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Metabolic Impact of Vitamin D on the Context of Metabolic Syndrome

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Abstract

Vitamin D deficiency is one of the major affecting factors on metabolic syndrome, cancer, cardiovascular diseases, and type 2 diabetes mellitus. So, this study aimed to show the anti-diabetic effects of vitamin D on type 2 diabetic rats. Therefore, 45 rats were divided into three groups (15 rats per each group). The first group served as a control and fed on a standard chow diet while the other two groups served as diabetic groups as they fed on high fructose, high fat, and high sucrose diet and for 12 weeks then they injected with intraperitoneal single dose (45 mg/kg b.wt) of STZ dissolved in cold 0.01 M citrate buffer (pH 4.5) to develop type 2 diabetes mellitus. After one week of injection the third diabetic group was treated for 4 weeks with two intramuscular (20,000 IU/Kg) of vitamin D dissolved in sesame oil. The obtained results demonstrated that administration of vitamin D could improve serum glucose and insulin levels with an increase in serum calcitonin and calcium in correlation with the decrease in parathyroid hormone, phosphorus, and lipids levels in the presence of significant upregulation of gene expression in liver (PPARα, GLP-1, and IGF-1) and in adipose tissue (Ptch, Smo, Gli-1, and hhip). In conclusion, vitamin D administration can improve insulin resistance by improving blood glucose and insulin levels.

KEYWORDS
Vitamin D, Metabolic syndrome, Type 2 diabetes, Rats

INTRODUCTION

Metabolic syndrome (MetS) is known as a Syndrome X, which is a cluster of risk factors that cause central obesity, insulin resistance (IR), dyslipidemia, hypertension type 2 diabetes mellitus (T2DM) and coronary vascular disease (CVD) (Gunawan *et al.*, 2021).

Vitamin D is a fat-soluble vitamin that considered also as a steroid hormone. It is derived from 7-dehydrocholesterol in skin cells after exposure to ultraviolet radiation (UVB), and it is activated in the liver and kidneys by their hydroxylases enzymes in the form of (1,25-dihydroxycholecalciferol). It is obtained from dietary products such as salmon and egg yolks. The active form of vitamin D3 is regulated by parathyroid hormone (PTH), calcitonin, calcium, and phosphorus (Mallya *et al.*, 2016). Vitamin D deficiency is associated with obesity, insulin resistance, and hepatic lipid accumulation through promoting fatty acid oxidation, hypertension, cardiovascular diseases, infectious, and autoimmune diseases, albuminuria, and cancer (Lontchi-Yimagou *et al.*, 2020).

Insulin like growth factor-1 (IGF-1) is known as a somatomedin C and act as anabolic growth hormone produced by liver. It promotes the effect of growth hormone (GH). It has a similar structural and function like insulin as they bind to the same cell membrane receptor it improves insulin resistance by regulating glucose homeostasis through promoting the glucose uptake in peripheral tissues, suppressing the hepatic glucose release and increasing insulin sensitivity (Derakhshanian *et al.*, 2017).

Peroxisome proliferator activated receptors (PPARs α) are ligands used to activate transcriptional factors of nuclear hormone receptor. They found on liver, heart, BAT and skeletal muscle tissues which has high fatty acid oxidation (FAO). They can be activated by higher intake of high fat diet (Villarroel-Vicente *et al.*, 2021).

They have an important role in lipid and glucose metabolism through metabolic competition (the glucose/fatty acid cycle) including fatty acid binding and activation, fatty acid transport and desaturation, mitochondrial fatty acid oxidation, peroxisomal fatty acid oxidation fatty acid oxidation, synthesis and the breakdown of triglycerides (TAGs).They reduce abdominal fat and improve insulin sensitivity (Xu *et al.*, 2022).

Glucagon like peptide (GLP-1) is produced by pancreatic α cells and intestinal enteroendocrine L cells in response to nutrient ingestion, such as protein, glucose, fatty acids, and dietary fiber. GLP-1R is located in the hypothalamus, intestine, liver, and pancreas. The peripheral and the brain (hypothalamic ARC, PVN

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and brainstem areas) they help in body weight loss through inhibiting food intake and improving insulin sensitivity. GLP-1 improves pancreatic β-cell proliferation, inhibits cell apoptosis, it reduces the postprandial glycemic response by stimulating insulin secretion (Pinyo *et al*., 2019).

Hedgehog (Hh) proteins, including Shh, Indian hedgehog (Ihh), and Desert hedgehog (Dhh), Hh bind to the inhibitory receptor Patched (PTCH) leading to the activation of the cell-surface receptor Smoothened (Smo) and subsequently drives the transcription of Gli target genes (Gu *et al*., 2021). It has a role in nonalcoholic fatty liver disease by regeneration of the liver from progenitor cells. They regulate epithelial and cell expansion during early pancreas morphogenesis when hedgehog signaling pathway is highly activated or has any defects that will impair cell function and insulin secretion which lead to insulin resistance by signaling in myeloid cells of adipose tissue which may be the cause of type 2 diabetes mellitus (Garg *et al*., 2022).

This study was designed to investigate the possible palliative effect of vitamin D administration on obese type 2 diabetic rats with addressing the underlying mechanism.

MATERIALS AND METHODS

Animals

Forty five adult male Sprague–Dawley rats (9 weeks old; 150 -200 g) were obtained from the laboratory animal's farm, Faculty of Veterinary Medicine, Zagazig University, Egypt. Animals were kept under controlled environmental conditions 12 h light/ dark cycle at 24oC and free access to water and diet. All research procedures were complied with the NIH recommendations for the Care and Use of Laboratory Animals and approved by the Ethics of Animal Use in Research Committee (IACUC), Zagazig University, Egypt, with the reference number (ZU-IACUC/2/F/131/2023).

Rats were divided into three groups (15 rats per each group). The first group served as a control and fed on standard chow diet, while the other two groups served as diabetic groups as they fed on high fructose and high fat and high sucrose diet for 12 weeks, then they injected with intraperitoneal single dose (45 mg/kg b.wt) of STZ dissolved in cold 0.01 M citrate buffer (pH 4.5) to develop type 2 diabetes mellitus. After one week of injection the third diabetic group was treated for 4 weeks with two intramuscular (20,000 IU/kg) doses of vitamin D dissolved in sesame oil (de Moura *et al*., 2008; Derakhshanian *et al*., 2019; Gunawan *et al*., 2021).

Collection of blood samples

Fasting blood samples were collected by cardiac puncture then centrifuged immediately to separate blood sera and plasma.

Lipid Profile

Serum total cholesterol and TGs were measured using enzymatic colorimetric methods (N.S. BIOTEC, Wellkang Ltd, UK). HDL-C was analyzed using NS Biotec HDL-precipitating reagent. LDL-C was calculated using Friedewald formula (Friedewald *et al*., 1972): LDL-C (mg/dl) = TC – HDL-C – (TGs/5).

Serum hormonal and calcium and phosphorus levels

Serum hormonal (parathyroid hormone, calcitonin, insulin, 1, 25 dihydroxy cholecalciferol) levels were evaluated using commercially available kits according to the manufacturer's guidelines. Serum calcium and phosphorus levels were analyzed using calcium & phosphorus commercial kits (SPINREACT).

Hepatic MDA and antioxidants assays

Liver samples were collected after rat sacrifice, such samples were divided into two parts; one used for hepatic antioxidants assay and the second for histopathology.

The MDA (MyBioSource, USA), CAT (MyBioSource, USA), GSH (Biodiagnostic, USA), and SOD (Abcam, USA) were evaluated following the manufacturer's instructions.

Gene expression

Isolation of total RNA from liver and adipose tissue was performed according to the manufacturer's instructions using TRIZOL reagent (Invitrogen, Germany), quantification was performed using a NanoDrop® ND-1000 spectrophotometer, and the RNA was used in the cDNA synthesis reaction using a Maxima first strand cDNA synthesis kit from Thermo Fisher Scientific (MA, USA). Real-time quantitative PCR was performed on a Stratagene (MX3005P, USA) using the QuantiTect SYBR Green PCR Master Mix (Qiagen). Primers for the targeted genes are included in Table 1. Using the Stratagene MX3005P program, we determined the amplification curves and cycle threshold (CT) values. Fold change in gene expression was reported as as $2^{-\Delta\Delta CT}$ relative to control after normalization to GADPH as a housekeeping gene (Darwish *et al*., 2010).

Histopathological examination

After eighteen weeks, the rats were anesthetized and euthanized with i.p injections of 50 mg/kg ketamine and 30 mg/kg xylazine. Tissue specimens from liver, kidney, testes, brain, heart and retina were fixed in neutral buffered formalin 10% for 24 hours, dehydrated in ascending grades of alcohol (70%-100%), cleared in xylene, embedded in paraffin wax. 5µm thickness of

Table 1. Primers used in the present study.

Gene	Forward Primer	Reverse Primer	bp	accession no.
Smo	TTCCTCATCCGAGGGGTCAT	ATTGATCTTGCTGGCTGCCT	87	NM_012807.1
Gli-1	CCTCCACCCCAGTATCTCCA	ACAATTCCTGCTGCGACTGA	163	NM_001191910.1
Ptch-1	TCCCCTCCTCCTCCTCTTTC	CTTGTTCTCCTCACCGACCC	192	NM_053566.3
Hhip	GCTCTTTGGTCCTGATGGCT	GCTGGTTGGTGCTGTTGAAG	191	NM_001191817.1
PPAR-α	GTCCTCTGGTTGTCCCCTTG	GTCAGTTCACAGGGAAGGCA	176	NM_013196.2
Gapdh	GCATCTTCTTGTGCAGTGCC	TACGGCCAAATCCGTTCACA	74	NM_017008.4
GLP-1	CTCAGCTCAGTCCCACAAGG	AGCTGCCTTGTACCAGCATT	87	NM_012707.3
IGF-1	GACCCGGGACGTACCAAAAT	GAACTGAAGAGCGTCCACCA	162	NM_178866.4

paraffin sections were obtained by using automated microtome then stained with routine Hematoxylin and Eosin (H & E) (Suvarna *et al*., 2018).

Statistical analysis

The GraphPad Prism 8 (GraphPad software Inc., San Diego, CA 18940, USA) was used to analyses the data. The acquired information is presented as mean values ± SE. The Shapiro-Wilk test was used to check for data normality. One-way analysis of variance (ANOVA) was used for group comparisons. The Tukey post hock test was used to analyses the data, and differences between groups were declared significant when the p-value was less than 0.05.

RESULTS

Effect of vitamin D administration on lipid profile of type 2 diabetic rats

The obtained results revealed that the diabetic group had a significant (P <0.01) increase in the mean value of cholesterol, triglycerides, LDL, and VLDL and a decrease in the mean value of HDL compared to the control group (Fig. 1). On the other hand, the administration of vitamin D caused a significant (P <0.01) reduction in the mean value of serum level of cholesterol, triglycerides, LDL, and VLDL with an increase in the mean value of the serum level of HDL compared with diabetic group (Fig. 1)

Effect of vitamin D administration on the hormonal and biochemical parameters in type 2 diabetic rats

The results of the study illustrated that the diabetic group provoked a significant (P <0.01) decrease in the mean value of the serum level of calcium, calcitonin, 1, 25 dihydroxy cholecalciferol and insulin, with an increase in the mean value of the serum level of parathyroid hormone and phosphorus compared to control group. On the other hand, the administration of vitamin D caused a significant (P <0.01) increase in the mean value of the serum level of calcium, calcitonin, 1, 25 dihydroxy cholecalciferol and insulin with a decrease in the mean value of serum level of (parathyroid hormone and phosphorus) compared with diabetic group (Fig. 2)

Effect of vitamin D administration on the oxidative stress and hepatic antioxidants level in type 2 diabetic rats

The outcomes we obtained from our study showed that the diabetic group had a significant (P <0.01) decrease in the mean value of the hepatic level of CAT, SOD, GPX, and an increase in the mean value of MDA compared to control group. Conversely, vitamin D administration caused a significant increase in the mean value of the hepatic level of CAT, SOD, GPX, and a decrease in the mean value of MDA compared with diabetic group (Fig. 3).

Effect of vitamin D administration on the relative expression level of PPARα, GLP-1 and IGF-1 in the hepatic tissue of type 2 diabetic rats

Relative mRNA expression was calculated with ΔCt method using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the housekeeping gene. The results showed a significant down regulation in the relative expression of PPARα, GLP-1 and IGF-1 in the hepatic tissue of the diabetic rats when they compared with the control group. On contrast, vitamin D administration caused a clear upregulation in the relative expression of PPARα, GLP-1 and IGF-1 in the hepatic tissue of the diabetic rats when they compared with the diabetic non treated group (Fig. 4).

Effect of vitamin D administration on the relative expression level of Ptch, Gli-1, Smo and Hhip in the adipose tissue of type 2 diabetic rats

The results presented in Fig. 5 showed an extreme upregulation in the relative expression of Ptch, Gli-1 and Smo with a drastic down regulation in the relative expression of Hhip in the adipose tissue of the diabetic rats when they compared with the control group. On the contrary, vitamin D administration caused

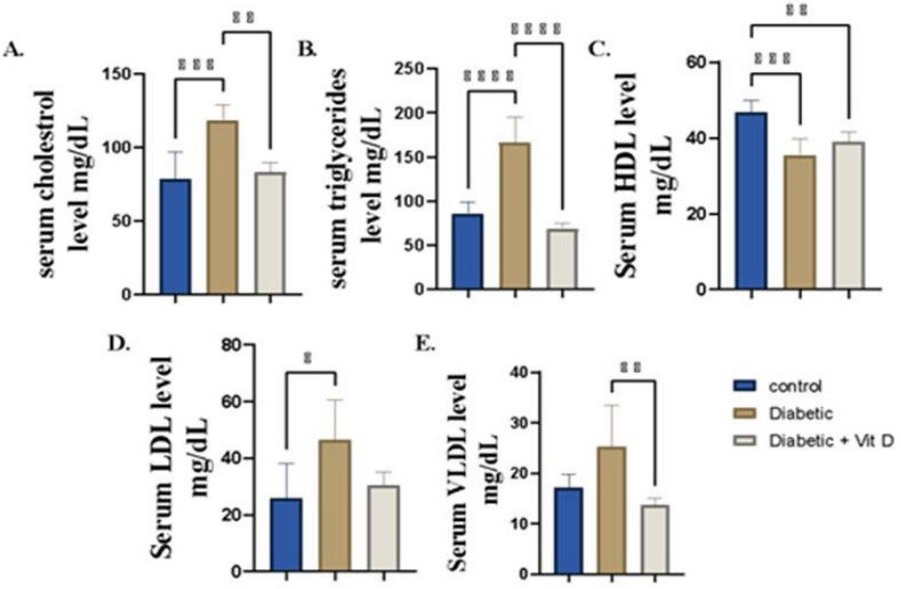

Fig. 1. Effect of vitamin D administration (20,000IU/Kg, IM), for 4 weeks on serum level of (A) cholesterol., (B) triglycerides., (C) HDL., (D) LDL, (E) VLDL. In type 2 diabetic rats in control, diabetic and diabetic + Vit D groups.
Data are expressed as mean value ± SE, n = 6 rats per each group. Each bar carrying the Significance which may be significant at * means when *p < 0.05, **p < 0.01 *** p < 0.001, **** p < 0.0001.

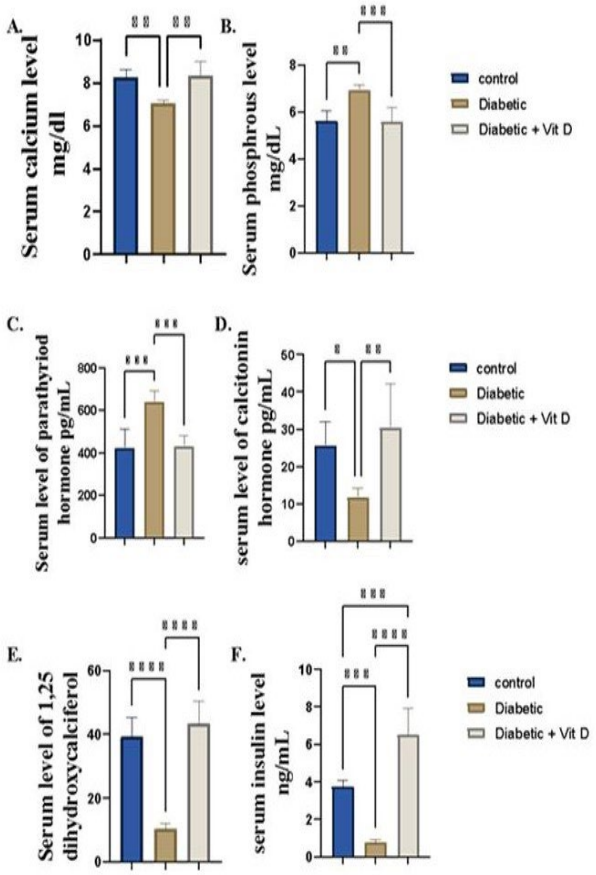

Fig. 2. Effect of vitamin D administration (20,000IU/kg i.m), for 4 weeks on serum level of (A) calcium, (B) phosphorus, (C) parathyroid hormone, (D) calcitonin, (E) 1,25 dihydroxy cholecalciferol, (F) Insulin in type 2 diabetic rats in control, diabetic and diabetic + Vit D groups. Data are expressed as mean value ± SE, n = 6 rats per each group. Each bar carrying the Significance which may be significant at * means when *p < 0.05, **p < 0.01 *** p < 0.001, **** p < 0.0001.

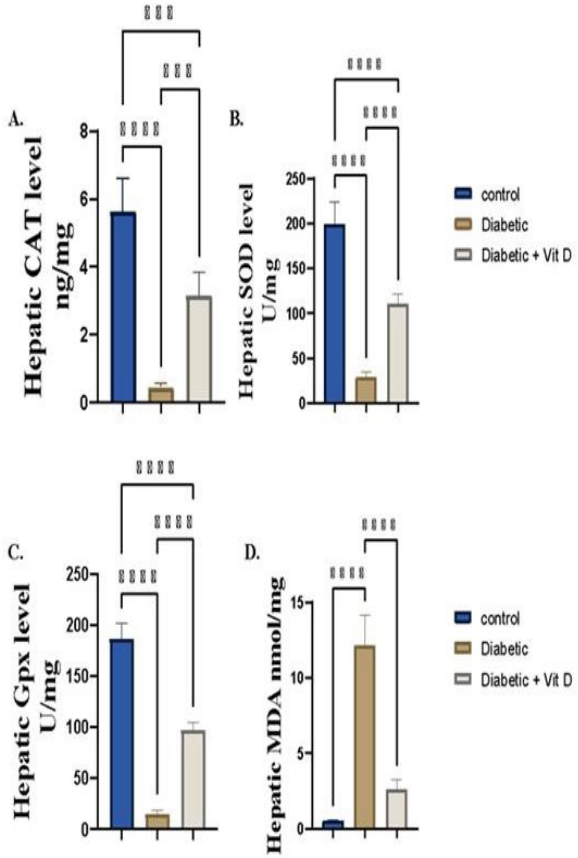

Fig. 3. Effect of vitamin D administration (20,000IU/kg i.m), for 4 weeks on serum level of hepatic antioxidants (A) CAT, (B) SOD, (C) GPX (D) MDA in type 2 diabetic rats in control, diabetic and diabetic + Vit D groups
Data are expressed as mean value ± SE, n = 6 rats per each group. Each bar carrying the Significance which may be significant at * means when *p < 0.05, **p < 0.01 *** p < 0.001, **** p < 0.0001.

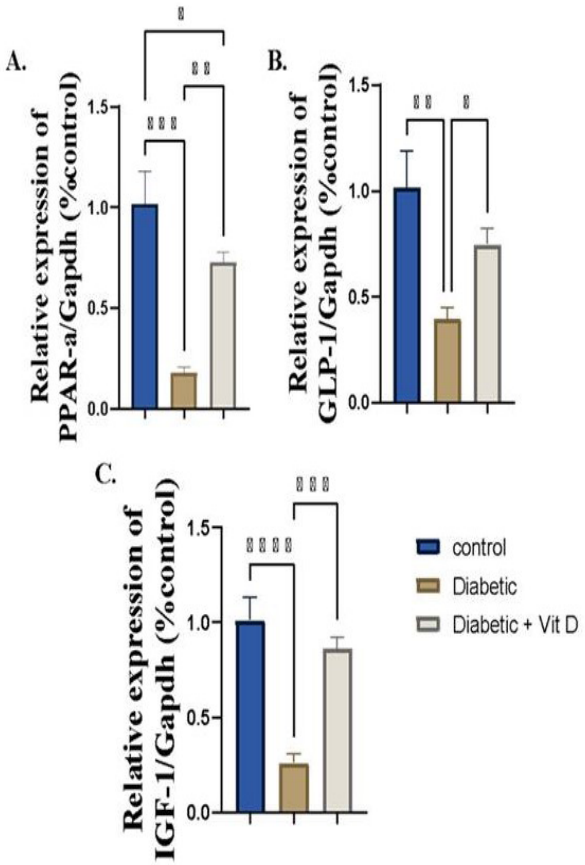

Fig. 4. Effect of vitamin D administration (20,000IU/kg i.m), for 4 weeks on the relative expression level of (A) PPARα, (B) GLP-1, (C) IGF-1 in the hepatic tissue of type 2 diabetic rats in control, diabetic and diabetic + Vit D groups.
Data are expressed as mean value ± SE, n = 6 rats per each group. Each bar carrying the Significance which may be significant at * means when *p < 0.05, **p < 0.01 *** p < 0.001, **** p < 0.0001.

a marked down regulation in the expression of Ptch, Gli-1 and Smo and a significant upregulation of Hhip in their adipose tissue when they compared with the diabetic group (Fig. 5).

Figure 6 shows significant increase in the body weight associated with an increase in the blood glucose level.

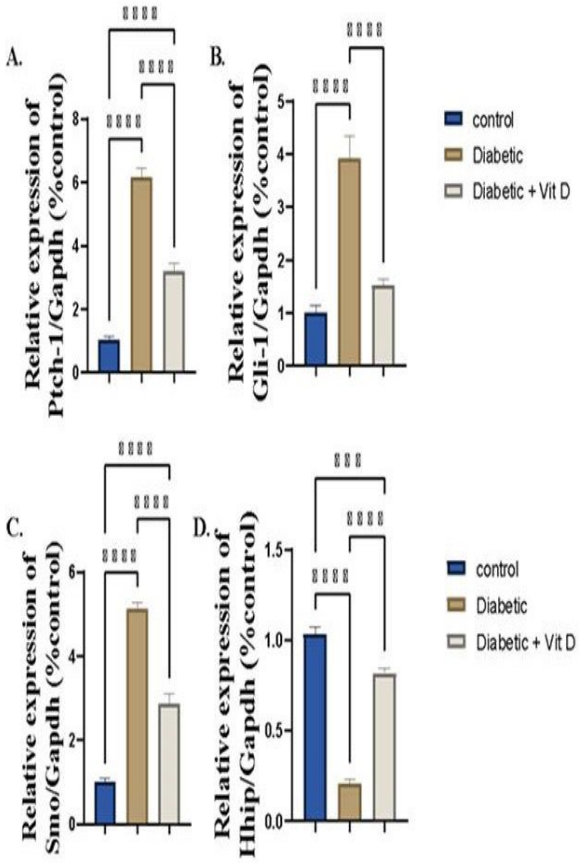

Fig. 5. Effect of vitamin D administration (20,000IU/kg i.m), for 4 weeks on the relative expression level of (A) Ptch, (B) Gli-1, (C) Smo, (D) in the adipose tissue of type 2 diabetic rats in control, diabetic and diabetic + Vit D groups. Data are expressed as mean value ± SE, n = 6 rats per each group. Each bar carrying the Significance which may be significant at * means when *p < 0.05, **p < 0.01 *** p < 0.001, **** p < 0.0001.

Histopathological findings

Liver

Control group (Fig. 7A) showed normal histological structures of hepatic lobules, portal triads, and central veins. Diabetic group (Fig. 7B, 7C) revealed randomly distributed areas of vacuolated hepatocytes, multifocal areas of co-agulative necrosis encircled by number of inflammatory cells, beside presence congested vasculatures. But, most hepatic cords and stromal structures were apparently normal in vitamin D treated group (Fig. 7D) except minute areas of leukocytic aggregates that were seen.

Kidney

Control group (Fig. 8A) showed normal architectures of the glomerular corpuscles and renal tubules. The diabetic group (Fig. 8B, 8C) revealed focal tubular necrosis with pyknotic nuclei and pale eosinophilic cytoplasm beside atrophied some glomerular tufts. The latter were congested in some examined sections. Vitamin D-treated group (Fig. 8D) displayed preserved structures of glomerular tufts and cloudy swelling in some renal tubular epithelium.

Testes

Control group (Fig. 9A) showed normal histology of seminepherous tubules and interstitial tissues. The seminepherous tubules were lined by a germinal epithelium which formed spermatogonia, primary spermatocytes, secondary spermatocytes, spermatid and mature sperms. The diabetic group (Fig. 9B) revealed atrophied some seminepherous tubules and edema within interstitial tissue. The atrophied tubules represented by degenerated and necrotic spermatocytic elements with pyknotic nuclei. In the other hand, the majority of seminepherous tubules and interstitial tissue were apparently normal in vitamin D-treated group (Fig. 9C, 9D). But there were minute areas of interstitial edema and few number of spermatocytes giant cells within some seminepherous tubules.

Cerebral cortex

Control group (Fig. 10A) showed normal histological structures of neurons, glia cells, vasculatures, and neutrophils. However, the diabetic group (Fig. 10B) revealed large numbers of degenerated or necrotic neurons, vacuolated neutrophils and congested cerebral blood vessels. While Vitamin D-treated group (Fig. 10C) showed few number of degenerated neurons and preserved structures of most neurons, glia cells and cerebral vasculatures.

Heart

Control group (Fig. 11A) showed normal histomorphology of striated branched cardiomyocytes with centrally located oval nuclei. But, the diabetic group (Fig. 11B) exhibited hyaline degener-

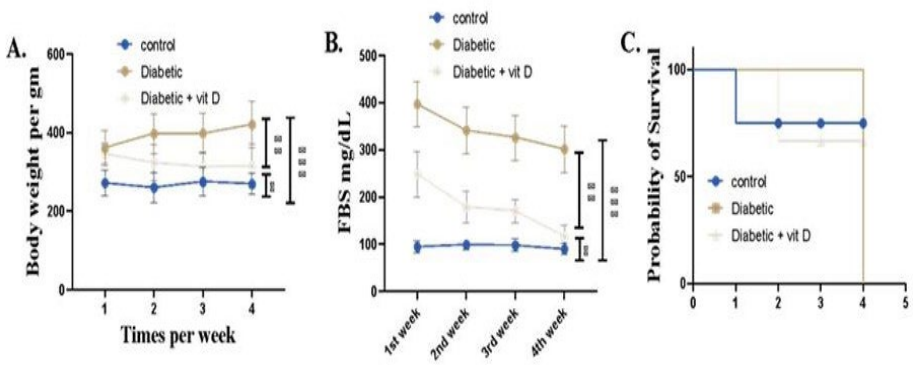

Fig. 6. (A) Body weight (g/week), (B) Fasting blood glucose (FBS (mg/dl), (C) probability of survival to the control, diabetic, diabetic + VD group during the experimental period. Data are expressed as means ± S.E.M.

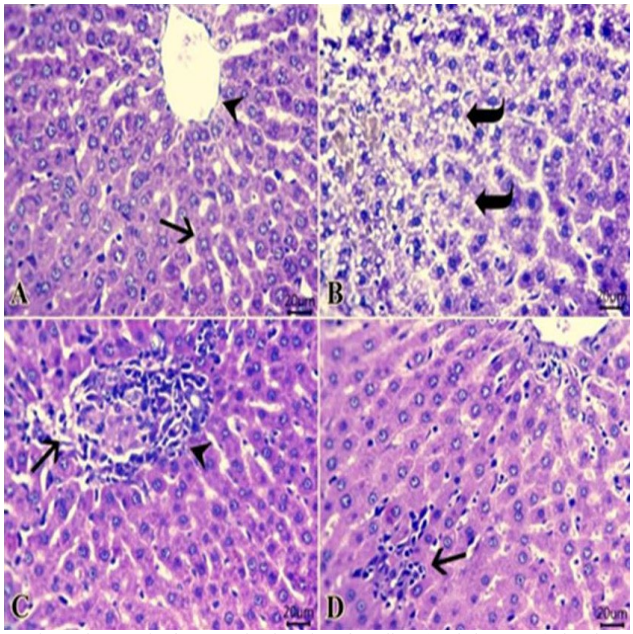

Fig. 7. Photomicrograph of H&E stained sections from liver (Scale bar 20µm) showing: normal histological structures of hepatic cords (arrow) and central vein (arrow) in control group (A). Randomly distributed areas of vacuolated hepatocytes (curved arrows), multifocal areas of co-agulative necrosis (arrow) encircled by number of inflammatory cells (arrowhead) in diabetic group (B, C). Apparently normal most hepatic cords with presence minute areas of leukocytic aggregates (arrow) in treated group (D).

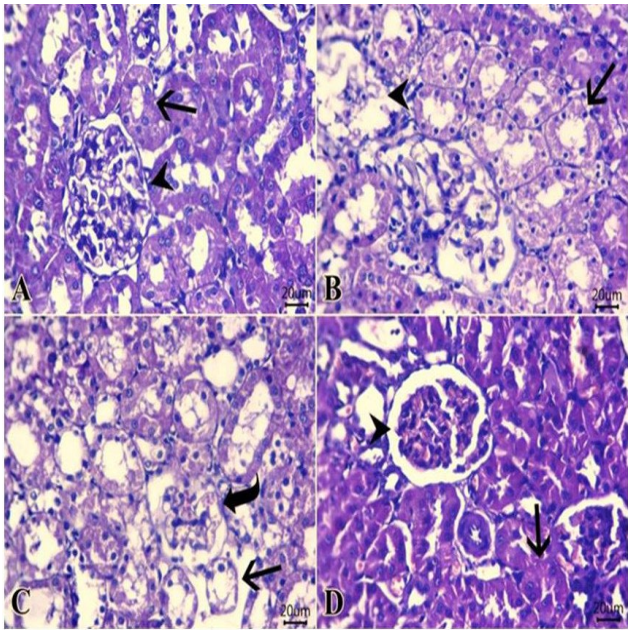

Fig. 8. Photomicrograph of H&E stained sections from kidney (Scale bar 20µm) showing: normal architectures of glomerular corpuscles (arrowhead) and renal tubules (arrow) in control group (A). Focal tubular necrosis (arrows) atrophied some glomerular tufts (arrowhead) and congested some glomerular tufts (curved arrow) in diabetic group (B, C). Preserved structures of glomerular tufts (arrowhead) and cloudy swelling in some renal tubular epithelium (arrow) in treated group (D).

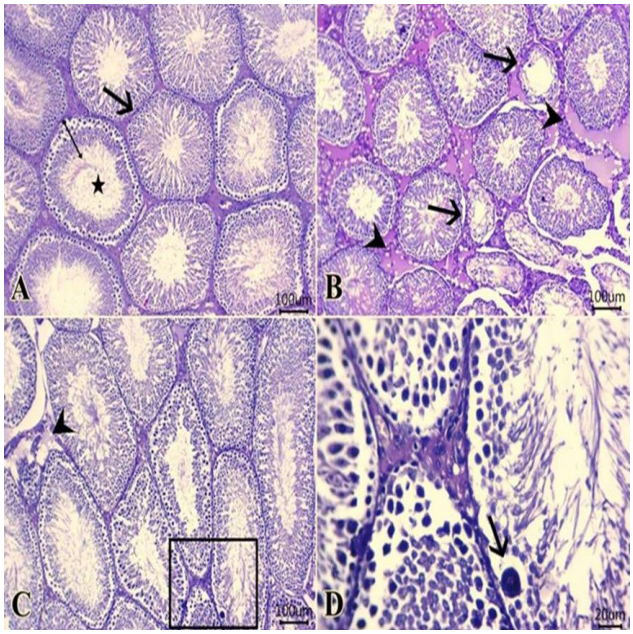

Fig. 9. Photomicrograph of H&E stained sections from testes showing: normal histopathology of seminepherous tubules (arrow) lined by germinal epithelium (double headed arrow) and filled with mature sperms (star) in control group (A) (Scale bar 100µm). Atrophied some seminepherous tubules with necrotic spermatocytes elements (arrows) and edema within interstitial tissue (arrowheads) in diabetic group (B) (Scale bar 100µm). Minute areas of interstitial edema (arrowhead) and spermatocyte giant cell (arrow) within some seminepherous tubules in treated group (C, D) (Scale bar 100, 20µm).

ations of most cardiac muscles which appeared more eosinophilia in cytoplasm with absence of their striations. In the other hand, Most cardiac muscles were apparent normal in treated group (Fig. 11C) but few zenker,s degenerated muscles were also seen.

Retina

Control group (Fig. 12A) showed normal histology of retinal layers which formed from: ganglion cell layer (GCL), inner nuclear layer (INL), outer nuclear layer (ONL), inner segment (IS) and outer segment (OS). The diabetic group (Fig. 12B) showed dilated retinal capillaries, dissociated and apoptotic INL and vacuolated inner and outer segment layers. Vitamin D- Treated group (Fig. 12C) revealed swelling in ganglion cell layer (GCL) and mildly dilated retinal capillaries and normal remaining retinal layers.

Abdominal fat

Control group (Fig. 13A) showed normal histology of sharp, clear vacuolated adipocytes with peripherally located nucleus and less amount of extracellular matrix. The diabetic group showed closely packed enlarged fat cells with number of ruptured adipocytes (Fig. 13B). Clear, empty adipocytes with compressed nucleus at one side and an intact cell membrane were

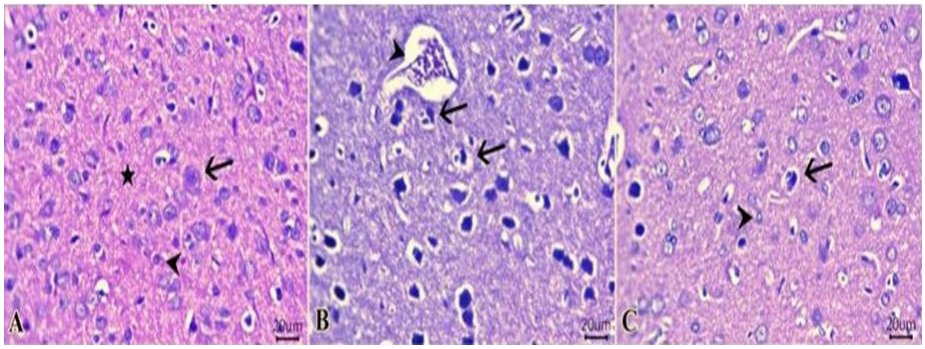

Fig. 10. Photomicrograph of H&E stained sections from cerebral cortex (Scale bar 20 µm) showing: normal histological structures of neurons (arrow), glia cells (arrowhead), vasculatures and neutrophil (star) in control group (A). Degenerated or necrotic large number of neurons (arrows), vacuolated neutrophil and congested cerebral blood vessels (arrowhead) in diabetic group (B). Few numbers of degenerated neurons (arrow) and preserved structures of most neurons and glia cells (arrowhead) in treated group (C).

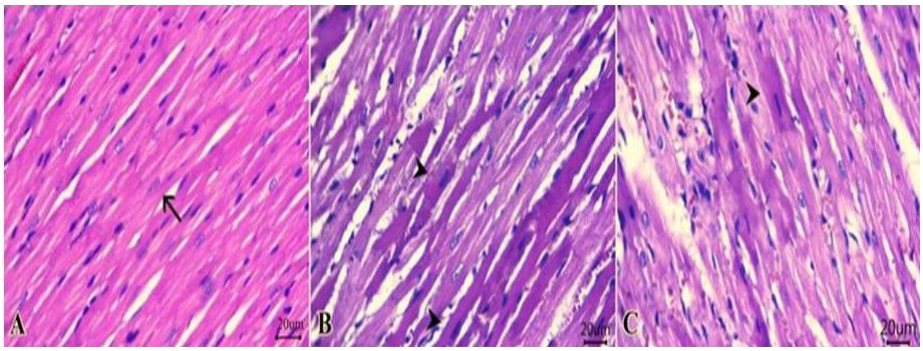

Fig. 11. Photomicrograph of H&E stained sections from heart (Scale bar 20µm) showing: normal histomorphology of striated branched cardiomyocytes with centrally located oval nuclei (arrow) in control group (A). Hyaline degenerations of most cardiac muscles (arrowheads) in diabetic group (B). Few zenker,s degenerated muscles (arrowhead) in treated group (C).

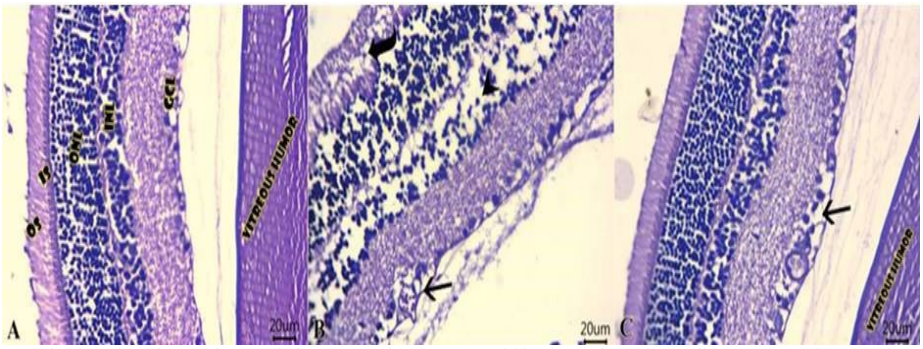

Fig. 12. Photomicrograph of H&E stained sections from retina (Scale bar 20µm) showing: normal histology of ganglion cell layer (GCL), inner nuclear layer (INL), outer nuclear layer (ONL), inner segment (IS) and outer segment (OS) in control group (A). Dilated retinal capillaries (arrow), dissociated and apoptotic INL (arrowhead) and vacuolated inner and outer segment layers (curved arrow) in diabetic group (B). Swelling in ganglion cell layer (GCL) (arrow) with mildly dilated retinal capillaries and normal remaining retinal layers in treated group (C).

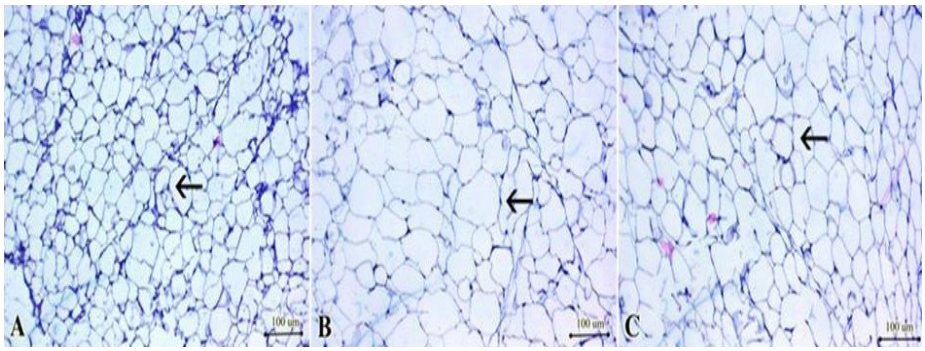

Fig. 13. Photomicrographs of H&E stained sections from abdominal fat (Scale bar 100 µm) showing: normal histology of sharp, clear vacuolated adipocytes with peripherally located nucleus (arrow) and less amount of extracellular matrix in control group (A). Closely packed enlarged fat cells (arrow) with number of ruptured adipocytes in diabetic group (B). Clear, empty adipocytes with compressed nucleus at one side and an intact cell membrane (arrow) in treated group (C)

observed in vitamin D-treated group (Fig. 13C) with minimal interstitial connective tissue bands. Moreover, the size of adipocytes in treated group become lesser in diameters in comparison with the diabetic group.

DISCUSSION

The study evaluated the improving actions of vitamin D administration on obesity, hyperlipidemia, insulin resistance, oxidative stress, and inflammation as they are the major risk factors of metabolic syndrome. In the present study, we showed that the high caloric intake of high fructose, high fat and high sucrose diet for 12 weeks obviously induced obesity, while on 13th week, rats was injected by STZ to develop type 2 diabetes mellitus. This caused lipotoxicity and glucotoxicity leading to dysfunction of pancreatic islet β cell causing decreasing in insulin action and secretion leading to elevation in the blood glucose level and increased body weight in the diabetic group compared with the control group. The deficiency of vitamin D is a risk factor of hyperlipidemia by increasing in serum level of cholesterol, triglycerides, VLDL and LDL and a significant decrease in serum level of HDL (Karhapää *et al*., 2010, Vitezova *et al*., 2015).

The obtained results of the present study agreed with that of Liu (2015) who recorded a significant improvement in the blood glucose level and obesity record upon administration of vitamin D. Likely, elevation in serum HDL with a reduction in serum cholesterol, TAGS, VLDL, and LDL were also reported in the same study.

The elevation of the serum level of 1, 25 dihydroxy cholecalciferol, calcium and calcitonin and the decrease in the serum level of PTH agree with a previous report (Santulli and Marks, 2015).

It was suggested that hyperparathyroidism is associated with T2DM (Danescu *et al*., 2009). Likely, we found in our study in diabetic group a significant increase in serum level of PTH with a decrease in calcium and calcitonin level compared with the control group. Using STZ to induce T2DM initiated a defensive inflammatory mechanism against free radical oxidative stress-mediated hyperglycemia (Wahlqvist, 2013).

The significant increase in the hepatic lipid peroxidation marker MDA and a decrease in antioxidant activity (GPX, SOD, and CAT) in the diabetic group compared with the control group was similarly reported before (Zhao *et al*., 2014). Vitamin D administration could significantly activate the antioxidation system

(Nugent, 2008).

The effect of vitamin D administration on the insulin sensitivity by upregulation of the expression of peroxisome proliferator-activated receptor (PPAR) has a crucial role in the regulation of glucose and lipid metabolism by fatty acid beta oxidation that needed in high energy-requiring tissues (Ferré, 2004). Vitamin D administration caused upregulation of IGF-1, which by turn elevated IGF-I/IGFBP-3 ratio in the obesity due to the relationship between insulin and IGF-I in their intracellular activities. That caused an improvement in blood glucose, hyperinsulinemia and hyperlipidemia (Derakhshanian, 2017). Vitamin D administration upregulated the expression of GLP-1, which improved insulin secretion, suppression of glucagon secretion, inhibition of gastric emptying, promotion of glycogen synthase activity in the liver, and protection and proliferation of pancreatic β-cells. GLP-1 receptor is widely expressed in peripheral organs, such as the pancreatic islets, heart, kidneys, and gastrointestinal tract, and the central nervous system (Holst, 2007).

In our study we presented the novel effect of vitamin D administration on the sonic hedgehog. That caused an up regulation in the expression of relative proteins (Ptch, Smo, Gli-1, and Hhip), which have a serious role in obesity and insulin resistance.

CONCLUSION

Based on the previous outcomes, it could be speculated that vitamin D could improve insulin sensitivity and glucose utilization via modulating expansion of the hepatic IGF-1, GLP-1, PPAR α, and sonic hedgehog signaling pathway in the adipose tissue which provides a new insight tangent for any improvement in the blood glucose level.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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