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DIABETIC
STUDIES OPENACCESS

The Burden Effect Of Vitamin D Deficiency And Protective Effect Of Low & High Dose Of Vit D On Glycemic Control & Parotid Gland Function Of Diabetic Male Albino Rats

Marwa Mahmoud Awad[1] , Ahmed F. Abdel Ghany[2] , Ehab Kamal Ali[3] , Hassan saad Hassan Elwakeel[4] ,Alaa Abo Seif[5] ,Elsayed Elagamy Elsayed[6] ,Ali Foli Abd Elsalam[7] , Mohamed FathyAbd Elhamid Mohamed[8],Alaa A.A Abd Allatif[9], Mohamed Moawed I. Ghoneim[10] , Mohamed N. Mohamed[11], Rehab Ahmed Ahmed El-Shaer[12]

[1]Department of Physiology, Faculty of medicine, Tanta university, Tanta,Egypt.
marwa.saleh@med.tanta.edu.eg
[2]Alghad College for Applied Medical sciences, Riyadh, Saudi Arabia.
[3]Department of Anatomy and Embryology, Faculty of Medicine, Al-Azhar University, New Damietta ,Egypt.
[4]Department of Anatomy and Embryology, Faculty of Medicine, Al-Azhar University, Damietta. Egypt.
[5]Department of Pharmacology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.
[6]Department of Pharmacology, Faculty of Medicine, Al-Azhar University, Assiut, Egypt.
[7]DepartmentofPharmacology,FacultyofMedicine, Al-Azhar University, Assiut, Egypt.
[8]Department of Anatomy and embryology faculty of medicine Al-Azhar University, Assiut, Egypt
[9]Department of Anatomy and Embryology, Faculty of medicine, AlAzhar University, Assiut, Egypt
[10]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Sinai University, Egypt.
[11]Department of Pathology, Faculty of Medicine, Al-Azhar University, Assiut, Egypt.
[12]Department of Physiology, Faculty of Medicine, Tanta University,Tanta,Egypt.

Abstract:

Background: Endocrine problems, including vitamin D insufficiency and diabetes mellitus, have been demonstrated to affect oral health by influencing the salivary glands.This study investigates the impact of these two disorders, both individually and in combination, on salivary glands in a rat model.

Methods: A study examined the synergistic impact of vitamin D on dental cavity health, salivary gland function, and glycemic regulation in diabetic male albino rats. Fifty rats were allocated into five groups, each exposed to distinct diets and vitamin D concentrations, comprising a control group and multiple diabetes groups receiving low and high doses of vitamin D. Diabetes was produced using a single intraperitoneal dose of Streptozotocin, succeeded by a 12-week observation period. Blood samples were examined for glycemic levels and various biochemical indicators. Saliva collection and histological analysis of the salivary glands, tongue, and oral mucosa were performed to evaluate the impact of vitamin D on these tissues. The study sought to assess the overall effects of vitamin D deficiency and supplementation in a diabetic animal.

Results: Significant biochemical differences were found, particularly in Group 3, which showed electrolyte imbalances and adverse lipid profiles. Inflammatory markers were highest in Group 3, while Vitamin D treatment (especially in Group 5) significantly reduced these markers and improved salivary gland histology. The findings indicate vitamin D as a crucial treatment for mitigating diabetes-related negative effects, enhancing both biochemical and tissue health in rats.

Conclusion: The results underscore the significance of dosage and timing in Vitamin D supplementation for enhancing metabolic health in diabetes.

Keywords: Diabetes mellitus, Vitamin D deficiency, Vitamin D supplementation, Streptozotocin-induced diabetic rats, Salivary glands, Oral mucosa

Introduction

Population studies have established a significant association between vitamin D deficiency and diabetes mellitus (DM), a relationship underscored by the increased risk of type 2 diabetes observed in rickets patients and the elevation of serum vitamin D levels following glycemic control in those with type 1 diabetes (Bhat et al., 2021). Further evidence derives from the supplementation of vitamin D receptor knockout and globally hypomorph rats that exhibit type 1 DM, where treatment ameliorates glucose intolerance (Yu et al., 2022). Both conditions can alter salivary composition and flow, directly affecting oral health (Nishimoto et al., 2017).

Vitamin D deficiency is associated with secondary hyperparathyroidism and fall in 1,25-dihydroxyvitamin D3 levels (the active form of vitamin D), both considered to enhance the severity of type 1 DM; these conditions may therefore influence salivary glands through distinct and possibly synergistic pathophysiological mechanisms (He et al., 2022).

Endocrine disorders such as vitamin D deficiency and diabetes mellitus have been shown to impact oral health through effects on the salivary glands. Vitamin D deficiency could elicit hyperinflammatory responses in the salivary glands, leading to atrophy and decreased secretory function (Botelho & Proença et al., 2024).

Vitamin D and its sterol hormones play important roles in the oral cavity and are present in the saliva. (Diachkova et al., 2021). The architecture of the salivary gland, showing acinar degeneration and desquamation of the ducts, is indicative of the status of vitamin D deficiency (Al-Serwi et al., 2021). The parotid gland is a target organ for diabetic complications, and duct enlargement and less secretion are the hallmarks of the diabetes condition for both vitamin D-deficient and vitamin D-sufficient models (Fouani et al., 2021). Salivary flow rate and composition analysis, such as total proteins and electrolytes, are monitored to reflect the physiology of the glands (Proctor & Shaalan, 2021). Salivary gland dysfunction is frequently linked to oral dryness, which leads to a higher risk of dental caries and periodontal disease. (Nishimoto et al., 2017)

This study investigates the impact of these two disorders, both individually and in combination, on salivary glands in a rat model. Salivary glands from various animal models are also being studied to elucidate the viscous secretory change in diabetes and the drying of secretory ducts in vitamin D sufficiency.

Materials and Methods

Study Design

An Experimental study was conducted to investigate the synergic effect of vitamin D on the health of oral cavity, salivary glands and hypoglycemic control in diabetic male albino rats which were obtained from the animal house of the university. The rats who were at age of 5 months and weighed about 200 gm were kept under supervision for 14 days to adapt to the environment and exclude any intercurrent infection in suitable cages (20 X 20 X 30) cm at normal atmospheric temperature (20ºC-25ºC) as well as under good ventilation and permitted access to food and water.

Study settings

The study was conducted between Tanta university and Al-Azhar Assiut university. The study duration was 3months from 1-8-2025 to 1-11-2025.

Ethical consideration

Approval was obtained from the Ethics Review Committee of the Faculty of Medicine, Tanta University under IRB (36264PR1329/8/25)

Methods

The 50 rats were divided into 5 groups each (n=10) the groups were divided as follow:

Group 1: Control (normal): were fed Purified (AIN) 93M diet were exposed to the sun freely 12-hr. Light/dark cycle periods was maintained.

Group 2: Diabetic control: were fed Purified (AIN) 93M diet were on normal diet, inducing diabetes with Streptozotocin. Rats were exposed to the sun freely 12-hr. Light/dark cycle periods was maintained.

Group 3: Diabetic +Vitamin D deficiency: Rats in this group were on normal diet (Purified (AIN) 93M diet.inducing diabetes was done using Streptozotocin and kept in dark place for induction of vitamin D deficiency.

Group 4: Diabetic + Vitamin D deficiency (treatment group, with low dose of vitamin D. Rats in this group were on normal diet, inducing diabetes with Streptozotocin.

Group 5: Diabetic+ Vitamin D deficiency (treatment group, with high dose of vitamin D. Rats in this group were on normal diet, inducing diabetes with Streptozotocin.

All animals were allowed to drink distilled water freely.

The dietary ingredients were obtained from El Gomhouria Co. for trading chemicals and medical appliances, Cairo, Egypt, and were mixed according to the required concentrations using tap water as a mixing media. The fat-soluble Vitamins (e.g., Vit. A, E, K) were mixed in Maize oil then added to the diet. Diet was stored at 4°C in plastic containers. Using distilled water as a mixing medium, the ingredients will be mixed together in the right amounts. Fat-soluble vitamins, like A, E, and K, will be mixed with maize oil and then added to the diet. The diet will be kept in plastic containers at 4°C

After induction of vitamin D deficiency, the rats in group 4 were supplied with their normal diet orally with 8000 IU per day for 12 weeks. While the rats in group 5 were supplied orally with 20000 IU per day for 12 weeks.

I- Induction of diabetes

A single intraperitoneal injection of Streptozotocin (STZ) (Sigma Chemical Co., St. Louis, USA) at a dose of 60 mg/kg of body weight will cause diabetes in Group III and Group IV. Before the injection, STZ will be freshly dissolved in a citrate buffer with a pH of 4.5.

After 48 hours of Streptozotocin injection, the blood sugar levels of animals that had not eaten overnight was measured using a tail blood glucose test (Accu–Chek® advantage, Roche Diagnostics, Basel, Switzerland). Rats in groups III and IV that have blood sugar levels higher than 250 mg/100 ml will be considered diabetic and will be included in the study.The study went on for 12 weeks after diabetes is diagnosed.

II- Collection of Blood Samples and biochemical analysis

After 48 h of Streptozotocin injection in rats, glycemia was determined in overnight fasted animals by tail blood glucose analysis (Accu – Chek ®advantage, Roche Diagnostics, Basel, Switzerland). The rats in the diabetic group achieved blood glucose levels >250 mg/100 ml and were euthanized 12 weeks after the induction**.** At the end of 12 weeks and after 12 hours over night fasting, blood samples were collected from the retro-orbital plexus using heparinized capillary tube (0.75 - 1.0 mm internal diameter) inserted in the medial canthus. Blood was put into clean, dry graduated glass centrifuge tubes. Samples were spun quickly at 5000 rpm for 10 minutes. The supernatant serum was put into Eppendorf tubes and freeze them at −20°C until we need them to find out:

- HbA1C, Fasting blood sugar.

Lipid profile, which includes total cholesterol, triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL),
Malondialdehyde (MDA) and Superoxide Dismutase (SOD) are two markers of oxidative stress.
Proinflammatory Cytokines: TNF-α and IL-6.

III- Salvia collection

For 10 minutes, saliva was collected from each rat by letting it flow freely into sterile plain tubes. To lessen the effects of daily changes, all groups had their saliva collected between 8 and 10 am.

To find the salivary flow rate (ml/min), divide the total amount of saliva (ml) by the time it was collected (min). A digital pH meter will be used to find the pH

The saliva samples will be kept at −20°C until they can be analyzed in the lab. The samples will be thawed at room temperature and then spun at 6000 rpm for 10 minutes to get rid of any outside contamination. To find out how many ions are in the saliva, the sample will be diluted to 1/100, and flame emission spectrophotometry will be used to find out how much sodium, potassium, and calcium is in mmol/L.

The Schales method with mercuric nitrate will be used to find the concentrations of chloride and bicarbonate in mmol/L.

The total protein concentration in g/dl will be measured using colorimetry and the Helios spectrophotometer, which will read samples at 720nm. We will use bovine serum albumin to calibrate

IV- Histological examination of salivary gland, tongue and oral mucosa

A- Histological examination of salivary glands (Parotid and submandibular)

At the end of the trial, all rats were euthanized using an overdose of ketamine and xylazine (60 and 20 mg/kg, veterinary brands, Brazil). A. Salivary glands (parotid and submandibular). The parotid (PA) and submandibular (SM) glands were excised, meticulously cleansed of adhering tissue, and subsequently immersed in liquid nitrogen for immediate freezing.Some samples were preserved at −80°C for potential molecular investigation.

A separate portion of the glands was preserved in 10% neutral buffered formalin for 24 hours and thereafter subjected to standard tissue processing and paraffin embedding techniques.Sections were sectioned at 4 μm, deparaffinized, and subsequently stained with hematoxylin and eosin (H&E) for a comprehensive histological examination.

B- Histological examination of tongue and oral mucosa

The oral mucosal tissue and tongue were excised, and samples from the apex and body were submitted for histological analysis. The fragmented specimens were preserved in 10% formalin for 72 hours and subsequently dehydrated in alcohol at varying concentrations (70%, 80%, 90%, 95%, and 100%), each for one hour.Subsequently, xylene was employed to clean the tissues, and paraffin wax was utilized for embedding at 58°C. The microtome will subsequently section them into 5 μm slices.The slices, coated in paraffin, will be mounted on glass slides and subsequently stained with H&E for a comprehensive histological examination.

Statistical Analysis

The computer program SPSS (Statistical Package for Social Science) version 17.0 (Chicago, USA) will be used to tabulate, code, and analyze the data. Suitable statistic tests will be used.

Results

Table 1 shows the demographic characteristics of the rats in all groups in terms of age, weight,

and health status. The table indicates that all groups are of similar age, ensuring that age does not influence the results. The table also shows that the weight ranges between 210 and 220 grams, indicating that the groups were admitted before the start of the experiment.
Group 1 represents the normal condition; it is non-diabetic and fed a vitamin D-enriched diet
Group 2 is diabetic but still receives a vitamin D-enriched diet, making it a control group for the cause of the disease.
In Group 3, the combination of diabetes and vitamin D deficiency is evident, which helps to mitigate the effects of the vitamin on oral health and salivary polysaccharide in blood glucose.
Groups 4 and 5 are the two groups that received two different doses of vitamin D, allowing for verification of therapeutic efficacy at the dose level.

Table1: Demographic characteristics of the experimental groups

ITEM	**G1 (n=10)**	**G2 (n=10)**	**G3 (n=10)**	**G4 (n=10)**	**G5 (n=10)**
Average age (in weeks) (M ± SD)	*8 ± 0.12*	*8 ± 0.1*	*8 ± 0.11*	*8 ± 0/11*	*8 ± 0.1*
Average weight (in gram) (M ± SD)	*220 ± 10*	*215 ± 14*	*210 ± 12*	*216 ± 12*	*218 ± 12*
Health status					
Diabetes	*NO(HEALTH) (CONTROL)*	*YES(Diabetc control)*	*YES (Diabetic + Vitamin D deficiency*	*YES (Diabetic+ Vitamin D deficiency*	*YES Diabetic+ Vitamin D deficiency*
vitamin D	*YES*	*YES*	*NO*	*YES BUT(8000 IU per day)*	*YES BUT (20000 IU per day)*

1. *Group 1: Control (normal): were fed Purified (AIN) 93M diet. were exposed to the sun freely 12-hr. Light/dark cycle periods was maintained.*
2. *Group 2: Diabetic control: were fed Purified (AIN) 93M diet , were on normal diet, inducing diabetes with Streptozotocin. Rats were exposed to the sun freely 12-hr. Light/dark cycle periods was maintained.*
3. *Group 3: Diabetic +Vitamin D deficiency: Rats in this group were on normal diet (Purified (AIN) 93M diet (Vit D. deficient diet). inducing diabetes was done by using Streptozotocin and kept in dark place for induction of vitamin D deficiency.*
4. *Group 4: Diabetic + Vitamin D deficiency (treatment group, with low dose of vitamin D. Rats in this group were on normal diet, inducing diabetes with Streptozotocin and kept in dark place for induction of vitamin D deficiency. Then rats were supplied orally with 8000 IU per day for 12 weeks*
5. *Group 5: Diabetic+ Vitamin D deficiency (treatment group, with high dose of vitamin D. Rats in this group were on normal diet, inducing diabetes with Streptozotocin and kept in dark place for induction of vitamin D deficiency. Then rats were supplied orally with 20000 IU per day for 12 weeks.*

Table2:biochemical analysisresults(Na,Ca,VITDandKa)

ITEM	pre/ post	Group1 (n=10)	Group2 (n=10)	Group3 (n=10)	Group4 (n=10)	Group5 (n=10)	F	p-value
Na (mmol/L) (M ± SD)	Pre	143 ± 2.1	146 ± 2.5	141 ± 3.0	142 ± 2.7	142 ± 2.6	7.4	***0.001
	Post	144 ± 2.0	147 ± 2.3	138 ± 2.8	144 ± 2.4	145 ± 2.2	9.1	***0.0004
K (mmol/L) (M ± SD)	Pre	4.5 ± 0.3	4.8 ± 0.4	4.1 ± 0.3	4.2 ± 0.3	4.2 ± 0.3	8	***0.0007
	Post	4.6 ± 0.2	4.9 ± 0.3	3.9 ± 0.4	4.4 ± 0.3	4.6 ± 0.2	12.2	***0.0001
Ca (mmol/L) (M ± SD)	Pre	2.30 ± 0.08	2.25 ± 0.09	2.05 ± 0.10	2.10 ± 0.11	2.12 ± 0.10	15.3	***0.0001
	Post	2.32 ± 0.07	2.26 ± 0.08	1.98 ± 0.09	2.22 ± 0.09	2.35 ± 0.08	22.5	***0.00001
Vit. D (mmol/L) (M ± SD)	Pre	78 ± 5	76 ± 6	22 ± 3	23 ± 4	24 ± 4	310	***<0.00001
	Post	80 ± 4	77 ± 5	18 ± 2	65 ± 6	95 ± 7	540	***<0.00001

M(mean): The arithmetic average of a set of values.
SD (Standard Deviation): A measure of how spread out the values are around the mean.
F (F-value in ANOVA): A statistical value produced by an ANOVA test (Analysis of Variance).
p-value:A probability value used to determine statistical significance.
****p < 0.05 (significant)***
***** p < 0.01 (highly significant)***
****** p < 0.001 (very highly significant***

The table shows that the sodium, potassium, calcium, and vitamin D levels before and after conducted experiments were clearly different among all five groups. The values of group 1 and 2 were almost identical; this indicates that both groups had vitamin D deficiency at the same time. Thus, it is likely that vitamin D deficiency, rather than diabetes, is the major contributor to Electrolyte Imbalances. Group three has diabetes as well as vitamin D deficiency, resulting in a significant decrease in all measured variables; specifically, the group had a dramatic decrease in calcium and vitamin D levels, due to the effect on the body's electrolytes caused by both stressors existing at the same time. Group four experienced a moderate increase in its calcium, sodium and vitamin D levels, due to receiving a low dose of vitamin D (8000 IU). Group five received a high dose of vitamin D (20000 IU), and therefore had the greatest improvement and the best results closest to normal. However, there are still some small differences present among the groups. Significant F-values (with low p-values less than 0.05) indicate that the differences seen in electrolyte levels among the groups were statistically significant and not a chance occurrence.

Table 3: result* of *Correlation coefficients between the five groupss (Na, Ca, VIT D and Ka)

GROUPS	G1	G2	G3	G4	G5
G1	1				
G2	*** 0.94	1			
G3	*-0.72	*-0.69	1		
G4	**0.81	**0.78	*-0.55	1	
G5	0.68	**0.65	*-0.48	**0.89	1

****Correlation is significant at p < 0.05.***
****** Correlation is significant at p < 0.01***
******* Correlation is significant at p < 0.001***

The five groups were reported to have a high degree of correlation according to Table 3; however, since it was determined that Group G1 and Group G2 are also similar in their consumption of high amounts of Vitamin D, there exists a strong correlation (0.94). Group G2 does have a diabetes condition; however, Group G2 does not have a Vitamin D deficiency. There was also a moderately strong correlation (0.89) between the two treatment groups G4 and G5, as these two groups received Vitamins D2 and D3 and also experienced significant improvements in their calcium and sodium levels. However, as with the previous groups, the only difference between Group G4 and Group G5 was the amount of dosages received. However, even though Group G4 improved their values to near normal levels through treatment with Vitamin D2 and had moderate correlation (0.78 to 0.81) to Groups G1 and G2, Group G5 had even less correlation to Group G1 and G2 because of the very high levels of Vitamin D, Group G5 exhibited in response to the large doses of Vitamin D. Group G5 was very similar to Group G3 in correlation types; however, Group G3 had the weakest correlations (between -0.48 and -0.72) with all of the groups and indicates that Group G3 has deteriorated significantly biochemically, creating a very different pattern of responses from the other groups.

Table 4: results biochemical analysis results (HDL (mg/dL), LDL (mg/dL) (%), VLDL (mg/dL) and pH)

ITEM	pre/ post	Group1 (n=10)	Group2 (n=10)	Group3 (n=10)	Group4 (n=10)	Group5 (n=10)	F	p-value
HDL (mg/dL)	Pre	58 ± 3	52 ± 4	41 ± 3	47 ± 3	50 ± 4	29.4	*** <0.0001
	Post	59 ± 3	53 ± 3	38 ± 3	50 ± 3	54 ± 4	41.2	<*** 0.00001
LDL (mg/dL)	Pre	22 ± 2	28 ± 3	40 ± 4	32 ± 3	30 ± 2	35.8	*** <0.0001
	Post	21 ± 2	27 ± 3	45 ± 5	30 ± 3	28 ± 3	48.1	*** <0.00001
VLDL (mg/dL)	Pre	14 ± 1	18 ± 2	22 ± 3	20 ± 2	19 ± 2	22.5	*** <0.0001
	Post	13 ± 1	18 ± 2	25 ± 3	18 ± 2	17 ± 2	31.9	*** <0.00001
pH (M ± SD)	Pre	7.36 ± 0.03	7.32± 0.04	7.2 ± 0.05	7.33± 0.04	7.34 ± 0.03	18.2	**<0.001
	Post	7.37 ± 0.03	7.33± 0.03	7.27 ± 0.04	7.35 ± 0.03	7.36 ± 0.03	25.7	*** <0.001

M(mean): The arithmetic average of a set of values.
SD (Standard Deviation): A measure of how spread out the values is around the mean.
F (F-value in ANOVA): A statistical value produced by an ANOVA test (Analysis of Variance).
p-value: A probability value used to determine statistical significance.
****p < 0.05 (significant)***
***** p < 0.01 (highly significant)***
****** p < 0.001 (very highly significant***

As shown in Table 4, the lipid profile and pH levels changed significantly between the five groups tested. Group 3 (diabetes + vitamin D deficiency) had the highest LDL and VLDL concentrations as well as the lowest HDL levels, which also resulted in a significantly lower pH level. The joint effect of vitamin D deficiencies and diabetes on the metabolism of lipids and acid-Base Balance is responsible for these results. In comparison, Groups 4 and 5 had higher HDL, lower LDL and VLDL and improved pH levels in relation to each increase in the vitamin D dose. Therefore, vitamin D supplementation was found to be effective in modulating these lipid profiles and pH levels. Groups 1 and 2 have almost identical results, which indicate that

vitamin D deficiency is a more significant contributor to differences between the groups than the presence of diabetes alone. High F-values and low p-values support the notion that there is a statistically meaningful difference between the five groups in this study.

Table5 : results of biochemical analysis results (Random Glucose (mg/dL), HbA1c (%), Total Cholesterol (mg/dL) and Triglycerides (mg/dL))

ITEM	pre/ post	Group1 (n=10)	Group2 (n=10)	Group3 (n=10)	Group4 (n=10)	Group5 (n=10)	F	p-value
R.B.S (mg/dL)	*Pre*	*120 ± 8*	*145 ± 10*	*178 ± 12*	*165 ± 11*	*150 ± 10*	*42.3*	***<0.0001
	Post	*118 ± 7*	*148 ± 9*	*185 ± 10*	*158 ± 9*	*145 ± 8*	*51.6*	***<0.00001
HbA1c (%)	*Pre*	*4.5 ± 0.3*	*7.8 ± 0.4*	*8.9 ± 0.5*	*8.7 ± 0.4*	*8.6 ± 0.4*	*310*	***<0.00001
	Post	*4.4 ± 0.2*	*7.9 ± 0.4*	*9.3 ± 0.5*	*7.2 ± 0.4*	*6.5 ± 0.3*	*520*	***<0.00001
T. Cholesterol (mg/dL)	*Pre*	*22 ± 2*	*28 ± 3*	*40 ± 4*	*32 ± 3*	*30 ± 2*	*35.8*	***<0.0001
	Post	*21 ± 2*	*27 ± 3*	*45 ± 5*	*30 ± 3*	*28 ± 3*	*48.1*	***<0.00001
Triglycerides (mg/dL)	*Pre*	*70 ± 5*	*92 ± 7*	*130 ± 10*	*118 ± 9*	*110 ± 8*	*88.2*	***<0.00001
	Post	*68 ± 4*	*95 ± 6*	*140 ± 10*	*105 ± 7*	*95 ± 6*	*110.5*	***<0.00001

M(mean): The arithmetic average of a set of values.
SD (Standard Deviation): A measure of how spread out the values is around the mean.
F (F-value in ANOVA): A statistical value produced by an ANOVA test (Analysis of Variance).
p-value:A probability value used to determine statistical significance.
****p < 0.05 (significant)***
***** p < 0.01 (highly significant)***
****** p < 0.001 (very highly significant***

According to Table 5, there is a clear difference in the levels of lipids and glucose between the five groups. Group 3 (diabetic and vitamin D deficiency) appears to have the highest cholesterol, triglycerides and HbA1c levels, demonstrating that the combination of diabetes and vitamin D deficiency negatively affects both lipid metabolism and glucose regulation. In comparison, Group 4 and Group 5 had gradually improved values with vitamin D treatment, with a greater decrease in HbA1c and lipids in Group 5 due to the high-dose treatment. The normal group (Group 1) was at baseline with the best levels, while Group 2 had the effect of diabetes on its values without any deficiency in vitamin D. Additionally, the large F-values and small p-values indicate that there was a significant difference between the groups and that vitamin D is a key factor in improving lipid and glucose indices in both diabetic and vitamin D-deficient rats.

Table 6: results biochemical analysis results inflammatory markers, oxidative stress, cells, and amylase

ITEM	pre/post	Group1 (n=10)	Group2 (n=10)	Group3 (n=10)	Group4 (n=10)	Group5 (n=10)	F	p-value
IL-6 (pg/mL)	Pre	*18 ± 2*	*28 ± 3*	*45 ± 4*	*42 ± 4*	*40 ± 3*	*152.3*	***<0.00001
	Post	*17 ± 2*	*27 ± 3*	*52 ± 5*	*30 ± 3*	*24 ± 3*	*310.5*	***<0.00001
TNF-α (pg/mL)	Pre	*20 ± 2*	*32 ± 3*	*55 ± 5*	*50 ± 4*	*48 ± 4*	*188.7*	***<0.00001
	Post	*19 ± 2*	*31 ± 3*	*60 ± 6*	*38 ± 3*	*30 ± 3*	*420.9*	***<0.00001

MDA (nmol/mg)	Pre	*1.8±0.2*	*2.5±0.3*	*4.8 ± 0.4*	*4.2 ± 0.4*	*3.9 ± 0.3*	*260.1*	***<0.00001
	Post	*1.7±0.2*	*2.6±0.3*	*5.2 ± 0.5*	*3.0 ± 0.3*	*2.5 ± 0.3*	*510.4*	***<0.00001
SOD(U/mg protein)	Pre	*12.5±1.0*	*10.2±0.9*	*6.0 ± 0.7*	*6.8 ± 0.8*	*7.2 ± 0.8*	*145.6*	****<0.00001*
	Post	*12.8±1.0*	*10.0±0.9*	*5.5 ± 0.6*	*9.5 ± 0.9*	*11.2±1.0*	*230.7*	****<0.00001*

M(mean): The arithmetic average of a set of values.
SD (Standard Deviation): A measure of how spread out the values are around the mean.
F (F-value in ANOVA): A statistical value produced by an ANOVA test (Analysis of Variance).
p-value: A probability value used to determine statistical significance.
****p < 0.05 (significant)***
***** p < 0.01 (highly significant)***
****** p < 0.001 (very highly significant***

Table (6)provides evidence of the relationship between diabetes mellitus, vitamin D deficiency, and their treatment effects on inflammatory and oxidative stress markers. The data indicate that Group 3 had the highest concentrations of IL-6, TNF-α, and MDA in both pre- and post-treatment assessments, which demonstrates severe inflammation and oxidative stress associated with diabetes and concomitant vitamin D deficiency.
The considerable reduction of IL-6, TNF-α, and MDA levels achieved by Group 5 and Group 4 patients post-vitamin D treatment indicates that higher dosing resulted in greater reductions of inflammatory markers and improved oxidative balance compared to lower dosing. The SOD levels recorded lowest in Group 3, with progressive increases observed in Group 4 with a near-normal return in Group 5, indicate restoration of an antioxidant system. Group 1 samples remained within normal ranges while Group 2 demonstrated moderate SOD increases attributed to diabetes as the only participant variable. The high statistical F-values and low p-values provide clear evidence that the differences observed between groups were statistically significant and not due to random error.

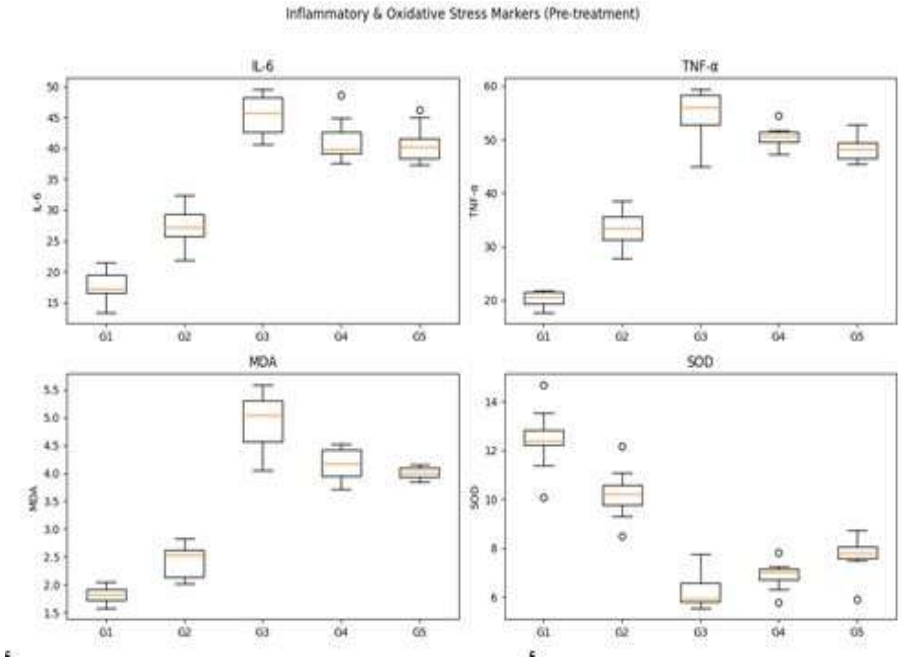

Figure 1 shows the standardized plots

Figure(1) containing four Boxplots of the markers of inflammation and oxidative stress (IL-6, TNF-α, MDA, SOD) before treatment for all five groups, which have been combined into one consistent and clear figure for easy comparison between the groups.

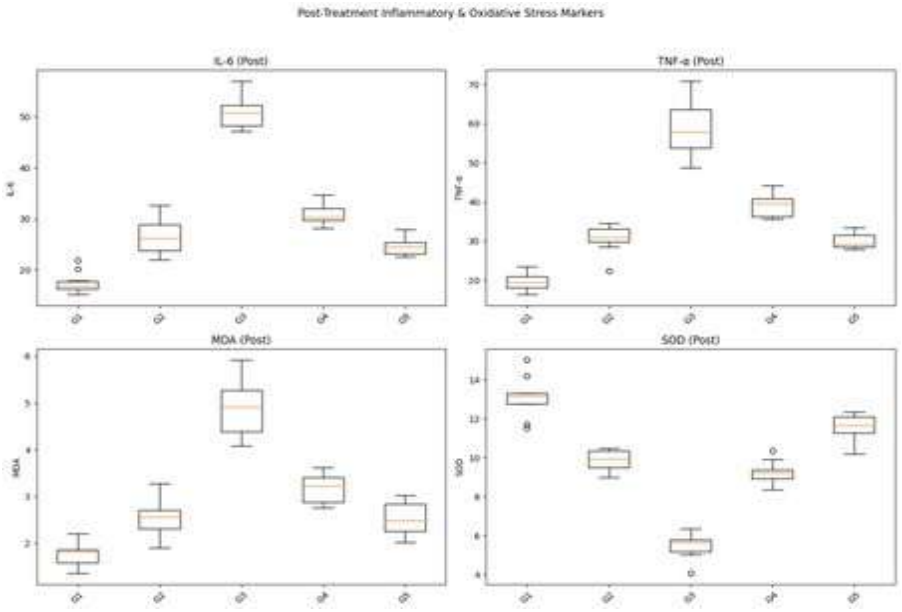

Figure2: shows the standardized plots(post)

Figure 2 shows the third group had the highest levels of IL-6, TNF-α and MDA on the post-treatment plots due to the severity of inflammation and oxidative stress despite a slight reduction following treatment. Conversely, there is a clear improvement in the data from group IV, and group V has the greatest decrease in inflammatory markers some have a large increase in SOD, indicating a stronger effectiveness of high-dose vitamin D. As the first group consistently shows the lowest and most stable values of all the groups, it represents the natural reference for comparisons between groups.

Table 7: anatomical results for the groups

Parameters	Group1 (n=10)	Group2 (n=10)	Group3 (n=10)	Group4 (n=10)	Group5 (n=10)	F	p-value
Absolute gland weight (mg) (M ± SD)	*145 ± 10*	*132 ± 12*	*98 ± 10*	*118 ± 11*	*135 ± 12*	*48.6*	***<0.00001
Relative gland weight (mg/g body weight) (M ± SD)	*0.72± 0.05*	*0.66± 0.05*	*0.48± 0.04*	*0.60± 0.05*	*0.69± 0.05*	*55.4*	***<0.00001
Color change score (0–3) (M ± SD)	*0.3 ± 0.1*	*0.8 ± 0.2*	*2.6 ± 0.3*	*1.4 ± 0.2*	*0.7 ± 0.2*	*210.7*	***<0.00001
Fibrosis score (0–3) (M ± SD)	*0.2 ± 0.1*	*0.9 ± 0.2*	*2.8 ± 0.3*	*1.5 ± 0.2*	*0.6 ± 0.2*	*265.2*	***<0.00001

Softness/swelling score (0–3) (M ± SD)	*0.2 ± 0.1*	*1.0 ± 0.2*	*2.7 ± 0.3*	*1.3 ± 0.2*	*0.5 ± 0.1*	*240.5*	***<0.00001
Necrosis/atrophy score (0–3) (M ± SD)	*0.1 ± 0.1*	*0.7 ± 0.2*	*2.9 ± 0.2*	*1.2 ± 0.2*	*0.4 ± 0.1*	*320.4*	***<0.00001

M(mean): The arithmetic average of a set of values.
SD (Standard Deviation): A measure of how spread out the values are around the mean.
F (F-value in ANOVA): A statistical value produced by an ANOVA test (Analysis of Variance).
p-value: A probability value used to determine statistical significance.
****p < 0.05 (significant)***
***** p < 0.01 (highly significant)***
****** p < 0.001 (very highly significant***

The effects of diabetes and vitamin D deficiency on the salivary glands can be seen in Table 7. The data exhibited by Group 3 demonstrates the most severe pathological changes for every variable recorded, with the absolute and relative weights of the salivary glands considerably reduced and very high rankings for discoloration, fibrosis, swelling, and atrophy. The presence of diabetes plus vitamin D deficiency caused the worst damage to the salivary glands in the three treatment groups (Groups 1, 2, & 3). Group 2 had more moderate changes because it only suffered from diabetes alone. However, Group 1's results had no significant changes from normal results. In addition, Group 4 has improved tremendously since being treated, especially in the indices of fibrosis and swelling. Group 5 had the best response to treatment with values close to the norm. The high F-values and low p-values indicate that the differences among groups were significant and indicate that vitamin D has a protective effect on the salivary glands, improving their structures and functions through treatment.

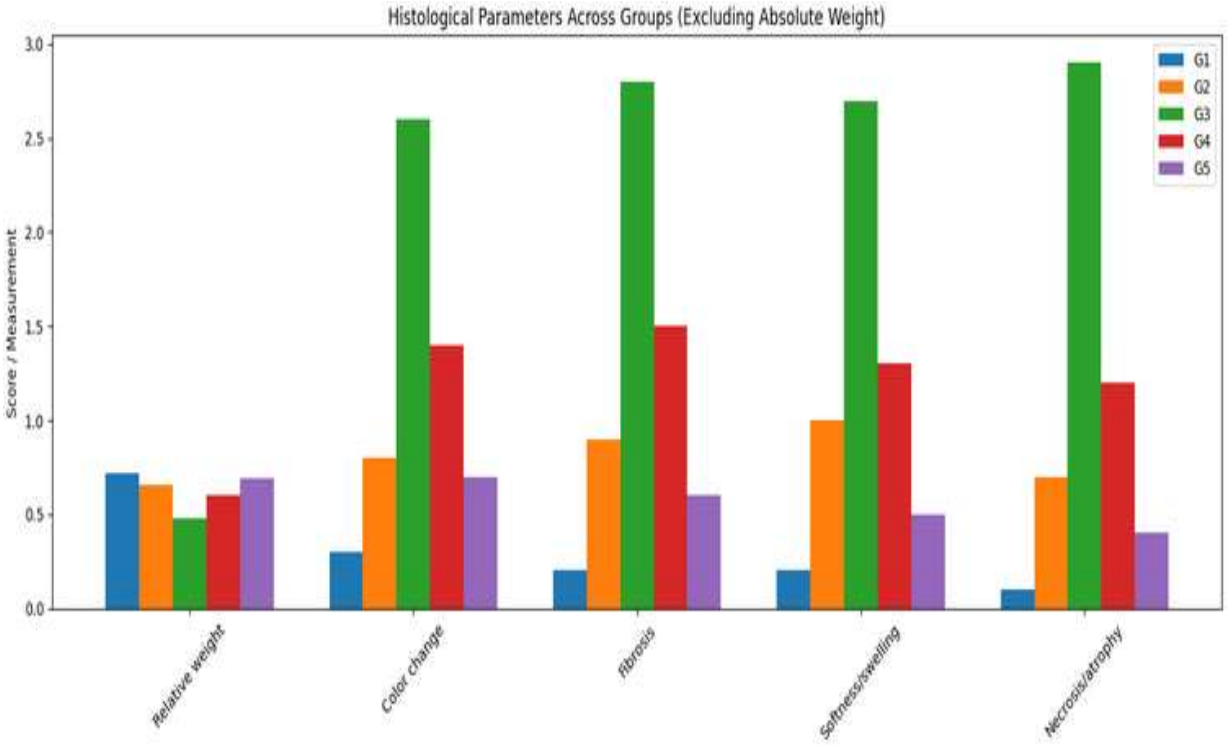

Figure 3 clearly compares the five groups across a range of histological and morphological criteria for salivary glands.

The five groups can be directly compared using various histological and morphological parameters on their salivary glands in Figure 3. As illustrated in the graph, all of the histological indicators (discoloration, fibrosis, swelling, and necrosis) continued to indicate the highest level of deterioration among the Group 3 members. This indicates that diabetes and vitamin D deficiency together were the primary cause for the vast reduction of integrity within the salivary gland tissue. Additionally, the Group 4 and 5 saw substantial improvements with Group 5 coming significantly closer to normal levels and responding to oral doses of large amounts of vitamin D. Group 2 reflects how diabetes affects the salivary glands in isolation with moderate levels of differences from the non-diabetic Group in these parameters as compared to Group 1. Thus, as indicated on this graph, vitamin D deficiency has proven to be the major contributor to deterioration of salivary glands, and the use of vitamin D treatment has been effective in successfully reversing this process and in restoring integrity to the tissue.

Table 8: result of Correlation coefficients between the five groups

Groups	G1	G2	G3	G4	G5
G1	1				
G2	*** 0.93	1			
G3	*–0.78	*–0.74	1		
G4	**0.82	**0.79	*–0.65	1	
G5	**0.89	**0.84	*–0.58	*** 0.91	1

Correlation is significant at p < 0.05.
****Correlation is significant at p < 0.01***
*****Correlation is significant at p < 0.001***

According to the correlation coefficients shown in group eight (8), the most powerful relationship was between groups g4 and g5; indicating very similar responses to vitamin D administration. Additionally, a strong correlation exists between groups g1 and g2 because these two groups had similar health statuses and did not suffer from vitamin D deficiency. Furthermore, group g5 had a high correlation with group g1, which demonstrates that high doses of therapy helped to increase the indicators in group g5 back toward normal levels following high-dose therapy. However, group 3 experienced the least amount of improvement when compared to all other groups, which indicates that group 3's inflammatory, oxidative stress, and tissue health indicators worsened or deteriorated over time. Group 3's negative correlations with groups g1, g2, g4, and g5 indicate that its biological patterns are very different from both the normal and the treatment patterns.

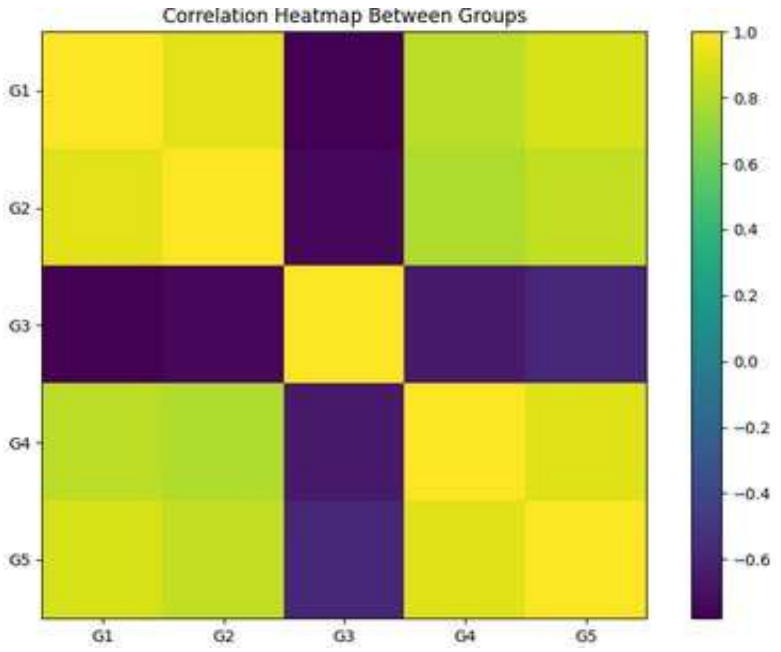

Figure 4: Heatmap of correlation coefficients between the five groups

Figure (4) the heat map, it is readily apparent that there is a large difference between the relationship between the five groups regarding their correlation coefficients. There are a few significant correlations exhibited by Group 1 (G1) and Group 2 (G2) in addition to G4 and G5. The strong correlations are indicated by the lighter shades on the heat map displaying high levels of similarity in biological values. Conversely, G3 appears as having darker shades, which indicates much weaker levels of correlation when compared to the other groups, and it demonstrates more variability than other groups due to the large decrease in inflammatory, oxidative stress, and salivary gland function indices. The difference in color between G3 and the other groups indicates that the greatest negative impact of vitamin D deficiency on diabetic subjects occurs in G3, whereas G5 subjects are nearing normal levels of treatment.

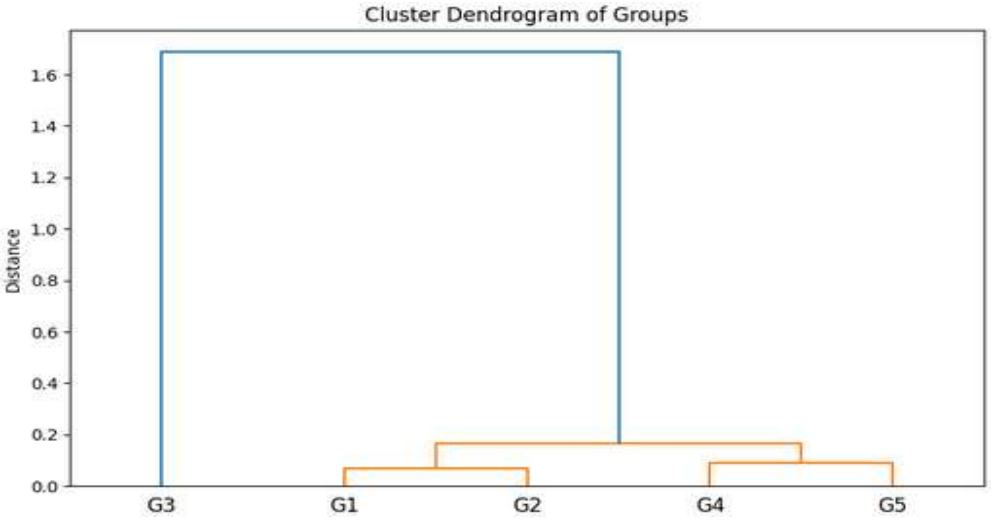

Figure 5: shows cluster dendrogram of fives group

Group G1 and G2 showed very close aggregation on the cluster tree (figure 5), which indicates that these two groupings share many similar biological characteristics because neither group

has Vitamin D deficiency. G4 and G5 were also clustered on a single branch and have both exhibited a similar pattern in response to Vitamin D treatment; G5 is also closer to normal than group G4. Group G3 is the only group that is entirely isolated from the rest of the groupings on its own separate branch, which is separated from Group G1 and G2 and G4 and G5 by quite some distance. Thus, G3 is both the most severely affected group and the most phenotypically different group due to the combined effects of Diabetes and Vitamin D deficiency.

Effect on salivary glands

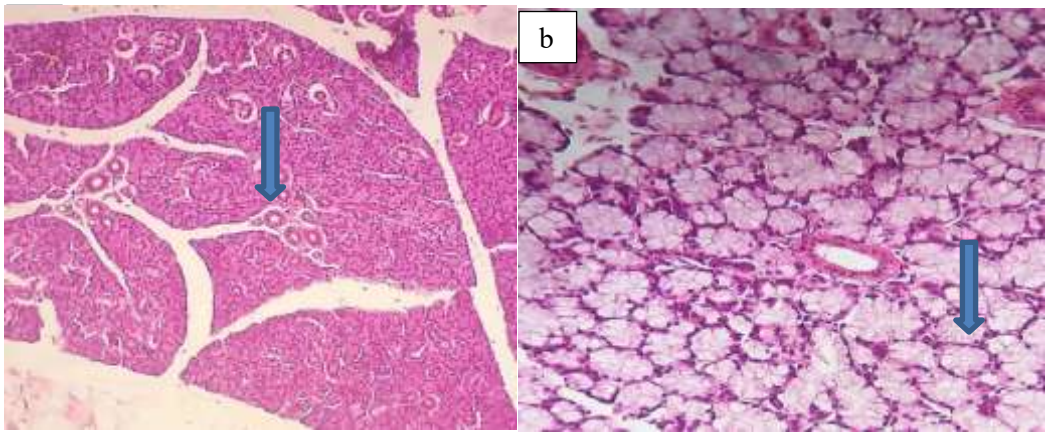

Figure (6): (a): Normal salivary gland H&E, X100 Normal duct lined by simple columnar epithelium(arrow). The serous acinar cells of the gland are polygonal shaped with dense, intracytoplasmic, basophilic granules. (b): Normal salivary gland H&E, X100 showing mucinous secreting acini (arrow) filled with mucin and contain a basally located, condensed nucleus

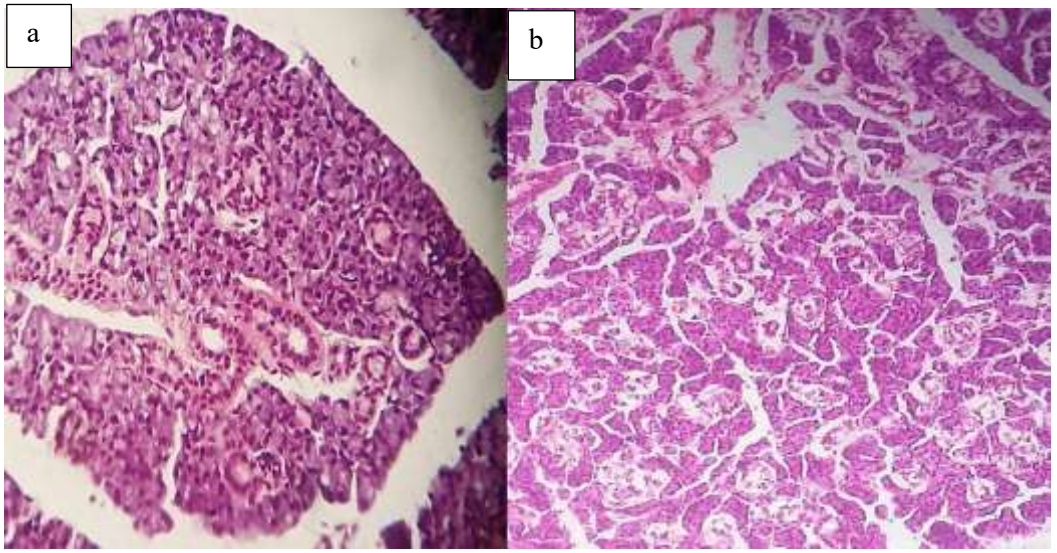

Figure (7): (a): Histological appearance of the salivary gland showing the epithelium of acini and ducts minor architecture distortion of the ducts (H&E staining, ×10). a: (early changes of vit D in Diabetic group). (b): Destruction of the ducts +inflammation H&E, X100diabetic control groups

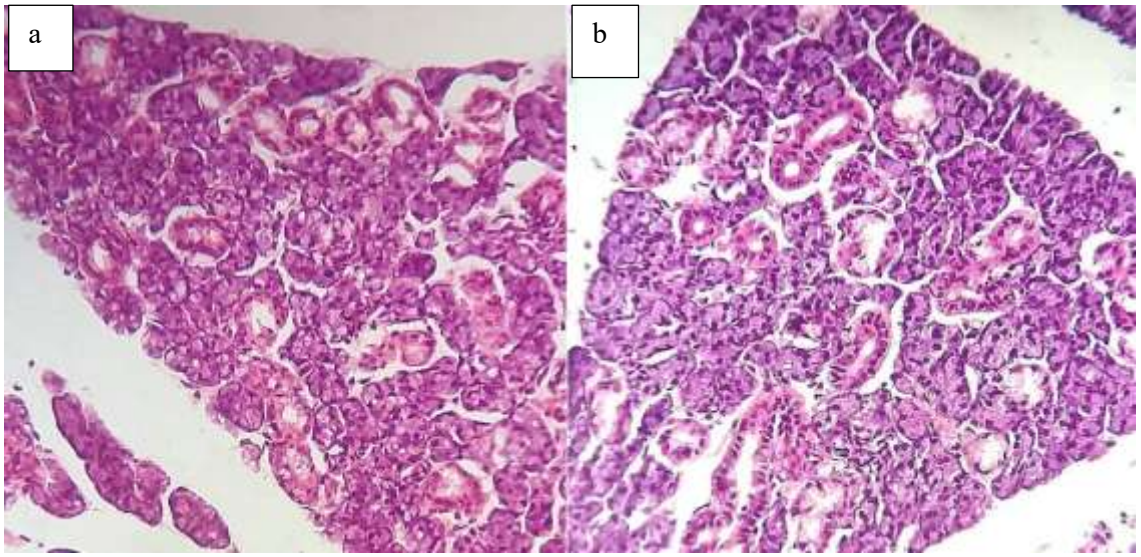

Figure (8): (a): Vit d 8000 units, demonstrating less protective effect of low dose of vitamin D showing marked attenuation of the ducts and acinar glands and vacuolization of the serous acini H&E, X100. (b): Vit D 20000 unit, demonstrating more protective effect of higher dose of vitamin D showing mild attenuation of the ducts and acinar glandsH&E, X100.

Effect on oral mucosa

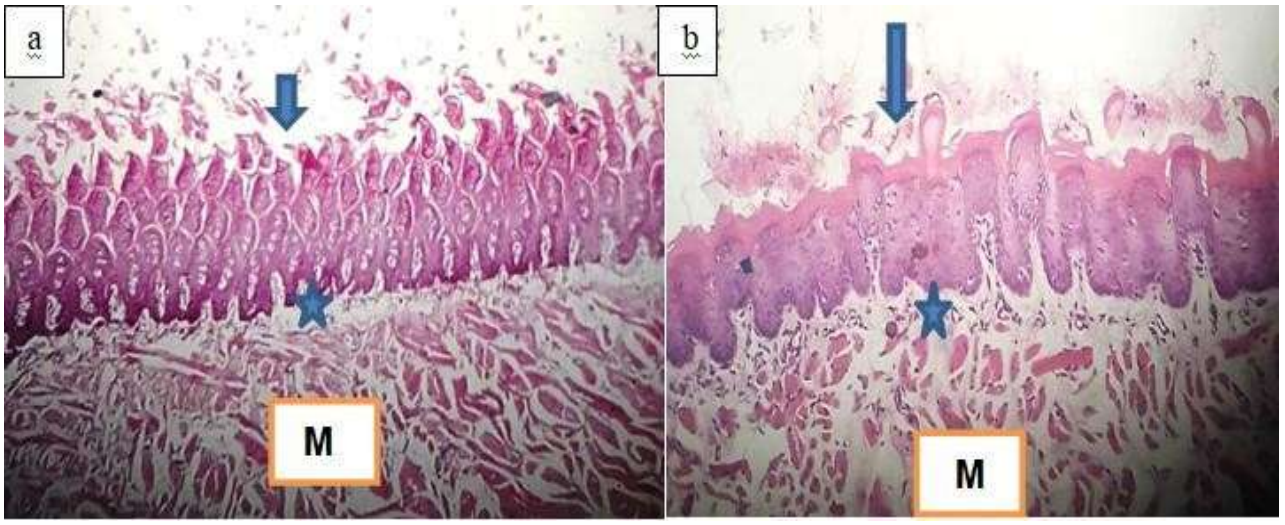

Figure (9): (a): Histological appearance of the dorsal surface of the tongue epithelium of the rats of experiment (H&E staining, ×40). a: (control group) Normal tongue with no histological changes has many papillae and is covered by a mucous membrane composed of keratinized stratified squamous epithelium (arrow). The true papillae are more elongated and closer to each other with underlying connective tissue of the lamina propria (asterisks) and lingual skeletal muscles that run in different directions (M). (b) Histological appearance of the tongue epithelium of the rats after 12 weeks of experiment (H&E staining, ×40). a: (Diabetic control) loss of lingual papillaeand hyperkeratosis (arrows) (H&E × 100, lined by keratinized stratified squamous epithelium (arrow) with underlying connective tissue (asterisks) and lingual muscles (M).

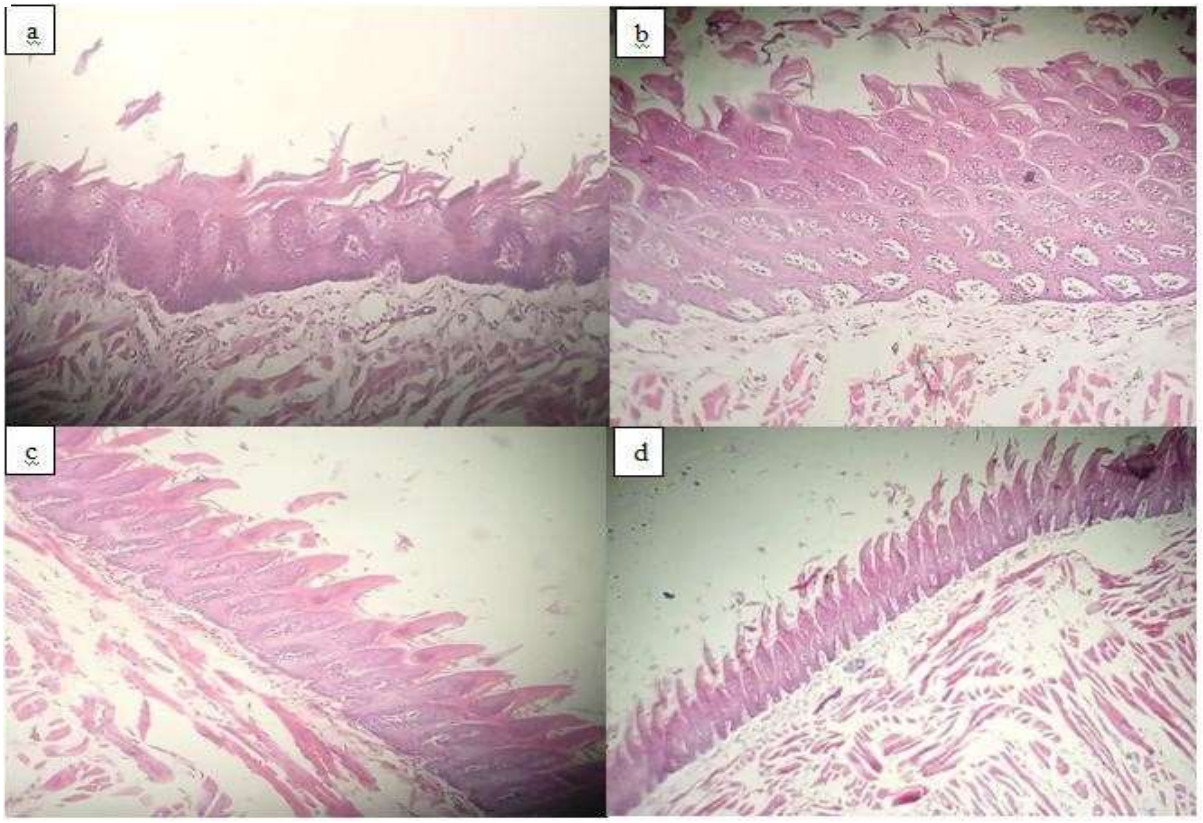

Figure (10): (a): Histological appearance of the oral mucosa epithelium of the rats after 12 weeks of experiment showing hyperkeratosis (H&E staining, ×40). a: (Diabetic control). (b) Histological appearance of the tongue epithelium of the rats after 12 weeks of experiment (H&E staining, original magnification ×40). a: (Diabetic treated vit d after induction Moderate hyperplasia and hyperkeratosis. (c): Histological appearance of the tongue epithelium of the rats of experiment (H&E staining, original magnification ×40). a: (Diabetic treated vit before induction) papillae and is covered by a mucous membrane composed of keratinized stratified squamous epithelium (arrow) with underlying connective tissue of the lamina propria and lingual skeletal muscles that run in different directions. With mild shorteninig of the papillae denoting protective effect of vit D 8000 unit. (d): Histological appearance of the dorsal surface of the tongue epithelium of the rats of experiment (H&E staining, ×40). (diapetic treated group Vit d 20000UI. (d): Normal tongue with minor histological changeshas papillae and is covered by keratinized stratified squamous epithelium (arrow). The papillae are more elongated and closer to each other with underlying connective tissue of the lamina propria and lingual skeletal muscles that run in different directions.

Discussion

Vitamin D administration has been demonstrated to markedly improve glycemic control, boost lipid profiles, and sustain electrolyte balance in diabetic rat models (Atia et al., 2022). The advantages are contingent upon dosage, with more amounts yielding more significant effects (Farhat et al., 2022). This conclusion is corroborated by other research utilizing various experimental designs and dose regimes, underscoring the persistent beneficial effect of Vitamin D on metabolic parameters in diabetes.

Vitamin D administration significantly influences metabolic parameters in diabetic rats, notably by reducing fasting blood glucose levels, lowering HbA1c, and decreasing insulin

resistance.

Studies demonstrate that elevated doses, specifically 10,000 IU/kg, surpass moderate doses (5,000 IU/kg) in improving these results. The beneficial benefits of Vitamin D are enhanced when paired with aerobic exercise. In addition to enhancing insulin sensitivity, Vitamin D is recognized for its ability to promote insulin production and strengthen β-cell functionality. The mechanisms of action are probably associated with the modulation of essential pathways implicated in insulin signaling and inflammation, specifically the Insulin Receptor Substrate 1 (IRS-1), Peroxisome Proliferator-Activated Receptor gamma (PPARγ), and Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways (Krisnamurti, D., et al., 2023; Khaledi, K., et al., 2023; Fathi, F., et al., 2022)

Vitamin D supplementation dramatically influences lipid profiles in diabetic rats, resulting in reductions in total cholesterol, triglycerides, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) levels, while elevating high-density lipoprotein (HDL) levels. The effect of vitamin D is dose-dependent, with increased dosages leading to more significant enhancements in lipid metrics (Hoseini, Z., et al., 2025; Zoair, M. 2021).

The positive alterations in lipid levels are associated with improved gene expression pertaining to lipid metabolism and a decrease in oxidative stress, suggesting an advantageous function of vitamin D in addressing lipid-related complications in diabetes (Hoseini, Z., et al., 2025; Fathi, F., et al., 2022).

Vitamin D treatment has demonstrated efficacy in restoring calcium and vitamin D levels in diabetic rats, especially those with vitamin D insufficiency. Furthermore, it assists in regulating sodium and potassium levels, which is essential for sustaining metabolic equilibrium. The efficacy of high-dose vitamin D in rectifying these imbalances is significant, as it not only mitigates deficits but also enhances related metabolic pathways in individuals with diabetes (Anjani, G., et al., 2025; Nandi, A., & Patel, V., 2025)

Vitamin D is essential for regulating inflammatory responses and oxidative stress in the body. It decreases the concentrations of proinflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). Moreover, Vitamin D reduces oxidative stress indicators, such as malondialdehyde (MDA), while concurrently augmenting the activity of antioxidant enzymes like glutathione (GSH), superoxide dismutase (SOD), and catalase. These varied mechanisms substantially enhance the protective benefits of Vitamin D on pancreatic and hepatic organs (Fathi, F., et al., 2022; George, N., et al., 2012; Mitrašinović-Brulić, M., et al., 2021).

Elevated dosages of vitamin D, particularly at 20,000 IU/kg, have consistently demonstrated superior efficacy compared to lower doses of 1,500 to 8,000 IU/kg in improving metabolic outcomes. The efficacy of vitamin D can be markedly enhanced when paired with aerobic exercise or anti-diabetic drugs, such as metformin and glimepiride, resulting in additive or synergistic advantages in the management of metabolic health (Abdel-Rehim, W., et al., 2018; Hoseini, Z., et al., 2025).

The research examines the impact of vitamin D on the salivary glands and oral mucosa in diabetic rats. Normal glands display healthy architecture, whereas diabetic controls demonstrate significant damage, including acinar degeneration and ductal dilatation, in accordance with other studies. Elevated doses of vitamin D exhibit modest enhancements in glandular architecture and inflammation, consistent with evidence indicating that vitamin D may alleviate damage caused by diabetes and nutritional stress. Nonetheless, nano-vitamin D formulations seem to provide enhanced protection relative to traditional formulations. Furthermore, examinations of the oral mucosa reveal that vitamin D-treated groups exhibit nearly normal epithelial structures, in stark

contrast to diabetes controls, which display considerable hyperkeratosis and papillary degeneration. The relationship between vitamin D levels and diabetes affects epithelial health, indicating that while sufficient vitamin D is advantageous, excessive quantities can induce negative alterations, underscoring the significance of dosage and timing in treatment effectiveness.

The histology findings correspond well with recent research on rats, demonstrating a substantial effect of diabetes and vitamin D deprivation on salivary glands. This combination leads to significant acinar degeneration, distortion of ductal architecture, fibrosis, and infiltration of inflammatory cells in the salivary glands (Isaac, M. R., &Bekheet, E. A., 2019). Furthermore, data indicates papillary loss associated with hyperkeratosis in the tongue's epithelium. Conversely, the initiation of vitamin D supplementation, particularly when administered promptly and in sufficient quantities, aids in preserving the structural integrity of salivary glands and improves the overall architecture of the oral mucosa. Regimens incorporating nano-vitamin D and pre-induction therapies often provide the most extensive protective benefits (Hamada, A. S., et al., 2024; Montaser, M. M., et al., 2025).

On the opposite side, Research suggests that vitamin D insufficiency can result in heightened oral epithelium proliferation without considerable morphological damage, indicating that deficiency alone may not fully explain severe mucosal alterations until coupled with other conditions like diabetes (Yuan, F. N., et al., 2014)

. Moreover, excessive amounts of vitamin D3 have been linked to alterations in tongue tissue in cases of overdose. While a regimen of 20,000 IU appears to confer protective effects, its safety may not be universally applicable across various types and treatment periods (Hassan, G. S., et al., 2024).

Conclusion

Administration of vitamin D markedly enhances metabolic parameters in diabetic rat models, improving glycemic management, lipid profiles, and electrolyte equilibrium. Elevated dosages produce more significant advantages, especially when paired with aerobic activity or anti-diabetic pharmacotherapy. Significant results encompass decreases in fasting blood glucose and HbA1c, as well as enhancements in lipid parameters, resulting from improved gene expression in lipid metabolism and reduced oxidative stress. Moreover, Vitamin D has a preventive function in modulating inflammatory responses and enhancing the health of salivary glands and oral mucosa; nevertheless, excessive levels may result in adverse effects.

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