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RESEARCH ARTICLE

# Vitamin D regulates insulin and ameliorates apoptosis and oxidative stress in pancreatic tissues of rats with streptozotocin-induced diabetes

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## Abstract

This study was designed to evaluate the potential therapeutic efficacy of vitamin D (Vit D) in averting the harmful effects of type 2 diabetes mellitus (T2D). Forty male Wistar rats were allotted into four groups: (1) the control, (2) Vit D, (3) streptozotocin (STZ), and (4) STZ + Vit D groups. Rats co-treated with Vit D had significantly ($p < 0.05$) decreased levels of cortisol; proinflammatory cytokines, including interleukin-6 (IL-6); and malondialdehyde (MDA). Meanwhile, the levels of insulin significantly ($p < 0.05$) increased, whereas the activity of the antioxidant system, including glutathione (GSH), superoxide dismutase (SOD), catalase, and total antioxidant capacity (TAC), significantly ($p < 0.05$) decreased. Histopathological examination revealed the destruction of beta cells in the islets of Langerhans in rats with diabetes. Meanwhile, immunoexpression revealed an increase in the immunoreactivity of caspase-3 and endothelial nitric oxide synthase and a reduction in the immunoreactivity of insulin in rats with diabetes. In conclusion, Vit D ameliorated the harmful biochemical impact of diabetes mellitus, probably by increasing insulin secretion and sensitivity, ameliorating β-cell function, and decreasing cortisol levels; also, the anti-inflammatory effect of Vit D reduces the number of proinflammatory cytokines (e.g., IL-6) and increases the activity of the antioxidant system, such as GSH, SOD, TAC, and catalase while reducing lipid peroxidation enzymes (e.g., MDA).

**Keywords** Diabetes mellitus · Vitamin D · Caspase-3 · Inducible nitric oxide synthetase · Oxidative stress

## Introduction

Diabetes mellitus (DM) is a chronic disease characterized by hyperglycemia, which impairs the metabolism of carbohydrates, proteins, fats, and electrolytes (Richardson and Pugliese 2021). It can disrupt the nervous and vascular systems (Lotfy et al. 2017) by damaging the capillary endothelium in several organs, including the retina, renal glomerulus, and central and peripheral nerves, because of the excessive accumulation of glucose in these cells (Malone and Hansen 2019). The proportion of DM is rapidly increasing in both developed and developing countries, particularly type 2 DM (T2DM), which is associated with modern lifestyle habits, such as fast-food consumption, obesity, and reduced physical activity, and genetic factors. Untreated DM can lead to numerous diseases and long-term complications, leading to death (Lotfy et al. 2017). Recent evidence confirms that DM-induced chronic hyperglycemia can induce the formation of oxidative stress and free radicals in humans and animal models (Sadek et al. 2017; Abouzed et al. 2018; Alsenosy Abdel-wahab et al. 2019; Abouzed et al. 2020). Oxidative stress is the main factor that induces the secondary complications of DM, such as injuries and foot ulceration (Sivitz and Yorek 2010).

Vitamin D (Vit D) is a fat-soluble vitamin that has hormone-like actions and plays a key role in calcium and bone metabolism. The biological role of the normally active form

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of Vit D [1,25(OH)2D3] extends to influence various systemic processes, such as cell differentiation, immune regulation, and inflammation (Baeke et al. 2010; Labudzyns'kyĭ et al. 2014). Recently, Vit D has drawn more attention as a non-enzymatic antioxidant compound (D'Aurizio et al. 2015). Studies by Foroozanfard et al. (2015) and Sadek and Shaheen (2014) showed that Vit D supplementation significantly decreased the plasma level of malondialdehyde (MDA) and increased the levels of total antioxidant capacity (TAC). Panigrahy et al. (2017) reported that hyperglycemia disrupts electron transfer and activation in the mitochondria and causes an excessive production of reactive oxygen species (ROS) via the polyol pathway. ROS in conjunction with nitric oxide synthase (NOS) shifts some intracellular compounds of the membrane components of protein, lipid, and carbohydrate structure. In contrast, Vit D plays a vital role in promoting free radical generation in the liver and pancreatic tissues in rats with diabetes by binding to Vit D receptors in the cell nucleus (Labudzynskyi et al. 2015).

In DM, β-cells increase insulin secretion to counteract the reduced effectiveness of insulin action. However, the continuous rise in glucose leads to glucotoxicity, which increases hyperactivity and induces β-cell apoptosis. This decline in the release of insulin excessively elevates blood glucose levels, which characterizes DM (Wimalawansa 2016). The emerging evidence suggests that Vit D is necessary for the normal release of insulin by protecting pancreatic β-cells from oxidative stress and apoptosis, which results in insulin resistance, which is a major contributor to T2DM (Pittas et al. 2006).

Islet apoptosis has been recently shown as an essential event in both type 1 DM (T1DM) and T2DM; however, the specific in vivo role of individual caspases in cell apoptosis and disease progression in DM models still requires more studies. Cermisoni et al. (2018) concluded that 1,25(OH)2D3 might control various pro-apoptotic mechanisms, including the downregulation of anti-apoptotic genes and the upregulation of pro-apoptotic genes. Therefore, this study was designed to illustrate the picture of the pathological, immunohistochemical, and biochemical alterations in rats with streptozotocin (STZ)-induced DM treated with Vit D.

## Materials and methods

### Ethics statement

The experimental procedures were performed according to the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health, and the study protocol was approved by the local authorities (Faculty of Veterinary Medicine, Damanhur University, Egypt). Precautions were taken during sampling and throughout the entire experiment to minimize animal suffering.

### Chemicals

STZ (CAS number: 18883–66-4) and chemical reagents were purchased from Sigma Chemicals (St Louis, MO). Vit D (cholecalciferol) (Vidrop®) was obtained from Medical Union Pharmaceuticals (Abu Sultan, Ismailia, Egypt).

### Animals

Forty male Wistar albino rats (age: 6 weeks; average weight 140 ± 10gm) were obtained from the Department of Animal Science, Faculty of Science, Damanhur University, to be used in this experiment. The rats were kept in galvanized metal cages in a well-ventilated house, with a temperature range of 27–30 °C and a 12-h natural light and 12-h darkness cycle, with free access to tap water and dry rat pellets. They were allowed to acclimate for 15 days before the experiment.

### Experimental designs

The rats were randomly assigned into four equal groups ($n = 10$), as follows: (1) the control (CTR) group where the rats were maintained on a normal basal diet without any treatment; (2) the Vit D group where the rats were fed with a basal diet supplemented with Vit D (Vidrop®; 10 IU/kg (Pittas et al. 2006) daily via an intragastric tube; (3) the STZ group where the rats received a high-fat diet (HFD), followed by 2 successive intraperitoneal doses of STZ (35 mg/kg) (nongenetic model of T2DM in rats; Guo et al. 2018); and (4) the STZ + Vit D) group where the rats were fed with an HFD, followed by 2 successive intraperitoneal doses of STZ (35 mg/kg), and they were gastrogavaged with Vit D (Vidrop®, 10 IU/kg) 3 days after the induction of T2D, which was continued daily throughout the experiment. The experimental period lasted for 60 days. The induction of T2D was monitored by measuring the plasma levels of glucose and insulin before starting the experiment.

### Evaluations of biochemical parameters

At the end of the experimental period (60 days), blood samples (3 mL) were collected from the orbital venous plexus of overnight (10–12 h)-fasted rats in all groups (i.e., the CTR, Vit D, STZ, and STZ + Vit D groups). After that, the samples were centrifuged at 3000 rpm for 10 min to separate blood serum for measuring insulin according to the method described by Dudley et al. (1985). Additionally, the serum level of cortisol was determined using Rat Cortisol ELISA kits (OKEH0254; Biocompare Company).

### Histopathological evaluations

The rats were euthanized and eviscerated, and pancreatic tissues were harvested from the carcasses. The collected tissues were washed with phosphate-buffered saline (PBS) solution (pH 7.4) containing 0.16 mg/mL heparin to remove any erythrocytes and clots and fixed in 4% paraformaldehyde for 2 days at 4 °C. After fixation, the samples were embedded in paraffin blocks, sectioned using microtome to the desired thickness (3–5 µm), and routinely stained with hematoxylin and eosin (Bancroft and Gamble 2013). Semiquantitative lesion score of pancreatic tissues was performed according to El-Far et al. (2022). In brief, 10 fields/slide/rat were randomly chosen and blindly examined and averaged. The lesions were scored as follows: score scale: 0=normal; 1≤25%; 2=26–50%; 3=51–75%; and 4=76–100%.

### Estimation of lipid peroxidation parameters and interleukin-6

The pancreatic tissues were homogenized in 5 mL cold PBS per gram of tissue (1:5 dilution). The samples were centrifuged at 4000 rpm for 15 min at 4 °C. The supernatant was collected and stored at − 20 °C until the estimation of lipid peroxidation (expressed as MDA) according to the method described by Ohkawa et al. (1979), reduced glutathione (GSH) as assayed by Beutler et al. (1963), superoxide dismutase (SOD) in accordance with Nishikimi et al. (1972), catalase (CAT) according to the method described by Aebi (1984), and TAC as assayed by Koracevic et al. (2001). Additionally, the anti-inflammatory marker interleukin-6 (IL-6) was estimated as previously described by Chan and Perlstein (1978).

### Immunohistochemistry

According to Noreldin et al. (2018), additional tissue Sects. (4 µm) were obtained and mounted on positively charged gelatin-coated slides. Then, the slides were dried by incubation at 45 °C for 2 h and used for immunohistochemical localization of caspase-3, endothelial nitric oxide synthase (eNOS), and insulin antibodies in pancreatic tissues. Briefly, the slides were deparaffinized, rehydrated in descending grades of ethyl alcohol, washed with PBS, deactivated for endogenous peroxidase by 3% $H_2O_2$ in absolute methanol, then blocked for non-specific reaction, and incubated with 10% normal goat serum. After that, the sections were incubated overnight at 4 °C with rabbit polyclonal anticleaved caspase-3 (1:100, BioCare Medical, catalog number: CP229C, Concord, CA, USA), rabbit monoclonal anti-eNOS (Abcam, catalog number: ab300071, Cambridge, UK), and rabbit monoclonal anti-insulin antibody (Abcam, catalog number: ab181547, Cambridge, UK). The streptavidin–biotin complex was visualized using 3,3′-diaminobenzidine tetrahydrochloride (DAB)-$H_2O_2$ solution. Then, the slides were counterstained with Mayer's hematoxylin solution. Original representative micrographs were captured using a digital camera (EC3, Leica, Germany) linked to a Leica microscope (DM500). The quantification of immunoexpression intensities was performed by the Image J software (National Institutes of Health, Bethesda, MD, USA) as described by Khafaga et al. (2019 and 2021). Ten randomly chosen fields from various sections were chosen; the inverse mean density was determined as reported by Vis et al. (2000).

### Statistical analysis

Data were collected and analyzed using SAS (2004) via one-way analysis of variance. Duncan's test was applied for determining the significant treatment effects. *P*-values of less than 0.05 were used to denote statistical significance. The results are expressed as the means ± standard errors.

## Results

### Evaluation of serum insulin, cortisol, and interleukin-6

As summarized in Table 1, treatment with STZ significantly decreased ($p < 0.05$) insulin levels compared with those in control rats. However, rats co-treated with Vit D showed significant upregulation ($p < 0.05$) of the insulin levels compared with those of their counterparts in the STZ group. Furthermore, rats that received Vit D alone and control rats showed non-significant differences.

Regarding cortisol levels, data showed that the STZ group exhibited a significant increase ($p < 0.05$) in cortisol levels compared with the CTR group. However, the STZ + Vit D group showed significant downregulation ($p < 0.05$) of the cortisol levels compared with those of the STZ group. In

**Table 1** Effect of diabetes mellitus (DM) and vitamin D supplementation on serum levels of insulin, serum cortisol, and interleukin-6

| | Control | Vitamin D | Diabetes | Vitamin D + diabetes |
|---|---|---|---|---|
| Insulin (µIU/mL) | $22.65 \pm 1.71^{ab}$ | $29.44 \pm 2.67^{a}$ | $8.15 \pm 1.26^{c}$ | $18.62 \pm 2.26^{b}$ |
| Cortisol (m IU/dL) | $7.03 \pm 0.34^{c}$ | $9.22 \pm 0.12^{c}$ | $29.47 \pm 1.77^{a}$ | $15.42 \pm 1.74^{b}$ |
| IL-6 (pg/mL) | $5.33 \pm 0.58^{c}$ | $7.45 \pm 0.35^{c}$ | $27.74 \pm 2.11^{a}$ | $14.79 \pm 0.36^{b}$ |

The data presented as mean ± standard error. Means bearing different superscript letters within the same row are significantly different ($P < 0.05$)

contrast, rats that received Vit D alone and control rats did not show significant differences (Table 1).

Additionally, the evaluation of IL-6 levels in different groups showed that the STZ group showed a significant increase ($p < 0.05$) in IL-6 levels compared with the CTR group. However, rats treated with Vit D in concurrent with Vit D (STZ + Vit D) showed a significant reduction ($p < 0.05$) in their IL-6 levels compared with their counterparts in the STZ group. Moreover, rats that received Vit D alone and control rats did not show significant differences (Table 1).

## Evaluation of lipid peroxidation parameters

Data summarized in Table 2 showed that STZ-treated rats exhibited a significant increase in MDA levels compared with control rats. In contrast, the STZ + Vit D group showed a significant reduction in MDA levels compared with the STZ group. Moreover, the Vit D and CTR groups did not show significant differences in MDA levels.

Regarding GSH, SOD, CAT, and TAC levels, a significant reduction was reported in STZ-treated rats compared with those in control rats. In contrast, rats treated with STZ + Vit D showed a significant reduction in the levels of GSH, SOD, CAT, and TAC compared with STZ-treated rats. Additionally, rats treated with Vit D alone and control rats did not show significant alterations in these levels.

## Histopathological and immunohistochemical findings

Histopathological examination of pancreatic tissues revealed the destruction of β-cells in the islets of Langerhans and

**Table 2** Effect of diabetes mellitus (DM) and vitamin D supplementation on lipid peroxidation (expressed as malondialdehyde; MDA) and antioxidant enzymes (reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC))

| Groups | MDA (nmol/g tissue) | GSH (nmol/g tissue) | SOD (U/g tissue) | CAT (U/g tissue) | TAC (mmol/g issue) |
|---|---|---|---|---|---|
| Control | $27.54 \pm 1.46^{c}$ | $24.23 \pm 2.67^{a}$ | $15.13 \pm 1.48^{ab}$ | $18.89 \pm 2.68^{a}$ | $10.67 \pm 0.64^{b}$ |
| Vitamin D | $25.72 \pm 2.84^{c}$ | $24.65 \pm 1.94^{a}$ | $17.92 \pm 1.30^{a}$ | $22.97 \pm 2.99^{a}$ | $17.54 \pm 2.15^{a}$ |
| Diabetes | $61.73 \pm 5.25^{a}$ | $6.13 \pm 1.01^{c}$ | $7.26 \pm 0.83^{c}$ | $6.41 \pm 1.20^{c}$ | $5.06 \pm 0.38^{c}$ |
| Vitamin D + diabetes | $40.15 \pm 2.21^{bc}$ | $12.62 \pm 1.04^{bc}$ | $11.18 \pm 0.44^{bc}$ | $9.39 \pm 0.66^{bc}$ | $8.49 \pm 1.08^{bc}$ |

The data presented as mean ± standard error. Means bearing different superscript letters within the same column are significantly different ($P < 0.05$)

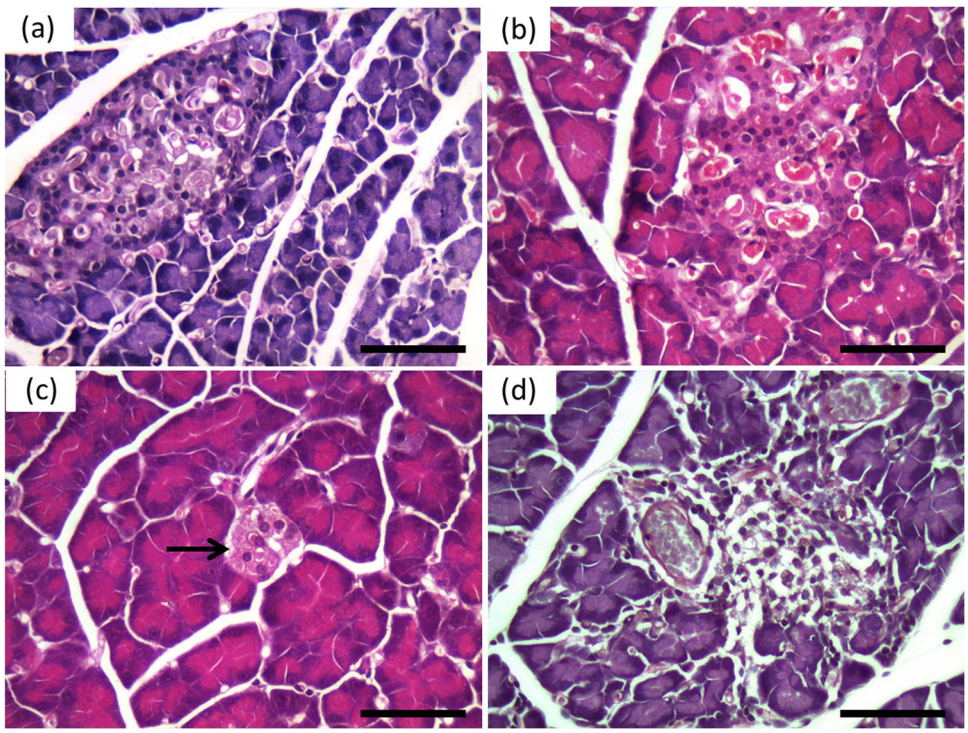


**Fig. 1** Representative photomicrograph for pancreatic tissues from control (**a**), Vit D-treated (**b**), diabetic (**c**), and Vit D-treated diabetic rats (**d**) (H&E stained section, scale bar = 50 µm) showing: (**a**, **b**) normal pancreatic lobules and Langerhans. (**c**) Destruction of beta cells in islets of Langerhans and amyloidosis-like deposition (arrow). **d** Improved histologic picture of β-cells in the islets of Langerhans

amyloidosis-like deposition in the diabetic group (Fig. 1c) compared with the CTR and Vit D-treated group (Fig. 1a and b; respectively). However, diabetic rats treated with Vit D showed improved histomorphologic picture of pancreatic tissues and β-cells in the islets of Langerhans (Fig. 1d). The semiquantitative scoring of pancreatic lesions including vacuolation, necrosis, and congestion revealed no significant changes in Vit D-treated group compared to control group. On contrary, rats in diabetic group showed significant increase in all scored pancreatic lesions compared to control group. However, diabetic rats treated with Vit D showed significant reduction in scored lesions compared to diabetic non-treated rats (Fig. 3a).

The immunohistochemical expression of caspase-3 is illustrated in Fig. 2 (a1–c1). The CTR group showed negative to mild immunoreactivity (a1), whereas rats in the diabetic group showed moderate immunoexpression of caspase-3 (b1). In contrast, rats with diabetes treated with Vit D showed mild immunoreactivity in the hyperplastic islets of Langerhans (c1). Additionally, intense immunoreaction was detected in the interlobar pancreatic duct, intralobar duct, and intercalated duct in the diabetic groups with or without Vit D treatment. No reactivity in the pancreatic acinus was detected in any group. The quantification of caspase-3 antibody in different groups revealed no significant changes in Vit D-treated group compared to control group. On contrary, rats in diabetic group showed significant increase in

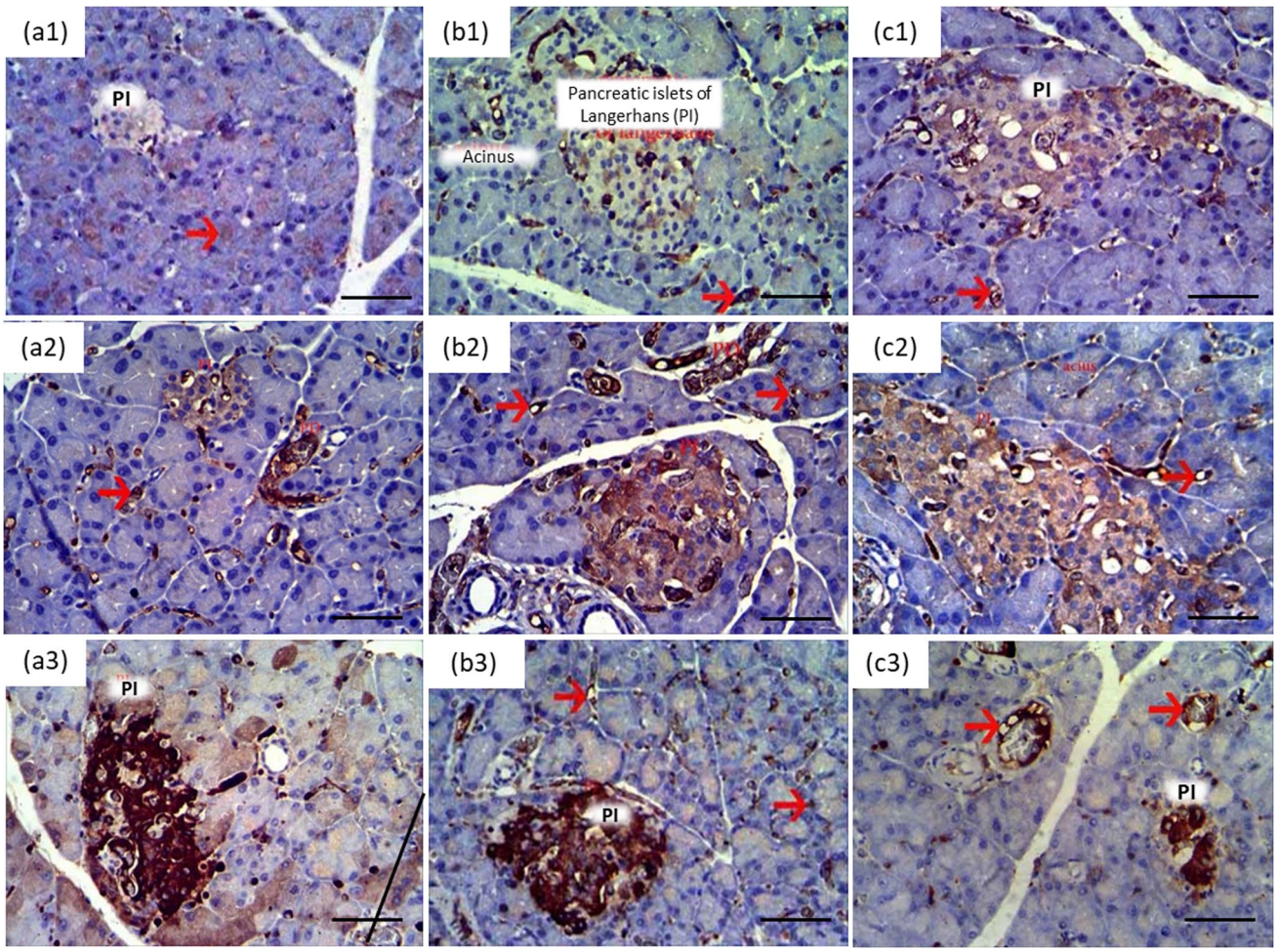


**Fig. 2** Representative photomicrograph for immunohistochemical expression of caspase-3, eNOS, and insulin in pancreatic tissues from control (**a1**-**a3**), diabetic (**b1**-**b3**), and Vit D-treated diabetic rats (**c1**-**c3**) (scale bar = 50 μm) showing: (**a1**) negative to mild immunoreactivity of caspase-3 (**b1**) moderate immunoexpression of caspase-3. **c1** mild immunoreactivity of caspase-3 in hyperplastic islets of Langerhans and intense immunoreaction in interlobular pancreatic duct (arrows). **a2** Negative to mild immunoexpression of eNOS. **b2** Intense immunoexpression of eNOS. **c1** Moderate immunoreactivity of eNOS in hyperplastic islets of Langerhans and intense immunoreaction was detected in interlobar pancreatic duct (arrows). **a3** Intense immunoreactivity of insulin in pancreatic islet of Langerhans. **b3** Moderate immunoreactivity of insulin in pancreatic islet of Langerhans. **c3** Intense immune reaction of insulin in pancreatic islet of Langerhans and intense immunoreaction in interlobular pancreatic duct (arrows). PI = pancreatic islets; PD = pancreatic duct

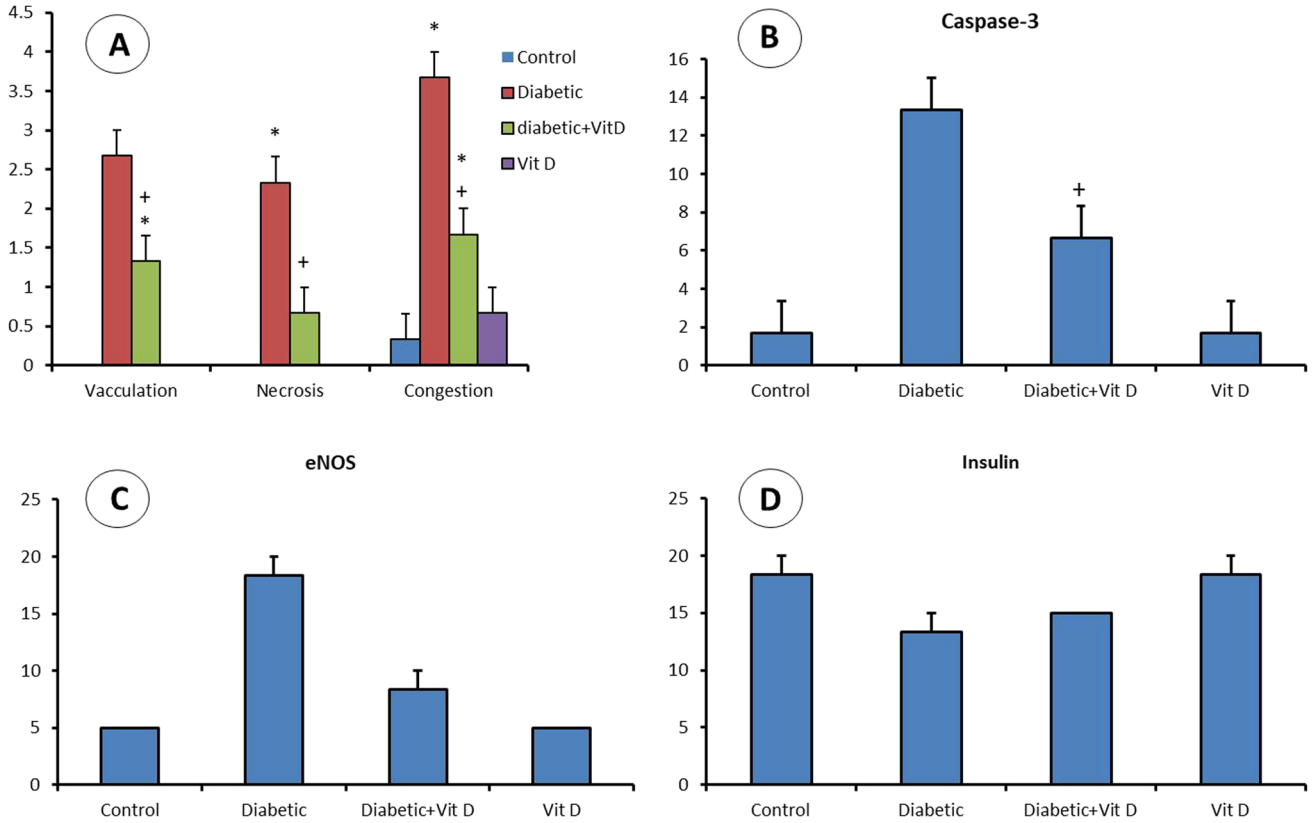


**Fig. 3** **a** Hematoxylin and eosin (H&E) semiquantitative scoring of pancreatic vacuolation, necrosis, and congestion. **b** Quantification of caspase-3 immune expression in the pancreatic islets of Langerhans in different groups. **c** Quantification of eNOS immune expression in the pancreatic islets of Langerhans in different groups. **d** Quantification of insulin immune expression in the pancreatic islets of Langerhans in different groups. Data were analyzed with one-way ANOVA followed by Tukey's multiple comparison test. * $p < 0.05$ vs. control. $^{+}$ $p < 0.05$ vs. Diab + Vit D. Error bars represent mean ± SD

caspase-3 immune expression compared to control group. However, diabetic rats treated with Vit D showed significant reduction in caspase-3 immunoreactivity compared to diabetic non-treated rats (Fig. 3b).

Regarding the immunoreactivity of eNOS, as shown in Fig. 1 (a2–c2), the CTR group showed negative to mild immunoexpression of eNOS (a2), whereas rats in the diabetic group showed intense immunoexpression of eNOS (b2). In contrast, rats with diabetes treated with Vit D showed moderate immunoreactivity in the hyperplastic islets of Langerhans (c2). Additionally, intense immunoreaction was detected in the interlobar pancreatic duct, intralobar duct, and intercalated duct in all groups. No reactivity in the pancreatic acinus was detected in any group. The quantification of eNOS antibody in different groups revealed no significant changes in Vit D-treated group compared to control group. On contrary, rats in diabetic group showed significant increase in eNOS immune expression compared to control group. However, diabetic rats treated with Vit D showed significant reduction in eNOS immunoreactivity compared to diabetic non-treated rats (Fig. 3c).

The immunoreactivity of the insulin antibody in pancreatic tissues is presented in Fig. 1 (a3–c3). Intense immunoreactivity of the pancreatic islets of Langerhans was noticed in control rats (a3). However, rats with diabetes showed moderate immunoreactivity of insulin in the pancreatic islets of Langerhans (b3). Additionally, rats with diabetes treated with Vit D showed an intense reaction in the pancreatic islet of Langerhans (c3). The quantification of insulin antibody in different groups revealed non-significant changes in all treated groups compared to control group (Fig. 3d).

## Discussion

The findings of this study revealed that Vit D supplementation regulates insulin levels. These results are consistent with those obtained by Hutchinson et al. (2011), who observed an inverse association between fasting blood sugar levels in adults and children and the effects of Vit D on insulin secretion and sensitivity. Vit D acts to reduce insulin resistance and the inflammatory mechanisms responsible for T1DM

and T2DM (Mathieu et al. 2005; Takiishi et al. 2010). Moreover, Boucher (2011) demonstrated that the mechanism of action of Vit D in T2DM is not only through the regulation of calcium trafficking in pancreatic β-cells, which regulate insulin synthesis, secretion, and sensitivity, but also by direct action on pancreatic β-cell function mediated by the binding of the active form of 1,25(OH)2D to its receptor (VDR), which is expressed in pancreatic β-cells. The direct effects of Vit D on insulin synthesis and secretion are supported by the promoter and transcriptional activation of the human insulin gene caused by 1,25(OH)2D. Another important finding was the significant increase in serum cortisol levels in rats with diabetes and significant reduction in their levels after co-treatment with Vit D. This finding is consistent with those obtained by Petramala et al. (2014), who observed that a reduction in Vit D level is associated with the excess production of both aldosterone and glucocorticoids (GCs). GCs increase gluconeogenesis by increasing the cellular levels of enzymes and substrates that increase the production of hepatic glucose. GCs in the pancreas attenuate insulin release and adversely affect the cells. Furthermore, GCs in peripheral tissues impair glucose uptake by adipose tissue and skeletal muscle (Delaunay et al. 1997). This study demonstrated that the rise in proinflammatory cytokines favors diabetes-related glucose toxicity, leading to oxidative stress, mitochondrial dysfunction, and hepatocellular death. A growing body of epidemiological, genetic, and experimental evidence demonstrated that IL-6 plays a significant role in the pathogenesis of inflammation, insulin resistance, and DM and its complications (Bastard et al. 2006). Additionally, DM may induce IL-6 expression, which can promote pancreatic inflammation (Yang et al. 2014). Interestingly, the repletion of serum Vit D downregulates IL-6 expression; thus, we can speculate that Vit D deficiency may facilitate the activity of these proinflammatory factors. Similarly, Sanchez-Nio et al. (2012) demonstrated that Vit D and its analogs 1,25(OH)2D3 and 25(OH)D3 inhibit lipopolysaccharide production, IL-6 expression, and tumor necrosis factor-alpha production by human monocytes.

Hyperglycemia causes oxidative stress by increasing the mitochondrial production of superoxide anion. In this study, GSH and SOD activities decreased in the pancreas of rats with diabetes, suggesting that pancreatic oxidative stress was stimulated. Similar results have also been reported in different animal models (Roza et al. 1995; Gupta et al. 2012). Different mechanisms have been proposed for the increase in intracellular and extracellular glucose concentrations and the resultant oxidative stress, including glucose auto-oxidation, glucose non-enzymatic glycosylation, and increase in glucose levels derived from the final product of advanced glycosylation. Additionally, Karunakaran and Park (2013) reported that the activities of pancreatic antioxidative enzymes (e.g., SOD and GPx) were diminished in the islet cells of animals with diabetes as β-cells are considered to be low in antioxidant defense and susceptible to oxidative stress. In this study, the levels of MDA were upregulated in rats with diabetes; MDA is one of the stable products of lipid peroxidation, which serves as a biomarker for the peroxidation of oxidative stress and polyunsaturated fatty acids (Naveed et al. 2018; Abd El-Hack et al. 2021). As a result, the excessive production of MDA leads to the inactivation of many cellular receptors (Wasef et al. 2021; El-Far et al. 2022). The findings of this study revealed a significant reduction in CAT and TAC in rats with diabetes. CAT is the second line of defense in the antioxidant system, which is located in peroxisomes and decomposes hydrogen peroxide ($H_2O_2$) to water ($H_2O$) and oxygen ($O_2$). CAT is a heme enzyme that reacts with $H_2O_2$ and other peroxides to protect the cell membrane from oxidative damage. A decrease in CAT activity could be attributed to the increased production of ROS. CAT scavenges excess free radicals via enzymatic and chemical mechanisms, which resulted in its depletion (Winterbourn 1993). Additionally, TAC is used as a new biomarker for diagnosing and preventing many diseases, including DM (Kusano and Ferrari 2008). It is an indicator of all oxidative stress agents and the defensive effect of antioxidants that can be used as the first step to evaluate patients' health status (Deeb et al. 2007). In contrast, the administration of Vit D significantly improved MDA, CAT, and TAC levels. Vit D can control the generation of free radicals and to reduce oxidative stress depending on the type of cell, which may be either by binding to VDR in the nucleus or through the hydrophobic parts of Vit D. In liver cells of mice with diabetes, Vit D plays an important role in controlling the generation of free radicals by binding to the VDR in the cell nucleus (Labudzynskyi et al. 2015). Furthermore, a direct correlation was found between serum vitamin D levels and TAC (Riachy et al. 2002). This vitamin can protect the cell membrane by inhibiting lipid peroxidation and exert its antioxidant effects by changing antioxidant enzymes (Kostoglou-Athanassiou et al. 2013). It acts as a scavenger of free radicals in the early stages, before the activation of pathways against oxidative stress (Anvari et al. 2016; Saif-Elnasr et al. 2017). Similarly, Bhat and Ismail (2015) and Foroozanfard et al. (2015) concluded that at physiological concentrations, Vit D plays a crucial role in protecting cells from oxidative stress and cellular damage by increasing the plasma levels of TAC and reducing the levels of MDA.

Regarding histopathological and immunohistochemical findings, in previous studies (Komers et al. 2001; Ozmen et al. 2007), STZ induces severe and permanent DM with a decrease in insulin levels 3 days after STZ administration in mature rats to produce cytotoxic models of diabetes, which are very similar to T2DM. Similar findings were reported in this study, where hyperglycemia was detected 3 days after STZ administration. Cell

death is the last stage of cellular damage, which can occur by necrosis or apoptosis where apoptosis is defined as programmed cell death. Caspase-3 is the main marker of apoptosis (Slauson and Cooper 2002). This study reported an increased expression of caspase-3 in the pancreatic islets. In the same line, Russo et al. (2006) demonstrated that defective apoptosis plays a major role in the development and progression of DM. Several in vitro studies have suggested that caspase-dependent apoptotic pathways are essential for β-cell apoptosis (Maedler et al. 2001). Vitamin D 1,25(OH)2D3 might promote various pro-apoptotic mechanisms, including the upregulation of pro-apoptotic genes and the downregulation of anti-apoptotic genes (Deeb et al. 2007).

eNOS is an important enzyme in the cardiovascular system. It catalyzes the production of NO, which is the key regulator of blood pressure, angiogenesis, and vascular remodeling (Shaul 2002). In a study, Szabo (2009) reported that a reduction in NO synthesis has a clear contribution to diabetic vascular complications, whereas an increase in NO has been associated with DM (Abou-Seif and Youssef 2004). Recently, it has been shown that NOS, primarily eNOS, plays an important role in the pathogenesis of diabetic cardiovascular diseases and nephropathy (Dellamea et al. 2014). Nathan and Xie (1994) initially intended to compensate for the downregulation of eNOS by inducing oxidative stress (Weldy et al. 2011). In fact, a high level of glucose induces an uncoupling of eNOS, and although membrane translocation is activated, this might be an inactivated form of the enzyme (Komers et al. 2006). In contrast, Labudzynskyi et al. (2015) concluded that the ability of Vit D to control free radical generation in liver and pancreatic cells of rats with diabetes and reduce oxidative stress may be exerted either by binding to the VDR in the nucleus or through Vit D hydrophobic parts.

Glucose homeostasis is regulated by hormones. Elevations in blood glucose levels during feeding stimulate insulin release from pancreatic β-cells through a glucose sensing pathway (Straub and Sharp 2002). Insulin is synthesized as a precursor molecule (proinsulin), which is processed before its secretion. Insulin stimulates glucose uptake from the blood into the adipose tissue and skeletal muscle (Concannon et al. 1998). Insulin antibodies detect endogenous levels of total insulin protein. Vit D induces autophagy, suppresses apoptosis of pancreatic β-cells, and prevents insulitis. Ding and Choi (2015) reported that Vit D increases insulin secretion and resistance of β-cells to cellular stress encountered during T2DM. Mechanistically, circulating 1,25(OH)2D can bind directly to VDRs in β-cells, and Vit D directly stimulates the expression of insulin receptors and promotes insulin-mediated glucose transport in vitro (Wolden-Kirk et al. 2011). A study by Bourlon et al. (1999) showed that 1,25(OH)2D could activate the de novo biosynthesis of insulin in the islets from Vit D-deficient rats after stimulating the islets with glucose, which increases the rate of conversion of proinsulin to insulin.

## Conclusion

Vit D ameliorated and mitigated the harmful biochemical and immunohistochemical impacts of T2DM most likely by increasing antioxidant efficiency, which is responsible for increasing insulin secretion and sensitivity. Additionally, ameliorating the function of β-cells and improving the immunoreactivity of caspase-3, eNOS, and insulin antibodies, which are essential for insulin-mediated intracellular processes, could attribute partly to the antidiabetic activities of Vit D.

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**Author contribution** The authors contributed to the present study as follows: data curation: Fatima El Zahra M. Fathi; formal analysis: Kadry M. Sadek, Asmaa F. Khafaga; resources: Abdel Wahab Al senosy, Hanan A. Ghoniem; software: Sahar Fayez, Mohamed F. Zeweil; writing—original draft: Fatima El Zahra M. Fathi; writing—review and editing: Asmaa F. Khafaga.

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**Data availability** The data used to support the findings of this study are included within the article and the coding of the data is available from the corresponding author upon reasonable request.

## Declarations

**Ethics approval and consent to participate** The experimental procedures were performed according to the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health, and the study protocol was approved by the local authorities (Faculty of Veterinary Medicine, Damanhur University, Egypt). Precautions were taken during sampling and throughout the entire experiment to minimize animal suffering.

**Consent for publication** All the authors agree for consent for publication and the current article does not contain data from any individual person.

**Competing interests** The authors declare no competing interests.

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