In vivo mobilization of bone marrow stem cells versus injection of stem cells on experimentally induced diabetes in adult albino rats

Ashraf Mahmoud Moustafa[1], Salama Sayed Said[1], Ayman Fathy Elshaarawy[1], Rania Galhom[2] and Ibrahim Mohammed Fayed[1]

[1]Department of Histology, Faculty of Medicine, Al-Azhar University, Egypt.
[2]Department of Anatomy and Embryology, Faculty of Medicine, Seuz Canal University, Egypt.
ebrahimfayed@gmail.com

Abstract: Background: diabetes mellitus is a major health problem affecting more than 200 million of adult populations worldwide and is expected to affect at least 5 % of global population by the year 2025. **Objective:** this work was planned to evaluate the role of StemEnhance in mobilizing naturally occurring bone marrow stem cells in addition to the effect of this mobilization in improving streptozotocin-induced diabetes mellitus in rats in comparison with injection of stem cells in the adult albino rats. Histological techniques were applied in this study. **Materials and Methods: Streptozotocin (STZ):** is an antibiotic and it was purchased from Sigma Company (St. Louis, Mo, USA) in the form of powder. **StemEnhance:** capsules were purchased from STEM Tech Health Sciences (San Clemente, CA, USA) in the form of a bottle contained 50 capsules each contained 500 mg of L-selectin ligand enriched fractions of *Aphanizomenon flos-aquae (AFA)*. This study included forty four adult male albino rats (160-200 g). **Results:** the mean values of blood glucose level of diabetic (GII), diabetic-StemEnhance (GIV) and stem cells treated group (GV) were significantly increased in comparison with the control (Gs I & III). However it was increased in group GV in comparison with group GIV. This decrease may be related to insulin secreted by the newly differentiated β cells from the mobilized HSCs. However, blood glucose level decreased after StemEnhance administration. **Conclusion:** StemEnhance and endogenous injection of stem cells mobilized BM stem cells were differentiated into pancreatic islet β cells who ever its more occur in exogenous injection in comparison with endogenous mobilization.

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1. Introduction

Diabetes mellitus has two forms: type I (insulin-dependent) caused by an autoimmune destruction of the insulin-producing β-cells and type II (noninsulin-dependent), results from a combination of reduced insulin sensitivity of tissues and impaired function of the insulin-secreting β-cells **(1).** Treatment of diabetes by different regimens of insulin injections failed to offer complete cure and could not prevent secondary complications associated with diabetes, such as diabetic retinopathy, nephropathy and neuropathy. Moreover, insulin therapy was frequently associated with severe hypoglycemic episodes **(2).** Early treatment of patients by restoration of their β-cell function through pancreas or islet transplantation can relieve the patients from their dependency on insulin, achieve lifelong normoglycemia and prevent related complications **(3).**

A study concerned with using highly purified mice bone marrow stem cells for islet beta-cells repopulation was done by **Ianus *et al.*** **(4).** Another study concerned with differentiation of rat bone marrow mesenchymal stem cells into pancreatic islet beta-cells was done by **Chen *et al.*** **(5).**

StemEnhance™ capsules contained a blend of the cytoplasmic and cell wall fractions of *Aphanizomenon flos-aquae* (AFA) plant which were enriched by L-selectin ligand (LSL)**.** L-selectin ligand supports the release of stem cells from the bone marrow **(6).**

Aim of the Work

This work was planned to evaluate the role of StemEnhance in mobilizing naturally occurring bone marrow stem cells in addition to the effect of this mobilization in improving streptozotocin-induced diabetes mellitus in rats in comparison with injection of stem cells in adult albino rats. Histological techniques were applied in this study.

2. Material and Methods

A-Material

Drugs

Streptozotocin (STZ): is an antibiotic and it was purchased from Sigma Company (St. Louis, Mo, USA) in the form of powder.

StemEnhance: capsules were purchased by STEM Tech Health Sciences (San Clemente, CA, USA) in the form of a bottle contained 50 capsules each contained 500 mg of L-selectin ligand enriched fractions of *Aphanizomenon flos-aquae* (AFA).

Animals:

This study included fifty five adult male albino rats (160-200 g). They were housed in Al- Azhar animal house and they were categorized into four groups:

Group I: (the control group = GI) 10 rats received no treatment.

Group II: (the diabetic group = GII) 10 rats. Diabetes was induced by a single intraperitoneal (i.p.) injection of streptozotocin (STZ) 60 mg/kg body weight [(7)]**;** then this group was left untreated. Diabetes was confirmed by measuring the blood glucose level. The animals were considered diabetic if their blood glucose level was higher than 200 mg/dL [(8)].

Group III: (positive control-StemEnhance group = GIII) 10 rats treated daily orally with StemEnhance 270 mg/kg body weight dissolved in distilled water by gastric gavage till the day of sacrificing.

Group IV: (diabetic-StemEnhance group = GIV) 10 rats. Diabetes was induced by streptozotocin (STZ) 60 mg/kg body weight, by single i.p. injection then after the blood glucose test confirmed that the animals became diabetic (nearly on the third day) rats received daily StemEnhance (270 mg/kg body weight) dissolved in the distilled water orally by gastric gavage till the day of sacrificing. This dose was equivalent to the human dose of 6 capsules 3000 mg/day as recommended by STEM Tech Health Sciences (San Clemente, CA, USA). The dose was calculated as follows: human equivalent dose (mg/kg) =animal dose (mg/kg) multiplied by animal km divided by human km. Km factor is the representative surface area (m^2) to the body weight (kg) ratio. km of rat =5.9 while, km of adult human =37 [(9)]**.**

Group V: (diabetic-Stem cells group = GV) 10 rats. Diabetes was induced by streptozotocin (STZ) 60 mg/kg body weight, by single i.p. injection then after the blood glucose test confirmed that the animals became diabetic (nearly on the third day) rats were injected with 500000 cells of MSCs via rat's tail vein for each rat. The rats were sacrificed by using chloroform inhalation, 10 rats from the diabetic-StemEnhance group (group IV) in addition to10 rats from stem cells treated group (group V)in addition to ten rats from each of the groups I, II and III.

Methods

Induction of D.M:

STZ 60 mg/kg was solubilized in sodium citrate buffer in Biochemistry Department, Faculty of Medicine, Al-Azhar University. The solution was prepared at pH 4.5 and injected i.p. within 15 min of its preparation [(7)]. This procedure aimed to induce type I DM [(8)]**.**

Treatment of D.M:

StemEnhance was solubilized in distilled water and was given by using gastric gavage at the dose of 270 mg/kg. StemEnhance was given daily after the blood glucose test confirmed that the animals became diabetic (nearly on the third day) and continued till the day of sacrificing.

Laboratory Investigation:

Random blood sugar was measured daily for rats of all groups in the Histology Department (Al-Azhar University). The blood samples were obtained from the rat tail vein.

Histological Study:

The animals were sacrificed by using chloroform inhalation and the pancreas was immediately dissected out, fixed in 10 % formol saline for 24 hours at the room temperature then dehydrated in ascending grades of alcohol (70%, 95%, 100%), cleared in xylene then embedded into paraffin wax (Histology Department, Faculty of Medicine, Al Azhar University), Sections of 5 μm thickness were stained with Hematoxylin and Eosin.

3. Results

Normal levels of glucose in healthy adult rats were measured as 135±5 mg/dl,). But, in the diabetic rats the levels of glucose were significantly increased (measured as 544±21 mg/dl, $p<0.05$) all over the study period (**Charts 1, 2**).

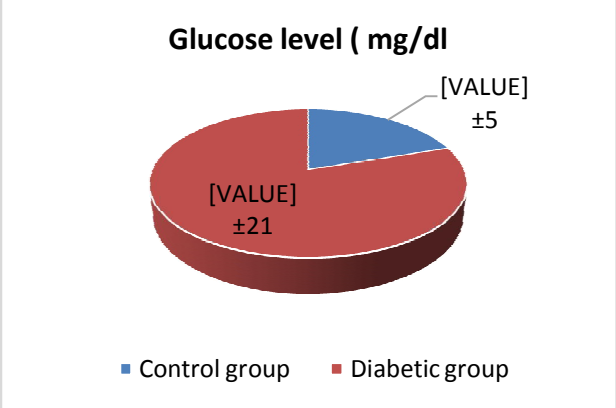

Chart 1: mean glucose level (mg/dl) in the studied groups during 14 days after induction of diabetes

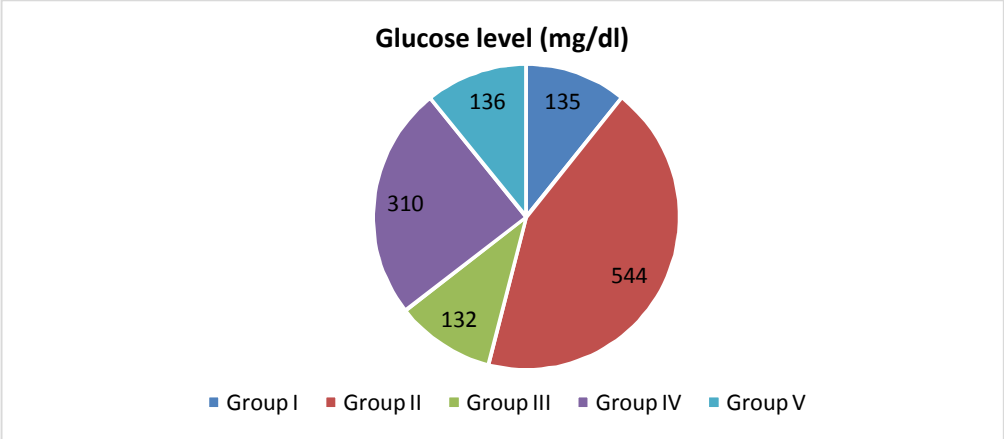

Chart 1: Mean ±SD of glucose level in the animals of the study groups

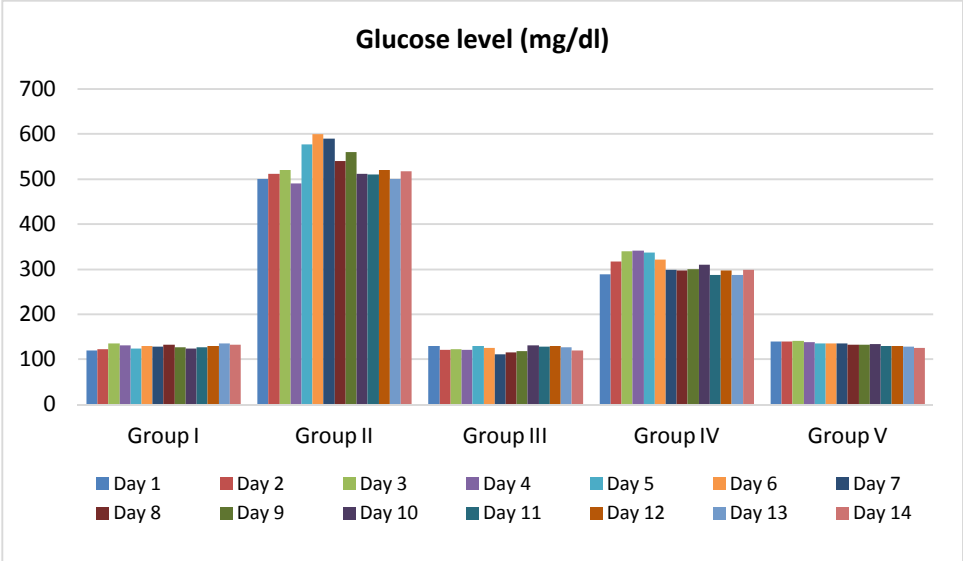

Chart 2: Mean ±SD of glucose level in the animals of the study groups during the following 2 weeks after induction of diabetes.

Hematoxylin and Eosin stained pancreatic sections

Group I (Control group):

Histological examination of pancreatic sections of rats of the control group (GI) with H & E staining revealed normal structure of the pancreas.

The exocrine component of the pancreas was consisted of closely packed secretory acini separated by inconspicuous supporting connective tissue containing numerous capillaries. Each acinus was made up of an irregular cluster of pyramidal secretory cells; their nuclei were basally surrounded by basophilic cytoplasm. The apical parts were packed with acidophilic secretory granules encircling a minute central lumen which represented the terminal end of the duct system **(Fig. 1).** The islets of Langerhans (the endocrine part) were scattered throughout the exocrine glandular tissue and varied in size and they were composed of groups of secretory cells supported by a fine collagenous network that contained numerous fenestrated capillaries. A delicate capsule surrounded each islet. Some endocrine cells were small with a pale basophilic cytoplasm while, others were large with a pale acidophilic cytoplasm. In contrast, the larger cells of the surrounding exocrine pancreatic acini stained strongly. The endocrine pancreas contained secretory cells of several types: however, in H & E stained preparations, some cell types were indistinguishable from one another. Few cells were seen with deeply acidophilic cytoplasm and small dark nuclei **(Fig. 1)**.

Group II: (The diabetic group):

Histological examination of rat's pancreatic sections of GII stained with H & E stain revealed swelling, vacuolation and degranulation of the stained cytoplasm of some islet cells **(Figs. 2).**

Group III (Positive control-StemEnhance group):

Histological examination of rat pancreatic sections (from rats treated with StemEnhance) and stained with H & E revealed normal structure of the pancreas. Islet cells showed the same features mentioned in relation to GI **(Fig. 3)**.

Group IV: (Diabetic-StemEnhance group):

Histological examination of rat pancreatic H & E-stained sections of GIV (which received StemEnhance for one week) showed some swollen and vacuolated cells. Congested capillaries were detected in some locations of the examined slides **(Fig. 4).**

Group V: Diabetic-stem cells injected group:

Histological examination of pancreatic H & E-stained sections of rats of group GV (which received stem cells) showed some swollen and vacuolated cells. Less congested capillaries were detected in some locations of the examined slides **(Fig.5).**

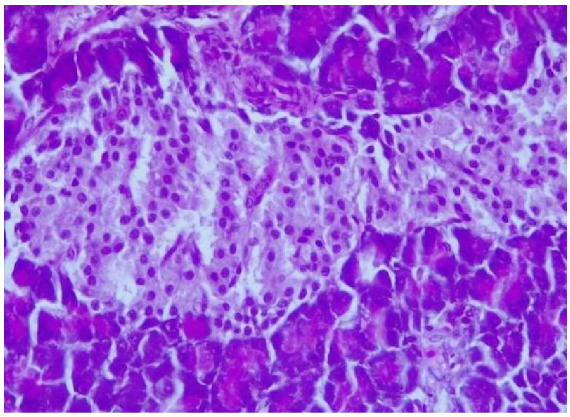

Fig. 1: a photomicrograph of a section in the pancreas of a control adult male rat (GI) showing pale stained islets rich in capillaries surrounded by many deeply stained pancreatic acini, The islet contains large acidophilic cells and small basophilic cells**. (H & Ex400)**

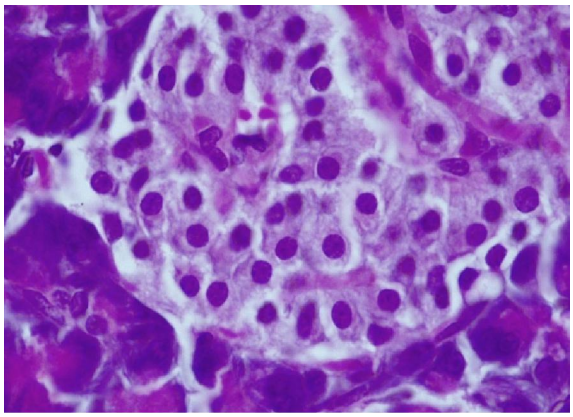

Fig. 2: a photomicrograph of a section in the pancreas of a diabetic rat GII showed atrophied islet with pale disintegrated nuclei and the intact dark cells at the periphery of the islets with normal structure of the exocrine pancreas and degranulation in the cytoplasm of many cells of an islet of Langerhans **(H & E X400)**

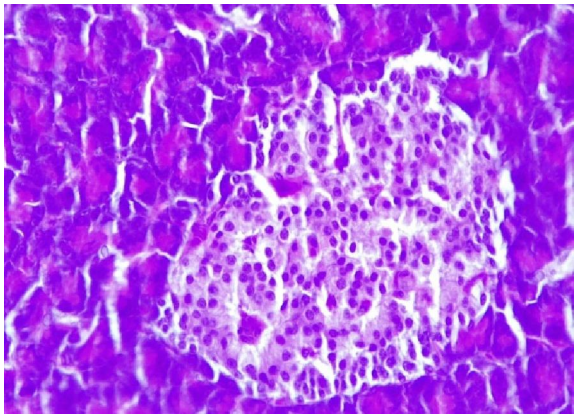

Fig. 3: a photomicrograph of a section in the pancreas of an adult male rat (GIII); pale stained islets rich in capillaries are surrounded by many deeply stained normal pancreatic acini. **(H & EX400)**

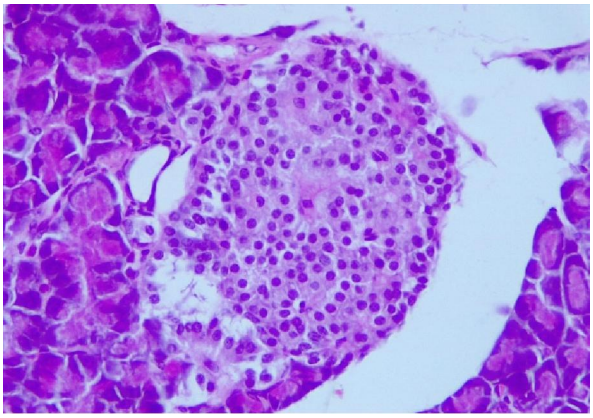

Fig. 4: a photomicrograph of a section in the pancreas of an albino rat from GIV showing pale stained islets with hypocellularity cellularity and poor vascularity between islet cell. Some islet cells have dark nuclei while, others have paler nuclei. **(H & E, x400)**

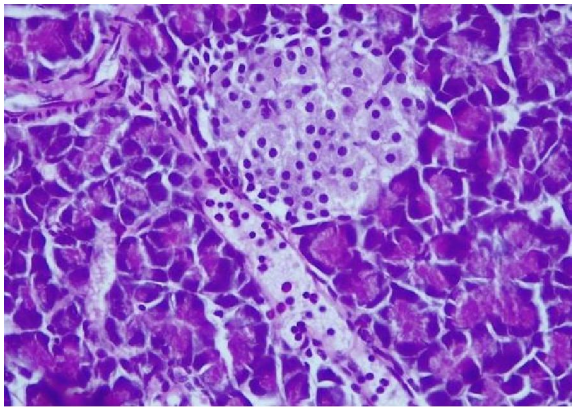

Fig. 5: a photomicrograph of a section in the pancreas of an albino rat from GV showing pale stained islets rich in capillaries with less vacuolation**. (H & EX400)**

4. Discussion

This study was an attempt to investigate the changes which occurred in the pancreas of streptozotocin-induced diabetic rats after induction of bone marrow stem cell mobilizer (StemEnhance) in comparison with induction of stem cells. Concerning the blood glucose level, the level of blood glucose level of rats of the diabetic group were significantly increased in comparison with the control group, indicating that the STZ induced DM in group II.

In accordance to our findings **Akbarzadeh *et al.*** [(11)] induced experimental DM by STZ in normal adult Wistar rats and confirmed it through several laboratory changes including increased blood glucose level. Apoptosis is a programmed individual cell death which may be induced by physiological or pathological stimuli. It includes energy-dependent fragmentation of DNA by endogenous endonucleases with intact lysosomes and cell membrane. The cell shrinks and fragments to form apoptotic bodies with dense chromatin. There is noinflammatory response and dead cells are cleared by phagocytic cells [(12)]**.** In this study, the rats of the diabetic and diabetic-StemEnhance groups to avoid interference with the differentiation of stem cells to pancreatic islet β cell. **Rajagopal *et al.*** [(13)] reported that insulin staining could overestimate β cell differentiation when exogenous insulin was used.

Endogenous or *in situ* ASCs (most notably bone-marrow stem cells) can be mobilized from their niches in the body and migrate to various organs to engage in tissue repair or regeneration [(14)]**. Jensen and Drapeau** [(15)] and **Bickford *et al.*** [(16)] stated that there were several factors that could induce endogenous ASCs and promote them to differentiate into the desired cell types in order to treat specific organ damage. StemEnhance is a recently used stem cell mobilizing agent extracted from *Aphanizomenon flos aquae* (AFA). Their effect was detected on B.M stem cell mobilization and on muscle regeneration [(9, 15)]**.** The main objective of this study was to differentiate the ability of StemEnhance to induce stem cells mobilization from the Bmin compared to exogenous injecion of stem cells and promote their migration to the diabetic pancreas. The previous findings are in accordance with those of **Jensen *et al.*** [(15)]who performed a study on 12 patients. They revealed that the consumption of one gram of StemEnhance led to a significant increase in the circulating CD34+ve cells percentage after one hour. They also proved that mobilization of BM CD34+ve cells was related to L-selectin ligand contained in StemEnhance which caused down regulation of CXCR4 chemokine receptor. This interrupted the binding of SDF-1with CXCR4 chemokine receptor leading to mobilization of CD34+ve stem cells. Furthermore, **Cottler-Fox *et al.*** [(19)]emphasized that the previously mentioned compounds could only be used for short periods of time due to severe side effects. In our study, no deaths or abnormal behavior were observed in all rats treated with StemEnhance. This was in accordance with results of **Drapeau *et al.*** [(9)] who reported neither death nor toxicity and animal growth was normal. Histological examination of H & E stained pancreatic sections of diabetic-StemEnhance rats after one week revealed some swollen and vacuolated cells with loss of cytoplasmic details denoting cell necrosis. However, the increase in islet cell's area observed after one week significantly when compared to the control group denoting nonsignificant cell swelling. The islets appeared distorted in shape with congested capillaries. **Underwood** [(20)] demonstrated that necrosis is associated with inflammation. Moreover, **Homo-Delarche *et al.*** [(21)] reported that the inflammatory reaction which took place at the islet level in hyperglycemia associated with type 2 diabetes might results in microangiopathy with subsequent fibrosis leading to loss of islet architecture in stem cells treated group cell. The structure of the pancreas after StemEnhance and stem cells showed some improvements. The area of the islets of Langerhans decreased in both the diabetic stem cells treated and the diabetic-StemEnhance groups due to decreased cells mass mostly secondary to necrosis and apoptosis which occurred with sustained hyperglycemia. This was supported by **Donath and Halban**[(22)]**.** Another explanation was the development of fibrosis secondary to hyperglycemia since the latter was known to stimulate the secretion of fibronectin and collagen I and III by endothelial cells and/or vascular smooth muscle cells [(22)]. **Korbling *et al.*** [(14)] mentioned that diseased or degenerated cells produce a variety of chemical messengers (e.g., cytokines) or have alterations of cell surface receptors that may attract reparative ASCs**.** This is in accordance with results of

Dong *et al.* [7] who demonstrated that a small amount of allogeneic MSCs from STZ-diabetic rats homed to the diabetic pancreas and survived after transplantation. **Sordi *et al.*** [23] provided evidence that BM-MSCs were attracted by pancreatic islets *in vitro* and *in vivo* and confirmed that CXCL12 (SDF-1α) and its ligand CXCR4 played an important role in the homing process. The MSCs were found mainly inside and around islets.

Urbán *et al.* [25] reported that administration of BM cells with MSCs normalized the blood glucose level and serum insulin levels in STZ-induced diabetes in mice. This allowed regeneration of recipient-derived pancreatic insulin-secreting cells. They also stated that neither BMC nor MSC transplantation was effective alone and successful treatment of diabetic animals was not due to the reconstitution of the damaged islet cells from the transplant. They suggested that two aspects of this successful treatment regimen operate in parallel and synergistically in their model. First, BMCs and MSCs induce the regeneration of recipient-derived pancreatic insulin-secreting cells. Second, MSCs inhibit T cell mediated immune responses against the newly formed beta-cells. Similar results were reported by **Ezquer *et al.*** [26] in the same model of STZ-induced type I diabetes. Reversion of hyperglycaemia and glycosuria was observed after injection of 0.5×10^6 MSCs, with increased morphologically normal pancreatic islets β cells.

5. Conclusion

In conclusion, StemEnhance and eogenous ingection of stem cells mobilized BM stem cells and they differentiated into pancreatic islet β cells who may occur in exogenous injection in comparison this endogenous mobelization.

Recommendations

1-Increasing the uses of StemEnhance administration or combining it with antioxidants may give better results that improve the state of DM.

2-The injection of stem cells potentiate healing faster than endogenous mobilaization.

3-Extension of this work in a clinical trial on humans is recommended as it has been proved that StemEnhance is a safe drug and it improved DM.

Correspondence author:
Name: Ibrahim Mohammed Fayed
Address: Department of Histology, Faculty of Medicine, Al-Azhar University, Egypt.
E-mail: ebrahimfayed@gmail.com

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