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Effects of Mesenchymal Stem Cells Derived Microvesicles Therapy on Pancreas in Experimentally–Induced Diabetes in Adult Male Albino Rats

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ABSTRACT

Background: Mesenchymal stem cell-derived extracellular vesicles [MSC-sEVs] have potentials that can be utilized for mitigating the adverse effects associated with diabetes mellitus [DM].

The aim of the work: To test the effects of MSC-sEVs on pancreas with DM.

Patients and Methods: The study was carried out on forty adult male albino rats at Faculty of Medicine, Al-Azhar University. The rats were randomly allocated into four equal groups [10 rats each]: Group I: control. Group II: Rats received IP alloxan [80 mg / kg bodyweight] twice [one week apart] to induce DM. Group III: After induction of DM, rats received 200 mg/ml IV culture media [1 ml / rat]. Group IV: After DM induction rats received 200 mg/ml MSC-sEVs IV three times [once per week]. Rats were sacrificed and pancreatic sections from all groups were subjected to hematoxylin & eosin and immunohistochemical stains with tumor necrosis factor-α [TNF-α] and Bax.

Results: The obtained results showed that the diabetic rats had marked decrease in number and atrophy of islets, congested blood vessels, interstitial haemorrhage, and perivascular inflammatory cellular infiltrate. MSC-sEVs treated group showed marked restoration of normal pancreatic architecture in the form of normal islets of Langerhans with unremarkable cell vacuolation surrounded by normal pancreatic acini in addition to marked reduction of vascular congestion and interstitial haemorrhage. In diabetic group immunostained sections for BAX and TNF-α showed high BAX and TNF-α expression in islet cells and acinar cells. MSC-sEVs -treated group showed lower BAX and TNF-α immunoreactivity.

Conclusion: Bone marrow-MSCs can be considered as treatment modality for DM.

Keywords: Mesenchymal Stem Cells; Pancreas; Diabetes Mellitus; BAX; Tumor necrosis factor-α.

INTRODUCTION

High blood glucose levels due to insufficient insulin production by pancreatic -cells characterize diabetes mellitus, a chronic metabolic disorder. People with type 1 diabetes may experience the aforementioned behavior due to the autoimmune destruction of β-cells [1]. Reduced insulin sensitivity [non-insulin dependent diabetes] and impaired β-cell functionality both contribute to the development of type 2 diabetes [2].

Diabetes has the ability to be inherited, or produced by autosomal dominant gene mutations leading to the impairment of insulin manufacturing. Although less common than type 2 diabetes, gestational diabetes is becoming more of a risk factor for the later stages of pregnancy and the postpartum period [3].

The effects of diabetes mellitus are widespread throughout the human body, but the kidneys, heart, pancreas, and eyes are particularly hard hit. Disease tends to reveal itself more obviously in these systems earlier due to their heightened susceptibility. According to **Gupta *et al.*** [4], microvascular and macrovascular complications arise as a result of persistently high blood glucose levels in diabetics.

In the treatment of a wide range of human diseases, mesenchymal stem cells [MSCs] have emerged as a leading cell-based therapeutic method. The therapeutic potential of MSCs is the subject of much speculation. Paracrine molecules like proteins, peptides, and hormones play a role in these processes, as do the transmission of exosomes and microvesicles [EVs] containing a wide variety of compounds. **Fan *et al.*** [5] stated that paracrine chemicals contained within vesicles are likely responsible for the therapeutic efficacy of MSCs.

Bioactive lipids, proteins, and RNAs are transmitted between cells via EVs, which have been recognized as important mediators in intercellular communication [6]. As signaling molecules found in many bodily fluids, exosomes are crucially involved in a wide variety of physiological and pathological processes. The purpose of this research was to examine the effects of MSC-sEVs therapy on the pancreas of male albino rats that had been artificially engineered to develop diabetes.

MATERIALS AND METHODS

The current study was conducted at Al-Azhar University's School of Medicine in Cairo. All procedures involving the care and testing of animals have been carried out in compliance with the guidelines for the care and use of laboratory animals, as set out by the Experimental Animal Ethics Committee of Al-Azhar University.

Experimental Design and Animal Groups

The research was conducted on a sample of forty adult male albino rats of the Sprague-Dawley strain. These rats had an average body weight ranging from 200 to 250 grams and an average age between 6 and 8 weeks. The rats were obtained from the animal house located at the Faculty of Medicine, Al-Azhar University. The rats had a period of acclimatization at the laboratory of the department of physiology, Faculty of Medicine, Al-Azhar University, lasting two weeks prior to the commencement of the experiment. The rats were accommodated in enclosures subjected to a light/dark cycle lasting twelve hours, while maintaining a temperature of 24 °C [±3 °C] and a humidity level ranging from 50% to 70% in a regulated environment. Adequate supplies of uncontaminated water and a standardized meal for rodents were provided without restriction throughout the duration of the study.

The rats were randomized into four groups of similar size [n=10] using a random allocation method. The groupings were as follows:

Group I, often known as the control group, the rats were administered 1 cc of distilled water by intraperitoneal injection. These animals were utilized as negative controls**.**

The rats in Group II, are known as the Diabetic Group, were administered an IP of alloxan at a dosage of 80 mg per kilogram of bodyweight. The alloxan was diluted with sterile saline immediately prior to administration. The injection was administered on two occasions, with a one-week interval between each administration. This was sufficient to establish type-1 DM in the animals, so serving as a positive control [7].

Group III [DM + Culture medium]: Following the development and verification of DM, the rats in this experimental group were administered the culture medium, namely the Dulbecco's Modified Eagle Medium [DMEM], via intravenous injection into the caudal vein of each rat at a dosage of 1 ml per rat.

In the Group IV, rats were administered MSC-sEVs following the establishment and confirmation of DM. The administration of MSC-sEVs was performed via intravenous injection into the caudal vein of each rat. This procedure was

repeated three times, with a frequency of once per week. The dosage of MSC-sEVs administered was 200 mg/ml.

Materials: MSC-sEVs from tibia of white female albino rat, Phosphate-Buffered Saline [PBS] containing ethylene diamine tetra acetic acid [EDTA], pH 7.4 [Sigma-Aldrich], Ficoll-Paque [Gibco-Invitrogen, Grand Island, NY], 10% Fetal Bovine Serum [FBS, Thermo Fisher Scientific], Roswell Park Memorial Institute [RPMI]-1640 medium [Thermo Fisher Scientific], 0.5% penicillin, streptomycin [Sigma-Aldrich], 10% formalin solution as tissue fixative [Sigma-Aldrich], serum-free medium 199 containing N-2-Hydroxy Ethyl Piperazine-N' 2-Ethane Sulfonic acid [HEPES] 25 mM [Sigma], Lugol's iodine by equal parts of 0.5% $KMNO_4$ and 0.5% sulphuric acid and sodium thiosulphate by 2% sodium bisulphite, BAX antibodies, TNF antibodies.

Instruments: Glucometer [GlucoDr, Korean], Tissue culture flask [Thermo Fisher Scientific, USA], Laminar air flow [1300 series A2, Thermo scientific, USA], CO_2 incubator [Innova CO 170, USA], Automated pipette [precision, UK], Cooling centrifuge for falcon [Sigma 2 – 16 PK, Germany], Ultra-centrifuge [Beckman Coulter Optima L-90 K], Inverted microscope [Leica Microsystem, USA], Light microscope [Leica, USA], Flow cytometer [Beckman coulter, FL, USA], Fluorescent microscope [Leica Microsystem, USA], Tissue homogenizer [RT, Miccra, Germany], Spectrophotometer [Cary 4000 UV – Vis, Agilent Technologies, USA].

Induction of diabetes: With the exception of the control group [Group I], the remaining three groups were rendered diabetic through the administration of two doses of alloxan [Sigma Co, USA] via intraperitoneal [IP] injection. The injections were given with a one-week interval, following a 12-hour fasting period. The dosage administered was 80 mg/kg of body weight, diluted with sterile saline immediately prior to injection. Diabetic animals were selected based on fasting glucose level of ≥ 250 mg/dL in two consecutive determinations, conducted 1 and 2 weeks after diabetes induction. In order to maintain the desired number of rats in each group, any animals that died during the post-induction period or during follow-up were replaced. This was done to ensure that the final number of rats in each group was not compromised [7].

Verification of diabetes: Blood samples were collected from tail vein under general anesthesia, 7 days after diabetic induction. The glucose level measured using AGM-2200 Gluco Dr. Super sensor. Hyperglycemia was confirmed 1 week after injection of alloxan, the fasting glucose level was ≥ 250 mg/dl [7]. From the beginning of diabetic induction until one month the glucose level was monitored periodically. At the same time isolation of BM MSCs and culturing procedures were began.

Isolation of BM-MSCs

Preparation, isolation, culture and identification of BM-MSCs: In this study, rat bone marrow [BM] was utilized as a source for the isolation of mononuclear cells [MNCs]. BM cells were extracted from the tibia of female white albino rats [Cux1: HEL1] that were of the same age [6 months] and weight [150~200 g]. The extraction was performed using PBS. A volume of 15 ml of the flushed bone marrow cells was meticulously placed on top of a 15 ml layer of Ficoll-Paque [Gibco-Invitrogen, Grand Island, NY]. Subsequently, the mixture was subjected to centrifugation at a speed of 400 ×g for a duration of 35 minutes at a temperature of 5 ℃. The top layer was removed by aspiration, leaving the MNC layer unaltered during the interphase. The MNC layer was carefully removed and rinsed twice in PBS containing 2 mM ethylene diamine tetra acetic acid [EDTA]. It was then centrifuged for 10 minutes at a speed of 200 ×g and a temperature of 5℃. The isolated BM-MSCs [cell pellet] were subsequently cultured and expanded in 25 ml culture flasks using Roswell Park Memorial Institute [RPMI]-1640 medium supplemented with 10% Fetal Bovine Serum [FBS], 0.5% penicillin, and streptomycin. The cells were incubated at a temperature of 37℃ and a carbon dioxide [CO2] concentration of 5% until they reached a confluence of 80~90% within a period of 7 days. The cultured MSCs were evaluated based on their adherence, morphology, and Fluorescent Activated Cell Sorting [FACS] analysis. This analysis involved determining the presence of cluster of differentiation markers CD90＋ and CD29＋, which are known to be positive in MSCs, as well as the absence of CD45−, which is distinctive to MSCs.

Preparation and identification of MVs derived from BM-MSCs: Microvesicles [MVs] were acquired from the supernatants of mesenchymal stem cells [MSCs] that were grown for a period of one night in RPMI medium without fetal bovine serum [FBS]. To isolate microvesicles [MVs], the cell-free supernatants were subjected to a two-step centrifugation process. Initially, the supernatants were centrifuged at 10,000 ×g for 20 minutes at 4 ℃ to eliminate debris. Subsequently, the resulting supernatants were further centrifuged at 100,000

×g for one hour at 4 °C using a Beckman Coulter Optima L-90 K ultracentrifuge. The MVs were then washed in serum-free medium 199 containing N-2-Hydroxy Ethyl Piperazine-N'- 2-Ethane Sulfonic acid [HEPES] at a concentration of 25 mM [Sigma] and subjected to a second ultra-centrifugation under the same conditions. The surface markers of MSC-MV [CD44, CD73] were analyzed by flow cytometry in the study conducted by **Tang *et al.*** [8].

Histopathological study

Following duration of 6 weeks, the rats from each experimental group were euthanized in order to examine the adverse impacts of hyperglycemia on the pancreatic tissue of untreated animals [Group II]. Additionally, the potential effects of treatment on pancreatic tissue in the treated groups [III and IV] were investigated through histopathological and immunohistochemical examinations. The whole pancreas was excised and subsequently cleansed. Random segments were extracted from each experimental group, with 5 animals per subgroup designated for sacrifice. These segments were then immersed in buffered 10% formalin, subjected to paraffin processing, and subsequently sectioned at a thickness of 3-5 µm for histological analysis. Histological analysis was conducted subsequent to the fixation of rat pancreases in a 10% formalin solution. The specimens were then subjected to processing and embedding in paraffin wax. The tissue blocks were sliced into sections that were 5 µm in thickness and subsequently subjected to staining using haematoxylin and eosin [H&E].

1. Haematoxylin and Eosin stain: The portions were subjected to deparaffinization using xylene. The process of hydration was conducted using a series of alcohol solutions arranged in declining order of concentration. The sections were immersed in a haematoxylin solution for duration of ten minutes. The surplus blue color was rinsed out using a continuous flow of tap water for duration of five minutes. The process of desiccation was conducted using a hot plate set at a temperature of 40 °C, until the tissue reached a state of dryness characterized by a dried and stretched appearance. A counterstaining procedure was performed using a 1% solution of eosin for duration of 10 minutes. The process of rinsing away extra pigment under a stream of flowing tap water was conducted. The experimental procedures of dehydration in increasing concentrations of alcohol, followed by clearing in xylol and mounting, were executed. The findings of the study are as follows: The nuclei have a blue/black coloration. The cytoplasm exhibits a range of pink hues. The red blood cells were dyed with a pink colloidal solution, resulting in an orange/red coloration [9].

2. Immunohistochemistry: Tissue sections, fixed in paraffin and measuring four microns in thickness, were produced. The immunohistochemical staining procedure was performed in accordance with manufacturer. The tissue sections were subjected to deparaffinization and subsequently treated with a 3% hydrogen peroxide solution for duration of 20 minutes. This was followed by incubation with specific antibodies, namely anti-Bax and TNF-α [11379, Thermo Fisher Inc.] at the same dilution. The incubation was carried out at 4 °C overnight. After incubation, the sections were washed with PBS, the sections were incubated with diaminobenzidine [DAB] for 10 minutes. The specimens were washed using PBS and subsequently counterstained with hematoxylin. Following this, the specimens were dehydrated, cleaned in xylene, and finally covered. The technique of immunostaining offers a means of detecting and characterizing chemicals inside tissues through the utilization of antigen-antibody interactions. These reactions may be rendered visible under a microscope by integrating an appropriate label. The immunohistochemical staining procedure involved the utilization of the Avidin-Biotin immunoperoxidase polyclonal kit, which was given by NEO Marker's Laboratories [Lab. Vision Corporation] located in Westinghouse, Dr. Fremont, CA, USA [10]. The staining was performed for anti Bax and TNF-α.

RESULTS

Hematoxylin and Eosin stained sections: In Figure [1], light microscopic examination of pancreatic sections of the control group [group I] showed the normal histological structure of the pancreas, pancreatic acini and the islets of Langerhans [Figure 1-A]. While pancreatic sections of the diabetic group showed marked decrease in number and atrophy of islets, congested blood vessels, interstitial haemorrhage, and perivascular inflammatory cellular infiltrate [Figure 1-B]. Also, pancreatic sections of the DMEM-treated group showed vacuolated and degenerated islet cells, acinar necrosis, and interstitial haemorrhage [Figure 1-C]. In contrast; MSC-sEVs treated group showed marked restoration of normal pancreatic architecture in the form of normal islets of Langerhans with unremarkable cell vacuolation surrounded by normal pancreatic acini marked reduction of vascular congestion and interstitial haemorrhage [figure 1-D].

Immunohistochemical stained sections: In figure [2], concerning [BAX] BAX Concerning immunohistochemical staining of the pancreatic sections in the current study; Healthy rats showed negative BAX immunohistochemical expression in pancreatic islet and acinar cells [2-A]. While those of diabetic group revealed high BAX expression in both islet and acinar cells [2-B]. Pancreatic sections of the DMEM-treated immunostained for BAX showed high BAX expression in pancreatic acinar cells [2-C]. Pancreatic sections of the MSC-sEVs treated group immunostained for BAX lower BAX immunoreactivity compared to groups II, III [2-D].

In Figure [3], concerning Immunohistochemical study [TNF-α] stained pancreatic sections in the current study; pancreatic sections of the normal control group immunostained with TNF-α showed negative TNF-α immunostaining to weak TNF-α expression in pancreatic acinar cells [3-A]. Pancreatic sections of the diabetic group immunostained with TNF-α show high TNF-α expression in pancreatic acinar cells [3-B]. Pancreatic sections of the DMEM-treated group immunostained with TNF-α show high TNF-α expression in pancreatic acinar cells [3-C]. Pancreatic sections of the MSC-sEVs -treated group, immunostained with TNF-α show lower immunoreactivity to TNF-α in pancreatic acini and islet cells [3-D].

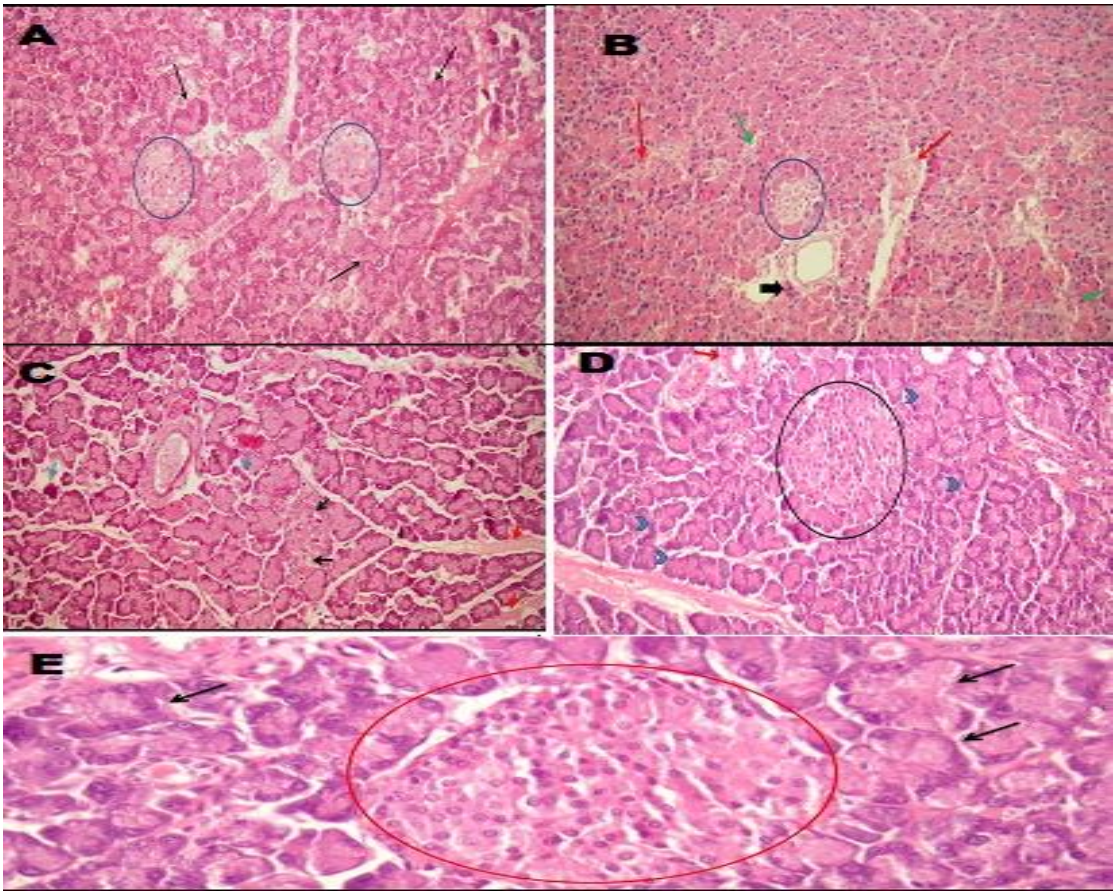

Figure [1]: Hematoxlin and eosin-stained sections in the pancreas showing a photomicrograph of: a section of the pancreatic tissue of control group [I], pancreatic acini show alveolar [round] shape and lobulated architecture. The acini are densely arranged in close proximity to each other and are subsequently emptied by a network of ductules and ducts. The acinar cells exhibit a blue staining at their base when treated with hematoxylin due to their elevated RNA content and the presence of nuclei. The luminal side exhibits a pink staining pattern as a result of its elevated concentration of digesting enzymes. The lining of ductules and ducts consists of a monolayer of cuboidal or cylindrical epithelial cells. The wall of major ducts, such as the main pancreatic duct, contains a substantial quantity of collagen. The cells inside the islets of Langerhans [blue circles] have a polygonal to round morphology, characterized by a substantial pale to slightly eosinophilic cytoplasm. These cells also possess monomorphic round nuclei, which display heterogenous chromatin with a salt and pepper appearance [black arrows] [X 200] [1-A], a section of the pancreatic tissue of diabetic group [II] showed marked decrease in number and atrophy of islets [blue circle], congested blood vessels [red arrows], interstitial haemorrhage[green arrows], perivascular inflammatory cellular infiltrate[black arrows], [X200] [1-B], a section of the pancreatic tissue of DMEM-treated group [III] showed vacuolated and degenerated pancreatic cells [black arrows], and acinar necrosis [blue arrows] [X200] [1-C], a section of the pancreatic tissue of MSC-sEVs treated group showed marked restoration of normal pancreatic architecture in the form of normal islets of Langerhans with unremarkable cell vacuolation [black circle] surrounded by normal pancreatic acini [blue arrow heads] marked reduction of vascular congestion [Red arrow] and interstitial haemorrhage [X200] [1-D], a section of the pancreatic tissue of MSC-sEVs treated group [IV]showed normal pancreatic architecture; remarkable increase in size and density of pancreatic islet [red circle] as well as normal looking pancreatic acini [black arrows] [X400] [1-E].

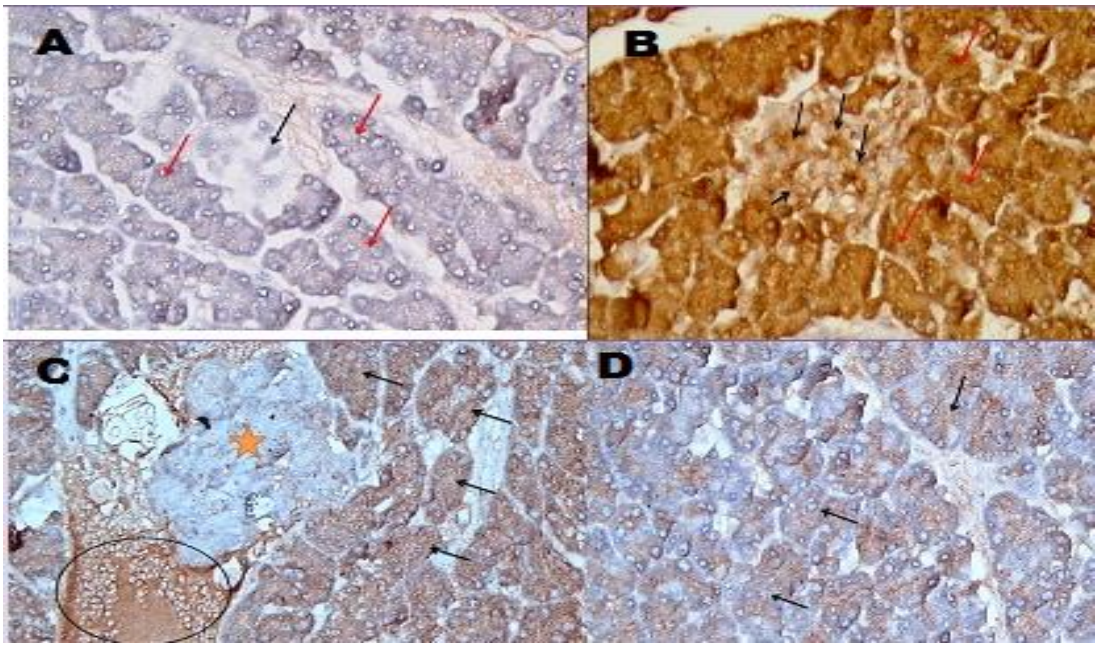

Figure [2]: Photomicrograph of immune localization of Bax in the pancreatic sections of all the study groups; Control group [I] shows negative BAX immunohistochemical expression in islet [black arrows] and acinar cells [red arrows] [BAX immunostaining X400] [2-A]. Diabetic group [II] shows high BAX immunexpression in both islet [black arrows] and acinar cells [red arrows] [BAX immunostaining X400] [2-B]. DMEM-treated group [III] showing high BAX expression in pancreatic acinar cells [black arrows] [BAX immunostaining X200] [2-C]. BM- MSCs -MVs treated group [IV] displaying lower BAX immunoreactivity in pancreatic acinar cells [black arrows] compared to groups II, III [BAX immunostaining X400] [2-D].

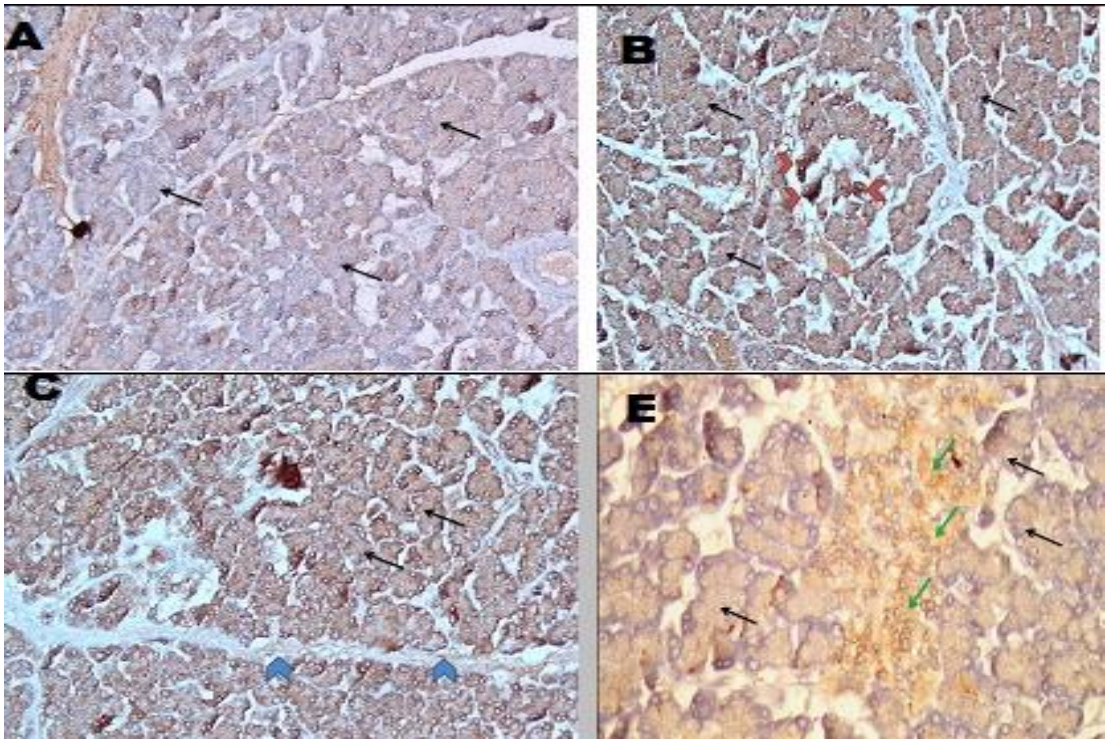

Figure [3]: Photomicrograph of immune localization of TNF-α in the pancreatic sections of all the study groups showing; control group [I] displaying negative to weak TNF-α expression in pancreatic acinar cells [black arrows] [TNF-α immunostaining X400] [3-A]. Diabetic group [II] shows high TNF-α expression in pancreatic acinar cells [black arrows] and apoptotic islet cells [arrows head] [TNF-α immunostaining X200] [3-B]. DMEM-treated group [III] shows high TNFα expression in pancreatic acinar cells [black arrows] and areas of fibrosis [arrows head] [TNF-α immunostaining X200] [3-C]. BM- MSCs –MVs treated group [IV] shows lower immunoreactivity to TNFα in pancreatic acini [black arrows] and islet cells [green arrows] [TNF-αimmunostaining X400] [3-D].

DISCUSSION

The prevalence of Type 2 diabetes mellitus [T2DM] has showed a continuous growth in recent years, comprising a considerable majority of 90% among individuals diagnosed with diabetes [11].

T2DM is defined by an abnormal control of glucose homeostasis, resulting from deficiencies in both insulin synthesis and insulin action within the body. Depletion of β-cell function due to excessive insulin production, worsening insulin resistance [IR], and the onset of many complications, such as cardiovascular disease, nephropathy, neuropathy, and retinopathy, are all associated with long-term hyperglycemia. Current pharmaceutical therapies for the treatment of T2DM, said by **Domouky *et al.*** [12], are fraught with side effects and offer only temporary control of blood glucose levels to lessen the severity of complications.

It has been suggested that stem cell [SC] therapy might be used to repair damaged pancreatic cells and restore glucose homeostasis in diabetics, leading to the complete reversal of the disease. The safety worry of probable tumorigenicity linked with MSC-based therapy continues despite the positive results demonstrated in various trials [13].

MSCs have been found to play a fundamental role in tissue repair and regeneration through the production of paracrine substances. These signals are commonly provided by extracellular vesicles [EVs]. According to **Jing *et al.*** [14], EVs are a kind of nanoparticles that are produced spontaneously by lipid bilayer cells but lack the capacity to proliferate.

There is a large variety of mediators involved in EVs that are required for both short- and long-range cellular communication. The mediators include not just proteins and lipid metabolites but also different types of DNA and RNA. EVs have been linked to both normal physiological processes and aberrant disease situations, as shown by a number of studies. EVs carry proteins and miRNAs. Therefore, academia is now investigating the use of EVs for various purposes [15].

The purpose of this research is to evaluate the effects of treating adult male albino rats with experimentally induced diabetes using microvesicles derived from BM-MSCs. In the current study, slices of pancreas were examined under a light microscope to serve as healthy rats [group I]. Pancreatic acini and islets of Langerhans were inspected, and their histological structure was confirmed to be normal. Diabetic pancreatic sections were characterized by a decrease in islet number and size, arterial narrowing, interstitial bleeding, and inflammatory cell infiltration into the perivascular spaces. Pancreatic slices from the DMEM group also showed acinar necrosis, interstitial hemorrhage, and vacuolated and damaged islet cells. In comparison, the pancreas of the group given MSC-sEVs looked much more as it should, with normal islets of Langerhans and no obvious cell vacuolation. Interstitial hemorrhage and vascular congestion in the pancreatic tissue also diminished.

The present results are congruent with those of **Sun *et al.*** [16], who found that hMSCs-derived EVs may reduce pancreatic damage and promote islet regeneration in rats with STZ-induced diabetes. This study's findings suggest that hMSCs-derived EVs might have a protective or regenerative effect on the pancreas of diabetic rats, reducing the severity of diabetes-related pancreatic damage.

Furthermore, in line with the findings of **Mazen *et al.*** [17], as indicated by the present study's observations, H&E-stained sections in the pancreas of the pancreatitis group treated with microvesicles, a considerable improvement was evident in the overall structure of the majority of the acini, characterized by the restoration of central acidophilia and peripheral basophilia. However, in a small percentage of acini, the nuclei are strongly stained. The Langerhans islands looked like normal islets.

When MSC-sEVs were given to diabetic rats induced by streptozotocin [STZ], a more significant hypoglycemic response and a faster commencement of action was observed compared to those treated by MSCs alone **Sabry *et al.*** [18]. Accelerated islet cell regeneration, improved islet number and size, decreased fibrosis and inflammation, and considerable up-regulation of genes related with islet regeneration were all seen in the MSC-sEVs-treated group.

Similar results were found by **Dong *et al.*** [19], who found that the control group showed no signs of edema, infiltration of inflammatory cells, or necrosis in the pancreatic tissue. Edema

development and inflammatory cell infiltration were both significantly reduced in the BM-MSC infusion group. **Elshemy *et al.*** [20]'s investigation showed that rats in the MSC-treated groups had pancreases that seemed structurally normal, retained tissue architecture and undamaged pancreatic islets of Langerhans, suggesting the lack of any injury. Unlike pancreatic necrosis, damage, hemorrhage and lymphocytes infiltration in diabetic rats.

Yap *et al.* [21] noticed analogous findings to those of the current experiment, wherein the pancreatic sections of non-diabetic rats revealed the usual anatomical features of the pancreatic islet. Pathological changes, including disorganization and reduction of the Langerhans islets, were nevertheless seen in the untreated diabetic rats. In addition, inflammation was present, as evidenced by infiltrating leukocytic cells, in the diabetic rats that were not treated. Hydropic degeneration of endocrine cells was seen in the glibenclamide treatment group, followed by regeneration to the pre-treatment state. Pancreatic islet architecture was restored in the experimental group at doses of 0.25 and 1 mg/kg after treatment with MSC-sEVs derived from hUC-MSCs.

In a recent experiment done by **Samy *et al.*** [22], it was observed that pancreatic sections collected from persons with type II diabetes exhibited abnormal pancreatic acini characterized by considerable cytoplasmic vacuolization and a decrease in basal basophilia. There are acini that do not have nuclei. Blood artery dilation and congestion, as well as interlobular septal enlargement and inflammatory cell infiltration, are among the pathological features that have been reported. There are interstitial spaces between the cords of cells in an islet of Langerhans. Blood vessel dilation and congestion accompany a remarkable decrease in the islet's numbers. However, in the exosomes treated rats, a noticeable increase in the quantity and size of the islets of Langerhans was presented, indicated the regeneration of islet cells.

Sayed *et al.* [23] conducted a research that demonstrated enhanced oral glucose tolerance following injection of MSC-sEVs. Improvements were seen because of efforts to lessen inflammation, revive -cells, and lessen insulin resistance.

It has been observed that MSC-sEVs improved the performance of pancreatic beta cells, suggesting that this approach might be used to treat diabetes. MSC-sEVs, as reported by **Li *et al.*** [24], show a specific chemotactic action upon transplantation, moving to and attracting the damaged islets. This procedure enhances the repair and the renewing of beta cells inside the injured islets by suppressing beta cell apoptosis and boosting the proliferation of beta cells.

Bax has a critical function as the principal effector in the process of apoptosis. When endothelial cells are dysfunctional due to diabetes, as shown by the overexpression of Bax, apoptosis of those cells is triggered. It is theorized that higher levels of plasma glucose and HbA1C are caused by an increase in Bax expression, which in turn exacerbates endothelial dysfunction in people with diabetes. In the absence of remedial interventions for chronic hyperglycemia, more blood vessels malfunction will die over time [25].

In the current study; pancreatic islet cells and acinar cells lacked BAX immunohistochemistry expression in the first group's pancreatic sections, according to the results of the current study. Immunostaining for BAX in pancreatic sections from the diabetes cohort revealed substantial increase of BAX expression in both islet cells and acinar cells. Immunostaining for BAX in DMEM-treated pancreatic sections revealed an increase in BAX expression in pancreatic acinar cells, in addition to fibrosis and interstitial hemorrhage. There was less BAX positivity in the pancreatic acinar cells in the immunostained pancreatic sections of MSC-sEVs treated with BAX compared to groups II and III.

Immunological localization of Bax in the pancreas of the healthy rats revealed low expression of Bax in the islet of Langerhans, as established by **Mazen *et al.*** [17]. Bax immunostaining in the islet of Langerhans was much more intense in the pancreatitis diseased rats. Microvesicle-treated rats, showed just a modest immunological response in a handful of islets of Langerhans cells.

Chen *et al.* [26] used the bTC-6 murine B-cell line to examine how hypoxia affected the expression of apoptosis-related proteins, finding that, beta cells were protected from hypoxia-induced apoptosis due to the EVs action. When it comes to boosting islet cell survival and function, MSC-sEVs are on par with their parent MSCs. MSC-sEVs have been demonstrated to have a cytoprotective impact [27], which may be due to the presence of VEGF within the EVs.

In a different experiment done by **Kordelas *et al.*** [28], it was found that MSC-sEVs had the capacity to increase angiogenesis and boost the survivability of transplanted pancreatic islets. This discovery indicates that MSC-sEVs may be used to improve the efficiency and success of pancreatic islet transplantation. Research shows that people with diabetes can benefit from using MSC-sEVs because they improve the viability and functionality of the encapsulated islets. Comparable results were observed by **Abdel Aziz *et al.*** [29], who found that MSC treatment significantly increased angiogenic growth factor VEGF, as well as the anti-apoptotic protein bcl2, and decreased the pro-apoptotic protein Bax.

The Bax/Bcl-2 ratio in adipose-derived mesenchymal stem cells [ADSCs] was shown to be significantly elevated in a study by **Aminzadeh *et al.*** [30]. Based on their research, **Sharifi *et al.*** [31] concluded that elevated glucose levels cause apoptosis in PC12 cells via increasing the Bax/Bcl-2 ratio.

The expression levels of BAX were significantly raised in the pancreatic tissue of the T2DM group compared to the healthy ones. The mRNA and protein levels of BAX were significantly lower in the hUC-MSCs therapy group compared to the rats with DM, but Bcl-2 levels were significantly higher in the study of **Wang *et al.*** [32].

One of the hallmarks in the onset and progression of diabetes is an impaired immune system. TNF-α, a cytokine released in response to inflammation, is released by inflammatory cells and contribute significantly to IR in chronically inflamed tissues. Therefore, MSC-sEVs are viewed as an important strategy for improving the milieu of the body and modulating the immune response in the treatment of diabetes [16, 33].

In the current study, pancreatic slices from the normal control group showed either no TNF-α immunostaining or mild TNF-α expression in pancreatic acinar cells, as determined by immunohistochemical analysis. TNF-α immunostaining was performed on pancreatic sections from the diabetic rat. TNF-α expression was shown to be significantly elevated, but only in pancreatic acinar cells. TNF-α immunostaining was performed on pancreatic sections from the DMEM group. The findings demonstrated the existence of fibrotic areas and an increase in TNF-α expression in the pancreatic acinar cells. TNF-α immunostaining was performed on pancreatic sections from the MSC-sEVs-treated group. Both the pancreatic acini and islet cells showed reduced immunoreactivity to TNF-α.

Using a STZ-induced diabetic rat model, **Sun *et al.*** [16] found that tail vein infusion of hucMSC-sEVs suppressed the pro-inflammatory cytokine TNF-α. T2DM is characterized by IR, however its suppression led to a reversal of IR. Similarly, **Abdel Aziz *et al.*** [29] found that TNF-α was suppressed in response to MSC therapy.

The simultaneous inhibition of Fas and miR-375 significantly reduced apoptosis of pancreatic islets in response to inflammatory cytokines, and this was followed by an enhancement of insulin output. Immunosuppressive effects of intravenous injection of BM-MSCs co-cultured with sEVs in a mouse model. These results are accomplished by lowering circulating peripheral blood mononuclear cells [PBMC] proliferation and increasing Treg activity. sEVs formed by BM-MSCs have showed promise as a possible method for improving islet function in the setting of inflammation, as reported by **Li *et al.*** [24].

Similar to what was shown by **Dong *et al.*** [19], TNF-α levels were significantly higher in the severe acute pancreatitis group in contrast to the control group. TNF-α levels, on the other hand, were shown to be reduced when BM-MSCs treatment was administered. In a rat model of pancreatic inflammation, MSCs isolated from BM were shown to influence the immune response. By upregulating expression of anti-inflammatory mediators and downregulating expression of inflammatory factors, this modulation is accomplished.

TNF-α was one of several inflammatory markers that **Elshemy *et al.*** [20] found to be significantly higher in the DM group compared to healthy rats. In contrast, it was revealed that all groups treated with mesenchymal stem cells-conditioned media [MSCs-CM] displayed considerable decreases in all inflammatory markers in compared to the DM group.

Liu *et al.* [34] and **Tu *et al.*** [35] reported that injecting MSCs into the tail vein of rats resulted in a number of positive outcomes. The structural integrity of acinar cells is maintained, pancreatic angiogenesis is promoted, inflammation is significantly dampened, and acinar cells are less likely to die.

Conclusions: Mesenchymal stem cells derived microvesicles therapy caused marked restoration of normal pancreatic architecture on pancreas in experimentally induced diabetes in adult male albino rats, decreased inflammation by lowering immunoreactivity to TNFα in pancreatic acini and islet cells and decreased apoptosis by lowering BAX immunoreactivity.

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