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The Possible Ameliorative Effect Of Vitamin D3 On The Histological Structure And Stem Cell Expressions In The Pancreas Of Streptozotocin-Induced Diabetic Mice

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ABSTRACT: Background: The use of stem cells in regenerative medicine gives a great promise for the treatment of many diseases, including diabetes mellitus. This study aimed to investigate the stem cell expression in adult pancreas of control and STZ-induced diabetes and to explore the possible ameliorative effect of vitamin D3 on their biochemical, histopathological and immunohistochemical changes. **Material and Methods**: 65 mice were purchased from the animal house of King Fahd Research Centre in King Abdul-Aziz University, Jeddah, Saudi Arabia. The mice were divided into 8 groups (control, STZ-induced diabetic, vitamin D3 supplemented control and diabetic). After 2 months, blood samples were collected from all groups and assessed for lipid profile and glycemic control and statistical analysis were performed using SPPS program. Then, the pancreata were extracted from scarified mice and were processed for histological examination and immunohistochemical stem cell markers expression CD56 and PAX8. **Results**: Variable degree of marker expression is identified in all groups ranging from mild, moderate to strong. Vitamin D3 supplementation to control groups and STZ diabetic groups stimulates the neogenesis of new islets of Langerhans. Vitamin D3 with metformin supplementation to STZ diabetic group stimulate the neogenesis of new functional islets of Langerhans. **Conclusion and recommendations:** a variable degree of CD56 and PAX8 immunomarkers expression of precursor stem cells in the pancreatic ducts, in between the acini and inside the islets are identified in all groups. Abundant stem cells are expressed mainly in the islets of Langerhans. Vitamin D3 supplementation to the control adults stimulates the neogenesis of new functional islets of Langerhans, so it can be probably used for prevention of diabetes 2 in prediabetic people. While vitamin D3 supplementation to STZ-diabetic group stimulates the neogenesis of new nonfunctional islets of Langerhan's, vitamin D3 supplementation in combination with metformin to STZ-diabetic group stimulates the neogenesis of new functional islets of Langerhan's. This suggests that vitamin D3 has a preventive and potential synergistic effect for the management of Type 2 Diabetes Mellitus with the antidiabetic drugs.

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Key Words: Pancreatic stem cells; Vitamin D3; STZ induced Diabetes; PAX8; CD56

1. INTRODUCTION

Diabetes Mellitus (DM) is one of the oldest disorders that is rapidly emerging as a global health problem. Little studies are investigating the expression of pancreatic stem in STZ- induced diabetes. The World Health Organization reported that estimated 90% of all cases of diabetes constitutes type 2 DM and approximately 15 million people globally suffer from Type 2 DM. Regeneration of insulin precursors cells of pancreas, Beta cells, is one of the methods to help in maintaining blood glucose in diabetic patients **(Hamza, Al-Eisa, and El-Shenawy, 2021)** and using stem cell therapy for Beta-cells reconstruction might be a successful therapy (**Pittas et al., 2020).**

Stem cells are undifferentiated cells capable of self-renewal and differentiate in specialized cell types.

The remarkable ability of stem cells to differentiate towards functional cells makes them suitable modalities for treating diabetes. Pancreatic stem cells were expressed in the ducts, intra-islets and in between the acini **(Jebaraji and Bhuvaneswari, 2020)**. Proposed two theories regarding the stem cell niche in adult pancreas, the 'Neogenesis theory' where the ß-islet cells arose from non-islet stem cells mainly from the ducts and the 'Expansion theory' where the ß-cell mass expanded from the existing intra-insular ß-cells. Pancreatic ducts contained endocrine stem cells and Pdx-1 marker expression became detectable in individual pancreatic ductal epithelial cells, raising the possibility of multipotent stem cells within the mature ductal epithelium (**Bonner-Weir and Sharma, 2002).** In the pancreas, nestin-positive cells are also numerous in the islets, in the stroma of the pancreatic ducts and in the connective tissue surrounding the acini (**Murtaugh and Kopinke, 2008; Zhou et al., 2007; Ku, 2008**). **Sadler et al. (2019)** had been proposed that intra-islet stem cells might serve as an extra source of new islet cells in addition to the replication of differentiated islet cells. Pancreatic stem cell markers in normal human tissues were studied, **Pax-8** was consistently noted in the pancreatic islet cells. The intracellular expression of Pax-8 was mostly concentrated in the nucleus (**Kakun et al., 2022 and Zhou et al., 2024**). **CD56** is a neural cell adhesion molecule expressed by pancreatic duct epithelium and islet cell of pancreas (**Bonner-Weir and Sharma, 2002 and Nugali et al., 2016**).

A study revealed extra skeleton activity of vitamin D3, including prevention from cardiometabolic diseases and cancer development as well as anti-inflammatory properties and diabetes mellitus (**Szymczak-Pajor and Śliwińska, 2019**). Vitamin D deficiency is associated with a decreased insulin release, insulin resistance and type 2 diabetes in many experimental and epidemiological studies. Vitamin D has a role in modulating diabetes risk and over the last years, low blood vitamin D level had emerged as a risk factor for type 2 diabetes, and vitamin D supplementation had been hypothesized as a potential intervention to lower diabetes risk. Vitamin D deficiency in mice leads to reduced insulin secretion that can be restored by vitamin D supplementation (**Pittas et al., 2020; Benedik, 2022 and Park et al., 2024).**

The β-cell proliferation from islet regeneration following injury is important as it indicates the existence of stem cells in the adult pancreas. Study of the β-cell derivation from the expressed stem cells might benefit both basic and clinical researchers. Following the restricted use of embryonic stem cells, Type 2 diabetes patients might benefit from the transplantation of cells expanded from their own duct cells since they would not need any immunosuppression (**Hamad et al., 2021; Liu et al., 2021 and Salib et al., 2022).** As the expression of the pancreatic stem cells in control and STZ-induced diabetic models with respect to their existence and location is important for the progress of transplantation research. Therefore, this study aimed to investigate the stem cell expression in adult pancreas of control and STZ-induced diabetes and to investigate the possible ameliorative effect of vitamin D3 on their biochemical, histopathological and immunohistochemical changes.

2. MATERIAL AND METHODS

Ethical approval:

All procedures used in this study were conducted in accordance with the ethical guidelines of medical ethics committee of the King Abdulaziz University, Jeddah, Saudi Arabia, and were approved by the research ethics committee at King Abdulaziz University (KAU).

Material:

Experimental animals

A total of 56 male adult mice at age of two months and of average weight ± SD (37 – 41 gm) were used in this study for two months. Mice were purchased from the experimental animal unit of KFCMR, KAU. Mice were housed in standard animal laboratory conditions; temperature ranged between 24-26° C, relative humidity was between 50 % and 70% and a 12-hour light/dark cycle. All animals were allowed to one week acclimatizing in animal housing conditions before being used for the experiment and were fed with a regular diet and drinking water ad libitum during the adapting period. After the adaption period, mice were divided into 8 groups (n= 7):

Group 1: Control group (received Corn Oil orally).

Group 2: Vitamin D3 treated group (dose: 500 IU/kg for 3 days/week for 2 months orally) (**Al-Zahrani et al., 2021).**

Group 3: Vitamin D3 treated group (dose: 1000 IU/kg for 3 days/week for 2 months orally) (**Al-Zahrani et al., 2021).**

The rest of the groups were STZ-induced diabetic animals according to (**Ghasemi et al., 2014**).

Group 4: STZ-induced diabetic group (received Corn Oil orally orally).

Group 5: STZ-induced diabetic group treated with Vitamin D3 (dose: 500 IU/kg for 3 days/week for 2 months orally).

Group 6: STZ-induced diabetic group treated with Vitamin D3 (dose: 1000 IU/kg for 3 days/week for 2 months orally).

Group 7: STZ-induced diabetic group treated with Metformin (dose: 400 mg/kg/day for 2 months orally) (**Ismail et al., 2015**) (standard group).

Group 8: STZ-induced diabetic group treated with Vitamin D3 (dose: 500 IU/kg and Metformin).

Induction of Diabetes:

Streptozotocin and Nicotinamide were obtained from Sigma Aldrich (CO., Saint Louis, MO, USA). After overnight fasting, mice were injected intraperitoneally by nicotinamide at a dose of 230 mg/kg (**Yan, 2022**) 15 minutes prior to STZ injection. Then, the diabetes was induced by using a single intraperitoneal injection of STZ at 100 mg/kg (**Ghasemi et al., 2014**) in citrate buffer and to avoid sudden death due to hypoglycemia, mice were supplemented with 5% glucose solution for the next 12 hours (**Saleh et al., 2016**). 72 hours after STZ injection, animals with fasting blood glucose (FBG) more than 250 mg/kg were included in the study (**Saleh et al., 2016; Pittas et al., 2020 and Magadmi et al., 2021**). Then, the animals were randomly divided into eight groups (n=7).

Vitamin D3 Supplementation:

Oral soft-gel capsules of cholecalciferol (**Vitamin D3** 1000 IU/capsule, JP Nutraceuticals) obtained from local pharmacy was used in this experiment. The capsules were dissolved in corn oil vehicle and prepared every week. It was given orally at a dose of 500 IU/Kg and at a dose of 1000 IU/Kg every other day/week for two consecutive months. (**Al-Zahrani et al., 2021**). Treatment was given after 72 hours of diabetes induction (**Hamza et al., 2021**) for two consecutive months.

Metformin dose:

Metformin tablets (Omformin in the form of metformin hydrochloride 500 mg, NPI pharma) obtained from local pharmacy. It was dissolved in normal saline and given orally at a dose of 400 mg/kg/day for two consecutive months (**Ismail et al., 2015**).

METHODS:

Blood analysis

Twenty-four hours after last medications, venous blood samples were collected from the retro-orbital veins in heparinized tubes and centrifuged at 3000 rpm for 15 minutes to separate the sera.

The sera of all groups were immediately frozen and stored at -70°C until analyzed for different biochemical analyses included fasting glucose, insulin and triglycerides levels. Concentration of different parameters in sera were determined by enzymatic immuno-assay methods using the following kits for measurements: **Mouse Glucose (GF),** Free ELISA Kit (My BioSource com, San Diego, USA, Catalogue No. MBS750459), **Mouse INS (Insulin),** ELISA Kit (My BioSource com, San Diego, USA, Catalogue No. MBS762025) and **Mouse Triglyceride (TG)**, ELISA kit (Competitive ELISA) 96 Tests (My BioSource com, San Diego, USA, Catalogue No. MBS726589).

Technique for histological study:

The mice were anaesthetized lightly by diethyl ether inhalation and the abdominal cavity was incised at the midline. The pancreata were removed and specimen were fixed by immersion in formaldehyde 10% for three days. The specimens were dehydrated in ascending grades of ethyl alcohol and cleared in benzene. The specimens were soaked for 3 changes in paraffin and were finally embedded in paraffin wax. A microtome was used to cut the paraffin blocks into serial sections at thickness of 6-8 µm. The sections were attached to an albumenized glass slide. They were stained with Hematoxylin and Eosin stain to study the general structure and Masson's trichrome stain to demonstrate the collagen fibres in the pancreas (**Aboul-Mahasen and Alshali, 2019 and Bancroft et al., 2019**).

Technique for immunohistochemical study:

Tissue sections for immunohistochemistry were cut at 4 µm on positively charged slide, baked for 1 hour at 60°C oven for antigen retrieval by heat treatment. An automated immunostaining was performed using a Ventana Benchmark GX Autostainer (Ventana Medical Systems, Roche Group, Tucson, AZ, USA). The Immunohistochemical marker expression of pancreatic stem cells markers (**PAX8 and CD56**) were processed in the different groups. Positive external controls were stained in parallel with study group tissues, consisting of sections from paraffin-embedded control pancreas specimen.

The slides were washed in PBS five times for 5 minutes. To reduce the background, non-specific antibody binding sites on the tissues were pre-blocked by blocking solution (10% Donkey serum in PBS) for 1 hour in a humid chamber. Blocking solution was tipped off and the sections were incubated with the prediluted primary antibody. The dilution was 1:100 for both **anti-PAX8** and **anti-CD56** and incubated in a humid chamber for 16 mins at 37°C. Omission of primary antibody was routinely used as a control. Then, primary antibody solution was tipped off and slides were washed in PBS five times for 5 minutes. The tissue sections were Counterstained with Hematoxylin II in a humid chamber at room temperature for 8 mins for PAX8 but 4 mins for CD56. After that, tissues were Post Counterstain with Bluing reagent for 4 minutes.

Finally, unbounded antibodies were removed by washing the slides in PBS five times for 5 minutes, and mounted in mounting medium with DAPI (Vectashield, Vector Laboratories, Peterborough, UK). These antibodies were used in immunohistochemistry techniques as recommended in the staining protocol with ultra-view Universal DAB Detection Kit.

They were **Mouse Monoclonal Primary Antibodies** as follow:

1. **PAX 8** (clone name MRQ-50) antibody: commercial antibodies raised in mouse. It is an IgG antibody **stains the nucleus.** It was purchased from Sigma-Aldrich Scientific Research (St. Louis, Missouri, USA. Catalogue No. 363M-1).

2. **CD 56** (clone name 123C3) antibody: commercial antibodies raised in mouse. It is an IgG antibody **stains the cell membranes and cytoplasm**. It was purchased from Thermo Fisher Scientific Inc. (Waltham, Massachusetts, USA. Catalogue No. 07-5603).

Finally, all slides were scanned by a Digital pathology slide scanner (Philips Intelli Site Pathology Solution). Photographs were captured from the scanned slides. The intensity of staining was assessed as negative or positive staining. The positive staining graded from mild, moderate and strong.

Statistical analysis:

Results presented as mean ± standard error of mean (SEM). Value analysis made using IBM Corporation's SPSS version 22 (Armonk, NY, USA). Shapiro-Wilk test employed to determine the normality of value distributions. Tukey's test is utilized to compare groups of normally distributed values after One-Way ANOVA has been used to analyze the data. Figures were made using Prism Graph pad soft were version 5. Statistical significance defined as a $p < 0.050$.

BIOCHEMICAL RESULTS and DISCUSSION

The data presented in **Table 1** and **Graphs (1&2)** illustrates the effects of vitamin D3 supplementation and metformin on fasting blood glucose (FBG) and insulin levels, in both control and diabetic groups. As expected, G1 (Control + Corn oil) group shows normal FBG at 100.43 mg/dL and insulin at 20.71 mI U/L. These values were within the typical range for non-diabetic individuals. Supplementation with 500 IU of vitamin D3 in G2 (Control + D3 500 IU) led to a slight reduction in FBG (97.14 mg/dL) suggesting that vitamin D3 might enhance glucose metabolism even in non-diabetic subjects. Vitamin D receptors were expressed in various tissues, including pancreatic beta cells, and vitamin D had been shown to influence insulin resistance, glucose tolerance, and inflammatory processes associated with the development of diabetes **(Takiishi et al., 2010** and **Pittas et al., 2020).** Surprisingly, higher doses of vitamin D3 (1000 IU) in G3 (Control + D3 1000 IU) resulted in slightly elevated FBG (102.14 mg/dL) more than G1, indicating that higher doses might not offer additional benefits and might even mildly impair glycemic control in non-diabetic individuals.

G4 (DM + Corn oil) group shows significantly elevated FBG (346.57 mg/dL) and decreased insulin (2.57 mIU/L) compared to control groups (G1, G2 and G3) (p <0.0001) confirming the severe impact of diabetes on glycemic control when left untreated. These findings are consistent with the well-established diabetogenic effects of STZ in inducing a state of severe insulin deficiency and hyperglycemia in experimental animal models **(Szkudelski, 2001 and Deeds et al., 2011). STZ** is a nitrosourea cytotoxic glucose analogue that selectively targets and destroys pancreatic beta cells, leading to a profound impairment in **insulin secretion**. This results in the characteristic metabolic disturbances observed in the G4 diabetic control group, including marked hyperglycemia, reduced circulating insulin, and elevated HbA1c levels, which are hallmarks of the diabetic state **(Szkudelski, 2001 and Lenzen, 2008).** The resultant loss of beta-cell mass impairs insulin secretion, which is critical for maintaining normal glucose homeostasis. Consequently, this leads to elevated FBG levels due to decreased insulin availability **(Alqasim et al., 2017 and Khodir et al., 2020).**

In diabetic groups, supplementation with 500 IU of vitamin D3 in G5 (DM + D3 500 IU) showed slight improvements in FBG (333.86 mg/dL) and insulin (3.29 mIU/L) but these values remained significantly higher than the control groups (G1, G2 and G3) (p <0.0001). This finding aligns with previous studies investigated the effect of vitamin D on STZ-induced type 1 DM **(Ning et al., 2015; Deng et al., 2016; Alqasim et al., 2017 and Khodir et al., 2020)**. On other hand, some studies showed that vitamin D improved glucose level and insulin resistance in STZ-induced type 2 DM **(Abdel-Rehim et al., 2019; Eltablawy et al., 2018).**

Increasing the dose to 1000 IU in G6 (DM + D3 1000 IU) led to a further improvement in FBG (285.00 mg/dL) though insulin levels remained low (2.86 mIU/L). The FBG was significantly decreased versus G5 (p <0.050). This suggests that vitamin D3 alone may have a relatively minor impact on managing severe hyperglycemia in the context of established, STZ-induced diabetes, where the underlying beta-cell destruction is a key driver of the metabolic disturbances **(Szkudelski, 2001 and Deeds et al., 2011).** Notably, the vitamin D3 supplementation improved the FBG levels in the diabetic groups, but

did not significantly increase the insulin levels. This indicates that the mechanism of action of vitamin D may involve enhancing glucose uptake or improving pancreatic function, rather than directly stimulating insulin release. The lack of corresponding increases in insulin levels suggests that vitamin D's mechanism of action may through non-insulin dependent pathways. The potential mechanisms **may include improving insulin sensitivity**, enhancing glucose transport, or modulating other regulatory pathways involved in glucose homeostasis **(El-Desouki, 2016).**

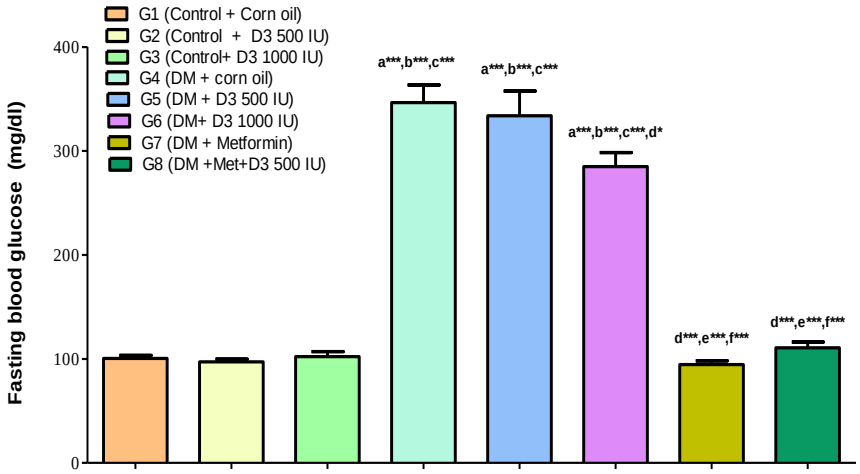
Metformin treatment in G7 (DM + Metformin**)** resulted in near-normal FBG (94.50 mg/dL) and insulin levels (21.00 mIU/L) highlighting its effectiveness in restoring glycemic control in diabetic subjects. The combination of metformin with 500 IU of vitamin D3 in G8 (DM + Metformin + D3 500 IU) showed improvement of FBG (110.71 mg/dL) and insulin levels (20.43 mIU/L) compared to (G4, G5 and G6) (p <0.001). This suggests that adding vitamin D3 (500 IU) to metformin therapy might slightly enhance the insulin, though its impact on FBG is less clear compared to G7.

In contrast, metformin treatment (G7) demonstrated a remarkable ability to almost completely normalize glycaemic parameters, including FBG and insulin, in the diabetic group. This finding is consistent with the well-documented mechanisms of action of metformin, which include reducing hepatic glucose production, enhancing peripheral glucose uptake, and improving insulin sensitivity **(Wulffelé et al., 2002 and Inzucchi et al., 2015).** The combination of metformin and vitamin D3 (G8) provided slightly enhanced insulin levels compared to G7, though its impact on FBG is less clear. This is suggesting **a potential synergistic effect of vitamin D3** with antidiabetic drug.

Table 1: Fasting Blood Glucose and Insulin of different studied groups.

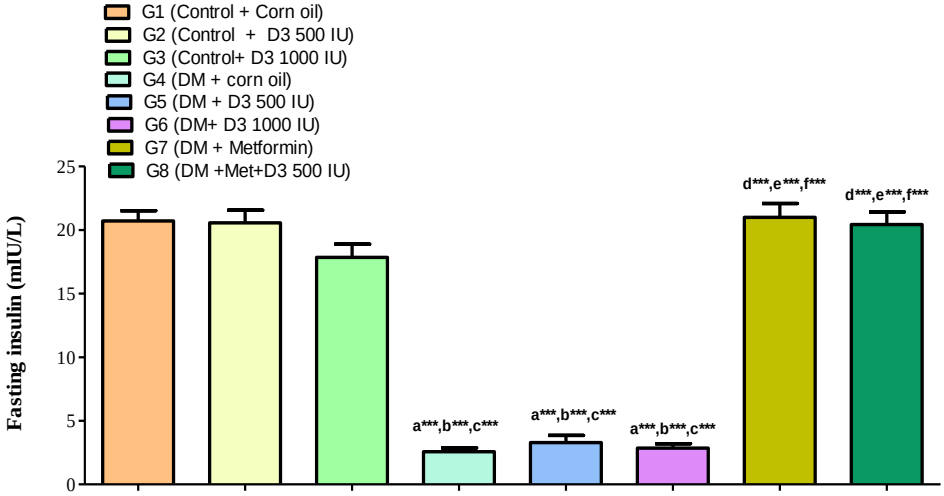
Groups	**FBG** (mg/dl)	**Insulin** (mIU/L)
G1 (Control + Corn oil)	100.43±3.03	20.71±0.81
G2 (Control + D3 500 IU)	97.14±2.59	20.57±1.00
G3 (Control + D3 1000 IU)	102.14±4.84	17.86±1.03
G4 (DM + corn oil)	346.57±16.88 ^{a***,b***,c***}	2.57±0.30 ^{a***,b***,c***}
G5 (DM + D3 500 IU)	333.86±23.70 ^{a***,b***,c***}	3.29±0.57 ^{a***,b***,c***}
G6 (DM + D3 1000 IU)	285.00±13.52 ^{a***,b***,c***,d*}	2.86±0.34 ^{a***,b***,c***}
G7 (DM + Metformin)	94.50±3.80 ^{d***,e***,f***}	21.00±1.08 ^{d***,e***,f***}
G8 (DM + Met + D3 500 IU)	110.71±5.50 ^{d***,e***,f***}	20.43±1.00 ^{d***,e***,f***}

FBG: Fasting blood glucose; Insulin. Data expressed as mean ± standard error of mean (SEM). a: significance versus G1 (Control + Corn oil); b: significance versus G2 (Control + D3 500 IU); c: significance versus G3 (Control+ D3 1000 IU); d: significance versus G4 (DM + corn oil); e: significance versus G5 (DM + D3 500 IU); f: significance versus G6 (DM+ D3 1000 IU). *: p <0.050, **: p <0.010, ***: p <0.001.



Graph 1: Fasting blood glucose (mg/dl) of different studied groups.

Data expressed as mean ± standard error of mean (SEM). a: significance versus G1 (Control + Corn oil); b: significance versus G2 (Control + D3 500 IU); c: significance versus G3 (Control+ D3 1000 IU); d: significance versus G4 (DM + corn oil); e: significance versus G5 (DM + D3 500 IU); f: significance versus G6 (DM+ D3 1000 IU). *: p <0.050, **: p <0.010, ***: p <0.001.



Graph 2: Fasting insulin (mIU/L) levels of different studied groups.

Data expressed as mean ± standard error of mean (SEM). a: significance versus G1 (Control + Corn oil); b: significance versus G2 (Control + D3 500 IU); c: significance versus G3 (Control+ D3 1000 IU); d: significance versus G4 (DM + corn oil); e: significance versus G5 (DM + D3 500 IU); f: significance versus G6 (DM+ D3 1000 IU). *: p <0.050, **: p <0.010, ***: p <0.001.

In the present study, **Table 2** and **Graph 3** shows the triglyceride (TG) data for different studied groups. G1 (Control + Corn oil) group has a normal triglyceride level at 75.86 mg/dL indicating a healthy balance of diet. Supplementation with 500 IU of vitamin D3 in G2 (Control + D3 500 IU) resulted in a minor reduction in triglycerides (72.29 mg/dL). Increasing the dose of vitamin D3 to 1000 IU in G3 (Control + D3 1000 IU) resulted in minimal changes in triglycerides, suggesting a potential slight impact on triglyceride levels at higher doses. G4 (DM + Corn oil) group exhibits a severely dysregulated triglycerides level at (129.00 mg/dL) of untreated diabetes. Dyslipidemia is a well-known complication associated with diabetes mellitus characterized by elevated total cholesterol, Triglyceride, and LDL, along with decreased HDL, is a common feature of the diabetic state and a major risk factor for the development of cardiovascular diseases in this patient population **(Taskinen, 2003 and Mooradian, 2009).** This is well-documented in the literature and is a critical component of the metabolic disturbances observed in diabetes **(Wu & Parhofer, 2014 and Schofield et al., 2016;).**

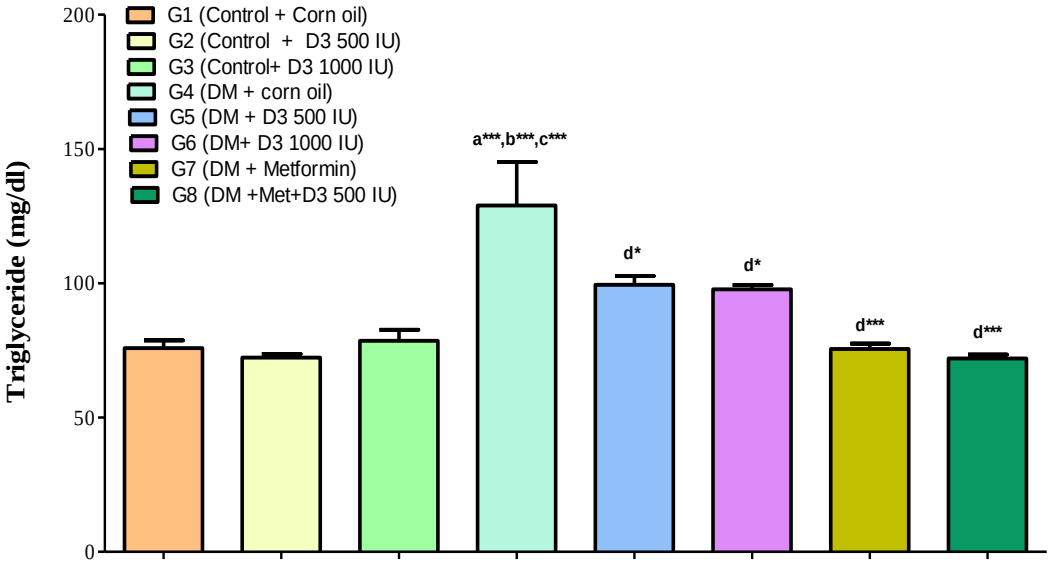
Supplementation with 500 IU of vitamin D3 to diabetic animals in G5 (DM + D3 500 IU) and 1000 IU of vitamin D3 to diabetic animals in G6 (DM + D3 1000 IU) resulted in a modest improvement in triglycerides versus control groups G1, G2 and G3 (p <0.0001). Meanwhile, triglyceride was significantly decreased versus G4 (p <0.050). Vitamin D3 supplementation, at both 500 IU and 1000 IU doses, showed a significant reduction in TG as compared to diabetic group. These results are in agreement with previous studies **(Ning et al., 2015; Deng et al., 2016 and Eltablawy et al.; 2018).**

Metformin treatment alone in G7 (DM + Metformin) or in combination with 500 IU vitamin D in G8 (DM + Metformin + D3 500 IU) significantly improves the triglycerides level as it was significantly decreased. The mechanisms underlying the TG-lowering effects of vitamin D3 supplementation in diabetic conditions include modulation of lipogenic pathways by downregulating the expression and activity of sterol regulatory element-binding protein-1c, a key transcriptional regulator of lipogenesis **(Wang et al., 2011 and Asano et al., 2017).** This leads to decreased synthesis of fatty acids and TG in the liver. Vitamin D can also activate the AMP-activated protein kinase (AMPK) pathway, which is a crucial cellular energy sensor **(Cheng et al., 2016).** Reduced gluconeogenesis can lead to decreased substrate availability for TG synthesis. Finally, vitamin D can enhance insulin sensitivity in peripheral tissues, such as adipose and muscle, which can lead to better regulation of lipid metabolism and reduced triglyceride levels. The more pronounced effects on triglycerides could be attributed to the specific mechanisms by which vitamin D modulates lipogenic and gluconeogenic pathways in the liver **(Alvarez and Ashraf, 2010).**

Table 2: Triglyceride levels of different studied groups.

Groups	Triglyceride (mg/dl)
G1 (Control + Corn oil)	75.86±2.91
G2 (Control + D3 500 IU)	72.29±1.41
G3 (Control + D3 1000 IU)	78.57±4.11
G4 (DM + corn oil)	129.00±16.16 ^{a***,b***,c***}
G5 (DM + D3 500 IU)	97.71±1.64 ^{d*}
G6 (DM+ D3 1000 IU)	100.29±2.73 ^{d*}
G7 (DM + Metformin)	75.50±2.02 ^{d***}
G8 (DM + Met + D3 500 IU)	72.00±1.41 ^{d***}

TG: Triglyceride level. Data expressed as mean ± standard error of mean (SEM). a: significance versus G1 (Control + Corn oil); b: significance versus G2 (Control + D3 500 IU); c: significance versus G3 (Control + D3 1000 IU); d: significance versus G4 (DM + corn oil). *: p <0.050, **: p <0.010, ***: p <0.001.



Graph 3: Serum levels of triglyceride (mg/dl) of different studied groups.
Data expressed as mean ± standard error of mean (SEM). a: significance versus G1 (Control + Corn oil); b: significance versus G2 (Control + D3 500 IU); c: significance versus G3 (Control+ D3 1000 IU); d: significance versus G4 (DM + corn oil). *: p <0.050, **: p <0.010, ***: p <0.001.

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL RESULTS and DISCUSSION

In the present study, histological structures of the pancreas in eight groups of adult mice, which are treated with different combinations of corn oil, vitamin D3, STZ -diabetes mellitus and metformin for two months were studied with special consideration on the **CD56** and **Pax8** marker expression of stem-cells like characters.

Histological Results and Discussion

Histological examination of the pancreas of the control group **G1** stained with **Hx and E** revealed that a normal pancreatic architecture is organized into lobes and lobules (Fig. 1a). The pancreas was formed of exocrine and endocrine parts. The acini represented the exocrine part and islets of Langerhans represented the endocrine part. The islets of Langerhans appear as lightly stained rounded or oval masses scattered among the darkly stained lobules of the pancreatic acini (Fig. 1a). The high-power examination showing each islet is consisted of anastomosing cords of cells separated by blood capillaries. The islets were occupied mainly by Beta cells which were centrally located and had basophilic rounded nuclei. While the Alpha cells were peripherally located and had small basophilic flat dark nuclei. The acini appeared having different sizes and shapes and most of them formed of a single row of pyramidal cells which have dark basophilic nuclei and apical acidophilic zymogen granules (Fig. 2a). These findings are consistent with previous studies **(Eroschenko and Di Fiore M, 2013; Aboul-Mahasen and Alshali, 2019 and Longnecker, 2021**).

Sections of **G1** stained with **Masson's trichrome** showed normal distribution of collagenous fibres were revealed among the capsule, around the ducts and around the islet's of Langerhans. These findings are consistent with other papers **(Eroschenko and Di Fiore M, 2013; Treuting, etal. 2017; Aboul-Mahasen and Alshali, 2019 and Longnecker, 2021).**

Histological examination of the pancreas of the control groups treated with vitamin D 500 IU and 1000 IU (**G2 & G3**) showed a normal architecture of the pancreas with a marked increase **in islets cell mass** especially in G3 treated with vitamin D3 1000 IU as there is a marked increase in number and size of the islets of Langerhans (Fig. 1b and Fig. 1c). Many islets of Langerhans appeared budding from intralobular ducts with an increase in the vascularity (Fig. 2b and Fig. 2c). These important findings indicate the presence of progenitor cells in the islets and ducts of the pancreas and suggest the ability of vitamin D3 to stimulate the neogenesis and/or expansion of the islets of Langerhans. These findings are in agreement with many studies **(Bonner-Weir and Sharma, 2002; Stoffers, 2004; Murtaugh and Kopinke, 2008 and Smukler et al., 2011)** confirmed the presence of adult stem cells in the pancreatic ducts based on several experimental models. **Smukler et al. (2011)** demonstrated that the adult mammalian pancreas contained a population of insulin positive multipotent stem cells and suggested that these cells might provide a promising line of investigation toward potential therapeutic benefit. **Jebaraji and Bhuvaneswari (2020)** proposed two theories regarding the stem cell niche in adult pancreas. The first theory is the 'Neogenesis' theory where the ß-islet cells arose from non-islet stem cells mainly from the ducts. The second one is the 'Expansion' theory where the B-cell mass expanded from the existing intra-insular B-cell.

Regarding the ability of vitamin D3 to stimulate the neogenesis and / or expansion of the islets of Langerhans, it is recommended to take vitamin D3 as a **prophylactic treatment** from diabetes especially in prediabetic people. These recommendations are confirmed by many researchers, (**Francesca et al., 2018**; **Rizzo et al., 2019 and Barbarawi et al., 2020),** as they mentioned that vitamin D treatment at moderate to high levels (≥1000 IU/day) substantially reduced the incidence of type 2 diabetes in people with prediabetes. **Park et al. (2024)** mentioned that vitamin D3 supplementation had shown mixed outcomes in the prevention of Type 2 Diabetes mellitus.

Histological examination of adult mice pancreas of the STZ-induced diabetic group treated with corn oil (**G4**) showed a complete distortion of the pancreatic architecture as it appeared shrunken. The pancreatic acini lost their arrangement into lobules, their nuclei appeared pyknotic. The interlobular spaces appeared wide and filled with blood cells and hemorrhage. The intralobular blood vessels and ducts appeared distorted and congested with thick walls. The islets of Langerhans appeared ill-defined and shrunken, as they lost their regular outlines with a marked reduction in their sizes and numbers. Most of B-cell appeared degranulated with small and degenerated nuclei (Fig.1d and Fig.2d). These results coincided with the results of pancreatic cells toxicity induced by STZ in several experimental models **(Mythili et al., 2004; Ozdemir et al., 2009; Kangralkar et al., 2010 and Hamza et al., 2021).** They mentioned that STZ caused diabetes by the rapid depletion of B-cells, which leads to a reduction in the insulin release.

In the present study, Type 2 Diabetes is induced by nicotinamide (NA) and streptozotocin (STZ) in accordance with **Yan (2022)**. He mentioned that the mechanism underlying diabetes induction by combining the two chemicals involved decreasing the toxic effect of STZ by NA so that only a percentage of cells were destroyed and the remaining viable cells could still respond to glucose stimulation.

Histological examination of adult mice pancreas of the STZ-induced diabetic group treated with vitamin D3 500 IU (**G5**) and vitamin D3 1000 IU (**G6**) showed a mild (G5) to moderate improvement (G6) of the pancreatic architecture with an obvious increase in islets cell mass than those of STZ-induced diabetic group. In spite of the improvement of pancreatic architecture, most of the intralobular blood vessels and ducts appeared distorted and congested with thick walls (Fig.1e and Fig.1f). Many pancreatic acini

restored their arrangement and shapes and many new islets of Langerhans especially in G6 appeared budding from the ducts. The newly formed islets of Langerhans appeared irregular in shape with ill-defined borders but with an increase in the vascularity. The newly formed B-cell are centrally located and showed faint or dark basophilic rounded nuclei (Fig.2e and Fig.2f). These results are in agreement with many researchers. **Barbarawi et al. (2020) and Zhang et al. (2020)** studied the preventive effects of vitamin D supplementation on Type 2 Diabetes in patients with prediabetes. **Ebrahim (2022**) mentioned that after employing the toxin streptozotocin to destroy the majority of the B-cells, they discovered another intra-islet precursor cells responsible for the B-cell regeneration. **Vasdeki et al. (2024)** gave the evidence supported the concept that vitamin D supplementation could be a valuable addition to pharmacological agents for the management of type 2 diabetes mellitus, potentially enhancing glycemic control and overall health outcomes in affected individuals.

Histological examination of adult mice pancreas of the STZ-induced diabetic group treated with metformin alone (**G7**) revealed a moderate improvement of the pancreatic architecture as compared with the STZ induced diabetic group (G4) with an increase in islets cell mass with restoration of nearly B-cell shape and size (Fig.1g and Fig.2g) but still not like the control group (G1). Consistent with these findings, restoration of nearly normal β-cell was observed by many studies. **Woods et al. (2009)** mentioned that the islet-protective effect of metformin might be partly correlated with its anti-inflammatory action. **Ismail et al. (2015)** revealed that metformin administration in STZ-diabetic model induced regenerative changes in the hepatocyte cytoplasm and parenchyma and induced positive signaling for insulin and the regeneration of pancreatic β cells. **Han et al. (2017)** studied the ameliorative effect of metformin on histopathologic changes of pancreatic islets in STZ-induced mice model of diabetes, they found that some pancreatic islets were moderately but significantly improved by metformin treatment. The evidence of reduction of proinflammatory cytokines, such as IL-1β and TNF-α, in the pancreatic tissues suggested that the anti-inflammatory actions of metformin might partly contribute to amelioration of insulitis.

Histological examination of adult mice pancreas of the STZ-induced diabetic group treated with vitamin D3 and metformin (**G8**) revealed a marked improvement of the pancreatic architecture (Fig.1h and Fig.2h) as compared with the STZ-induced diabetic groups (G4,5 ,6 & 7) and appeared nearly similar to the control groups (G1, 2 &3). These findings coincided with **Pittas et al. (2020) and Zhang (2020),** as they mentioned that vitamin D supplementation raised the rate at which prediabetes returned to normoglycemia and lowered the risk of type 2 diabetes.

Wu et al. (2023) mentioned that vitamin D3 regulated the insulin secretion in pancreatic islets and insulin sensitivity in multiple peripheral metabolic organs as in vitro studies in both Type 1 and Type 2 Diabetes Mellitus animal models. As vitamin D could improve glucose homeostasis by enhancing insulin secretion, reducing inflammation, reducing autoimmunity, preserving beta cell mass, and sensitizing insulin action. **Park et al. (2024)** concluded that a close relationship between vitamin D status and diabetes mellitus. vitamin D's innate immunity-enhancing effect could provide a protection against infections in prediabetic and diabetic people. In addition, the anti-inflammatory effects of vitamin D could ameliorate the complications associated with diabetes and islet dysfunction.

Histological examination of the pancreata of the control groups 1, 2 & 3 showed a normal distribution of collagen fibres as appeared as fine collagen fibres in the interlobular septa and around the acin. The collagen fibres are surrounding the interlobular vessels and ducts. Mild collagenous fibrous content appeared incompletely surrounding the islets of Langerhan's (Fig.3 a, b, & c). These findings are consistent with **Aboul-Mahasen and Alshali, (2019)** and **Longnecker, (2021).**

Histological examination of the pancreas of **G4** (STZ induced diabetes), showed a marked increase in the collagen fibres deposition in the interlobular septae around the thick-walled ducts and blood vessels with a marked collagen fibres deposition around the ill-defined islet of Langerhans and neighboring thick-walled distorted blood vessels and duct (Fig.3 d).These findings coincided with the findings of **(Mythili, 2004; El-Kordy and Alshahrani, 2015 and Yan, 2022).**

Histological examination of the pancreas of STZ-induced diabetic group treated with vitamin D3 500 IU (**G5**) and vitamin D3 1000 IU (**G6**) showed that in spite of the improvement of pancreatic architecture and the increase in islet cell mass, the thick collagenous fibrous deposition around the islets, vessels and ducts (Figs.3e & f) are still present as compared with those of G4. This might explain why the newly formed islets are nonfunctioning as proved by the biochemical results. These results are in agreement with many researchers. **Barbarawi et al. (2020) and Zhang et al. (2020)** studied the preventive effects of vitamin D supplementation on Type 2 Diabetes in patients with prediabetes. **Ebrahim (2022**) mentioned that after employing the toxin streptozotocin to destroy the majority of the B-cell, they discovered another intra-islet precursor cells

responsible for the B-cell regeneration. These cells expressing PDX-1 and somatostatin and progressing to cells expressing both PDX-1 and insulin. They did not develop **into actual insulin-positive cells**, though, there were relatively few somatostatin/insulin-positive cells.

Histological examination of adult mice pancreas of the STZ-induced diabetic group treated with metformin alone (**G7**) revealed a moderate improvement of the pancreatic architecture (Fig.1g and Fig.2g) as compared with the STZ induced diabetic group (G4) with an increase in islets cell mass but still not like the control group (G1). Consistent with these findings, restoration of nearly normal β cell was observed by many studies **(Kirpichnikov et al., 2002; Marchetti et al., 2004 and Han et al., 2017**). **Ismail et al. (2015)** revealed that metformin administration in STZ-diabetic model induced regenerative changes in the hepatocyte cytoplasm and parenchyma and induced positive signaling for insulin and the regeneration of pancreatic β cells. **Han et al. (2017)** concluded that the ameliorative effect of metformin on histopathologic changes of pancreatic islets in STZ-induced mice model of diabetes due to the reduction of proinflammatory cytokines, such as IL-1β and TNF-α, in the pancreatic tissues suggested the anti-inflammatory actions of metformin in sinusitis.

Histological examination of adult mice pancreas of the STZ-induced diabetic group treated with vitamin D3 500 IU and metformin (**G8**) revealed a marked improvement of the pancreatic architecture (Fig.1h and Fig.2h) as compared with the STZ-induced diabetic groups (G4, 5, 6 & 7) and appeared nearly similar to the control groups (G1, 2 & 3). These results are in agreement with many other studies. Regarding the restoration of nearly normal β cell mass, vitamin D3 action in the pancreatic β cells seemed to be exerted directly via binding of vitamin D to vitamin D receptor (**Szymczak-Pajor and Śliwińska 2019). Abdel-Rehim, et al. (2019) and Alaraj et al. (2022)** mentioned that both type 1 and type 2 diabetes could be prevented or treated with vitamin D and its analogues. As this vitamin was normalizing the function of the immune system, promoting B-cell survival and function, this vitamin had been demonstrated to normalize the action of B-cell.

Immunohistochemical Results and Discussion

Immunohistochemical marker expression **CD56** (Fig.4a) and **Pax8** (Fig.5a) of control group (**G1**) showed a mild CD56 and moderate PAX8 immunoreaction of most of the pancreatic acini. A strong CD56 and Pax8 immunoreaction were shown in the islets and the interlobular duct while it was negative CD56 in the intralobular ducts (Fig. 4a). **CD56** expression was consistent with (**Maestro et al., 2000**; **Bonner-Weir and Sharma, 2002)** as they mentioned that CD56 expressed the stem cells in the pancreatic duct epithelium and islet cell of pancreas**. Bernardo et al., (2009)** studied the postnatal developing pancreas in mice, they mentioned that from day 60, most islet cells had strong CD56 and PAX8 marker expression. **Pax 8** expression was in agreement with **Lorenzo et al. (2011); Kakun et al. (2022) and Zhou et al. (2024),** as they mentioned that Pax-8 is essential to the differentiation of cells and the generation of tissues throughout embryonic development. PAX8 is a transcription factor expressed by pancreatic islet and was consistently noted in pancreatic islet cells. Regarding subcellular localization, the intracellular expression of Pax-8 was mostly concentrated in the nucleus.

Immunohistochemical marker expression **CD56** (Fig.4 b & c) and **Pax8** (Fig.5 b & c) of control groups (**G2 and G3**) showed an increase of expression of stem cells in the newly formed islets, in the epithelial linning of the ducts and in between the acini (Figs.4 b & c). These important findings of the present work indicate the presence of progenitor cells in the islets and ducts of the pancreas and suggest the ability of vitamin D3 to stimulate the neogenesis and / or expansion of the islets of Langerhans due to activation of the silent stem cells in the ducts and in the islets. **Bonner-Weir and Sharma (2002)** mentioned that CD56 endocrine cells were numerous during the early stage of gestation and when islet neogenesis started. **Fujisawa et al. (2003)** mentioned that CD56 luminal cells might represent developmental and regenerative changes of pancreatic ducts.

Regarding the presence of progenitor cells in the islets and ducts of the pancreas, these findings are in agreement with many studies **(Bonner-Weir et al., 1993; Brelje T., 1994 and Bonner-Weir and Sharma, 2002; Stoffers, 2004 and Smukler et al., 2011).** They confirmed that pancreas were a source of adult stem cells as immunohistochemical analysis of these studies showed that in nearly 15% of all B cells were smaller and immature indicating the presence of immature progenitor cells in the islets that become differentiated into mature cells when it is required.

Regarding the suggestion that the **neogenesis** and / or **expansion** of the islets of Langerhans due to activation of the silent stem cells in the ducts and in the islets, these results are consistent with many studies. **Smukler et al. (2011**) identified pancreas derived multipotent precursor cells (PMPs) in the adult mouse with the intriguing capacity to generate progency in the pancreatic and neural lineage. **Jebaraji & Bhuvaneswari (2020)** proposed two theories (Neogenesis and Expansion) regarding the stem cell niche in adult pancreas.

Immunohistochemical marker expression **CD56** (Fig,4d) and **Pax8** (Fig.5d) of STZ induced diabetic group (**G4**) showed that inspite of the pancreatic atrophy and the decreased **CD56 marker expressions**, the stem cells expression still present in some ducts, in few small ill-defined islets of pancreas and in between acini (Fig.4d). These findings were in agreement with **Zanini et al., (2011); Guz, (2001) and Ebrahim, (2022)** who mentioned that after employing the toxin streptozotocin to destroy the majority of the B-cell, they discovered another intra-islet precursor cells responsible for the B-cell regeneration.

Immunohistochemical marker expression **CD56** (Figs.4 e & f) and **Pax8** (Figs.5 e & f) of STZ induced diabetic groups treated with vitamin D3 500 IU (**G5**) and vitamin D3 1000 IU (**G6**) showed an increase in CD56 and Pax8 expression in islets cell mass with increases stem cells expression (Figs.4 e&f and Figs.5 e&f). These results are in agreement with many researchers. **Barbarawi et al. (2020) and Zhang et al. (2020)** studied the preventive effects of vitamin D supplementation on type 2 diabetes in patients with prediabetes. **Ebrahim (2022**) mentioned that another intra-islet precursor cells repopulation responsible for the B-cell regeneration were present in STZ induced diabetic model. This repopulation occurred following the death of the B-cell core, beginning with cells expressing PDX-1 and somatostatin and progressing to cells expressing both PDX-1 and insulin. They did not develop into **actual insulin-positive cells**. **Cui et al. (2022)** mentioned that long-term, well-designed, interventional clinical trials should be conducted to achieve a better understanding of the therapeutic potential of supplementation in patients with pancreatitis with vitamin D deficiency with regards to doses, duration of therapy, side effects, and short-term and long-term results.

Immunohistochemical marker expression **CD56** (Fig.4 g) and **Pax8** (Fig.5 g) of STZ-induced diabetic group treated with metformin (**G7**) showed that more CD56 and Pax8 stem cells expression than those of G4, 5 & 6 especially in the ducts and the large islets of pancreas (Figs.4g and 5g). Consistent with these findings, restoration of nearly normal β cell was observed by many studies **(Kirpichnikov et al., 2002; Marchetti et al., 2004; Woods et al., 2009; Ismail et al., 2015 and Han et al., 2017**). **Woods et al. (2009**) concluded that the islet-protective effect of metformin in STZ -diabetic model might be partly correlated with its anti-inflammatory action.

Immunohistochemical marker expression **CD56** (Fig.4h) and **Pax8** (Fig.5h) of STZ-induced diabetic group treated with vitamin D3 and metformin (**G8**) showed strong CD56 and Pax8 expression of stem cells in the ducts, in between acini and especially in the large islets of pancreas (due to regeneration, neogenesis and /or expansion of the islets cell mass). In the present study, vitamin D3 administration with metformin resulted in the regeneration of the capacity of pancreatic β cells to secrete insulin. **Bonner-Weir and Sharma (2002)** confirmed that the pancreatic ducts contained endocrine stem cells as in the regenerating rat pancreas, after partial pancreatectomy, a mature duct cell could regress with replication to a less differentiated cell that regained the potential to differentiate into an islet, acinar, or mature duct cell. **Francesca etal. (2018) and Hamza et al. (2021)** studied the efficacy of mesenchymal stem cells (MSCs) and vitamin D in the treatment of diabetes in STZ-diabetic animal model. They demonstrated that treatment with MSCs and vitamin D improved the histological structure of the pancreas and prevented pancreatic injury via antioxidant and immune regulation in diabetic rats .

Hamad etal. (2021); Liu et al. (2021); Salib et al. (2022) and Cui et al. (2024) reviewed the promising applications of stem cells in the context of type 2 diabetes in the pancreatic β-cell failure, as two approaches, first approach in regenerative medicine for T2DM, is Beta Cell Replacement Therapy, which is the generation of insulin-producing β cells from embryonic and multipotent stem cells. The second approach**,** as mesenchymal stem cells (MSCs) which had immunomodulatory properties.

Vasdeki et al. (2024) gave the evidence supported the concept that vitamin D supplementation could be a valuable addition to pharmacological agents for the management of type 2 diabetes mellitus, potentially enhancing glycemic control and overall health outcomes in affected individuals.

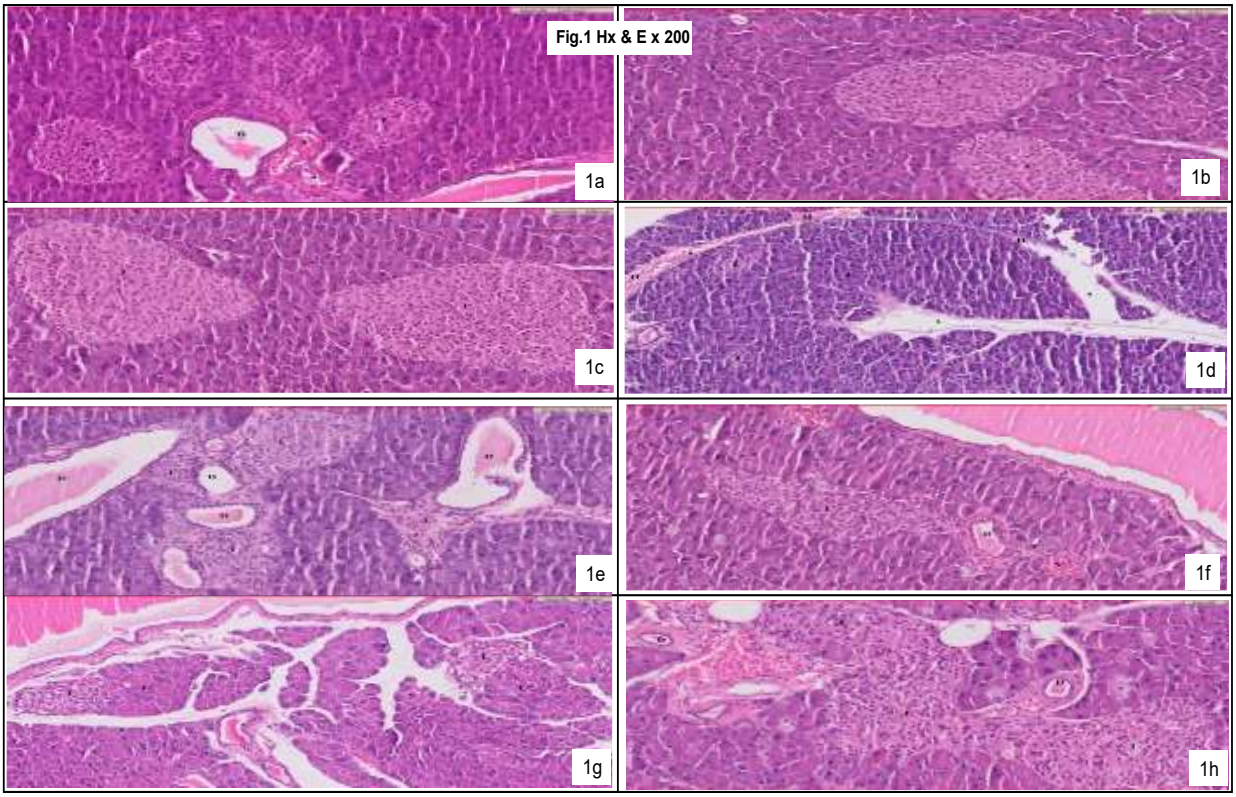


Figure 1 : Photomirograph of serial sections from adult mice pancreas stained with HX&E of :

a. **G 1** (control fed with corn oil) showing a a normal architecture of the pancreas , formed of exocrine and endocrine parts. The exocrine acini (C) and the endocrine islets of Langerhans (I). The islet of Langerhans appears as oval and round lightly stained cell masses (I). Notice also the thin pancreatic capsule (arrow), interlobular connective tissue septae (S) , the intralobular arteries (a), veins (v) and duct (D).
b. G 2 (Corn oil + Vit D3 500 IU) notice the increase in number and size of islet's of Langerhan's(I)
c. **G 3** (Corn oil + Vit D3 1000 IU) showing the increase in the vascularity of the large sized newly formed islets of Langerhans (I).
d. **G 4** (STZ induced diabetes) showing a distorted pancreatic acinus (C) losing their arrangement into lobules. The islets of Langerhans (I) appear ill defined and shrunken. The septae (S) appeared wide and filled with blood cells and hemorrhage (H).
e. **G 5** (STZ induced diabetes + Vit D3 500 IU)) showing the newly formed islets of Langerhans (I) budding from intralobular ducts (D). They appear expanded, irregular in shape with ill defined borders (I). Notice also the distorted thick wall intralobular ducts (D).
f. **G 6** (STZ induced diabetes + Vit D3 1000 IU) showing a newly formed expanded islet of Langerhans (I) budding from a congested thick walled intralobular duct (D). Showing also congested dilated blood vessels (a and v).
g. **G 7** (STZ induced diabetes +metformin) showing the budding of islets of Langerhans (I) from neighboring acini (c). Notice also the distorted dilated blood vessels (v).
h. **G 8**(STZ induced diabetes + Vit D3 500 IU + metformin) showing an expanded islet of Langerhans (I) budding from the intralobular ducts (D) with irregular outline. HX&E x200

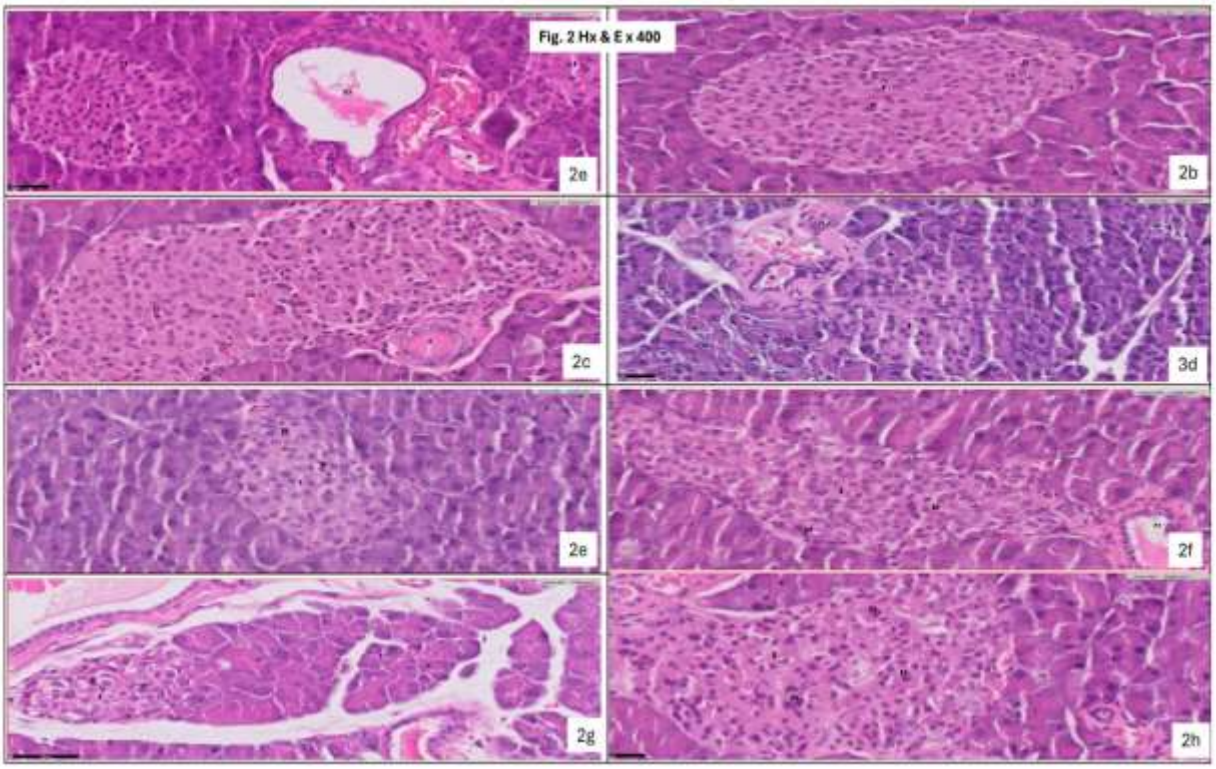


Figure 2 : Photomirograph of serial sections from adult mice pancreas stained with HX&E of :

a. **G 1** (control fed with corn oil) showing the normal acini (C) is formed of pyramidal cells with apical acidophilic zymogen granules (Z), and basal dark rounded nuclei (N). Showing two large masses of islet of Langerhans (I). They contain centrally located B-cells (B) and peripherally located A cells (A). The B – cells appear with large dark centrally located nuclei, while the A- cells with small oval dark nuclei. Showing a thin wall intralobular vein (v) and a thick walled intralobular artery (a). Notice the large intralobular duct is lined by cubical cells (D).
b. **G 2** (Corn oil + Vit D3 500 IU) showing a large islet of Langerhans (I) with many blood capillaries (ca). Shows many centrally located B-cells (B) with rounded dark nuclei and peripherally located A-cells (A) with flat dark nuclei
c. **G 3** (Corn oil + Vit D3 1000 IU) showing a large islet of Langerhans budding from a neighboring interlobular duct (d). Showing also many intra-islets blood capillaries (ca). Shows many centrally located B-cells (B) with rounded dark nuclei and peripherally located A-cells (A) with flat dark nuclei
d. **G 4** (STZ induced diabetes) showing the cells of pancreatic acini (C) having pyknotic nuclei (N). The intralobular blood vessels (a and v) and duct (d) are congested and showing thick irregular walls. The islets of Langerhans (I) have ill-defined border with a difficulty in distinguishing their cells.
e. **G 5** (STZ induced diabetes + Vit D3 500 IU) showing newly formed B cells of the pancreas are centrally located with faint or dark basophilic rounded nuclei. Notice the increased vascularity of the islets (I). Notice also most of the pancreatic acini (C) restore their arrangement and shape.
f. **G 6** (STZ induced diabetes + Vit D3 1000 IU) showing newly formed B cells of the pancreas are centrally located with faint or dark basophilic rounded nuclei. Notice the increased vascularity of the islets (I). Notice also the thick walled intralobular duct (D)
g. **G 7** (STZ induced diabetes +metformin) showing the pyramidal cells of the pancreatic acini (C) with apical acidophilic zymogen granules (Z), and basal basophilic nuclei (N). Notice the budding of an islet (I) from an acinus (c). Also showing the B-cells with dark rounded nuclei (B) and the A cells with dark oval nuclei (A).
h. **G 8**(STZ induced diabetes + Vit D3 500 IU + metformin) showing the normal pancreatic acini (C) with their nuclei (N) and zymogen granules (Z). Showing most of B- cells (B) in the central & peripheral regions of the islet with normal density. Notice also the increase in the intra-islet vascularity HX&E x400.

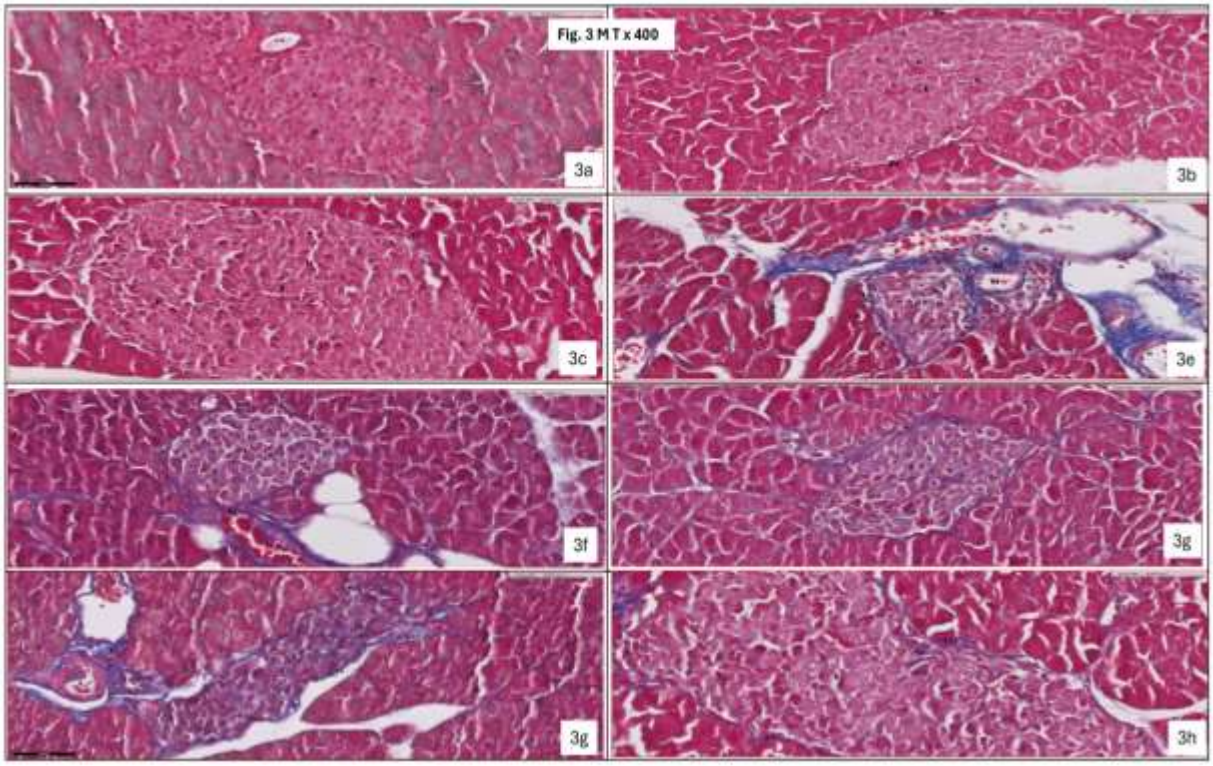


Figure 3 : Photomirograph of serial sections from adult mice pancreas stained with Masson's Trichrome (MT) of:

a. **G 1** (control fed with corn oil) showing fine collagenous fibres (G) around the islet (I). Notice the collagenous content around the small capillary (ca) inside the islet. Notice also the dark red B-cells (B).
b. **G 2** (Corn oil + Vit D3 500 IU) shows many B-cells (B) appeared dark red. Show also fine collagen fibres (G) around the islets (I) and around the walls of blood capillaries.
c. **G 3** (Corn oil + Vit D3 1000 IU) shows fine collagen fibres around the islets and around the walls of blood capillaries (G). Many B-cells (B) appear dark red.
d. **G 4** (STZ induced diabetes) showing a marked collagen fibres(G) deposition appeared around ill-defined islet of Langerhans (I) and neighboring thick-walled distorted blood vessels (a and V) and duct (D).
e. **G 5** (STZ induced diabetes + Vit D3 500 IU) showing the persistence of thick collagenous fibrous deposition (G) around the islet of Langerhans (I).
f. **G 6** (STZ induced diabetes + Vit D3 1000 IU) showing the thick collagenous fibrous deposition (G) around and inside the islet of Langerhans (I).
g. **G 7** (STZ induced diabetes +metformin) showing the collagen fibres (G) deposition around and inside an islet of Langerhans. Showing also the neighboring thick-walled distorted blood vessels (a and v) and duct (D).
h. **G 8**(STZ induced diabetes + Vit D3 500 IU + metformin) showing a nearly normal collagenous content (G) around and inside the islet of Langerhans (I). MT x400.

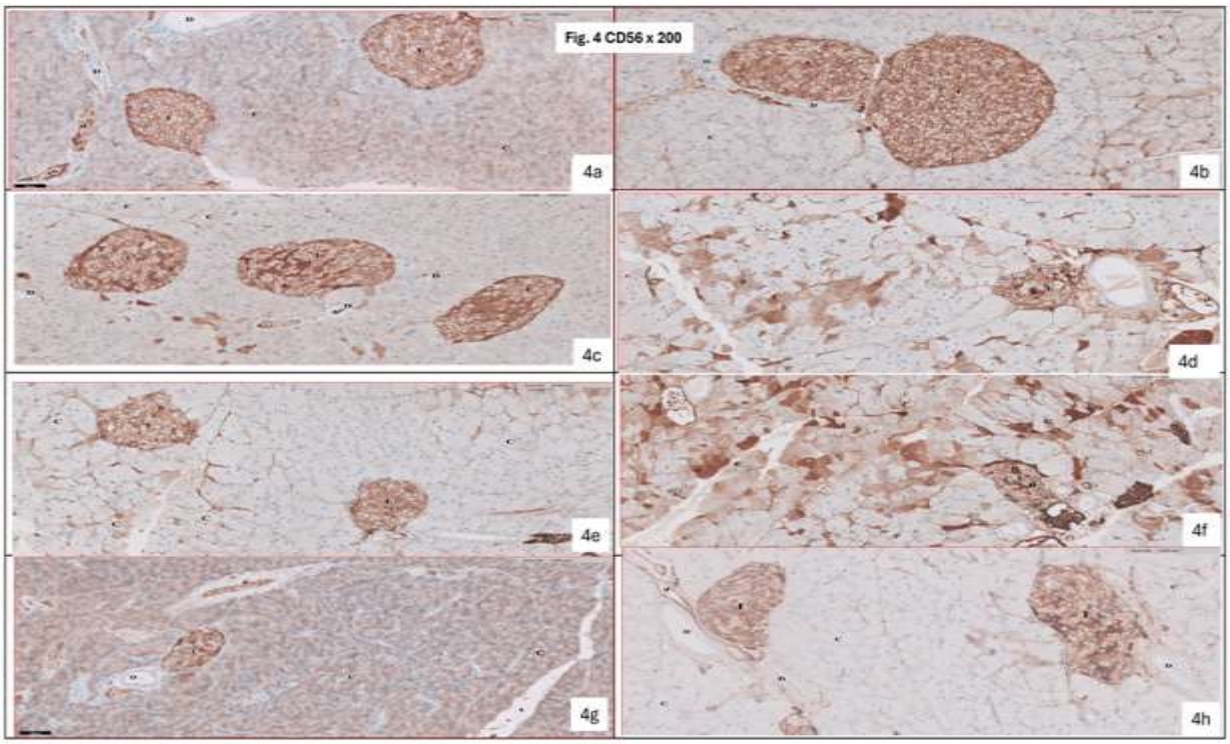


Figure 4: Photomirograph of serial sections from adult mice pancreas immunostained with CD56 of :

a. **G 1** (control fed with corn oil) shows a mild CD56 immunoreaction in pancreatic acini (C) , a strong CD56 immunoreaction in the islets (I) and interlobular duct (d). Notice a negative CD56 immunoreaction in the intralobular duct (D).
b. **G 2** (Corn oil + Vit D3 500 IU) shows two new islets of Langerhans (I) with strong CD56 reaction close to intralobular duct with a negative CD56 reaction (D). Show also the acini (C) give a moderate CD56 reaction.
c. G 3 (Corn oil + Vit D3 1000 IU) shows some B-cells (B) give strong CD56 reaction. Show also, some cells in between the acini give moderate CD56 reaction.
d. **G 4** (STZ induced diabetes) showing some cells in between the pancreatic acini (C) has a moderate CD56 immunoreaction. Some cells in the islet of Langerhans (I) showing a strong CD56 immunoreaction.
e. **G 5** (STZ induced diabetes + Vit D3 500 IU) showing the strong CD56immunoreaction in some cells and capillaries of the newly formed islets of Langerhans (I). Show most of the pancreatic acini (C) give mild CD56 immunoreaction.
f. **G 6** (STZ induced diabetes + Vit D3 1000 IU) showing the strong CD56immunoreaction in some B (B) cells and capillaries of the newly formed islets of Langerhans (I). Show most of the pancreatic acini (C) give a mild CD56 immunoreaction.
g. **G 7** (STZ induced diabetes +metformin) showing the pancreatic acini has a mild CD56 immunoreaction (arrows). The islets (I), which is budding from an intralobular duct (D), shows a strong CD56 immunoreaction. Notice the interlobular duct (d) shows a strong CD56 immunoreaction while the intralobular duct (D) shows a negative CD56 immunoreaction.
h. **G 8**(STZ induced diabetes + Vit D3 500 IU + metformin) showing a strong CD56 immunoreaction in many B (B) cells of the newly formed islets of Langerhans (I). Showing a mild CD56immunoreactionte in the pancreatic acini (C). The interlobular duct (d) shows a strong CD56 immunoreaction while the intralobular duct (D) give a negative CD56 immunoreaction. CD56x200

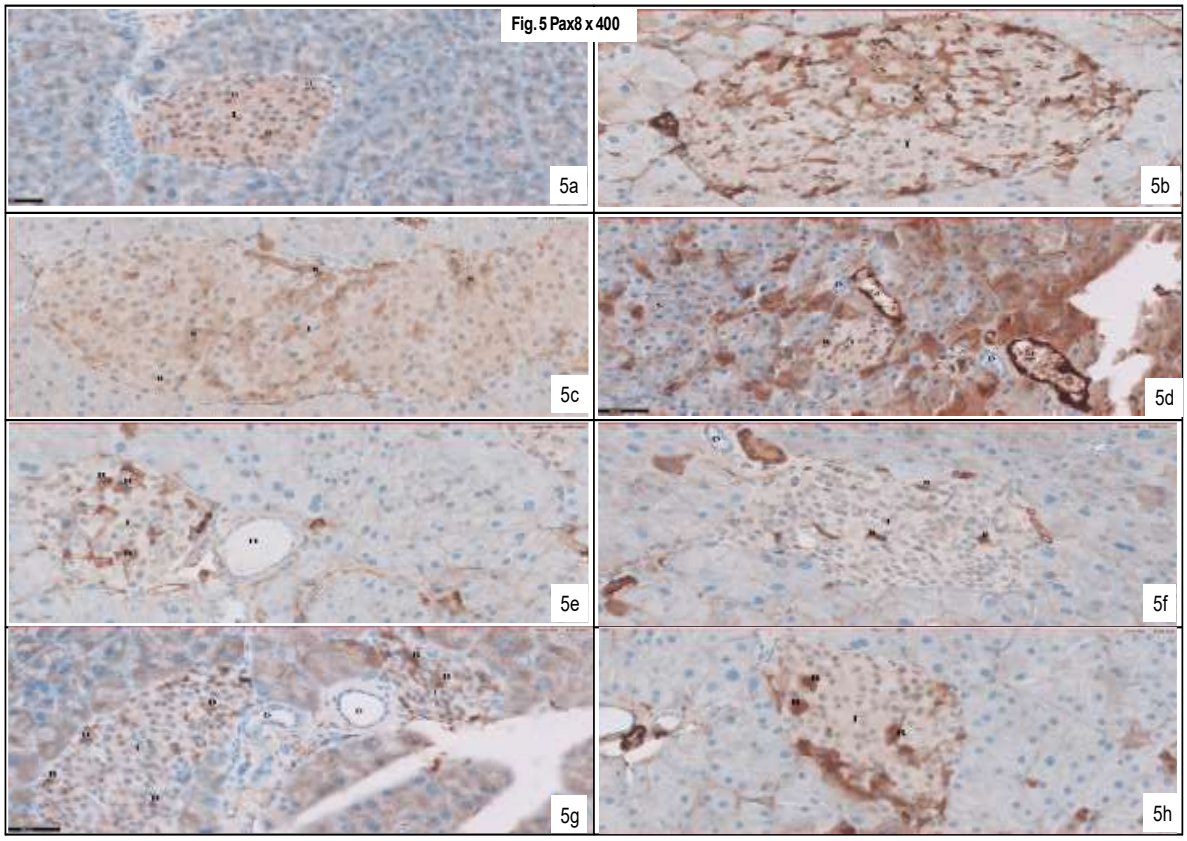


Figure 5: Photomirograph of serial sections from adult mice pancreas immunostained with Pax8 of :

a. **G 1** (control fed with corn oil) showing a strong Pax8 immunoreaction of many B-cells (B) of the islet of Langerhan's (I).
b. **G 2** (Corn oil + Vit D3 500 IU) showing a strong Pax8 immunoreaction of many B-cells (B) of the large islet of Langerhans (I).
c. **G 3** (Corn oil + Vit D3 1000 IU) showing a large islet of Langerhans (I) with a strong Pax8 immunoreaction and has many B-cells (B) with strong Pax8 immunoreaction.
d. **G 4** (STZ induced diabetes) showing a strong Pax8 immunoreaction in few B cells of a small islet of Langerhan's (I), and a strong Pax8 immunoreaction in the interlobular ducts (d) while the acini (C) gave negative Pax8 immunoreaction
e. **G 5** (STZ induced diabetes + Vit D3 500 IU) shows strong reaction in many B cells (B) of the islets of Langerhans (I). shows the intralobular duct (D) give a negative Pax8 reaction.
f. **G 6** (STZ induced diabetes + Vit D3 1000 IU) shows a strong reaction in some B (B) cells of the islet of Langerhans (I). Shows intralobular duct (D) give negative Pax8 reaction.
g. **G 7** (STZ induced diabetes +metformin) shows a strong Pax8 immunoreaction in some B cells (B) of the islets of Langerhans (I). The intralobular ducts (D) show a negative Pax8 immunoreaction. Notice also the islets of Langerhans (I) appeared budding from the intralobular ducts.
h. **G 8**(STZ induced diabetes + Vit D3 500 IU + metformin) showing a strong immunoreaction in many B (B) cells of the newly formed islets of Langerhans (I). ax8x400.

CONCLUSION and RECOMMENDATIONS:

- As the biochemical results showed that combination of vitamin D3 with metformin shows a potential synergistic role in managing hyperglycemia and dyslipidemia in diabetic groups so the combination of vitamin D3 with metformin provides additional synergistic benefits.
- As vitamin D3 improve the stem cells immune-expression in the adult pancreas of control and diabetic groups indicated the regeneration ability of islets of Langerhans. So, it is recommended for uses as a preventive drug in pre-diabetic persons and as synergistic with the antidiabetic drugs in diabetic patients.
- Since many islets of Langerhans appeared budding from the ducts, type 2 diabetic patients might benefit from the transplantation of cells expanded from their **own duct cells** as they would not need any immunosuppression.

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