

**Determination influence of Vitamin D3 on Beta cell of
Diabetic Rats.**

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Rasha Salih Nuhair

Email: dr.rasha-bio@sci.utq.edu.iq

Ali Naeem Salman

Email: dr.ali-n@utq.edu.iq

Faculty of Sciences, University of Thi-Qar

Hassan Raysan AL-Rekaby

**Faculty of Education for Pure Sciences, University of Thi-
Qar**

Email : [dr.hassan@ utq.edu.iq](mailto:dr.hassan@utq.edu.iq)

Abstract

Vitamin D has long been recognized for its beneficial role in regulating the secretion of insulin from pancreatic beta cells (**Holick, 2011**). The study included animal experimental during the period extended from November until December to investigate the role of the administration of vitamin D3 on beta cell of pancreas in alloxan induced toxic in mature male rats. 48 male rats were divided into 4 groups contain 12 animals in each as the following ; Group(1) Animals of this group administer D.W (0.2ml/kg)/day by gavages for 6 weeks orally as control group , Group(2) were injected intraperitoneally with 150mg/kg b.w of alloxan as diabetic group, Group (3) were injected with alloxan then administer 500 UI/kg/day of alpha 25-hydroxy vitamin D3 by gavages for 6 weeks orally, Group (4)were injected with alloxan then administer 1000 UI/kg/day of alpha 25-hydroxy vitamin D3 by gavages for 6 weeks orally. Cytological and histopathological examination of alloxan treated males showed that marked destruction in beta cells of pancreas and sever vacoulation of the cells in the Islets of Langerhans while a significant improvement in Cytological and histological changes of pancreas of alloxan- treated groups with two doses of Vit.D3 compared with control

sections. Our study suggests that 1, 25-(OH)₂ D₃ could play protective roles in pancreas. through reducing the toxicity of alloxan .

Keywords: Vitamin D₃, Cytological and histological changes of pancreas, Diabetic Rats, Beta cell, Alloxan .

تحديد تأثير فيتامين D₃ على خلايا بيتا في الجرذان المصابة بالسكري

رشا صالح نهير , علي نعيم سلمان , حسن ريسان الركابي

الخلاصة

تم التعرف على الدور المهم لفيتامين D₃ ودوره المفيد في تنظيم إفراز الأنسولين من خلايا بيتا البنكرياس منذ فترة. وهذا ماتحاول الدراسة الحالية التحري عنه ومعرفة الدور الحيوي لفيتامين D₃ في تحسين الاثار الناتجة عن تسمم خلايا بيتا البنكرياسية في ذكور الجرذان الناضجة المعاملة بالالوكسان . حيث شملت الدراسة تجريب مجموعة حيوانات من خلال تصميم تجربة حيوانية امتدت للفترة نوفمبر حتى ديسمبر . ولتحقيق هذا الغرض تم تقسيم ٤٨ من ذكور الجرذان البالغة إلى ٤ مجاميع وبواقع ١٢ حيوان في كل مجموعة حيث المجموعة (١) مجموعة السيطرة جرعت بالماء المقطر (٢ مليلتر / كغم من وزن الجسم يوميا) بواسطة الانبوب المعوي لمدة ٤٥ يوم ، المجموعة (٢) المعاملة بالوكسان والتي مثلت مجموعة السكري حقنت حيواناتها داخل الغشاء البريتوني ١٥٠ ملغم / كغم من وزن الجسم ، المجموعة (٣) تم معاملة بالالوكسان ثم ٥٠٠ وحدة دولية / كغم من وزن الجسم يوم من فيتامين D₃ ، المجموعة الرابعة والاخيرة (٤) عوملت الحيوانات فيها بالوكسان ثم ١٠٠٠ وحدة دولية / كغم / يوم من فيتامين D₃ بواسطة الانبوب المعوي لمدة ٦ أسابيع. أظهر الفحص الخلوي والنسجي المرضي للذكور المعالجة بالوكسان تدميرا واضحا في خلايا بيتا البنكرياسية اضافة الى التفجي المفرط للخلايا في جزر لانكرهانز سجلت المجموعات المعاملة بالالوكسان وفيتامين D₃ بالجرعتين تحسن وتصحيح كبير في التغيرات الخلوية والتغيرات النسيجية للبنكرياس. ومن خلال هذه النتائج اثبتنا الدور الحيوي والمهم لفيتامين من تقليل الاضرار الناجمة عن مرض السكري في بنكرياس الجرذان المصابة.

الكلمات المفتاحية : فيتامين D₃ , التغيرات الخلوية والنسيجية للبنكرياس , استحداث السكري في الجرذان , خلايا بيتا الالوكسان .

Introduction

Millions of people around the world have been diagnosed with Type II diabetes, and many more remain undiagnosed (Hagman 2016).

Vitamin D is a group of fat-soluble secosteroids or highly soluble in lipids and is transported to the blood by vitamin D binding protein. (Elmubarak and zsoy 2015). It has long been recognized for its beneficial role in regulating of the secretion of insulin from pancreatic beta cells, mineral metabolism (Holick, 2011) ,regulating cell growth, emerging evidence has suggested a role for vitamin D in a multitude of non-skeletal health outcomes including cancer, autoimmune disorders, cardiovascular disease and T2DM. The main defects of that determine the development of T2DM are insulin resistance, pancreatic β -cell dysfunction and systemic inflammation. Vitamin D promotes pancreatic β -cell function in numerous ways.1. Direct actions: Activation of vitamin D occurs in pancreatic β -cells by intracellular $1-\alpha$ -OHase enzyme. Vitamin D stimuli insulin secretion and promotes β -cell survival by modulating the generation and effects of cytokines and The anti-apoptotic action (Eliades and Pittas 2010). 2. Indirect actions: Insulin secretion is a calcium dependent process and is influenced by calcium flux through the cell membrane by RR.60 Vitamin,D regulates calbindin, a cytosolic calcium-binding protein found in β -cells. It acts as a modulator of depolarization-stimulated insulin release via regulation of intracellular calcium Thus, vitamin D could indirectly effect secretion of insulin additionally by regulating calbindin (Fujita and Palmieri 2000) . Most studies have reported an inverse association between 25(OH)D3 levels and T2DM, (Pittas *et al* .,2007) .Immune modulatory effects of vitamin D might provide additional protection against inflammation-triggered worsening of insulin resistance and, potentially, β -cell function (Norman 2006; Eliades and Pittas 2010) .

Materials and Method

Experimental design:

The present study was carried out at the College of Education for pure science, University of Thi-Qar , during the period extended from November until December. Forty eight adult male rats weighting (200-190 gm) were used for this study. The animals were kept in the animal

house for acclimatization fifteen days before the beginning of the experiments. After the period of acclimation, 48 male rats were equally divided into 4 groups with 12 animals in each as the following :

- 1- Group1: (control group)Animals of this group given D.W (0.2ml/kg)/day by gavages for 6 weeks orally.
- 2- Group 2:(diabetic group)Animals of this group have been injected intraperitoneally with (150mg/kg b.w of alloxan) (Al-Hilfy *et al.*, 2013).
- 3- Group 3: diabetic rats have been treated with low dose vita- D3 group (the rats first were injected with alloxan then administer 500 UI/kg/day of alpha 25-hydroxy vitamin D3 by gavages for 6 weeks orally).
- 4- Group 4: diabetic rats have been treated with high dose vita- D3 group (the rats first were injected with alloxan then administer 1000 UI/kg/day of alpha 25-hydroxy vitamin D3 by gavages for 6 weeks orally) (Alfawaz *et al.*,2014).

At the end of the 6 weeks, animals of each group were anaesthetized by chloroform and sacrificed. The tissues samples(pancreas) were excised and fixed in 10 % neutral buffer formalin for histopathological study.

The Histological Technique:

After fixation, the specimens have been washed off with tap water for 4-6 hours to remove the formalin solution, To remove all water from the specimen, the specimen has passed on a graded of ethyl alcohol concentration(70% -80%-90%-95%-100%) . then clearing in two stages of xylene and infiltrated with semi-liquid paraffin wax for 2 hours at 58C at two stages and the third change continues overnight. by using the rotary microtome The sectioning of tissue was done at (5-6 µm) FinallyThe histological sections have been transferred to water bath (52 °c) to plain the tissue and fixed on a slide containing mayers albumin (Mixture of

egg albumin with glycerin)The slides have been dried by an oven with 40°c for 24 hours (Luna, 1968).

Staining for Histology Study:

The histological sections have been stained by using different methods according to (Luna, 1968).Three types of stains have been used in this study as follows:

- 1- Gomori's Method for Pancreatic Islet Cells:** For the determination of Alpha cell, Beta cell and Delta cell (Luna, 1968).
- 2- Masson's Trichrome:** This stain is to display for appearance of the connective tissue fiber. The section has been deparaffinized and dehydrates with xylene to be fixed in bouin's solution for one hour at 65c, after that it has been washed in running water and rinsed in distal water and finally stained with weigert's iron hematoxylin (10 minutes) to be rinsed in distilled water the slide dip in biebric scarlet acid fuhsin solution continues for (2 minutes), then it has been rinse in distilled water. After dipping the section in phosphomolybdic – phosphor tungstic acid solution (10 minutes) it has been stained with aniline blue solution (5 minutes) rinsed in distilled water, and dipped in glacial acetic solution (3 minutes). The last steps include dehydrating in 95 then 100 % alcohol and then to be cleared by using xylen, mounted by using Canada balsam and covered by glass cover to be dried in hot plate .
- 3- Haematoxyline and Eosin Stain:** It is a routine stain in histological study which is used to display the general structure of the tissue.

Photographing of Tissue Sections

The histological sections were taken after the dyeing process was complete with special and ruotin dyes. Then the microscopic slides were observed by using light microscope under low and high magnification powers including X10, and X40 objective lenses respectively, and

subsequently photographed by digital Camera for histological changes examination.

Results

Cytological Examination with special stains

1- Histological Examination of Cells of pancreas

The pancreas is lobulated long gland and is located on the lower right side of the abdominal wall between the arms of the duodenum and linked by the dorsal mesentery.

The microscopic examination shows part of the internal secretion of the pancreas in four groups of study the existence of the three types of cells:

Beta cells.

Alpha cells.

Delta cells.

The Beta cells form rows of irregular polygonal cells with nuclei of spherical which is slightly larger than the nuclei of alpha cells. These cells are found in the center of islets of Langerhans.

The Alpha cells are spindle in shape, oval nuclei . and located periphery of islets of Langerhans.

The delta cells are few in number and dispersed between peripheral alpha cells and beta cells of the Centre with very low number. Figure (1) reveal islets of Langerhans of control group rats with normal beta cells ,alpha cells , Delta cells . The histopathological section in pancreas of diabetic male rats Figure (2) reveal marked destruction of beta cells (Vaculation cytoplasm and Necrosis).

While Figure (3) pancreas of DM+low dose Vit D₃ treated group , reveal destruction of some of beta cells and preservation of the others .

And Figure (4) pancreas of DM+ high dose Vit D₃ treated group , reveal most of beta cells is preserved in the sections .

2-Histological Examination of Collagen pancreas

The Mason dye was used to detect changes in the collagen fibers of pancreas for all groups. Sections of Collagen pancreas of treated and controlled groups don't show any significant difference in the amount of collagen fibers in the perivascular area for all groups figure (5,6,7).

Histological Examination with classical stains

1-Histological Examination of pancreas

sections of pancreas which stained with H&E stain reveals the normal appearance of both exocrine (EX) and Islets of Langerhans (IL) in control group figure (8). In contrast pancreas of diabetes group reveals sever vacuolation of the cells in the Islets of Langerhans (VIL) figure (9).figure (10) reveals sever vacuolation of the cells in the Islets of Langerhans (VIL)of DM+ low dose Vit D3 treatment group .But the internal section of pancreas of DM+ high dose Vit D3 treatment group reveals hyperplasia of the cells in the Islets of Langerhans figure (11).

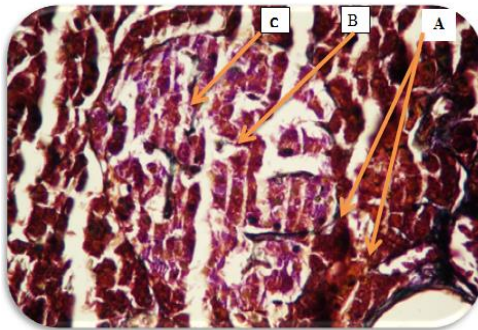


Fig. (1) islets of Langerhans of control group rats reveal normal beta cells (A) alpha cells (B) Delta cells (C) (Gomri stain 400X).

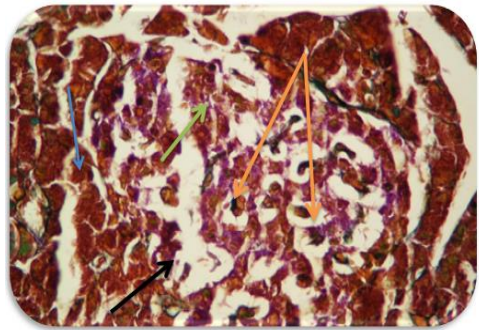


Fig. (2) islets of Langerhans of DM group reveal destruction of beta cells (Vaculation, Degeneration, Necrosis) (Gomri stain 400X).

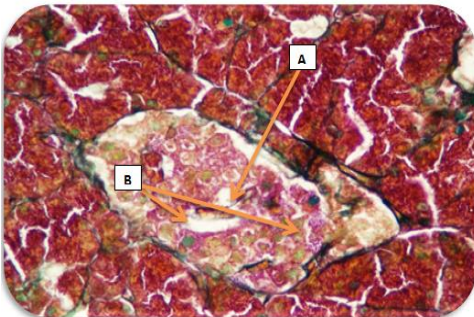


Fig. (3) islets of Langerhans of DM+Low dose VitD3 group reveal destruction of some of beta cells (A) preservation of the others (B) (Gomri stain 400X).

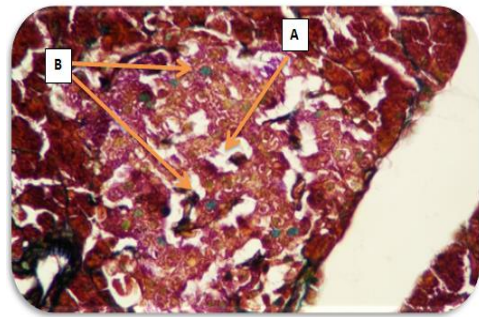


Fig. (4) islets of Langerhans of DM+ High dose VitD3 group reveal mild destruction of beta cells (A),(B) most of the beta cells is preserved (Gomri stain 400X).

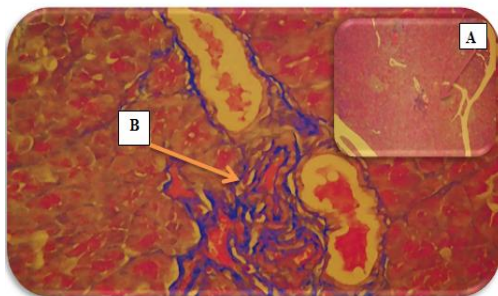


Fig. (5) section of pancreas of control group reveal the amount of perivascular collagen fibers (masson stain) (A) 125X, (B) 400X.

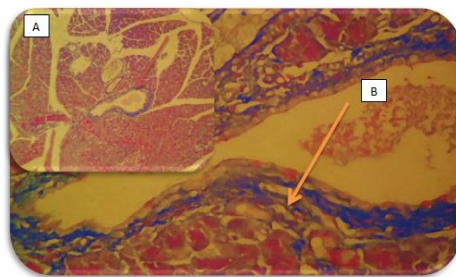


Fig. (6) section of pancreas of DM group reveal no difference in the amount of collagen fibers in the perivascular area in amount similar to that of normal. (masson stain) (A) 125X , (B) 400X .

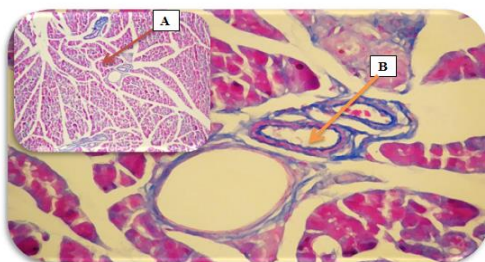


Fig. (7) section of pancreas of DM group reveal no difference in the amount of collagen fibers in the perivascular area in amount similar to that of normal. (masson stain) (A) 125X (B) 400X.

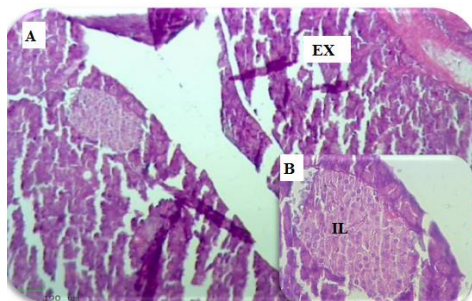


Fig. (8) section of pancreas of control group reveals the normal appearance of both exocrine (EX) and Islets of Langerhans (IL). (H&E) (A) 125X, (B) 400X

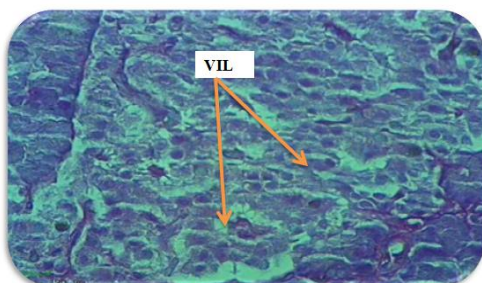


Fig. (9) section of pancreas of DM group reveals sever vacuolation of the cells in the Islets of Langerhans (VIL) (H&E 400X).

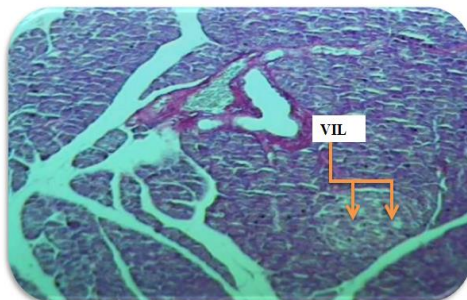


Fig. (10) section of pancreas of DM + low dose Vit D3 treatment group reveals sever vacuolation of the cells in the Islets of Langerhans (VIL) (H&E 400X).

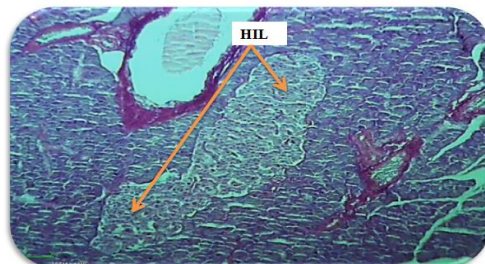


Fig. (11) section of pancreas of high dose treatment group reveals hyperplasia of the cells in the Islets of Langerhans (HIL) (H&E 400X).

Discussion

Cytological examination of pancreas of healthy male rats treated with alloxan revealed marked destruction of beta cells figure (5) . Sections of Collagen pancreas of treated group doesn't showed any difference in the amount of collagen fibers in the perivascular area .

These results are similar to those recorded by Helal *et al.*, (2013) who found that a definite vaculation, degeneration, karyolysis and pyknosis in beta cell of the diabetic group while pancreatic alpha and delta cells were not affected. Kessler *et al.*, (1999) who reported that vaculation of the islet in the most prominent with lesion associated with functional islet abnormality and development of hyperglycemia and also with Fischer and Homburger, (2011) who explained the vaculation by the diabetogenic action of alloxan which induced highly reactive oxygen radicals, which induced cytotoxic to β -cells.

The pancreas of Alloxane + high dose Vit D₃ treated group , reveal normal most of beta cells, these result agree with Su *et al.*, (2017) who reported that 1,25(OH)₂D₃ played certain protective roles in STZ-induced DM. Past studies showed that 1,25(OH)₂D₃ exerted its effects by restoring imbalanced Th1/Th2 and inducing immune tolerance.

Histological study :

Several studies reported that alloxan produces oxygen radicals, which destroy pancreatic β -cells and cause severe hypoinsulinaemia (type I diabetes) that is responsible for the hyperglycemia seen in alloxan-treated animals. However, its action is not directed to pancreatic β -cells only, as other organs such as the liver, kidney are also affected by alloxan administration (Sabu and Kuttan, 2002).

the oxidative stress of alloxan is responsible for causes the fatty degenerative and the aggregation of the inflammatory cells in these tissues (Al-Hilfy, 2013).

Pancreatic section of diabetes group reveals sever vacoulation , While hyperplasia of the cells in the Islets of Langerhans of high dose of vitamin D treatment group .

Alloxan administration led to degenerative and necrotic changes, reduced dimension of Islets of Langerhans as compared to control (Al-Hilfy, 2013; Walvekar *et al.*, 2016). Alloxan induces damage to β -cell DNA , mitochondria 24, lysosomes, and plasma membrane. It was noted that the destruction of 90% insulin secreting β cells of islets of Langerhans was caused by alloxan and hence high blood glucose level was detected (Walvekar *et al.*, 2016).

High circulating concentrations of glucose and fatty acids in diabetic states are attributed to loss of islet function and mass due to glucolipototoxicity, a process involving oxidative stress, ER stress, and inflammation (Groop, 2000).

Vitamin D might have dual antidiabetic influences: (1) modulation of hepatic glucose and lipid metabolism; and (2) promotion of pancreatic islet function and survival (Leung, 2016).

Vitamin D has been reported to act as an antioxidant; where, its antioxidant effect can be mediated through the -induction of expression of several molecules including reduced glutathione (GSH), GSH peroxidase and superoxide dismutase (SOD) enzymes, and –suppression of the NADPH oxidase expression; furthermore, such vitamin can be considered as -a powerful anti-inflammatory and -a promising anti-cancer agent (Mokhtari *et al.*, 2017).

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