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# Protective effects of vitamin D on diabetes-induced alterations in submandibular and sublingual salivary gland function; an experimental histological study in Albino rats

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## Abstract

**Introduction:** Diabetes mellitus is known to impair salivary gland structure and function, contributing to significant oral complications and reduced quality of life. Emerging evidence suggests that vitamin D plays an important regulatory role in immune modulation, epithelial integrity, and glandular homeostasis, yet its protective potential in diabetes-related salivary gland injury remains insufficiently understood.

**Objectives:** This experimental study investigates the extent to which vitamin D supplementation can mitigate diabetes-induced biochemical and histopathological alterations in the submandibular and sublingual glands of albino rats.

**Materials and Methods:** This experimental study was conducted at Al-Azhar university, Egypt in 2025, using 30 adult albino rats randomly assigned to three groups (n = 10 each): a healthy control group, a diabetic vitamin D-deficient group, and a diabetic vitamin D-deficient group treated with 20,000 IU vitamin D. Blood and saliva were collected weekly to assess serum and salivary electrolytes, lipid profiles (high-density lipoprotein [HDL], low-density lipoprotein [LDL], and very-low-density lipoprotein [VLDL]), salivary pH, total protein, and flow rate using standard biochemical assays. At study completion, parotid and submandibular glands were harvested for histological evaluation using hematoxylin and eosin staining. Data were collected and compared between groups.

**Results:** Compared to healthy control rats, diabetic vitamin D–deficient rats increased the levels of sodium, potassium, LDL, and VLDL, and salivary total protein, while decreasing the bicarbonate, HDL, salivary pH, and flow rate, with vitamin D treatment moderating, but not fully normalizing these alterations. Histopathological examination revealed extensive ductal damage, cytoplasmic vacuolization, and inflammatory changes in diabetic vitamin D–deficient glands, while vitamin D supplementation reduced the severity and frequency of these lesions.

**Conclusion:** Vitamin D supplementation exerted a protective effect against diabetes-related salivary gland dysfunction, partially improving biochemical disturbances and reducing histopathological injury. Although not fully restorative, vitamin D helped mitigate the severity of diabetes-induced alterations, underscoring its potential value as a supportive therapeutic strategy for preserving salivary gland health in diabetic conditions.

## Introduction

Submandibular and sublingual salivary glands are major paired exocrine glands that contribute substantially to unstimulated salivary flow and play key roles in lubrication, initial digestion, tooth remineralization, and mucosal defense through the secretion of water, electrolytes, enzymes, and mucins (1-3). Experimental work in rats has demonstrated functional specialization between these glands, with distinct patterns of glucose handling and ductal reabsorption that underscore their importance in maintaining oral homeostasis (3). Because of their high metabolic activity and rich microvasculature, submandibular and sublingual glands are particularly vulnerable to systemic metabolic disturbances that alter acinar and ductal cell

**Key point**

The study demonstrates that vitamin D plays a protective role in mitigating diabetes-induced salivary gland dysfunction, as evidenced by its ability to partially stabilize disrupted electrolyte balance, moderate adverse lipid alterations, and lessen reductions in salivary pH and flow rate. Vitamin D supplementation also reduced the severity of histopathological lesions, including ductal damage, cytoplasmic vacuolization, and inflammatory changes, highlighting its capacity to preserve glandular structure and function. Although the protective effects were incomplete, the overall findings indicate that adequate vitamin D status helps counteract the biochemical and structural deterioration associated with diabetes, supporting its potential value as an adjunctive strategy for maintaining salivary gland health in diabetic conditions.

structure and function (2,3).

Diabetes mellitus is associated with xerostomia and hyposalivation, and several experimental and clinical studies indicate that these symptoms reflect genuine structural and functional damage to major salivary glands (4-6). In streptozotocin-induced diabetic rats, submandibular glands show markedly reduced pilocarpine-stimulated saliva flow, decreased protein and amylase output, and profound disturbances in acinar $Ca^{2+}$ signaling and $Ca^{2+}$-ATPase activity, indicating intrinsic secretory dysfunction (5). Temporal analyses further revealed that diabetic rats develop reduced salivary flow together with increased oxidative damage markers and elevated oxidative stress indices in both parotid and submandibular glands, implicating reactive oxygen species as central mediators of glandular injury (6). Histopathological and stereological examinations of post-menopausal diabetic rat submandibular glands demonstrate neutrophilic infiltration, degeneration, and atrophy of seromucous acini, hypertrophy of granular ducts, and marked alterations in secretory content, consistent with chronic inflammatory and degenerative change (3). Complementing these animal data, morphometric analysis of human submandibular glands from well-controlled diabetic patients shows acinar and granule enlargement, mitochondrial alterations, and increased luminal microbuds, indicating that diabetes induces subtle but significant structural remodeling even when overt salivary dysfunction is not yet clinically evident (7).

Vitamin D has emerged as a potential modulator in the context of metabolic disease (8). Classic rat studies showed that severe vitamin D deficiency markedly reduces parotid salivary flow, while vitamin D3 replacement restores normal secretion without altering amylase output, suggesting a specific requirement for vitamin D in fluid and electrolyte secretion (9). More recently, a histological and ultrastructural study demonstrated that both conventional and nano-formulated vitamin D protect submandibular and sublingual glands from high-fat diet–induced fatty degeneration, with nano vitamin D more effectively normalizing acinar morphology and secretory granule profiles (10). In patients with type 2 diabetes, vitamin D supplementation improves systemic redox balance by increasing glutathione levels and reducing pro-inflammatory cytokines such as monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8), thereby attenuating oxidative stress and inflammation (11). Collectively, these findings support the hypothesis that vitamin D may counteract diabetes-induced oxidative and structural damage in submandibular and sublingual glands; however, direct experimental evidence in diabetic models remains limited. The present experimental histological study in albino rats was therefore designed to elucidate the protective effects of vitamin D on diabetes-induced alterations in submandibular and sublingual salivary gland structure and function.

## Objectives

The objective of this experimental study was to investigate the extent to which vitamin D supplementation can protect against diabetes-induced alterations in the submandibular and sublingual salivary glands of albino rats. Specifically, the study aimed to evaluate the impact of vitamin D on diabetes-related disturbances in salivary biochemical composition, glandular secretory function, and histopathological architecture, and to determine whether restoring vitamin D status can mitigate the metabolic and structural damage associated with diabetic conditions.

## Materials and Methods

### *Study design and samples*

This experimental laboratory-based study was conducted over an eight-week period at the research facilities of Al-Azhar university in Egypt in 2025. A total of 30 adult albino rats were enrolled and randomly assigned into three equal groups (n = 10 per group). Group 1 served as the non-diabetic healthy control and received a standard AIN-93M diet with adequate vitamin D under a normal 12-hour light/dark cycle. Group 2 consisted of diabetic rats with vitamin D insufficiency; these animals were maintained on a vitamin D-deficient AIN-93M diet, housed under continuous darkness to induce hypovitaminosis D, and diabetes was induced via a single intraperitoneal injection of streptozotocin. Group 3, also diabetic and vitamin D-deficient, was subjected to the same dietary and environmental conditions as group 2 but received a therapeutic vitamin D supplementation regimen of 20,000 IU throughout the study period.

### *Animal preparation method*

Throughout the 8-week experimental period, all animals were housed under controlled environmental conditions with regulated temperature, humidity, and ventilation to ensure physiological stability. Rats were acclimatized before the start of the study and monitored daily for general health, behavior, and food and water intake. Each group received its designated diet, either standard AIN-93M or vitamin D-deficient AIN-93M, and was maintained

under its assigned lighting conditions, including a standard 12-hour light/dark cycle for the control group and continuous darkness for vitamin D-deficient groups. Diabetes was induced in the designated groups using a single intraperitoneal injection of streptozotocin, followed by confirmation of hyperglycemia. Vitamin D-treated animals received 20,000 IU of vitamin D according to the planned supplementation schedule. Body weight, blood glucose, and clinical status were recorded regularly to ensure consistent monitoring of metabolic changes. All handling and procedures were performed in accordance with ethical guidelines to minimize stress and ensure animal welfare throughout the study.

### *Group classification*

Group 1 (n = 10) served as the non-diabetic healthy control group and was provided a nutritionally balanced AIN-93M diet containing adequate vitamin D, while being maintained under a standard 12-hour light/dark cycle. Group 2 (n = 10) comprised diabetic rats with vitamin D insufficiency; these animals received a vitamin D-deficient AIN-93M diet, were housed under continuous darkness to induce hypovitaminosis D, and diabetes was induced through a single intraperitoneal injection of streptozotocin. Group 3 (n = 10), also diabetic and vitamin D-deficient, was subjected to the same dietary and environmental conditions as group 2 but received a therapeutic vitamin D supplementation regimen consisting of 20,000 IU administered over eight weeks.

### *Sample collection and histological evaluation*

Blood and saliva samples were collected each week to the end of the 8-week experimental period to evaluate serum and salivary electrolytes (sodium, potassium, calcium, and bicarbonate), lipid profile parameters (high-density lipoprotein [HDL], low-density lipoprotein [LDL], and very-low-density lipoprotein [VLDL]), salivary pH, total salivary protein, and salivary flow rate using standardized biochemical techniques. Throughout the study, salivary samples were also obtained at predetermined intervals to monitor temporal changes in glandular function, and their mean concentration was calculated for final analysis. Following completion of the experiment, mice were euthanized, and the parotid and submandibular glands were excised, fixed, and processed for histological examination. Histopathological assessment was conducted by hematoxylin and eosin staining, which was used to assess duct formation, ductal injury, cytoplasmic vacuole development, hyalinosis, and inflammatory cell infiltration, enabling detailed evaluation of diabetes- and vitamin D-related structural alterations.

### *Outcome measurements*

The primary outcome of the study was the extent of diabetes-induced alterations in salivary gland function, assessed through changes in salivary electrolyte concentrations, lipid profile parameters, pH, total protein levels, and salivary flow rate. The secondary outcome was the degree of structural and histopathological damage in the submandibular and sublingual glands, evaluated by examining ductal morphology, cytoplasmic vacuolization, inflammatory changes, and combined lesion patterns. Together, these outcomes were used to determine the protective effect of vitamin D supplementation on both the biochemical and structural consequences of diabetes in salivary gland tissue.

### *Statistical analysis*

All statistical analyses were conducted using SPSS software version 27 (IBM Corp., USA). Data were checked for completeness and assessed for normality using the Shapiro–Wilk test. Descriptive statistics were generated for all biochemical, functional, and histopathological variables. Group differences were evaluated using one-way analysis of variance (ANOVA), followed by post hoc least significant difference (LSD) tests for pairwise comparisons when overall significance was detected. Categorical histopathological findings were analyzed using Fisher's exact test. Given that both parametric and nonparametric tests were of the same direction and had similar *P* values, parametric tests were used due to their high accuracy. Results were expressed as mean ± standard deviation, and a $P$ value $< 0.05$ was considered statistically significant.

## Results

Across all assessed biochemical parameters, the control group exhibited the most stable and physiologically coherent electrolyte profile, whereas the diabetic group with vitamin D deficiency demonstrated the greatest disturbances, indicative of marked impairment in electrolyte homeostasis. Sodium concentrations were elevated in the untreated diabetic animals relative to healthy controls, while vitamin D supplementation partially ameliorated this alteration. Potassium levels showed an inverse regulatory pattern, rising substantially in both diabetic groups compared with controls, although the increase was more moderate in vitamin D–treated rats, suggesting partial restoration of potassium handling. Calcium concentrations were consistently lower in both diabetic groups than in controls, with minimal differentiation between treated and untreated diabetic animals, reflecting a diabetes-driven decline that vitamin D was unable to fully correct. Bicarbonate levels were profoundly reduced in the vitamin D–deficient diabetic group, whereas vitamin D treatment produced an intermediate profile, consistent with partial attenuation of diabetes-associated acid–base dysregulation. Collectively, these findings indicate that diabetes in the context of vitamin D deficiency substantially disrupts salivary electrolyte regulation, and that vitamin D supplementation confers variable but measurable protective effects across different biochemical parameters (Table 1).

**Table 1.** Comparative analysis of biochemical parameters across experimental groups

| | | | | | |
|---|---|---|---|---|---|
| Na (mmol/L) | **Group** | | **Mean** | **SD** | ***P* value*** |
| | Control | | 140.45 | 2.19 | |
| | Diabetic +Vitamin D deficiency | | 151.72 | 2.69 | <0.001 |
| | Diabetic + Vitamin D deficiency treated with 20000 IU | | 148.6 | 3.84 | |
| | **Group** | | **Mean difference** | | ***P* value**** |
| | Control | Diabetic +Vitamin D deficiency | 11.27 | | <0.001 |
| | | Diabetic + Vitamin D deficiency treated with 20000 IU | 8.15 | | <0.001 |
| | Diabetic +Vitamin D deficiency | Diabetic + Vitamin D deficiency treated with 20000 IU | 3.12 | | 0.004 |
| K (mmol/L) | **Group** | | **Mean** | **SD** | ***P* value*** |
| | Control | | 4.29 | 0.55 | |
| | Diabetic +Vitamin D deficiency | | 5.05 | 0.4 | <0.001 |
| | Diabetic + Vitamin D deficiency treated with 20000 IU | | 5.22 | 0.31 | |
| | **Group** | | **Mean difference** | | ***P* value**** |
| | Control | Diabetic +Vitamin D deficiency | 0.76 | | <0.001 |
| | | Diabetic + Vitamin D deficiency treated with 20000 IU | 0.93 | | <0.001 |
| | Diabetic +Vitamin D deficiency | Diabetic + Vitamin D deficiency treated with 20000 IU | 0.17 | | 0.041 |
| Ca (mg/dl) | **Group** | | **Mean** | **SD** | ***P* value*** |
| | Control | | 9.61 | 0.67 | |
| | Diabetic +Vitamin D deficiency | | 8.28 | 0.46 | <0.001 |
| | Diabetic + Vitamin D deficiency treated with 20000 IU | | 8.19 | 0.31 | |
| | **Group** | | **Mean difference** | | ***P* value**** |
| | Control | Diabetic +Vitamin D deficiency | 1.33 | | <0.001 |
| | | Diabetic + Vitamin D deficiency treated with 20000 IU | 1.42 | | <0.001 |
| | Diabetic +Vitamin D deficiency | Diabetic + Vitamin D deficiency treated with 20000 IU | 0.09 | | 0.61 |
| HCO3 (mmol/L) | **Group** | | **Mean** | **SD** | ***P* value*** |
| | Control | | 22.96 | 1.37 | |
| | Diabetic +Vitamin D deficiency | | 17.45 | 2.28 | <0.001 |
| | Diabetic + Vitamin D deficiency treated with 20000 IU | | 19.45 | 2.77 | |
| | **Group** | | **Mean difference** | | ***P* value**** |
| | Control | Diabetic +Vitamin D deficiency | 5.51 | | <0.001 |
| | | Diabetic + Vitamin D deficiency treated with 20000 IU | 3.51 | | <0.001 |
| | Diabetic +Vitamin D deficiency | Diabetic + Vitamin D deficiency treated with 20000 IU | 2 | | 0.018 |

Na: Sodium; K: Potassium; Ca: Calcium; CL: Chloride; HCO3: Bicarbonate; Vit: Vitamin; SD: Standard deviation. *ANOVA, **Post hoc LSD.

The sodium concentration remained stable in the control group, whereas the diabetic group with vitamin D deficiency exhibited a marked increase, consistent with impaired electrolyte homeostasis. In contrast, the reduction in sodium levels was attenuated in the vitamin D–treated diabetic group, suggesting a partial protective effect on electrolyte regulation (Figure 1A). Potassium levels declined gradually in the control group, whereas the diabetic group showed a substantial increase, reflecting disrupted potassium regulation in diabetes. In the vitamin D–treated diabetic group, potassium levels also increased, but the rise was more controlled, indicating that vitamin D partially modulated the altered potassium handling associated with diabetes (Figure 1B). The levels of calcium in the control group showed a slight, progressive decline over the 8 weeks. In contrast, both diabetic groups exhibited a markedly greater reduction in calcium, with the untreated diabetic group demonstrating the most pronounced decrease. This pattern reflects the combined impact of diabetes and vitamin D deficiency on calcium homeostasis (Figure 1C). The pronounced and sustained decline in bicarbonate levels observed in the diabetic vitamin D–deficient group is consistent with the development of metabolic acidosis. In contrast, bicarbonate levels in the vitamin D–treated diabetic group remained comparatively stable and more closely aligned with those of the control group, indicating that vitamin D supplementation mitigated the diabetes-associated disturbances in salivary electrolyte balance over time (Figure 1D).

The comparative analysis between experimental groups indicated that the control group consistently demonstrated the most favorable lipid and salivary biochemical profile, whereas the diabetic group with vitamin D deficiency showed the greatest deviations, indicating substantial metabolic and functional impairment. The HDL levels were highest in the control group and markedly reduced in both diabetic groups, with vitamin D treatment producing only a modest improvement. The LDL concentrations displayed the opposite pattern, being lowest in the control group and substantially elevated in diabetic animals, although vitamin D supplementation attenuated this rise. The VLDL levels followed a similar trend, with the diabetic vitamin D–deficient group exhibiting the greatest increase relative to controls, and vitamin D treatment producing a partial reduction. Collectively, these findings indicate that

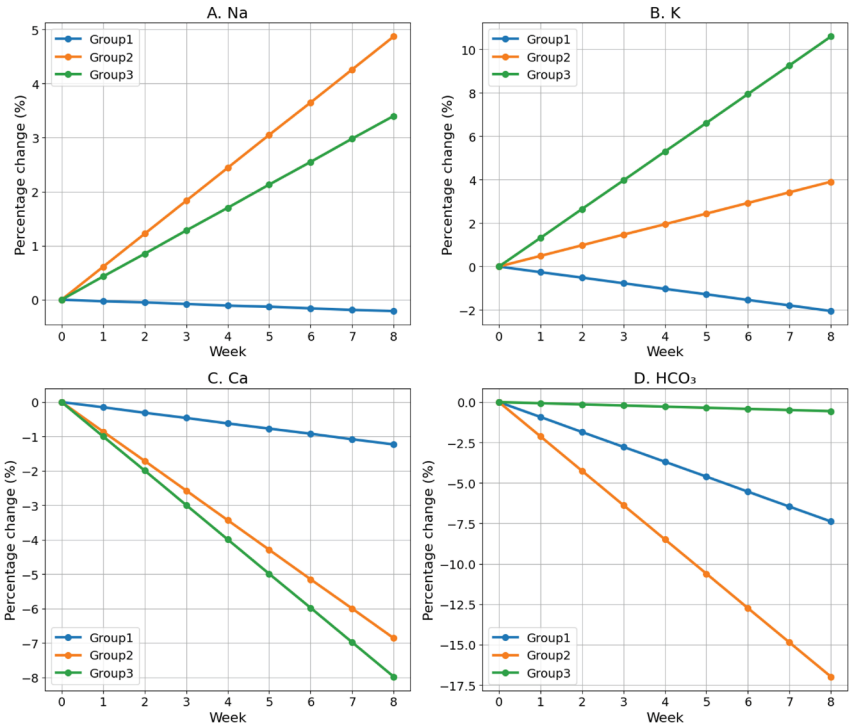


**Figure 1.** Percentage changes in salivary electrolytes over the 8-week period across the three study groups. Group 1: Healthy control rats, Group 2: Diabetic +Vitamin D deficiency, Group 3: Diabetic + Vitamin D deficiency treated with 20000 IU.

diabetes in the context of vitamin D deficiency profoundly disrupts lipid metabolism and salivary biochemical function, while vitamin D supplementation confers partial but measurable improvement across several parameters (Table 2).

The results demonstrated that the salivary pH was highest in the control group and markedly reduced in the diabetic vitamin D–deficient animals, with vitamin D treatment producing an intermediate pattern. Total protein concentrations followed an opposite trend, remaining

**Table 2.** Between-group comparison of lipid profile in the experimental groups

| | **Group** | | **Mean** | **SD** | ***P* value*** |
|---|---|---|---|---|---|
| HDL (mg/dL) | Control | | 53.95 | 3.98 | |
| | Diabetic +Vitamin D deficiency | | 32.27 | 3.37 | <0.001 |
| | Diabetic + Vitamin D deficiency treated with 20000 IU | | 34.6 | 5.71 | |
| | **Group** | | **Mean difference** | | ***P* value**** |
| | Control | Diabetic +Vitamin D deficiency | 21.68 | | <0.001 |
| | | Diabetic + Vitamin D deficiency treated with 20000 IU | 19.35 | | <0.001 |
| | Diabetic +Vitamin D deficiency | Diabetic + Vitamin D deficiency treated with 20000 IU | 2.33 | | 0.118 |
| LDL (mg/dL) | **Group** | | **Mean** | **SD** | ***P* value*** |
| | Control | | 88.13 | 7.31 | |
| | Diabetic +Vitamin D deficiency | | 183.64. | 5.55 | <0.001 |
| | Diabetic + Vitamin D deficiency treated with 20000 IU | | 138.1 | 6.52 | |
| | **Group** | | **Mean difference** | | ***P* value**** |
| | Control | Diabetic +Vitamin D deficiency | 95.51 | | <0.001 |
| | | Diabetic + Vitamin D deficiency treated with 20000 IU | 49.79 | | <0.001 |
| | Diabetic +Vitamin D deficiency | Diabetic + Vitamin D deficiency treated with 20000 IU | 45.54 | | 0.072 |
| VLDL (mg/dL) | **Group** | | **Mean** | **SD** | ***P* value*** |
| | Control | | 20.06 | 5.11 | |
| | Diabetic +Vitamin D deficiency | | 35.08 | 3.07 | <0.001 |
| | Diabetic + Vitamin D deficiency treated with 20000 IU | | 33.67 | 3.26 | |
| | **Group** | | **Mean difference** | | ***P* value**** |
| | Control | Diabetic +Vitamin D deficiency | 15.02 | | <0.001 |
| | | Diabetic + Vitamin D deficiency treated with 20000 IU | 13.61 | | <0.001 |
| | Diabetic +Vitamin D deficiency | Diabetic + Vitamin D deficiency treated with 20000 IU | 1.41 | | 0.148 |

HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low-density lipoprotein. *ANOVA, **Post hoc LSD.

lowest in the control group and substantially elevated in both diabetic groups, with no statistically significant difference between treated and untreated diabetic animals. Salivary flow rate was highest in the control group and markedly reduced in both diabetic groups, with vitamin D treatment failing to produce measurable improvement. Collectively, these findings demonstrate that diabetes profoundly disrupts salivary gland function, lowering pH, increasing protein concentration, and reducing flow rate, while vitamin D supplementation provides only partial correction for some parameters and no improvement for others (Table 3).

Across the 8-week study period, the control group consistently demonstrated the most stable patterns across all measured variables, whereas the diabetic group with vitamin D deficiency showed the greatest deviations, indicating substantial metabolic and functional disruption. The HDL levels declined progressively in the diabetic vitamin D–deficient group, while the control group maintained relative stability, and vitamin D treatment produced only partial improvement (Figure 2A). Meanwhile, LDL levels exhibited the opposite trend, rising sharply in the diabetic vitamin D–deficient rats and showing a more moderate increase with vitamin D supplementation, in contrast to the stable profile observed in controls (Figure 2B). The VLDL levels followed a similar pattern, with the diabetic vitamin D–deficient group showing the greatest elevation and the treated group demonstrating an attenuated response (Figure 2C). Salivary pH decreased markedly in the diabetic vitamin D–deficient group, with vitamin D treatment producing an intermediate trajectory between the control and untreated diabetic profiles (Figure 2D). Total protein concentrations increased substantially in both diabetic groups relative to controls, with vitamin D treatment offering limited correction (Figure 2E). Salivary flow rate declined sharply in both diabetic groups and remained consistently lower than in controls throughout the study, with no observable improvement following vitamin D supplementation (Figure 2F). Collectively, these temporal patterns illustrate that diabetes profoundly alters lipid metabolism and salivary gland function, while vitamin D supplementation provides only partial modulation of these disturbances over time.

The examined histopathological features indicated that the control group exhibited entirely normal glandular architecture with no detectable abnormalities, whereas the diabetic group with vitamin D deficiency demonstrated the highest frequency and widest range of structural alterations, indicating substantial tissue injury. These alterations included increased numbers of ducts, partial ductal destruction, cytoplasmic vacuolization, inflammatory changes, and combined lesions involving both ductal proliferation and degeneration. In contrast, the vitamin D–treated diabetic group, compared to the diabetic group with vitamin D deficiency, showed fewer and less severe abnormalities, with only occasional findings such as mild ductal increase, limited vacuolization, or partial ductal destruction. Notably, some complex lesions, such as combined ductal destruction with inflammation, were markedly reduced in the treated group compared with untreated diabetic animals. Overall, these

**Table 3.** Comparative analysis of salivary pH, total protein, and flow rate across experimental groups

| | | | | | |
|---|---|---|---|---|---|
| pH | **Group** | | **Mean** | **SD** | ***P* value*** |
| | Control | | 7.11 | 0.22 | |
| | Diabetic +Vitamin D deficiency | | 6.33 | 0.13 | <0.001 |
| | Diabetic + Vitamin D deficiency treated with 20000 IU | | 6.65 | 0.13 | |
| | **Group** | | **Mean difference** | | ***P* value**** |
| | Control | Diabetic +Vitamin D deficiency | 0.58 | | <0.001 |
| | | Diabetic + Vitamin D deficiency treated with 20000 IU | 0.46 | | <0.001 |
| | Diabetic +Vitamin D deficiency | Diabetic + Vitamin D deficiency treated with 20000 IU | 0.32 | | 0.097 |
| Total protein (mg/dL) | **Group** | | **Mean** | **SD** | ***P* value*** |
| | Control | | 124.58 | 16.16 | |
| | Diabetic +Vitamin D deficiency | | 189.4 | 15.66 | <0.001 |
| | Diabetic + Vitamin D deficiency treated with 20000 IU | | 189.99 | 12.21 | |
| | **Group** | | **Mean difference** | | ***P* value**** |
| | Control | Diabetic +Vitamin D deficiency | 64.82 | | <0.001 |
| | | Diabetic + Vitamin D deficiency treated with 20000 IU | 65.41 | | <0.001 |
| | Diabetic +Vitamin D deficiency | Diabetic + Vitamin D deficiency treated with 20000 IU | 0.59 | | 0.811 |
| Flow rate (mL/min) | **Group** | | **Mean** | **SD** | ***P* value*** |
| | Control | | 0.38 | 0.04 | |
| | Diabetic +Vitamin D deficiency | | 0.19 | 0.06 | <0.001 |
| | Diabetic + Vitamin D deficiency treated with 20000 IU | | 0.19 | 0.05 | |
| | **Group** | | **Mean difference** | | ***P* value**** |
| | Control | Diabetic +Vitamin D deficiency | 0.19 | | <0.001 |
| | | Diabetic + Vitamin D deficiency treated with 20000 IU | 0.19 | | <0.001 |
| | Diabetic +Vitamin D deficiency | Diabetic + Vitamin D deficiency treated with 20000 IU | 0 | | > 0.999 |

*ANOVA, **Post hoc LSD.

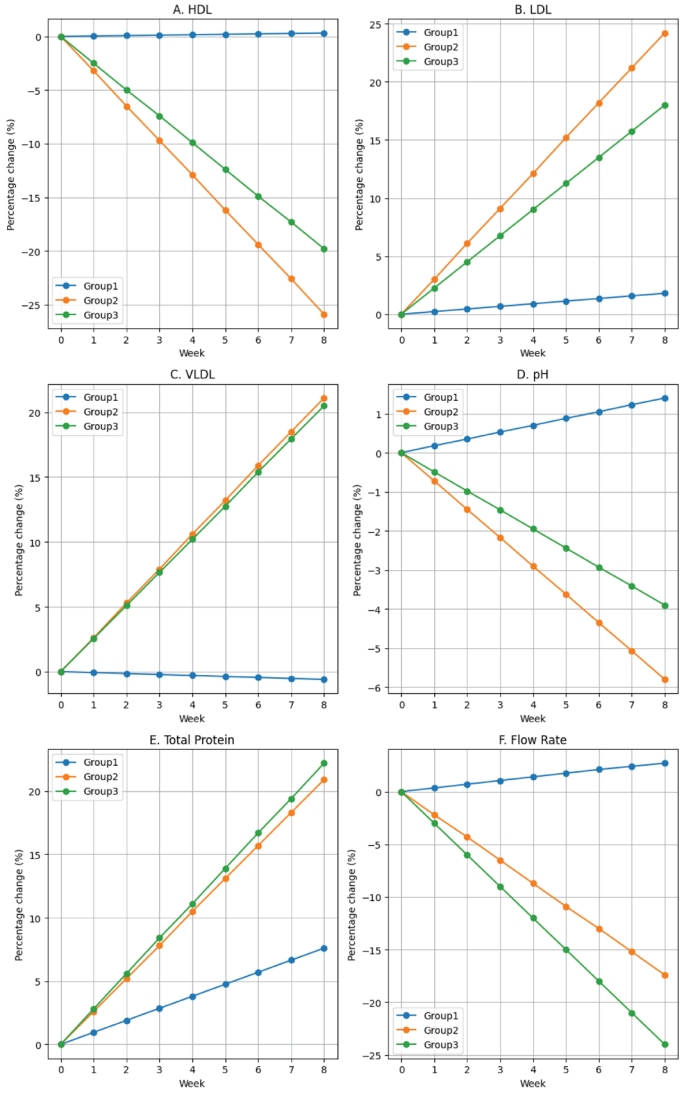


**Figure 2**. Percentage changes in lipid profile, salivary pH, total protein, and flow rate among experimental groups over the 8-week period of study. HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low-density lipoprotein.

histopathological patterns demonstrate that diabetes in the context of vitamin D deficiency induces pronounced structural damage in the parotid and submandibular glands, while vitamin D supplementation confers a measurable protective effect, reducing both the prevalence and severity of glandular injury (Table 4).

In the healthy control rats, which were maintained under a natural 12-hour light–dark cycle with unrestricted exposure to sunlight, the salivary glands exhibited normal histological architecture. In Figure 3A, the normal serous acini are composed of basophilic, granule-rich cells (star) arranged around well-defined interlobular excretory ducts (arrow). In Figure 3B, the mucinous acini display their characteristic morphology, consisting of mucin-filled cytoplasm with nuclei displaced basally, consistent with the typical appearance of mucous secretory units (Figure 3).

The glands exhibit marked structural deterioration characterized by destruction of the ductal epithelium accompanied by a noticeable reduction in cytoplasmic granularity, reflecting impaired secretory activity (Figure 4).

The acini and ductal structures show preservation of overall glandular architecture, with maintained cellular organization and reduced evidence of diabetes-related injury (H&E, ×100) (Figure 5A). The mucinous acini retain their characteristic morphology, displaying mucin-filled cytoplasm with basally positioned nuclei, and only mild structural alterations attributable to diabetes, indicating partial protection conferred by vitamin D supplementation (H&E, ×200) (Figure 5B).

## Discussion

In this experimental study, diabetes in the context of vitamin D deficiency was associated with marked disturbances in salivary biochemistry and major histopathological injury in the submandibular and sublingual glands, while vitamin D supplementation attenuated but did not completely reverse these alterations. Collectively, the findings support

**Table 4.** Comparative histopathological assessment of parotid and submandibular glands in the experimental groups

| Histopathological results | Experimental groups | | | *P* value |
|---|---|---|---|---|
| | Control (n = 10) | Diabetic +Vitamin D deficiency (n = 10) | Diabetic + Vitamin D deficiency treated with 20000 IU (n = 10) | |
| Increase the number of ducts | 0 (0%) | 4 (40%) | 1 (10%) | <0.001* |
| Partial destruction of the ducts | 0 (0%) | 3 (30%) | 1 (10%) | |
| Vacuolization of cytoplasm | 0 (0%) | 2 (20%) | 1 (10%) | |
| Partial destruction of the ducts + inflammation | 0 (0%) | 4 (40%) | 2 (20%) | |
| Partial destruction of the ducts + vacuolization of cytoplasm | 0 (0%) | 3 (30%) | 1 (10%) | |
| Increase the number of the ducts + Partial destruction of the ducts | 0 (0%) | 5 (50%) | 2 (20%) | |
| Partial destruction of the ducts + hyalinosis of the ducts | 0 (0%) | 0 (0%) | 1 (10%) | |

*Fisher's exact test.

a predominantly protective, rather than fully restorative, role of vitamin D against diabetes-induced salivary gland dysfunction in rats. Experimental and clinical studies have long indicated that diabetes mellitus compromises salivary gland function, leading to hyposalivation, altered salivary composition, and xerostomia, in parallel with structural injury of the major salivary glands (5,12-14). Streptozotocin-induced diabetes, reduced pilocarpine-stimulated salivary flow, and protein secretion have been linked to perturbations in calcium signalling and impaired $Ca^{2+}$-transporting pumps in submandibular acinar cells, providing a mechanistic basis for secretory dysfunction. Type 2 diabetes models have similarly demonstrated decreased basal and stimulated salivary flow rates, reduced amylase activity, and histopathological lesions in parotid and submandibular glands, including inflammatory infiltration, vacuolization, and acinar atrophy, which parallel the functional deficits observed in the present study (12-14).

The current biochemical data, showing increased salivary sodium, potassium, LDL, VLDL, and total protein, together with decreased bicarbonate, HDL,

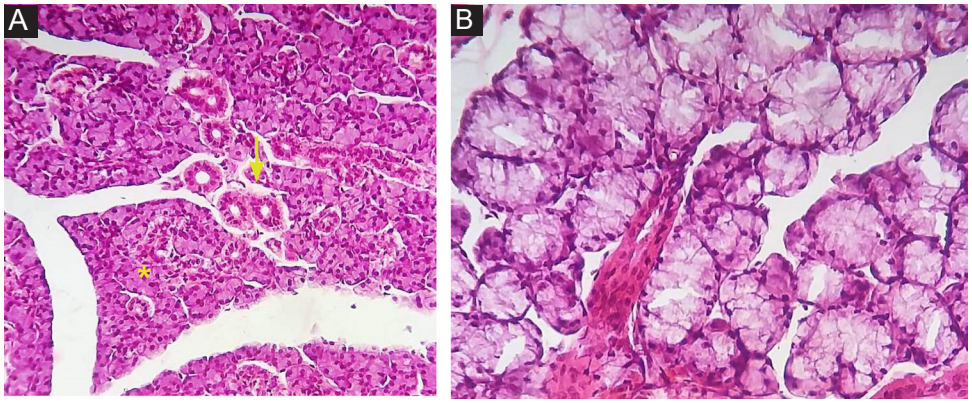


**Figure 3.** Histopathological assessment of parotid and submandibular glands in healthy control rats (H&E, ×200). Note: Arrow and asterisk signs show normal serous acini.

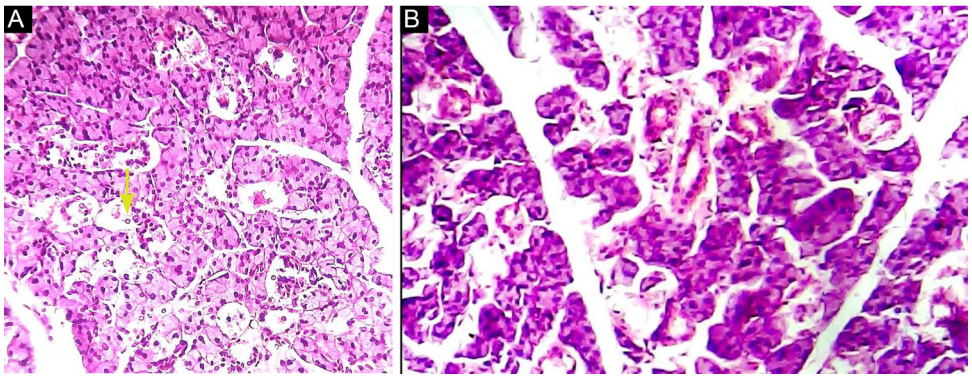


**Figure 4**. Histopathological assessment of parotid and submandibular glands in diabetic rats with vitamin D deficiency (H&E, ×200). Note: Yellow arrow sign shows glands.

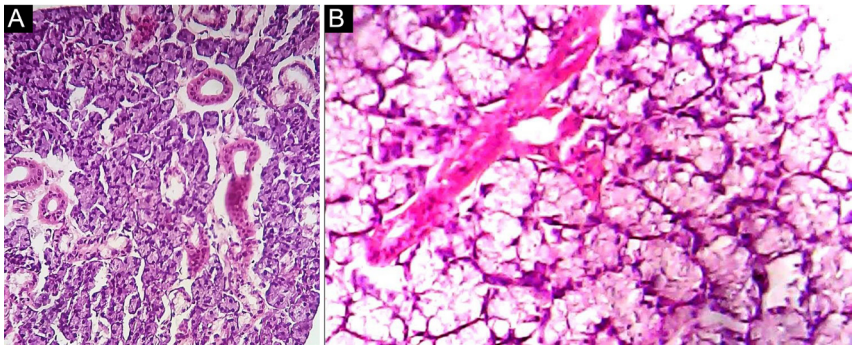


**Figure 5**. Histopathological assessment of parotid and submandibular glands in diabetic rats with vitamin D deficiency treated with 20000 IU vitamin D. (**A**: H&E, ×100), (**B**: H&E, ×200).

salivary pH, and flow rate in diabetic vitamin D–deficient rats, are broadly consistent with earlier reports of disrupted salivary composition and systemic dyslipidaemia in diabetes. Alterations in electrolytes and buffering capacity, reflected by reduced bicarbonate and lower salivary pH, are particularly relevant because they may contribute to mucosal dryness and increased caries risk, phenomena frequently described in diabetic cohorts (14). Beyond the oral cavity, vitamin D deficiency itself has been associated with unfavourable lipid profiles and heightened cardiometabolic risk, and supplementation has been reported to modestly improve triglycerides and HDL in some populations, suggesting that the partial correction of lipid disturbances observed here is biologically plausible but should be interpreted cautiously in the absence of direct mechanistic assays (15,16).

Histologically, diabetic vitamin D–deficient glands in this study exhibited extensive ductal damage, cytoplasmic vacuolization, and inflammatory changes, in line with previous descriptions of diabetes-related remodelling of the major salivary glands (12-14). Both short- and long-term type 1 diabetes have been shown to induce vacuolization of acinar cells, altered basement membrane components, and a reduction in specialized ductal structures, indicating that structural damage is an early and persistent feature of the disease (12,14). The observation that vitamin D supplementation reduced the severity and frequency of these lesions is conceptually similar to other experimental interventions, such as the use of salivary exosomes, that have mitigated xerostomia, improved salivary flow, and decreased inflammatory marker expression in diabetic rat salivary glands, reinforcing the notion that diabetes-induced damage is at least partially reversible under favorable conditions.

The partial protective effect of vitamin D observed in this study also aligns with earlier work in non-diabetic models demonstrating a role for vitamin D signalling in salivary gland physiology. Classic studies in vitamin D-deficient rats showed that parotid salivary flow is substantially reduced in the absence of vitamin D and can be restored by vitamin $D_3$ administration, indicating that normal fluid and electrolyte secretion from the parotid gland is vitamin D-dependent (9). More recently, vitamin D receptor (VDR) expression has been localized predominantly to ductal epithelium in murine and human salivary glands, and activation of the VDR pathway has been shown to decrease proliferation and promote a more differentiated ductal phenotype in salivary epithelial cells, suggesting a role in maintaining glandular homeostasis (17). Additional experimental work in calcium-deficient rats has reported that nano-formulated vitamin $D_3$ can better preserve parotid architecture and secretory granules than conventional vitamin $D_3$, further supporting a direct tissue-protective role of vitamin D within salivary glands (18).

Several methodological aspects of this work merit consideration when interpreting the findings. The use of clearly defined groups, healthy controls, diabetic vitamin D–deficient rats, and diabetic rats receiving a fixed high dose of vitamin D allowed discrimination between the effects of diabetes per se and the potential modifying influence of vitamin D status on salivary gland structure and function. At the same time, the single dose, route, and duration of vitamin D therapy, together with the focus on a single experimental diabetes model and on selected biochemical variables, limit the generalizability of the conclusions. The study did not directly assess serum vitamin D metabolites, VDR expression, inflammatory mediators, or oxidative stress markers within the glands, which constrains mechanistic inference and precludes definitive statements about the pathways mediating the observed protection.

Overall, this study indicates that diabetes in the context of vitamin D deficiency induces substantial biochemical and structural disturbances in the submandibular and sublingual salivary glands of rats, and that vitamin D supplementation confers a significant but incomplete protective effect. The pattern of partial normalization of salivary parameters and attenuation of histopathological injury is consistent with previous evidence that vitamin D supports salivary gland homeostasis and can mitigate, though not fully reverse, tissue damage in systemic

disease states. Future investigation integrating detailed mechanistic analyses and varying vitamin D regimens, as well as translational studies in human diabetes, will be essential to clarify the therapeutic potential and optimal use of vitamin D for preserving salivary gland function in diabetic conditions.

## Conclusion

Vitamin D demonstrated a clinically meaningful protective effect against diabetes-induced salivary gland dysfunction, mitigating disturbances in electrolyte balance, lipid metabolism, and glandular secretion. Although supplementation did not fully restore normal biochemical or structural integrity, it consistently reduced the severity of metabolic derangements and histopathological injury observed in vitamin D–deficient diabetic rats. These findings suggest that maintaining adequate vitamin D status may play an important supportive role in preserving salivary gland health in individuals with diabetes, potentially reducing the burden of oral complications associated with the disease.

## Limitations of the study

In case of findings interpretation, these limitations need to be considered. The use of an animal model, while valuable for controlled experimentation, may not fully replicate the complexity of human diabetes or vitamin D metabolism. Additionally, the study duration was restricted to eight weeks, preventing assessment of long-term biochemical or histopathological changes. Environmental manipulation to induce vitamin D deficiency, such as continuous darkness, may also introduce physiological stress that could influence outcomes. Finally, although both parametric and nonparametric analyses showed similar trends, reliance on parametric tests may still carry inherent assumptions that cannot be completely verified in small experimental samples.

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### Authors' contribution

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### Conflicts of interest

The authors declare no conflict of interest.

### Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declaration of generative artificial intelligence (AI) and AI-assisted technologies in the writing process

While preparing this work, the authors utilized AI (Grammarly, Perplexity, Copilot) to refine grammar points and language style. Subsequently, they thoroughly reviewed and edited the content as necessary, assuming full responsibility for the publication's content.

### Ethical issues

The study was conducted according to the ethical standards of animal studies and approved by the ethical committee of Al-Azhar University, Egypt, under registration number (0000017, dated February 2025). The experimental procedures were also conducted under the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). We also followed the guidelines related to animal experiments, approved by the United States National Institutes of Health (NIH, 1978). Besides, the authors have ultimately observed ethical issues (including plagiarism, data fabrication, and double publication).

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### References

1. Khoury ZH, Sultan AS. Prosthodontic implications of saliva and salivary gland dysfunction. J Prosthodont. 2023;32:766–75. doi: 10.1111/jopr.13725.
2. Takai N, Yoshida Y, Kakudo Y. Secretion and re-absorption of glucose in rat submandibular and sublingual saliva. J Dent Res. 1983;62:1022–5. doi: 10.1177/00220345830620100101.
3. Buyuk B, Parlak SN, Keles ON, Can I, Yetim Z, Toktay E, et al. Effects of Diabetes on Post-Menopausal Rat Submandibular Glands: A Histopathological and Stereological Examination. Eurasian J Med. 2015;47:199–207. doi: 10.5152/eurasianjmed.2015.80.
4. Stewart CR, Obi N, Epane EC, Akbari AA, Halpern L, Southerland JH, et al. Effects of Diabetes on Salivary Gland Protein Expression of Tetrahydrobiopterin and Nitric Oxide Synthesis and Function. J Periodontol. 2016;87:735–41. doi: 10.1902/jop.2016.150639.
5. Fedirko NV, Kruglikov IA, Kopach OV, Vats JA, Kostyuk PG, Voitenko NV. Changes in functioning of rat submandibular salivary gland under streptozotocin-induced diabetes are associated with alterations of $Ca^{2+}$ signaling and $Ca^{2+}$ transporting pumps. Biochim Biophys Acta. 2006;1762:294–303. doi: 10.1016/j.bbadis.2005.12.002.
6. Dyasnoor S, Kamath S, Khader NFA. Effectiveness of Electrostimulation on Whole Salivary Flow Among Patients with Type 2 Diabetes Mellitus. Perm J. 2017;21:15–164. doi: 10.7812/tpp/15-164.
7. Lilliu MA, Solinas P, Cossu M, Puxeddu R, Loy F, Isola R, et al. Diabetes causes morphological changes in human submandibular gland: a morphometric study. J Oral Pathol Med. 2015;44:291–5. doi: 10.1111/jop.12238.
8. Colombo C, Fabiano V, Labati L, Loiodice M, Ceruti D, Campi

I, et al. Vitamin D, adiposity, and cardiometabolic risk: Insights from a multivariable cross-sectional study. Obes Res Clin Pract. 2026;20:43–51. doi: 10.1016/j.orcp.2025.12.005.
9. Glijer B, Peterfy C, Tenenhouse A. The effect of vitamin D deficiency on secretion of saliva by rat parotid gland in vivo. J Physiol. 1985;363:323–34. doi: 10.1113/jphysiol.1985.sp015713.
10. Al-Serwi RH, El-Sherbiny M, Eladl MA, Aloyouny A, Rahman I. Protective effect of nano vitamin D against fatty degeneration in submandibular and sublingual salivary glands: A histological and ultrastructural study. Heliyon. 2021;7:e06932. doi: 10.1016/j.heliyon.2021.e06932.
11. Gu JC, Wu YG, Huang WG, Fan XJ, Chen XH, Zhou B, et al. Effect of vitamin D on oxidative stress and serum inflammatory factors in the patients with type 2 diabetes. J Clin Lab Anal. 2022;36:e24430. doi: 10.1002/jcla.24430.
12. Monteiro MM, D'Epiro TT, Bernardi L, Fossati AC, Santos MF, Lamers ML. Long- and short-term diabetes mellitus type 1 modify young and elder rat salivary glands morphology. Arch Oral Biol. 2017;73:40–7. doi: 10.1016/j.archoralbio.2016.08.028.
13. Mednieks MI, Szczepanski A, Clark B, Hand AR. Protein expression in salivary glands of rats with streptozotocin diabetes. Int J Exp Pathol. 2009;90:412–22. doi: 10.1111/j.1365-2613.2009.00662.x.
14. Chen SY, Wang Y, Zhang CL, Yang ZM. Decreased basal and stimulated salivary parameters by histopathological lesions and secretory dysfunction of parotid and submandibular glands in rats with type 2 diabetes. Exp Ther Med. 2020;19:2707–19. doi: 10.3892/etm.2020.8505.
15. Vasdeki D, Tsamos G, Dimakakos E, Patriarcheas V, Koufakis T, Kotsa K, et al. Vitamin D Supplementation: Shedding Light on the Role of the Sunshine Vitamin in the Prevention and Management of Type 2 Diabetes and Its Complications. Nutrients. 2024;16:3651. doi: 10.3390/nu16213651.
16. Akin F, Ayça B, Köse N, Duran M, Sari M, Uysal OK, et al. Serum vitamin D levels are independently associated with severity of coronary artery disease. J Investig Med. 2012;60:869–73. doi: 10.2310/JIM.0b013e31825457cb.
17. DeSantis KA, Robilotto SL, Matson M, Kotb NM, Lapierre CM, Minhas Z, et al. VDR in salivary gland homeostasis and cancer. J Steroid Biochem Mol Biol. 2020;199:105600. doi: 10.1016/j.jsbmb.2020.105600.
18. Montaser MM, Mahdy DM, Hamza SA. Prophylactic effect of nano vitamin D3 against fatty degeneration caused by calcium-deficient diet in parotid salivary gland: a histological and ultrastructural study. BMC Oral Health. 2025;25:1279. doi: 10.1186/s12903-025-06618-7.