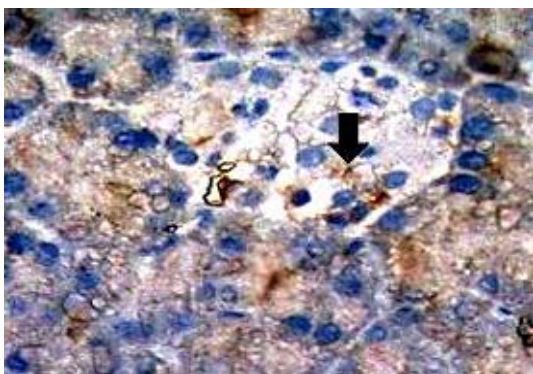
Fig.9 (A-D): Immunostained pancreatic sections of different treated rats for caspase-3. (x400).
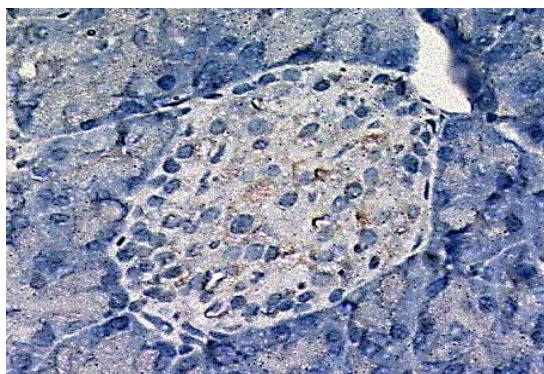

(A): Pancreatic sections of diabetic rats showing wide spread of positive immune reactivates for caspase-3 in islets of Langerhans (brown color).

(B): Pancreatic sections of diabetic rats treated with melatonin showing more positive immune reactivates for caspase -3 in Langerhans islet cells, brown color.

(C): Pancreatic section of diabetic rats treated with MSCs showing low expressions for caspase -3 in islet cells (arrow).

(D): Pancreatic tissue of diabetic rats treated with MSCs and melatonin showing lower expressions of caspase-3 reactivity in islet cells (arrows), (x400).

Fig.10 (A-D): Immunostained pancreatic sections of different treated rats for TNF-α. (x400).
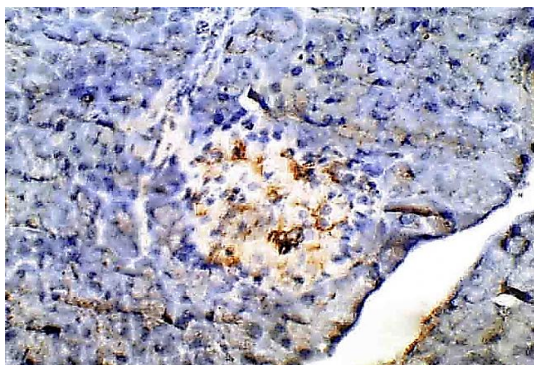
(A): Pancreatic section of diabetic rats showing more intensities of TNF-α expression in islet cells, brown color.
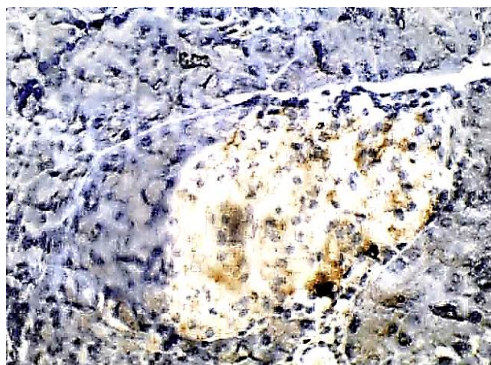
(B): Pancreatic section of rats treated with melatonin showing more expressions of TNF-α reactivates in islet cells, (brown color).

(C): Pancreatic sections of rats treated with MSCs showing moderate expressions of TNF-α reactivates in islet cells, brown colour.

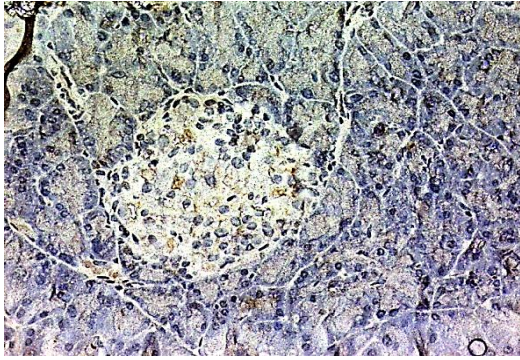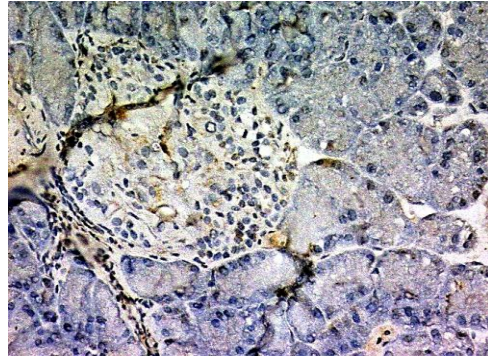**(B):** Pancreatic section of rats treated with MSCs and melatonin showing low expression of TNF-reactivity in islet cells brown colour.

Histopathological studies:

Diabetic Langerhans cells 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. 11(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. 11(C).

Pancreatic sections of rats receiving a single intravenous injection of stem cells (3×106 cell) appeared with nearly regular shape and regenerated cells in numerous islets. Fig. 11(D). Diabetic rats treated with both stem cells accompanied with melatonin for 2 month showed amelioration 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. 11(E).

Fig.11 (A-E): Pancreatic sections of different treated rats stained by H&E. (x400).
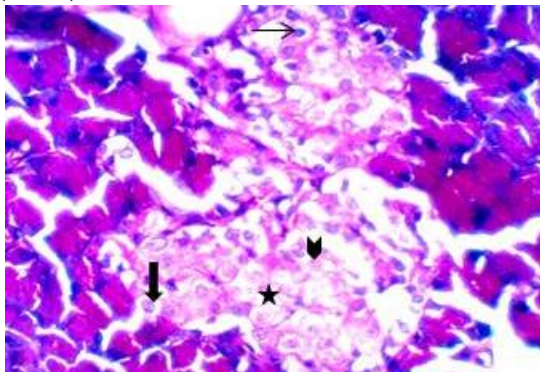
(A): Diabetic pancreas showed distortion of the general pattern of islets, deeply stained pyknotic nuclei (thin arrow), karyolysis of some nuclei (arrow head), patches of necrotic areas (star) and vacuolated cytoplasm in the islet cells
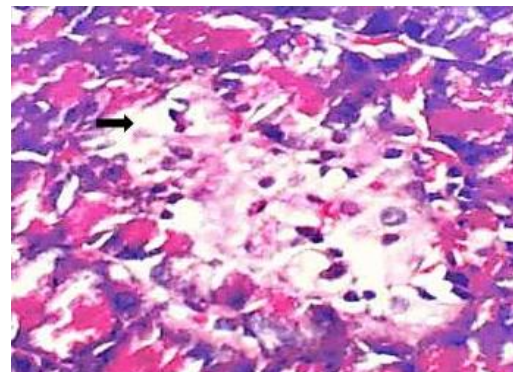
(B): Diabetic pancreas showed abnormal elongated islet with irregular margins, a reduction in the number of cells and diffused vacuolar degeneration (arrow).

(thick arrow).
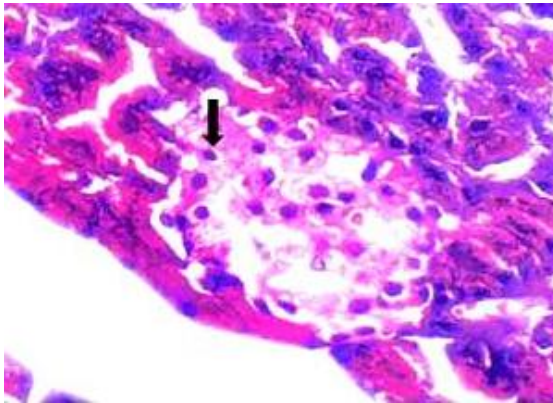
(C): Pancreatic tissue of diabetic rat treated with melatonin showing slight improvement with few pyknotic nuclei (arrow) diffused vacuolar degeneration and decreased number of islet cells.
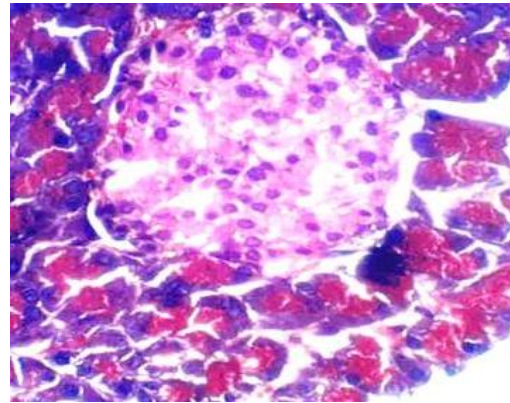
(D): Pancreatic tissue of diabetic rat treated with MSCs showing amelioration of islet architecture appearing nearly with regular shape and regeneration of some cells.

(E): Pancreatic tissue of diabetic rats treated with MSCs and melatonin showing restoration of some Langerhans islets appearing with normal architecture, increased number of more organized and less vacuolated islet cells.
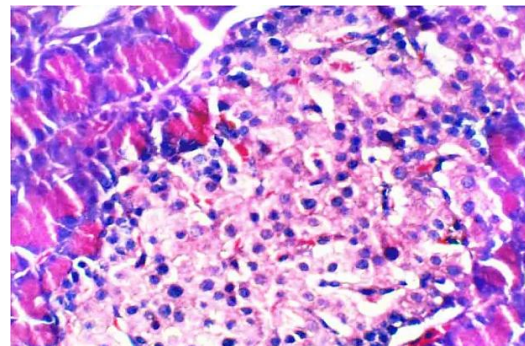
Discussion

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 (**Alai *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 statistical increase in the blood insulin, while revealed statistical decrease in blood glucose levels

as compared to diabetic model; with best observation in insulin level in case of combination injection. These results may be attributed to the moderate beneficial effects on β-cells functions **(Tajiri and Grill, 1999).** Also, **Sharma *et al.* (2015)** noticed the increasing 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.* (2013) 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.* (2012)** 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 work, treatment with melatonin or MSCs reduced MDA level and increased TAC level as compared to diabetic rats; the best result was in companied injection with both melatonin and MSCs together (p< 0.01). This may be attributed to 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.,* 1998**). It considered as an important component of the antioxidant profile of many tissues and cells (**Armagan *et al.,* 2006**). On the other hand, **Volarevic *et al.* (2016**) 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 *et al*., 2006**). Also TNF-α causes induction of apoptosis and necrotic cell death (**Laster *et al*., 1988 and Boyle *et al*., 2003).**

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, 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 statistical changes after the treatment, and the most observed reduction was recorded after the companied treatment.

Melatonin has been shown to possess anti-inflammatory effects. Its ability to directly scavenge toxic free radicals reduces macromolecular damage in all organs that contribute to the inflammatory response and associated tissue destruction **(Retier *et al.*, 2000).**

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. 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 disease, as well as in regenerative medicine **(Togel *et al.,* 2007).**

In the present study, the proliferative capacity of the islets cells of Langerhans (PCNA) increased meanwhile the apoptotic activity (caspace-3) decreased in MSC and/or Melatonin treated diabetic rats. These results are in agreement with **Arcolino *et al,* (2010), Gobbo *et al.* (2012) and Gobbo *et al.* (2013). Gobbo *et al.* (2015)** attributed the significant increase of PCNA and decrease in caspace-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.* (2013) 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 **(Togel *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 **Bhanduas and Gopal, (2016) and Kumar *et al.* (2016)** and **walvecar *et al.* (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 **(Maisaa and AL-Rawi, 2007; Vijayanand and Wesely, 2011; Tams, 2014 and Godam *et al.*, 2014)**

The present results indicated noticeable improvement of the pancreatic tissues after treatment of diabetic group with mescenchymal stem cells. These results are in agreement with **Nugroho *et al.* (2016)** who reported that the structure of pancreatic tissues recovered in one week after MSCs treatment. Recent results obtained by **Sato *et al.* (2016)** indicated that the sections from the pancreas after treatment with MSCs on day 28, showed 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.* (2013)** postulated that treatment with MSCs in diabetic rats resulted in reorganization of islets and partial restoration of β-cells as indicated by high insulin reactivity compared with the diabetic group.

Moreover, presented results showed increases in the body weight gain, insulin level, TAC, IL-10 and PCNA of diabetic rats treated with both melatonin and MSCs in relation to diabetic ones, while recorded decreases in glucose level, MDA, TNF-α and caspace-3 and detected highly improvement in histological pancreatic tissues.

More supporting result is that MSCs strongly homed in pancreatic tissues treated with MSCs and melatonin together than those treated with MSCs alone. These better results may be attributed to–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 **(Wang *et al.*, 2013)**.

This is in accordance with the work of **Farrell *et al.* (2014)** who showed that melatonin improved the survival of MSCs against oxidative stress. Another study showed that melatonin could exerts its anti-oxidant function on stem cells via its receptors **(Jones and Wagers, 2008)**.

In is worth mentioning that previous studies have shown that melatonin treatment improves MSCs cell therapy for various diseases. **Lee *et al.* (2014)** showed that melatonin treatment improves the mobility of umbilical cord blood-derived MSCs to improve skin wound healing **(Liu, *et al.*, 2013)**. **Lee *et al.* (2014)** reported that melatonin treatment improves adipose-derived MSCs therapy for acute lung ischemia-reperfusion injury.

In conclusion, the present results indicated that melatonin may improve the mobility and therapeutic role of MSCs in diabetic rats.

الدور المحتمل للميلاتونين على الخلايا الجذعية الميزنشيمية في علاج أنسجه البنكرياس في ذكور الجرذان المصابه بمرض السكري المستحث بواسطة ستريبتوزوتوسين.

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الملخص العربى

تهدف هذه الدراسة إلى تعيين تقييم دور الخلايا الجذعية والميلاتونين كل على حدة او كلاهما معا فى إستعادة الكفاءة

التركيبية والوظيفية لخلايا بيتا البنكرياسية فى الجرذان المصابة بمرض السكرى المحدث بواسطة استربتوزوتوسين وايضا

دور الميلاتونين فى تحفيز الخلايا الجذعية وزيادة فاعليتها.

فى هذه الدراسة تم إستخدام 52 من ذكور الجرذان وتم تقسيمهم الى 6 مجموعات كما يلى: المجموعة الاولى

(الضابطة): تم معاملتها بمحلول الفوسفات المتعادل عن طريق الحقن فى التجويف البريتونى مرة واحدة.المجموعة الثانية: تم

معاملتها بالميلاتونين (10ملجم/كجم/يوم) عن طريق الانبوبة المعدية لمدة شهرين.المجموعة الثالثة: مصابة بمرض السكرى وتركت دون معالجة طوال فترة التجربة . المجموعة الرابعة: مصابة بالسكرى وتم معالجتها بنفس الجرعة السابقة من الميلاتونين. المجموعة الخامسة: مصابة بالسكرى وتم معالجتها بالخلايا الجذعية (جرعة واحدة 10X3^6 خلية) عن طريق الحقن الوريدى فى الذيل. المجموعة السادسة: مصابة بالسكرى وتم معالجتها بكلا من الميلاتونين والخلايا الجذعية بنفس الجرعة وطريقة الحقن كما سبق.ولإحداث مرض السكرى تم معاملة الجرذان بجرعة واحدة من مادة استربتوزوتوسين(45 ملجم/كجم من وزن الجسم) عن طريق الحقن داخل التجويف البريتونى.

دلت النتائج على أن معالجة الجرذان المصابة بالسكرى بالخلايا الجذعية سواء منفردة او معها الميلاتونين أدت إلى حدوث زيادة فى وزن الجسم ووزن البنكرياس وحدوث تحسن ملحوظ فى محتوى كلاً من الجلوكوز والانسولين فى الدم ومضادات الاكسدة كما أدت إلى حدوث نقص فى محتوى المالوندايالدهيد فى السيرم. أيضاً أوضحت النتائج بإستخدام كيمياء الأنسجة المناعية إلى أن المعالجة بالخلايا الجذعية سواء منفردة او معها الميلاتونين أدت إلى تقليل معدل الإلتهابات والموت المبرمج للخلايا يقابله زيادة فى معدل إنقسامات خلايا البنكرياس ويرجع ذلك إلى تحسن مستوى كل من TNF-α و IL-10وPCNAوcaspase-3إلى مستويات قريبة من المجموعة الضابطة. أيضاً أظهر الفحص المجهرى لأنسجة البنكرياس و جزر لانجرهانز إلى أن المعاملة بكل من الميلاتونين والخلايا الجذعية إما منفردين أو مجتمعين معا أدتا إلى حدوث تحسن فى نسيج البنكرياس وقلة عدد خلايا بيتا التالفة.

أثبتت الدراسة أن المعاملة بالخلايا الجذعية لها دور ملحوظ فى إستعادة الكفاءة التركيبية والوظيفية لخلايا بيتا فى جزر البنكرياس. كما أثبتت النتائج أن المعالجة بالخلايا الجذعية مصحوبة بالمعالجة بالميلاتونين كانت اكثر تأثيراً وكفاءة من المعالجة بالخلايا الجذعية منفردة وقد يرجع ذلك الى دور الميلاتونين كمضاد للاكسدة فى زيادة كفاءة وفاعلية الخلايا الجذعية فى العلاج.

References

Aggarwal, S. and Pittenger, M. (2005): human mesenchymal stem cells modulate allogenic immune cell responses. *Blood, 105: 1815-1822.*

Alai, E.; Mir Zamohammadi, S.; Ghazanvi, Hi; Madjd, Z,; Larijani, B.; Ray egan, S. and Sharifi, A.M. (2014): A comparative study of mesenchymal stem cell transplantation with its paracrine effect on control of hyperglucenia in type 1 diabetic rats. *Journal of Diabetes & Metabolic disorders, 13 (76): 1-10.*

Anwer, T. (2014): Melatonin ameliorates hyperinsulinemia, glucose intolerance and insulin resistance in STZ-Nicotinamide induced type 2 diabetic rat. International Journal of pharmacy and pharmaceutical. *Science, 6 (2): 133-136.*

Arcolino, F. O.; Ribeiro, D. L.; Gobbo, M. G.; Taboga, S. R. and Goes, R. M. (2010): Proliferation and apoptotic rates and increased frequency of p63-positive cells in the prostate acinar epithelium of alloxan-induced diabetic rats. *International Journal of Experimental Pathology, 91(2):144–154.*

Armagan, Ai; UZ, E.; Xilmaz, H R.; Soyupek, S.; Oksay, T. and Ozcelik, N. (2006): Effects of melatonin on lipid peroxidation and antioxidant enzymes in Streptozotocin- induced diabetic rat testis. *Asian J. Androl., 8(5): 595-600.*

Asayama, J.; **Nakane, T.; Uchida, N.; Hayashibe, H.; Dobashi, K. and Nakazawa, S. (1994):** Serum antioxidant states in streptozotocin induced diabetic rat. *Horm.metab.Res., 26:313-315.*

Bhanduas, K.S. and Gopal, p. k. (2016): Histological structure of pancreas in normal control, diabetic control an extract treated albino rats. *Int. J. of Life Science, 4 (1): 78-82.*

Bhansali, S.; Kumar, V.; Saikia, U.N; Medhi, B.; Jha, V. and Dulta, p. (2015): Effects of mesenchymal stem cells transplantation on glycaemic profile & their location in streptozotocin induced diabetic wistar rats. *Indian J. Med. Res., 142: 63-71.*

Boyle, J.J.; Weissberg, P.L. and Bennett, M.R. (2003): Tumor necrosis factor-alpha by direct and autocrine mechanisms. *Arterioscler Thromb. Vasc. Biol., 23: 1553–1558.*

Brownlee, M. (2001): Biochemistry and molecular cell biology of diabetic complications. *Nature, 414: 813–820.*

Cantarelli, E.; Pellegrini, S.; Citro, A.; Sordi, V. and Piemonti, L. (2015): Bone marrow- and cord blood-derived stem cell transplantation for diabetes therapy. *CellR4, 3 (1): 1-21.*

Farrell, M.J.; Fisher, M.B.; Huang, A.H.; Shin, J.I.; Farrell, K.M. and Mauck, R.L. (2014): Functional properties of bone marrow-derived MSC-based engineered cartilage are unstable with very long-term in vitro culture. *J. Biomech., 47:2173-82*

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Galkina, E and Ley, K. (2006): Leukocyte recruitment and vascular injury in diabetic nephropathy. *J. Am. Soc. Nephrol., 17:368–377.*

Gobbo, M. G.; Taboga, S. R.; Ribeiro, D. L. and Góes R. M. (2013): Short-term stromal alterations in the rat ventral prostate following alloxan-induced diabetes and the influence of insulin replacement. *Micron., 43(2-3):326–333.*

Gobbo, M.G; Dizeyi, N.; Masitali, V.S. and Goes, M. R. (2015): Influence of melatonin on the proliferative and apoptotic response of the prostate under normal and hyperglycemic conditions. *Journal of Diabetes Research, 10 (1155): 1-18.*

Godam, E.T.; Samaila, M.O.A.; Ibegbu, A.O.; Hamman, W.O. and Musa, S. A. (2015): Histological effects of melatonin and *Azadirachta Indica*   administration on the pancreatic tissue in streptozotocin-induced diabetic wistar   rats. *Annals of Biological Sciences, 2 (2):27-35.*

Haasters, F.; Prall, W.C.; Anz, D.; Bourquin, C.; Pautke, C.; Endres, S.;   Mutschler, W.; Docheva, D. and Schieker, M. (2009): Morphological and   immunocytochemical characteristics indicate the yield of early progenitors   and represent a quality control for human mesenchymal stem cell culturing.   *J. Anat., 214:759–767.*

Javier, E.; Pariente, J.A. and Rodríguez, A.B. (2011): Bone marrow stem cells and liver disease. *World Journal of Diabetes*, , 2 (6), 82-91.

Johnston, B.; Hering, T.; Caplan, A.; Goldberg, V. and Yoo, J. (1998): *In vitro* chondrogenesis of bone marrow-derived mesenchymal progenitor cells. *Exp. Cell Res., 238: 265-72.*

Jones, B.E.; Lo, C.R.; Liu, H.; Pradhan, Z.; Garcia, L.; Srinivasan, A, Valentino, K.L. and Czaja, M.J. (2000): Role of caspases and NF-kappa B signaling in hydrogen peroxide- and superoxide induced hepatocyte apoptosis. *American Journal of Physiology, Gastrointestinal and Liver Physiology, 278: 693–699.*

Jones, D.L. and Wagers, A.J. (2008): No place like home: anatomy and function of the stem cell niche. *Nat. Rev. Mol. Cell Biol., 9:11-*21.

Kumar, O.; Rastogi, S.K.; Batra, M. pankaj, N.K. and Huozha, R, (2016): Pathological studies on the effect of melatonin administration in experimental diabetes in goats. *Veterinary Research International, 1 (1): 41-44.*

Laster, S.M.; Wood, J.G. and Gooding, L.R. (1988): Tumor necrosis factor can induce both apoptotic and necrotic forms of cell lysis. *J. Immunol., 141:2629–2634.*

Lee H.J., Lee J.K., Lee H., Shin J.W., Carter J.E., Sakamoto T., Jin H.K. and Bae J.S. (2010): The therapeutic potential of human umbilical cord blood-derived mesenchymal stem cells in alzheimer'disease. *Neurosci. Lett., 5: 30-35.*

Liu, X.; Gong, Y.; Xiong, K.; Ye, Y.; Xiong, Y. and Zhuang, Z. (2013): Melatonin mediates protective effects on inflammatory response induced by interleukin-1 beta in human mesenchymal stem cells. *Journal of pineal research, 55:14-25.*

Lui, H.; Tang, W. and Li, C. (2015): CdSe/ZnS Quantum dots-labeled mesenchymal stem cells for targeted fluorescence imaging of pancreas tissues and therapy of type 1 diabetic rats. *Nanoscale Research Letters, 10(265): 1-12.*

Maisaa, M. A. and Al- Rawi (2007): Effect of Antox on Paraquat - Induced Histological and Biochemical Changes in Kidney of Albino Rats. *Journal of Biological Sciences, 7(1): 10-18.*

Marina, M.; Casiraghi, F.; Azzollini, N.; Cassis, P.; Imberti, B.; Cugini, D.; Cavinato, R.A.; Todeschini, M. Solini, S.; Sonzogni, A.; Remuzzi, G. and Noris, M.(2008): Human bone marrow mesenchymal stem cells accelerate recovery of acute renal injury and prolong survival in mice. Stem cells, 26:2075-2082.

Meier, J. J.; Bhushan, A. and Butlar, P. C. (2006): The potential for stem cell therapy in diabetes. *International Pediatric Research Foundation, Inc., 95(4):65-73.*

Mokbel, A.; El Tookhy, O.; Shamaa, A. A.; Sabry, D.; Rashed, L. and Mostafa, A. (2011): Homing and efficacy of inta-articular injection of antilogous mesenchymal stem cells in experimental chondral detects in dogs. *Clinical and Experimental Rheumatology, 29: 275-284.*

Newman, R.E.; Yoo, Di; El Rou X, M.A. and Miagkora, A. (2009): Treatment of inflammatory disease with mesenchymal stem cells. *Inflammations & Allergy Drug Targets, 8(2), 10-12.*

Nugroho, W. S.; Liliek, D. and Suestya, H. (2016): The structural and functional recovery of pancreatic B -cells in type 1 diabetes mellitus induced mesenchymal stem cell-conditioned medium. *Veterinary World, 9(5):535-539.*

Reiter, R.J.; Tan, D.X.; Osuna, C. and Gitto, E. (2000c): Actions of melatonin in the reduction of oxidative stress. *J. Biomed. Sci., 7: 444–58.*

Ryan, E.A.; Paty, B.W.; Senior, P.A.; Bigam, D.; Alfadhli, E.; Kneteman, N.M.; Lakey, J.R. and Shapiro, A.M. (2005): Five-year follow-up after clinical islet transplantation. *Diabetes, 54:2060–2069.*

Sato Y., Araki H., Kato J., Nakamura K., Kawano Y., Kobune M. and Niitsu Y. (2005): Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion. *Blood, 106(2): 756-763.*

Sekkin, S.; Ipek, E. D.; Boyacioglue M. and Kum, C. (2015): DNA protective and antioxiadtive effects of melatonin in streptozotocin- induce diabetic rats. *Turk J. Biol., 39: 932-940.*

Seo, M.S.; Jeong, Y.H. and Park, J.R. (2009): Isolation and characterization of canine umbilical cord blood-derived mesenchymal stem cells. *J. Vet. Sci., 10: 181-187.*

Sharma, S.; Singh, H.; Ahmed, N.; Mishra, p, and Tiwari, A (2015): The role of melatonin in diabetes: therapeutic implications. *Arch. Endocrmol. Metab., 59(5): 391-399.*

Suzuki, S.; Hinokio, Y.; Komatu, K.; Ohtomo, M.; Onoda, M.; Hirai, S.; Hirai, M.; Hirai, A.; Chiba, M. and Kasuga, S. (1999): Oxidative damage to mitochondrial DNA and its relationship to diabetic complications. *Diabetes Research and Clinical Practice, 45: 161–168.*

Tajiri, Y. and Grill, V. E, (1999): Interactions between vitamin E and glucose on β – cell functions in the rat: an in vivo and in vitro study. *Pancreas, 18(3): 274-81.*

Tam E.M., Moore T.R., Butler G.S. and Overall C.M. (2004): Characterization of the distinct collagen binding, helicase and cleavage mechanisms of matrix metalloproteinase 2 and 14 (gelatinase A and MT1-MMP): the differential roles of the MMP hemopexin c domains and the MMP-2 fibronectin type II modules in collagen triple helicase activities. *J. Biol. Chem., 279: 43336-43344.*

Togel, F.; Weiss, K.; Yang, Y.; Hu, Z.; Zhang, P. and Westenfelder, C. (2007): Vasculotropic, paracrine actions of infused mesenchymal stem cells are important to the recovery from acute kidney injury. *Am. J. Physiol Renal Physiol., 292: 1626–35.*

Urban, V.V. (2009): A possible stem cell therapy for diabetes mellitus. *Cell biology International, 30: 401-4*

Vijayanand, S. and Wesley, E.G. (2011): International Journal of Current Pharmaceutical Research, *3(4): 37-4.*

Volarevic, V.; Al-Qahtani, A.; Arsenijevic, N.; Pajovic, S. and Lukic, M.L. (2010): Interleukin-1 receptor antagonist (IL-1Ra) and IL-1Ra producingmesenchymal stem cells as modulators of diabetogenesis. *Autoimmunity, 43:255–263.*

Wang, F.W.; Wang, Z.; Zhang, Y.M.; Du, Z.X.; Zhang, X.L.; Liu, Q.; Guo, Y.J.; Li, X.G. and Hao, A.J. (2013): Protective effect of melatonin on bone marrow mesenchymal stem cells against hydrogen peroxide-induced apoptosis in vitro. *J. Cell Biochem., 114(10):2346-55.*

Yagi H., Soto-Gutierrez A., Parekkadan B., Kitagawa Y., Tompkins R.G., Kobayashi N. and Yarmuch M.L. (2010): Mesenchymal stem cells: mechanisms of immunomodulation and homing. *Cell Transplant, 19: 667-679.*