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Journal of Bioscience and Applied Research

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Effect of anti-sca-1⁺ on diabetic rat: histological, immunohistochemical and biochemical evaluation

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Abstract

Diabetes mellitus is a disease of metabolic dysregulation most notable glucose metabolism accompanied by long term complications. The present study was carried out to investigate the potential effect of Anti-Sca-1⁺ stem cells on type 1 diabetes induced in albino rats. The study was carried on 7 male and 24 female Albino rats (150 ± 5g). The female albino rats were divided into three main groups: normal control, diabetic group and diabetic rats treated by Anti-Sca-1⁺. Induction of diabetic rats was carried out by single dose injection of fasting rats for 48 h with a diluted solution of 40 mg/kg of alloxan monohydrate (2%) in freshly prepared saline solution. Diabetic rats treated group was slaughtered into two phases; the first phase after 15 days of treatment, and the second phase after 30 days of treatment. Tissue samples were separated and kept in absolute 10% formalin solution for histological and immunohistochemical studies. SRY gene expression and biochemical study of glucose tolerance, c-peptide, insulin, glycated hemoglobin and glucose transporter 2 was evaluated. The results showed that, treatment of diabetic rats, with derivative bone marrow (Anti-Sca-1⁺) could greatly ameliorate glucose, insulin, c-peptide, HbA1c and glut2, Hafter 15 and 30 days of treatment, as compared to normal control group and these were confirmed by histological and immunological investigations, which indicate significant progress in the pancreas tissue, by marked significant increase of pancreatic islets size and improvement in the acinar cells after 30 days of treatment by Anti-Sca-1⁺.

1 Introduction

Diabetes has increasingly become a worldwide health problem, causing huge burden on healthcare system and economy (Wu, 2014). Diabetes has become one of the most serious threats to global public health, with an estimated worldwide prevalence of 171 million cases among the adult population (Hilary et al., 1998). The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, keto-acidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death (Craig et al., 2009).

Diabetes mellitus classified into; Type 1 or Insulin-Dependent Diabetes Mellitus (IDDM) also known as Juvenile Onset Diabetes, Type 2 or' Noninsulin-Dependent Diabetes Mellitus (NIDDM) also known as Adult Onset Diabetes and Gestational diabetes (Alberti et al. (1998). Type 1 diabetes is a chronic autoimmune disease that involves the progressive destruction of pancreatic β-cells, ultimately resulting in the loss of insulin production and secretion (Chhabra and Brayman, 2013). Insulin injection, the predominant treatment for T1D, is effective to ameliorate the hyperglycemia but incompetent to relieve the autoimmunity and to regenerate lost islets (Wu, 2014).

Alloxan (2,4,5,6-tetraoxy pyrimidine; 5,6-dioxyura-cil) has a diabetogenic properties reported many years later by Dunn and McLethie (1943), who studied the effect of its administration in rabbits and reported a specific necrosis of pancreatic islets. Since then, alloxan diabetes has been commonly utilized as an animal model of insulin-dependent diabetes mellitus (IDDM) (Szkudelski, 2001).

Adult stem cells, also called somatic stem cells, are stem cells which maintain and repair the tissue in which they are found. They can be found in children, as well as adults (Jiang et al., 2002). Bone marrow is a rich source of adult stem cells (Narasipura et al., 2008), which have been used in treating several conditions including spinal cord injury (William et al., 2011), liver cirrhosis (Terai et al., 2006) chronic limb ischemia (Subrammaniyan et al., 2011) and end stage heart failure (Madhu, 2007). Much adult stem cell research has aimed to characterize their potency and self-renewal capabilities (Gardner, 2002).

Stem cell antigen-1 (Anti-Sca-1^{+}) is an 18-kDa mouse glycosyl phosphatidyl inositol-anchored cell surface protein (GPI-AP) of the Ly6 gene family. Originally identified as an antigen upregulated on activated lymphocytes more than 30 years ago (Yutoku et al., 1974), Anti-Sca-1^{+} is the most common marker used to enrich adult murine hematopoietic stem cells (HSCs) (Okada et al., 1992) and can be used to isolate a nearly pure HSC population when used in conjunction with additional markers (Takano et al., 2004). The present work was designed to investigate the potential effect of Anti-Sca-1+ on type 1 diabetes, Alloxan-induced in rat.

2 Materials and Methods

2.1. Animals

The study was carried out on 7 male and 24 female Albino rats (150 ± 5 g) obtained from Biological Products and Vaccines Authority (BOVA), Helwan, Egypt. The animals were housed in cages in an environmentally controlled animal facility (room temperature about 25°C, 12 h light and 12 h dark cycle), with free access to a standard commercial diet and water was ad libration at the animal house of faculty of science, Menoufia University, Approval Number (MNI119). The weight gain, food and water intake were determined daily in the morning. The experiment was conducted for a period of (6) weeks. All aspects of animal care, complied with the ethical guidelines and technical requirements approved by the Institutional Animal Ethics Committee.

Experimental Design

Female albino rats were divided to 3 main groups; **Group (1)**: 6 rats served as a normal control, **Group (2)**: 6 diabetic rats untreated. Induction of diabetic rats was carried out by injection of fasting rats for 48 h with a diluted solution of 40 mg/kg of alloxan monohydrate (2%) in freshly prepared saline solution (Carvalho *et al.,* 2005). Diabetes was confirmed when blood glucose was equal to or higher than 16.7 mmol/l (300 mg/dl) on at least two consecutive occasions within the first week. Rats whose blood glucose remained lower than 16.7 mmol/dl after 1 week were withdrawn from the study. **Group (3)**: 12 diabetic rats treated by single dose of (10^8) undifferentiated male Anti-Sca1^{+} stem cells. 6 rats were randomly selected for sacrificed after 15 & the other 6 rats sacrificed after 30 days.

2.2. Separation of mononuclear cells from Bone Marrow Blood Cells.

Bone marrow samples were collected from femur and tibias by flushing the shaft with phosphate buffer saline using a syringe with 26G needle. Bone marrow blood was diluted with running buffer to 3:1, and 9 ml of diluted blood cells suspension was carefully layered over 3 ml Ficoll Hypaque (1.077 density) in a 15 ml conical tube and centrifuged at 1000 rpm for 30 minutes at +4°C in a swinging-bucket rotor without brake. The upper layer was aspirated off leaving the mononuclear cell layer undisturbed at the interphase, and then the interphase cells (lymphocytes and monocytes) were carefully transferred to a new 15 ml conical tube and filled with PBS containing 2 mM EDTA. Cells were mixed well and centrifuged at 1000 rpm for 10 minutes at +4°C then the supernatant was carefully removed completely. The pellet was resuspended in 15 ml PBS containing 2 mM EDTA and centrifuged at 1000 rpm for 10minutes at +4°C, then the supernatant was carefully removed completely. This step was repeated at the same condition. Viability test of mononuclear cells was carried out by mixing one drop of cell suspension with one drop of 0.4% trypan blue vital stain in separate eppendorf tube and cell counting was done using haemocytometer. The pellet was resuspended in a final volume of 300 μl / 10^8 total cells, and then magnetic labeling was proceeded (Ullah *et al.,* 2015).

Magnetic labeling of Anti-Sca-1^{+}and separation with auto MACS separator.

Cells were disaggregated by gently pipetting several times, and then passed through 30 μl nylon mesh (Pre-Separation Filters# 130-041-407) to remove cell clumps. Cell pellet was resuspended in 90 μL running buffer (MACS separation buffer containing 0.5% bovine serum albumin, phosphate-buffered saline, pH 7.2, and 2 mM EDTA and 0.09% sodium azide). 10 μl of Direct Anti-Sca-1^{+} Progenitor Cell Isolation kit was added to cell suspension and mix well then incubated for 30 minutes in the refrigerator at +4°C), washed by adding 1-2 mL of buffer and centrifuged again in +4°C cooling centrifuge at 1000 rpm for 10 minutes. Column was placed in the magnetic field of MACS Separator. Cell suspension was then applied on to the column. Anti-Sca-1^{+} cells were attached to the column while. After complete separation, the separation column was separated from the column and Anti-Sca-1^{+} was eluted by the use of running buffer to undergo proliferation in *vitro* (Bosio *et al.,* 2009).

2.3. Stem cells proliferation.

Anti-Sca1$^+$ stem cells were counted and there viability was insured by 0.4% trypan blue vital stain test before seed in cell culture flasks. Cells were re-suspended in MEM culture medium supplemented with $1X10^6$ unit penicillin, 1 g streptomycin and 1 g garamycin 80/ liter at concentration $5X10^6$ cells/ $75cm^2$ flask and incubated at 37 °C in 5% (v/v) humidified CO_2 for 15 days, for formation of large colonies for Anti-Sca-1$^+$ (hematopoietic stem cells) **(Rochefort et al., 2005)**.

Treatment of diabetic rats:

Treatment process was done by intravenous injection for diabetic rats by a single dose of (10^8 resuspended in 25µl running buffer) (10^8) Anti-Sca1$^+$ for each rat.

2.4. Histological investigation

After dissection, small pieces of pancreas tissue were immediately removed from each group and fixed in 10% neutral formalin. Specimens were dehydrated through ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraplast (m.p. 55-58 °C). Sections of five micrometer thickness using any adhesive media. Section were stained with Ehrlich's Hematoxyline and counter stained with eosin.

2.5. Immunohistochemical investigation

Pancreas was taken from animals of both control and experimental group that were treated for 15 & 30 days. Immediately after dissection, tissue fixed in 10% neutral formalin and then 5 micrometer thickness paraffin section was prepared. Sections were stained immunohistochemically for visualizing insulin positive cells, using suitable antibody in staining time according to the method of Hsu *et al.* (1981).

2.6. Molecular study

DNA fragmentation, and SRY gene were detected by Polymerase Chain Reaction (PCR), in pancreas tissue according to "salting out extraction method" of Aljanabi and Martinez (1997) with some technical modification according to (Hassab El-Nabi 2004, Hassab El-Nabi and Elhassaneen, 2008).

2.7. Biochemical analysis

Blood samples were collected from all animals after light anesthesia. The blood, cooled in clean tubes, contains heparin and centrifuged at 3000 rpm for reparation of plasma. Glucose was measured by using blood plasma samples and kits (Spinreact, Spain) according to the method of (Kaplan, 1984). Glucose transporter 2 (Glut2) was determined by Quantitative detection of glut2 gene expression using the method of (Pfaffl, 2001). Insulin was determined according to León and Stanley (2013) C-peptide was measured by the method of (Ashby and Frier, 1981).

2.8. Statistical analysis.

Data were expressed as mean ± SE and analyzed using the Statistical Package of Social Sciences (SPSS) program version 17 and Excel version 2007, (Chicago, IL, USA).

3 Results

3.1. Histological observations

Histological examination of pancreas of the control group showed both of exocrine and endocrine tissues. The exocrine tissue consists of closely packed acini, with a connective tissue in between. The cells of the acini are pyramidal in shape, with very small acinar lumen. They have rounded basally located nuclei and a cytoplasm which is characterized by the presence of zymogene granules. The pancreatic ducts can be classified into three types: intercalated ducts, intralobular ducts and the main pancreatic duct. The three ducts are formed of flattened cuboidal epithelium cells. The endocrine tissues are the islets of langerhans, appeared as pale and rounded or oval masses, scattered in between the acini and the containing alpha and beta cells, which could not be easily differenced in Hematoxyline and Eosin staining sections **(Figs. 1a & b)**. Examination of pancreas of diabetic rats revealed many histological alterations. The lobes are widely separated from each other and the blood vessels are congested **(Figs.1c & d)**. The acinar cells were degenerated and their nuclei were pyknotic and showed reduced zymogenic contents **(Fig. 2a)**, with leukocyte infiltration **(Fig. 2b)**. The pancreatic islets showed cytoplasmic vacuolation and the blood capillaries inside the islets were congested **(Fig. 2c)**. Animals of their groups still showed cytoplasmic vacuolation of islets cells **(Fig. 2d)** and large degeneration area were obtained in the acinar portion, which were degenerated **(Fig. 3a)**. Congestion of blood vessels were noticed **(Fig. 3b)**.

3.2. Immunohistochemical observations

Pancreas of control rats showed that Langerhans islet cells have very strong positive immunohistochemical reaction for insulin hormone in cytoplasm, with a normal cluster shape **(Fig. 4a)**. Pancreas of diabetic rats showed very week positive immunohistochemical reaction for insulin hormone in Langerhans islet β cells **(Fig. 4b)**. Pancreas of diabetic rats treated with Anti Sca-1$^+$ stem cells and examined after 15 days showed weak positive immunohistochemical reaction for insulin hormone **(Fig. 4c)** and those which were examined after 30 days showed mild positive immunohistochemical reaction for insulin **(Fig. 4d)**.

3.3. Molecular results
PCR products of *SRY* gene

As shown on **(Fig. 5)**, positive PCR product of *SRY* gene was expressed in male rats (lane 2, 104 bp), diabetic female rats with Anti-Sca-1$^+$ and examined after 15 days

(lane 4, 114 bp) and diabetic female rats with Anti-Sca-1$^+$ and examined after 30 days (lane 5, 119 bp).

DNA fragmentation

An increase in DNA fragmentation was recorded in diabetic rats (lane 2) compared to control ones (lane 1) **(Fig. 6)**. In case of treatment with Anti-Sca-1$^+$ an observable decreased of DNA damage was detected after 30 days of treatment (lane 4) when compared with rats after 15 days of treatment (lane 3).

3.4. Biochemical results

Administration of alloxan induced very high significant ($p<0.001$) increase in levels of plasma glucose **(Fig. 7)** and HbA1c **(Fig. 8)** as compared to control group. Treatment of diabetic rats by Anti-Sca-1$^+$ showed very high significant ($p<0.001$) decrease in glucose and HbA1c levels after 15 days and very high significant ($p<0.001$) decrease after 30 days of treatment comparable to control rats. The concentrations of C-peptide in blood plasma of diabetic rats show a very high significant decrease **(Fig. 9)** as compared to control group. Diabetic group treated with Anti-Sca-1$^+$ exhibited a significant increase in C-peptide concentration after 15 & 30 days of treatment comparable to diabetic group. The results revealed a very high significant increase in concentration of Glut2 **(Fig. 10)** and insulin **(Fig. 11)** levels in pancreas tissue of diabetic rats after 15 days and 30 days of treatment with Anti Sca-1$^+$ compared to diabetic group.

1(a)

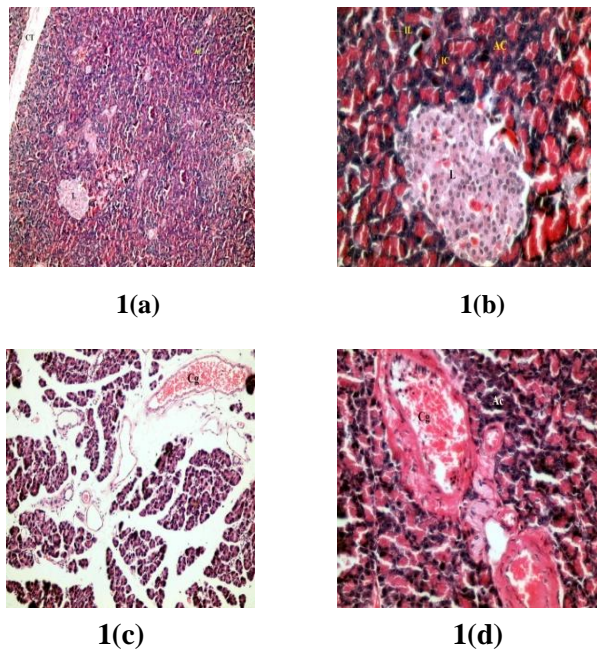**1(b)**

1(c)

1(d)

Figure (1a): Pancreas of a control rat showing acini (AC) and connective tissue (CT) between the lobes and Langerhans islet (L) (H&E stain, X 100), **1(b):** Higher-magnification of pancreas of a control rat showing acini (AC),intercalated ducts (IC), interlobular ducts (IL) and Langerhans islet (L) (H&E stain, X 400), **1(c):** pancreas of a diabetic rat showing congestion of blood vessels (Cg) and separation of the lobes from each other (H&E stain, X 100)

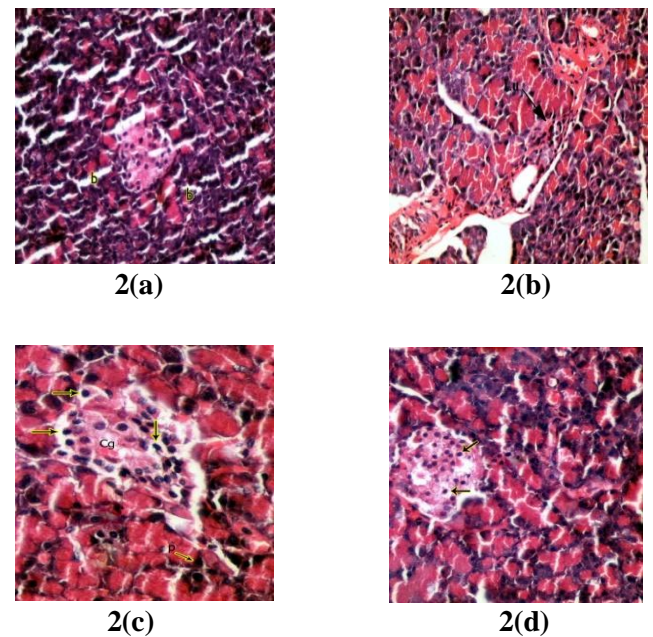, **1(d):** pancreas of a diabetic rat showing congestion of blood vessels (Cg) (H&E stain, X 200).

2(a)

2(b)

2(c)

2(d)

Figure (2a): Pancreas of a diabetic rat showing pyknotic nuclei (P) in acinar cells, (H&E stain, X200), **(2b):** Langerhans islet of a diabetic rat showing leukocyte infiltration (Lu) (H&E stain, X400), **(2c):** Langerhans islet of a diabetic rat showing pyknotic nuclei (arrows) (P), cytoplasmic vacuolation (V) and congestion in blood capillaries (Cg) (H&E stain, X 400)and **(2d):** Langerhans islet of a diabetic rat treated by Anti-Sca-1+ stem cells and examined after 15 days showing a vacuolation of cytoplasmic islet cells with pyknotic nuclei (arrows) (P) (H&E stain, X 200).
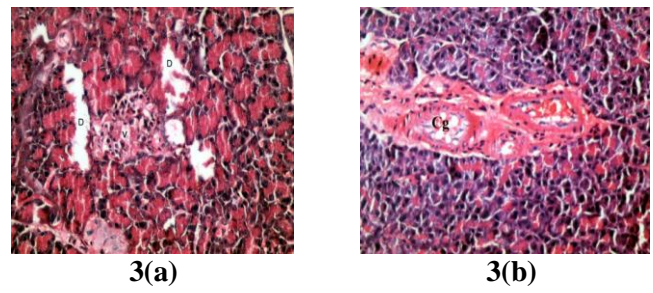

3(a)

3(b)

Figure (3a): Langerhans islet of a diabetic rat after 15 days of treatment with Anti-Sca-1$^+$ stem cells showing vacuolation in cytoplasm of islet cells with large area of degenerated acinar cells. (H&E stain, X 200) and **(3b):** langerhans islet of a diabetic rat after30 days of treatment with Anti-Sca-1$^+$ stem cells showing moderate blood vessel congestion (H&E stain, X 200).

4(a)   **4(b)**
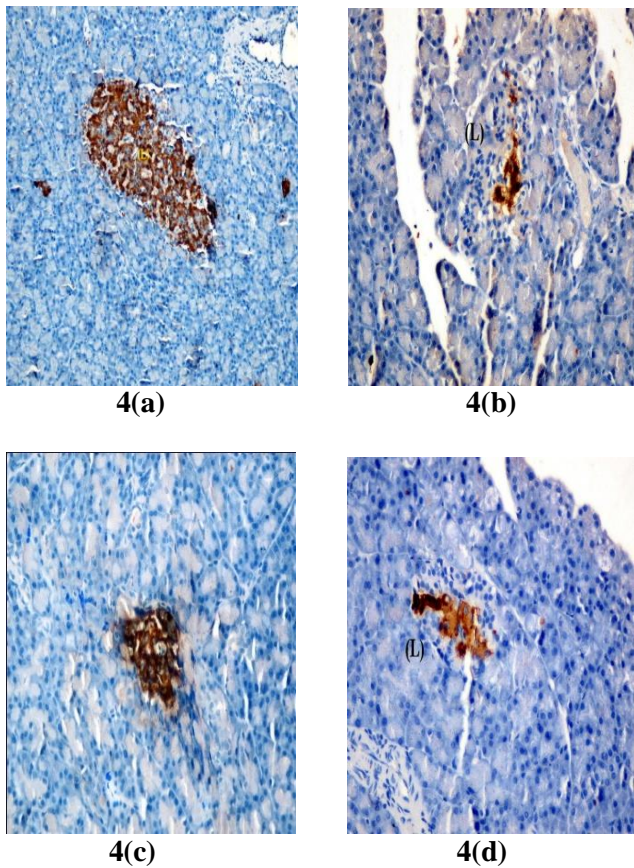

4(c)   **4(d)**

Figure (4a): Section of pancreas langerhans islet of a control rat showing insulin reactivity in langerhans islet, X200 **(4b):** Section of pancreas of a diabetic rat showing few cells that have very week positive insulin reactivity,X200 **(4c):** Section of pancreas langerhans islet of a diabetic rat treated with Anti-Sca-1$^+$ and examined after 15 days showing an increase in insulin reactivity in langerhans islet (L) X200 **(4d):** Section of pancreas langerhans islet of a diabetic rat treated with Anti-Sca-1$^+$ and examined after 30 days showing an improvement of insulin ,X200.
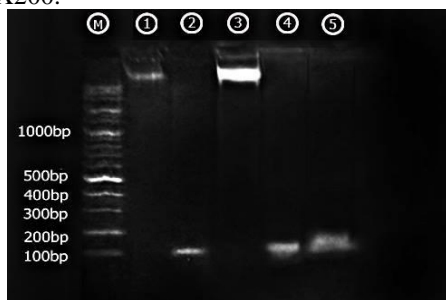

Figure (5): 1.8 % Agarose gel electrophoresis stained, with 0.5 µg µL-1 Ethidium Bromide of PCR product of *SRY* gene in different studied groups shows: **M-** DNA Ladder, **1-** Female control rats**, 2-** Male control rats, **3-** Female diabetic rats, induced by alloxan, **4-** Female treated rats by Anti-Sca-1$^+$ (after 15 days), **5-** Female treated rats, by Anti-Sca-1$^+$ (after 30 days)**.**
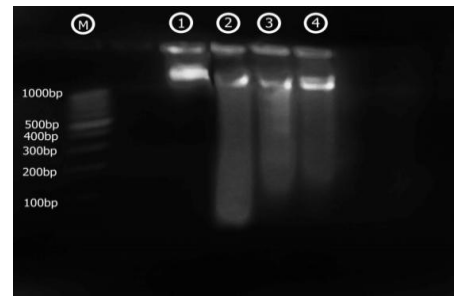

Figure (6): Agarose gel electrophoresis stained, with Ethidium Bromide of DNA fragmentation in different studied groups shows: **M-** DNA Ladder, **1-** Control rats, **2-** Diabetic rats, **3-** Female treated rats, by Anti-Sca-1$^+$ cells (after 15 days)**, 4-** Female treated rats, by Anti-Sca-1$^+$ cells (after 30 days)**.**
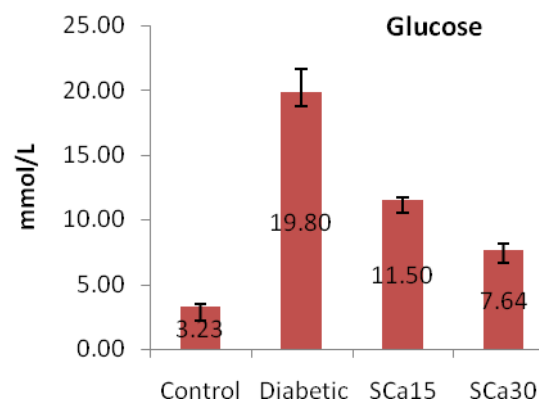

Figure (7): Showing the change of glucose level in diabetic rats induced by alloxan and treated with Anti-Sca-1$^+$ after 15 & 30 days.
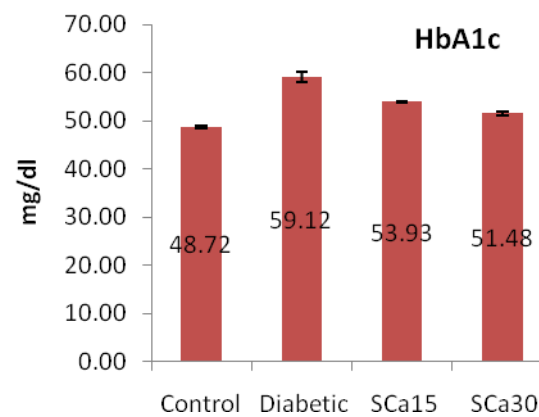

Figure (8): Showing the change of HbA1c level in diabetic rats induced by alloxan and treated with Anti-Sca-1$^+$ after 15 & 30 days.
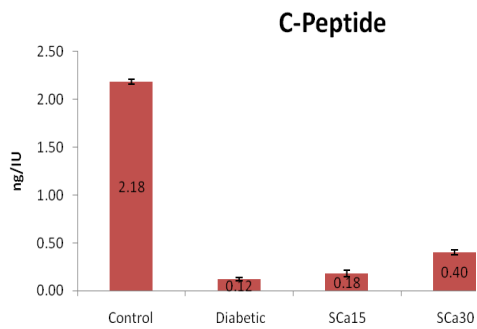
C-Peptide

Figure (9): Showing the change of C-peptide level in diabetic rats induced by alloxan and treated with Anti-Sca-1^{+} after 15 & 30 days.

Glut 2
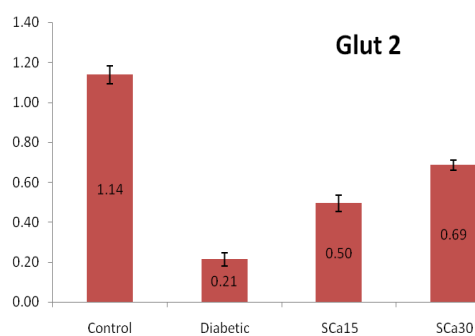

Figure (10): Showing the change of Glut2 level in diabetic rats induced by alloxan and treated with Anti-Sca-1^{+} after 15 & 30 days.
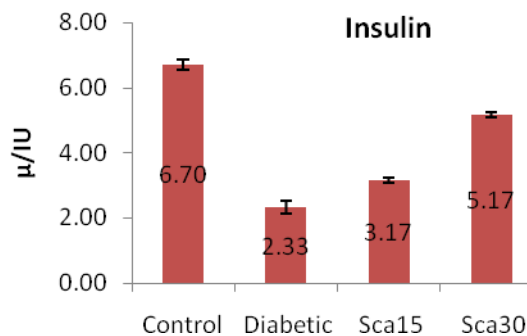
Insulin

Figure (11): Showing the change of insulin level in diabetic rats induced by alloxan and treated with Anti-Sca-1^{+} after 15 & 30 days.

4 Discussion.

Diabetes mellitus is a common disease affecting approximately 5% of the world population. The effects of diabetes mellitus include long-term damage, as well as dysfunction and failure of various organs. Diabetes mellitus may be present with characteristic symptoms, such as: thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, keto-acidosis or a non-ketotic hyperosmolar state may develop and lead to coma and, in absence of effective treatment, death (WHO, 1985). Diabetes mellitus is a disease of metabolic dysregulation most notable glucose metabolism accompanied by a long term complication. The complication that are specific to diabetes include retinopathy, nephropathy and neuropathy (Nathan, 1993).

The American Diabetes Association (2009) classified the diabetes into two broad categories; type 1 diabetes, accounts for ~5–10% of those with diabetes and is an autoimmune disease that results in the destruction of pancreatic β-cells, causing an absolute insulin deficiency, the other, the most common form is type 2 diabetes, which accounts for >90% of those with diabetes and is caused by a combination of insulin resistance, with a relative, but not absolute insulin deficiency. The control and management of blood glucose levels in both forms of diabetes is important for preventing ketoacidosis and diabetes-related complications. Peoples with type 1 diabetes, produce little or no endogenous insulin, administration of exogenous insulin is necessary for survival (Devendra *et al.,* 2004). Alloxan is the most commonly used chemical for induction of diabetes mellitus. It is a well-known diabetogenic agent widely used to induce type 1 diabetes mellitus in animals (Viana *et al.,* 2004).

The present study was designed to assess the effect of alloxan on β cells of pancreas and threw more light on the potential improvement in experimental diabetic rats by treatment with Anti-Sca-1^{+} stem cells.

Data of the present study showed that alloxan induced diabetes type1 which caused some biochemical, histological and molecular changes represented by destruction of β cells in pancreas tissue. The biochemical results showed very highly significant decrease in insulin, C-peptide and Glut2 levels, accompanied with increase of glucose and HbA1c concentrations after48 h of intravenous injection by alloxan as compared with normal control group.

These results are in agreement with Nurdiana *et al.,* 2013, which showed that alloxan induced diabetic rats, together with corresponding increase in plasma glucose level as well as with Tantawy and Haleem, 2014, who revealed that the injection of alloxan into rats resulted in a significant increase in serum glucose. Adeyi *et al.,* 2012, reported that alloxan is a cytotoxic agent to the insulin-secreting β-cells of the pancreas, effectively induces insulin dependent phenotypes that resemble type 1 diabetes or post-beta cell "burnout" type 2 diabetes in a wide variety of animal models. Thus, it allows elucidation of anti-hyperglycemic agents in the treatment of diabetes. Also, Davey *et al.,* 2014, showed that Type 1 diabetes mellitus (T1D) is caused by β-cell destruction. The pathogenesis of (T1D) is the autoimmune destruction of the pancreatic β-cells that leads to loss of insulin secretion and absolute insulin deficiency.

The histological investigation of this study showed that the diabetic rats revealed many histopathological alterations including wide separation between the lobes, congestion of

blood vessels and capillaries, reduction of zymogenic content degeneration of acinar cells and their nuclei were pyknotic. Similarly, Godam *et al.* 2014, showed that alloxan caused severe necrotic changes of pancreatic islets, especially in the centers of islets. Minamia *et al.* 1999 revealed that degranulation of zymogen and duct-like structures of exocrine cells and atrophy and disappearance of islet cells were observed in alloxan-injected. Qadori, (2011) reported that the pancreas of diabetic rats showed nuclear changes, karyolysis, disappearing of nuclei and in some places residues of destructed cells were visible. Relative reduction of size and number of islets, especially around the large vessels and severe reduction of β-cells were observed. Besides, shrinkage of islet Langerhans in size with necrosis of β-cells destruction and reduction of number and diameter of islets were noticed.

The immunohistochemical results of the present studies showed that the diabetic rats revealed irregular shape of langerhans islet, with a very weak positive immunohistochemical reaction for insulin hormone in β cells. These results are compatible with Abdul-Hameid and Moustafa, (2013), who reported that the immunoreactivity for anti-insulin antibodies was markedly decreased in number of insulin positive cells compared with control. The β-Cells of diabetic rats showed obvious vacuolation and decrease in secretory granules, fusion of some granules and pyknotic nuclei. Kikumoto *et al.* (2010) reported that a higher dose of alloxan accelerated severe atrophy of pancreatic islets and induced pancreatic β cell apoptosis in acatalasemic mice in comparison to a wild-type mice.

The molecular results in this study refers to a positive PCR product of SRY gene which showed a significant decrease of fragmented DNA after 30 days of treatment in diabetic rats, than those of the diabetic rats which examine after 15 days, as compared to control and diabetic groups. This result is in agreement with the study of Sigmon & Larcom, (1996), which showed that the mode of binding of ethidium bromide is intercalation between the base pairs. This binding changes the charge, weight, conformation, and flexibility of the DNA.

The obtained results revealed that Anti-Sca-1^{+} improved the histological, immunohistochemical and biochemical alterations caused in diabetic rats. There several studies indicated that under specific *in vitro* culture conditions, mesenchymal stem cells derived from bone marrow can be induced to differentiate into functional islet cells capable of normalizing hyperglycemia in a diabetic animal model (Tang *et al*. 2004; Chen *et al*. 2004). Shapiro *et al*. (2000), mentioned that the islets transplantation has been shown to restore normal glycemia in type 1 diabetes mellitus. Soria *et al*. (2000) stated that the use of a renewable source of stem cells capable of self renewal and differentiation as well as insulin production.

Ryan *et al*. (2002) mentioned that after initial report, follow-up studies results of stem cells transplanted patients continued to show positive results including significant decreases in fasting and postprandial glucose levels, normalized HbA1c levels, and improved fasting and postmeal C-peptide secretion as well as increased acute insulin responses to arginine and intravenous glucose tolerance test. Lin *et al*., (2009), reported that ELISA analyses showed that the transducer cells secreted an increasing amount of insulin in response to increasing concentration of glucose. Transplantation of these cells, under the renal capsule of diabetic rats, resulted in lowered blood glucose, higher glucose tolerance, smoother fur and less cataract.

Transplanted or co-transplanted MSCs decrease proliferation and activation of T-cells, dendritic cells, and NK cells in the recipient. Additionally, they act as immune-modulator and help in the establishment of a graft vascular network by secreting angiogenic paracrine factors and increase the expression of protective genes against hypoxia (Ding *et al.,* 2009). Borg *et al.*, (2014) reported that co-transplantation of MSCs with pancreatic islets in mice leads to improved islet function and survival, improved glucose homeostasis and reduced islet apoptosis. Tantawy and Haleem, (2014) found that the treatment of diabetic rats with MSCs stem cells significantly prevented alterations and attenuated alloxan-induced oxidative stress. Rat bone marrow harbors cells, that have the capacity to differentiate into functional insulin-producing cells, are capable of controlling glucose level. MSCs have well-characterized hypo-immunogenicity and immunomodulatory effects. These features make MSCs attractive for treating type 1diabetes (Wu. 2014).

Davey *et al.* (2014) found that concentration of insulin in blood serum was increased after MSC infusion. The damaged pancreatic islets were improved to near normal, with the ratio of insulin-positive cells per islet achieving near normal levels. Jiang *et al.* (2007) stated that the MSCs could be differentiated into insulin-producing cells following incubation under specific conditions.

The crosstalk between MSCs and pancreas in diabetic animals was revealed in some reports, even though the detailed mechanism is unclear. MSCs express a set of chemokine receptors, which may play critical roles in the pancreas homing/regeneration (Sordi *et al.,* 2005). Systemic administration of MSCs increased β-cell mass and reverted hyperglycemia in diabetic mice (Ezquer *et al.,* 2008). Moreover, MSCs merely served as a "tropic mediator" to support islet function in an indirect manner, such as promoting angiogenesis (Dor *et al.,* 2004). However, some reports showed that insulin-producing β cells can be regenerated from the transdifferentiation of α cells, pancreatic duct cells, and acinar cells (Furuya *et al.,* 2013). It is concluded from the present work that Anti-Sca-1^{+} treatment may act on the pancreas of diabetic rats by one or more of the above mentioned mechanisms.

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