Research Article Open Access

Vildagliptin-Omega-3 combination therapy mitigates the diabetic effects on rats sublingual salivary glands via anti-oxidative and anti-apoptotic mechanisms

Ahmed O. Galal[1], Lashin S. Ali[2,3], Osama I. Ramadan[4,5*], Reda S. Taha[6,7], Moaaz M. Awad[8,7], Osama M. Abdelhay[9,10], Ala'a Alasmar[11], Anas A. Darwish[12,13], Afnan I. Elgndy[14,15], Asmaa M. Meghawry[16], Mahmoud Diab[17], Nourelhuda A. Mohammed[18], Fatma M. Abd-Allah[19,5], Ibrahim M. Amr[5], Mohammed E. Elmitwalli[5], Nassar A. A. Omar[20,8], Ahmed E. Amer[7], Alsayed A. Abdel-Hady[21]

[1]Oral Surgery and Diagnostic Sciences Department, Faculty of Dentistry, Applied Science Private University, Amman, 19931 Jordan; [2]Department of basic medical science, faculty of Dentistry, Al-Ahliyya Amman University, Amman, 19328 Jordan; [3]Department of medical physiology, faculty of Medicine, Mansoura university, Mansoura 33516, Egypt; [4]Histology Department, General Medicine practice Program, Batterjee Medical College, Dammam, 21441 Saudi Arabia; [5]Histology Department, Damietta Faculty of Medicine, Al-Azhar University, Damietta 11884, Egypt; [6]Department of Basic Medical and Dental Sciences, Faculty of Dentistry, Zarqa University, Zarqa, 13132 Jordan; [7]Anatomy and Embryology Department, Damietta Faculty of Medicine, Al-Azhar University, Damietta 11884, Egypt; [8]Anatomy department, General Medicine Practice Program, Batterjee Medical Collage, Aseer, 62451, Saudi Arabia; [9]Department of Medicine & Surgery, Vision medical college, Jeddah, 23643 KSA; [10]Department of Physiology, Faculty of Medicine, Al-Azhar University, Cairo, 11651 Egypt; [11]Faculty of Applied Medical Sciences, Hashemite University, Zarqa 13115, Jordan; [12]Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, 21589 KSA; [13]Alghad colleges for applied medical science, Najran 66462, Saudi Arabia; [14]Department of Physiology, Faculty of Medicine, Benha University, Benha, 13518, Egypt; [15]Department of Physiology, General Medicine practice Program, Batterjee Medical College, Asser, 62451, Saudi Arabia; [16]Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Benha University, Benha 13511, Egypt; [17]Anatomy and Embryology Department, Faculty of Medicine, Al-Azhar University, Cairo 11754, Egypt; [18]Department of Physiology, Faculty of Medicine, Zagazig University, Zagazig 44511, Egypt ; [19]Department of Basic medical Sciences, College of Medicine, Shaqra University, Shaqra 11961, Saudi Arabia; [20]Department of Anatomy, Faculty of Medicine, Sohag University, Sohag 82524, Egypt; [21]Department of Anatomy and Embryology, Faculty of Medicine, Capital University (Formerly Helwan University), Cairo 11795, Egypt.

*** Corresponding author:** Osama Ramadan, Associate professor, General Medicine Practice Program, Batterjee Medical College, Dammam 32313, Saudi Arabia; Histology Department, Damietta Faculty of Medicine, Al-Azhar University, New Damietta 34711, Egypt. Email: dr.osama784@yahoo.com

Editorial Office: editor@ffhdj.com

Submission Date: February 12th, 2026**; Acceptance Date:** April 18th, 2026**; Publication Date:** April 22, 2026

Please cite this article as: Galas A. O., Ali L. S., Ramadan O. I., Tahoe R. S., Awad M. M., Abdelhay O. M., Alasmar A., Darwish A. A., Elgndy A. I., Meghawry A. M., Dian M., Mohammed N. A., Abd-Allah F. M., Amir I. M., Elmitwalli M. E., Omar N. A. A., Amer A. E., Abdel-Hady A. A. Vildagliptin-Omega-3 Combination therapy mitigates the diabetic effects on the rats' sublingual salivary glands via anti-oxidative and anti-apoptotic mechanism. *Functional Foods in Health and Disease* 2026; 16(4): 631 – 644. DOI: https://doi.org/10.31989/ffhd.v16i4.1937

ABSTRACT

Background: Patients with diabetes mellitus (DM) frequently have xerostomia (dry mouth) and hyposalivation. Whether xerostomia and hyposalivation are more common in DM patients than non-DM individuals is unclear**.**

Aim of the study: We aimed to detect the diabetic effects on the sublingual salivary gland and investigate the therapeutic potential of Vildagliptin (a dipeptidyl peptidase-4 (DPP-4) inhibitor) and omega-3 polyunsaturated fatty acids (PUFAs), alone and in combination, in protecting sublingual salivary glands in streptozotocin (STZ)-induced diabetic rats**.**

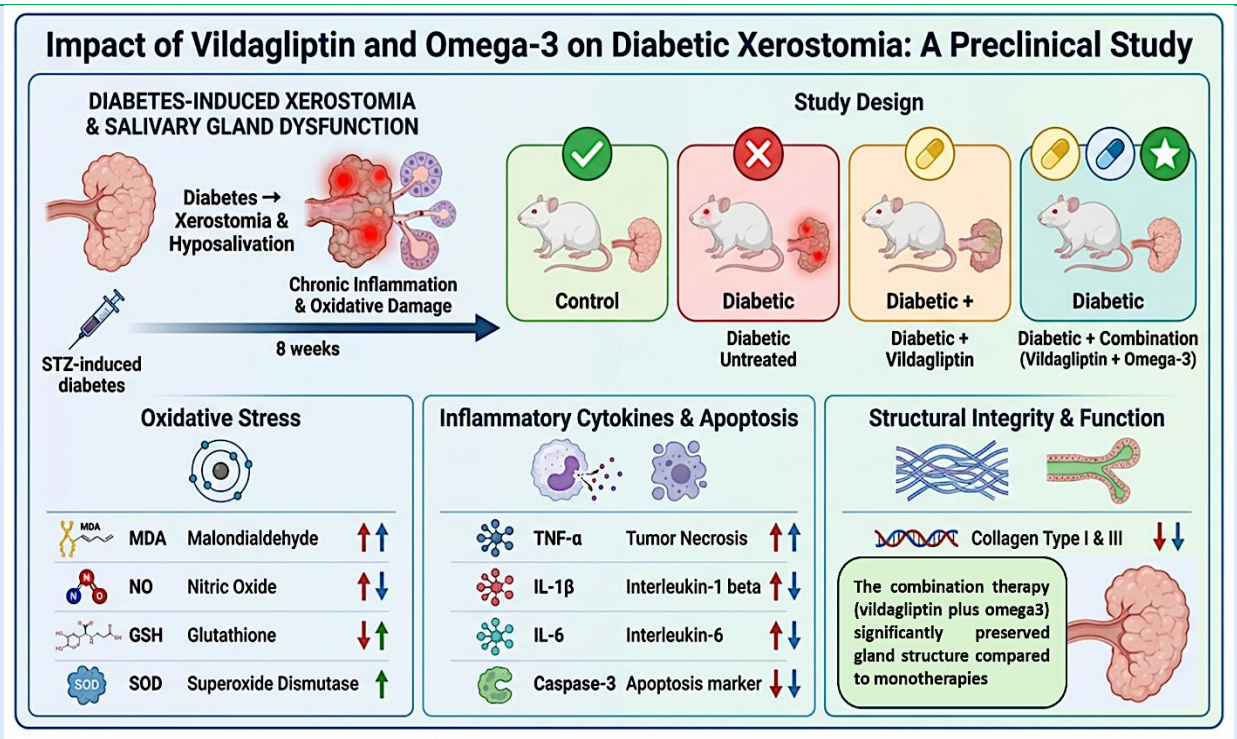
Methods: Forty male albino rats were randomly allocated into control, diabetic untreated, diabetic + Vildagliptin, diabetic + omega-3, and diabetic + combination groups. After 8 weeks, biochemical markers of oxidative stress including: Glutathione (GSH); Malondialdehyde (MDA); Nitric Oxide (NO); Superoxide Dismutase (SOD); Total Antioxidant Capacity (TAC), and inflammation markers as Interleukins ((IL-1β, IL-6) and Tumor Necrosis Factor (TNF) and histological outcomes (acinar integrity, mucin content, apoptosis) were analyzed**.**

Results: Induction of diabetes in rats caused structural changes in the sublingual gland associated with fibrotic, apoptotic and inflammatory changes**,** treatment of diabetes with vildagliptin enhanced the previous changes, but the combination therapy (vildagliptin plus omega 3) significantly reduced oxidative stress, suppressed inflammation, and preserved gland structure compared to monotherapies**.**

Conclusion: The synergistic efficacy of Vildagliptin-omega-3 in mitigating diabetic salivary gland damage, proposing a novel therapeutic strategy**.**

Novelty of the Study: Although Omega-3 and vildagliptin, a DPP-4 inhibitor, are both well-known for their metabolic and antioxidant advantages, this study is the first to show how they integrate to protect salivary gland architecture from diabetes-related damage via dual mechanisms (antioxidative and antiapoptotic). This provides a novel therapeutic approach for treating diabetic xerostomia (dry mouth) with glycemic control.

Keywords: Diabetes mellitus; salivary glands; Vildagliptin; Omega-3; xerostomia



Graphical Abstract: Vildagliptin-Omega-3 combination therapy mitigates the diabetic effects on rats sublingual salivary glands

INTRODUCTION

Diabetes mellitus (DM) is a major global health burden, as a chronic metabolic disease that affects millions of people worldwide [1, 2].

Prolonged hyperglycemia and related metabolic disorders cause major oral diseases as well as other systemic problems [3, 4].

In both human and experimental models, damage to the main salivary glands is generally recognized because of diabetes mellitus. Salivary gland dysfunction was manifested in patients with type 1 and type 2 diabetes, as hyposalivation and xerostomia (dry mouth)[5, 6].

This decrease in salivary flow raises the risk of dental caries, oral infections, and periodontal disease by compromising lubrication, digestion, and antibacterial action. The quality of life of a patient can be considerably reduced by these oral problems [7].

Diabetes mellitus and primary salivary gland dysfunction have a complicated and ambiguous association. Chronic inflammation, oxidative stress, and apoptosis could be the main suggested mechanisms of diabetic-induced damage to the salivary glands [8].

A popular and well-established experimental system for simulating important features of both type 1 and type 2 diabetes, the streptozotocin (STZ)-induced diabetic rat model offers a useful platform for researching possible treatment approaches [9, 10].

Significant oxidative damage as well as structural and functional alterations in the salivary glands of diabetic mice have been verified by studies using this paradigm [11].

Vildagliptin is a well-known oral antidiabetic drug that works by increasing the activity of the incretin hormone. It is an inhibitor of DPP-4 [12].

Vildagliptin has shown protective effects on multiple organs in diabetic models due to its strong antioxidant and anti-inflammatory qualities, which are separate from its glycemic effects [4, 13].

Similarly, through their bioactive components, functional foods were known to reduce the risk of chronic illnesses, promote metabolic health, and increase general well-being [14].

Omega-3 is one of the bioactive components, which are found in large quantities in fish oils, are well known for their potent antioxidant and anti-inflammatory properties [15]. Omega-3 supplementation has been proven to have a direct preventive impact by reducing age-related and other degenerative alterations in numerous organs as liver, kidney and the salivary glands [16-18].

The combined therapeutic potential of vildagliptin and omega-3 fatty acids for addressing salivary gland pathology in a diabetic setting is still largely unknown, despite their well-established preventive benefits on a number of diabetes problems.

To the best of our knowledge, this is the first investigation to assess D.M.'s effects on the sublingual salivary gland, and to detect the effect of combination therapy vildagliptin and omega-3. This could provide a synergistic protective strategy for the mucous salivary glands based on the different but complementary mechanisms of action of omega-3 and vildagliptin, which have anti-inflammatory and anti-diabetic effects.

MATERIAL AND METHODS

Ethical Considerations: The proposal for this research was approved by the institutional review board in our faculty of medicine (DFM-IRB 00012367-25-06-026).

Animal Model: 40 male albino rats (8–10 weeks old) weighting 130 g to 140 g were used because of their well-documented sensitivity to STZ and because they are frequently utilized in studies involving diabetes. Rats were kept in a temperature-controlled space (22± 2 °C) with a 12-hour light/dark cycle, and they were given unlimited access to food and water. Rats were randomly assigned to four groups (each consisting of ten rats) following acclimatization.

Drugs: Streptozotocin was provided from "Sigma Aldrich Chemical Company CO., St. Louis, MO, USA." Streptozotocin was diluted in "0.01 M sodium citrate buffer, pH 4.5" before to being administered.

Vildagliptin (Galvus® 50 mg tablet) was purchased from Novartis Pharma Company in Cairo

Omega-3: A soft gelatin capsule was purchased from (SEDICO Pharmaceutical Co., Egypt). The 1000 mg of fish oil in each capsule includes 13% eicosapentaenoic acid (EPA) and 9% docosahexaenoic acid (DHA).

Diabetes induction: After 1 week of acclimatizations in animal house, a single intraperitoneal injection of STZ (55 mg/kg), freshly dissolved in cold 0.1 M citrate buffer (pH 4.5), were administered to all groups except the control group to rats that had fasted for 12 hours. To stop the hypoglycemia that would have allowed STZ to enter the cells, a 5% sucrose solution was administered overnight [19]. The experimental period lasted for a specific duration (8 weeks) to observe both short- and long-term effects of diabetes and the treatment. Fasting blood glucose levels were measured from tail vein samples three to five days after the STZ injection using a glucometer. The study included rats with blood glucose levels greater than 250 mg/dL, which were categorized as diabetic [20].

Animal grouping was as follows:

1. **Control (non-diabetic):** rats receiving a vehicle (e.g., distilled water)
2. **Diabetic (STZ-induced, untreated):** a single intraperitoneal injection of STZ (55 mg/kg) then left for the remaining 8 weeks on normal diet.
3. **Diabetic + Vildagliptin:** after induction of diabetes, rats received Vildagliptin orally (via oral gavage) at a clinically relevant dose (10 mg/kg/day) for the remaining of 8 weeks [21].
4. **Diabetic + combination therapy:** after induction of diabetes, rats received both Vildagliptin (10 mg/kg/day) and Omega-3 (100 mg/kg b.w.) administered orally for the remaining 8 weeks [17].

Sample collection: At the end of the treatment period, animals were euthanized following the approved protocol. Blood was collected via tail vein. Serum was separated for biochemical analyses and stored at -80°C. fresh.

Salivary gland tissue: both sublingual salivary glands of every rat were excised. One gland was immediately frozen in liquid nitrogen and stored at -80°C for molecular and biochemical analysis. The other gland was fixed in 10% buffered formalin for histopathological evaluation.

Biochemical Assays

- **Serum glucose and insulin levels:** According to the manufacturer's instructions, serum insulin levels were assessed using a rat insulin ELISA kit (Bio Vendor Laboratory Medicine, Brno, Czech Republic) and glucose levels were determined using the glucose oxidase method (Spinreact, Girona, Spain).
- **Oxidative Stress:** Superoxide dismutase (SOD) activity, glutathione (GSH) concentration, Nitric Oxide (NO), and malondialdehyde (MDA), a lipid peroxidation indicator, were all assessed by homogenizing frozen salivary gland tissue using bio-diagnostic colorimetric kits (Giza, Egypt) according to the manufacturer's protocol.
- **Inflammation:** salivary gland homogenates were subjected to an enzyme-linked immunosorbent test (ELISA) to evaluate the amounts of pro-inflammatory cytokines, including tumor necrosis factor (TNF), and Interleukins -1β (IL-1β) and 6 (IL-6) using commercially available kits (Spinreact, Spain) according to the manufacturer's protocol.

Histological Analysis: The formalin-fixed sublingual salivary glands were paraffin-embedded, and sectioned to be 5 µm thick. Sections were stained with hematoxylin and eosin (H&E) to evaluate tissue architecture, acinar and ductal changes; Masson trichrome Staining to detect fibrotic effects; and anticaspase-3 to detect apoptotic cells. Then a histologist, blinded to the experimental groups, performed the histological analysis.

Statistical Analysis: The statistical analysis was conducted with the use of SPSS V.24. The mean plus standard deviation (SD) was used to express the results. The study employed a one-way analysis of variance (ANOVA) and a suitable post-hoc test (Duncan's) to examine group differences. P-values less than 0.05 were regarded as statistically significant.

RESULTS

Glycemic control & Total Antioxidant Capacity: Both the Vildagliptin-only group and the Combination group showed significant reductions in the fasting blood glucose levels and significant elevations in the Serum Insulin levels compared to the diabetic (STZ) group indicating improvement in the glycemic control. Also, the treated groups (the Vildagliptin-only group and the Combination group) showed significant elevations in the serum levels of total antioxidant capacity (TAC) compared to the diabetic (STZ) group, with the combined therapy showing the most enhancement.

Table 1. Effects of Vildagliptin-Omega-3 Combination therapy on the Glycemic control and Total Antioxidant Capacity.

Study variables	**Serum Fasting blood glucose** (mg/dl)	**Serum Insulin** (µIU/ml)	**Serum total antioxidant capacity** (mM/dl)
Cont.	80.28 ± 3.57	5.51 ± 0.47	267.39 ± 8.43
D.M	324.18 ± 31.95*	3.19 ± 0.38 *	161.71 ± 14.33 *
D.M+ Vildag.	183.49 ± 14.14 $^{\#}$	4.71 ± 0.68$^{\#}$	241.81 ± 13.58$^{\#}$
D.M+ (Vildag.+ Omeg.)	169.41 ± 11.17 $^{\#@}$	5.24 ± 0.42 $^{\#@}$	254.29 ± 11.74$^{\#@}$

Cont.: control; D.M: Diabetes mellitus; Vildag.: Vildagliptin; Omeg.: Omega-3); Data are shown as mean ± SD and showed; *Significantly changed in comparison to control group at p <0.05; $^{\#}$Significantly changed in comparison to diabetic group at p <0.05; $^{@}$ Significantly changed in comparison to vildagliptin only group at p <0.05.

Assay of tissue levels of the oxidative status: The diabetic group showed significant elevations in the tissue levels of oxidative markers (MDA and NO) and significant decrease in the tissue levels of the antioxidant markers (GSH and SOD) compared to the control group, indicating a significant decrease in oxidative stability. The treated groups (the Vildagliptin-only group and the Combination group) revealed enhancements of the previous parameters, indicating significant improvement in antioxidative properties, with the combined therapy showing the most enhancement (Table 2).

Table 2. Effects of Vildagliptin-Omega-3 Combination therapy on the tissue levels of oxidative stress parameters

Study variables	**MDA** (nmol/mg)	**NO** (mg/g.tissue)	**GSH** (mg/g.tissue)	**SOD** (u/g. tissue)
Cont.	3.14± 0.22	13.81± 1.39	4.30± 0.24	259.48± 4.39
D.M	9.72± 1.87*	34.29± 4.62*	2.15± 0.97*	139.29± 8.23*
D.M+ Vildag.	5.09± 0.61$^{\#}$	19.01± 2.54$^{\#}$	3.61± 0.51$^{\#}$	197.74± 5.34$^{\#}$
D.M+ (Vildag.+ Omeg.)	4.18± 0.41$^{\#@}$	15.62± 1.80$^{\#@}$	4.1± 0.74$^{\#@}$	235.48± 7.24$^{\#@}$

Assay of inflammatory markers: The diabetic group showed significant elevations in the serum levels of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) compared to the control group, indicating significant increase in inflammatory properties. The treated groups (the Vildagliptin-only group and the Combination group) revealed enhancements of the previous parameters, indicating significant improvement in the inflammatory properties, with the combined therapy showing the most enhancement (Table 3).

Table 3. Effects of Vildagliptin-Omega-3 Combination therapy on the tissue levels of oxidative stress parameters

Study variables	**TNF-α** (pg/ml)	**IL-1β** (pg/ml)	**IL-6** (pg/ml)
Cont.	14.37 ± 2.08	39.19±4.29	61.17±3.46
D.M	181.26± 14.61*	142.18± 11.67*	129.39± 9.11*
D.M+ Vildag.	58.28± 7.39$^{\#}$	61.55± 6.08$^{\#}$	95.15± 3.22$^{\#}$
D.M+ (Vildag.+ Omeg.)	20.91± 3.67$^{\#@}$	48.31± 5.49$^{\#@}$	71.05± 4.81$^{\#@}$

Assay of Collagen & Caspase-3 expression: Increased markers of fibrosis (percentage of Collagen deposition) and apoptosis (Caspase-3 expression) were found in the sublingual salivary gland tissue of diabetic animals. The treated groups (the Vildagliptin-only group and the Combination group) have shown decreased markers of fibrosis (percentage of Collagen deposition) and apoptosis (Caspase-3 expression), indicating antifibrotic and anti-apoptotic effects with the combined therapy showing the most enhancement (Table 4).

Table 4. Effects of Vildagliptin-Omega-3 Combination therapy on the area percentage of Collagen & Caspase-3 expression:

Study variables	Percentage area of Collagen density (%)	Anti-Caspase-3 immunexpression (area %)
Cont.	0.74	0.18
D.M	4.36*	3.12*
D.M+ Vildag.	1.91#	0.71#
D.M+ (Vildag.+ Omeg.)	1.09#@	0.44#@

Structural changes: Histopathological analysis of the sublingual salivary gland tissue of diabetic rats revealed significant morphological abnormalities in the salivary glands, including acinar cell shrinkage in size and impaired epithelial structure. Vildagliptin treatment showed a preservation of the tissue of sublingual salivary gland compared to the diabetic group manifested by enhanced acinar shrinkage in size, and improved duct morphology. Combination group (Vildagliptin-Omega-3): The combined therapy showed the most substantial histological preservation, with glands more closely resembling those of the healthy control group.

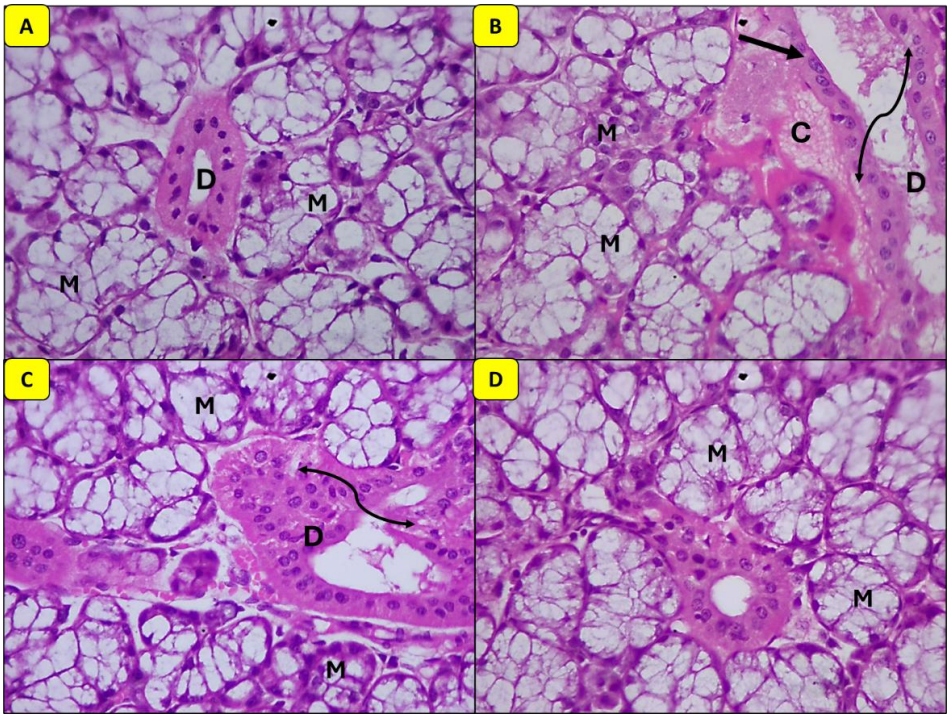


Figure 1: sections of sublingual salivary gland: **A**: control group specimen showed normal gland tissue in the form of large mucous acini (M) with pale basophilic spongy cytoplasm, flattened basal nuclei, and an interlobular duct (D) lined with cuboidal cells with central rounded nuclei and acidophilic cytoplasm. **B**: More degenerative alterations were seen in the diabetic group's specimens, particularly in the striated ducts (D). Congestion and dilatation of the capillaries surrounding the ducts (C); densely packed mucous acinar cells with deeply stained nuclei; and shrinkage in the ducts' cells with displacement of their nuclei (black arrow) and variable degree of vacuolation in their cytoplasm (curved double-sided arrow).**C**: After exposure to Vildagliptin, many mucous acini (M) appeared smaller, and ducts (D) appeared smaller with disorganized architecture and vacuolated cells (curved double-sided arrow). **D**: in Vildagliptin-Omega-3 treated group most of acini and ducts appeared intact (X400, Hx.&E.).

Fibrotic changes: There was an increase in collagen fiber deposition in the sublingual salivary gland tissue of diabetic animals compared to the control group, denoting fibrosis. Vildagliptin treatment has shown anti-fibrotic effects in the sublingual salivary gland tissue. The combination therapy demonstrated superior anti-fibrotic effects compared to the individual treatments.

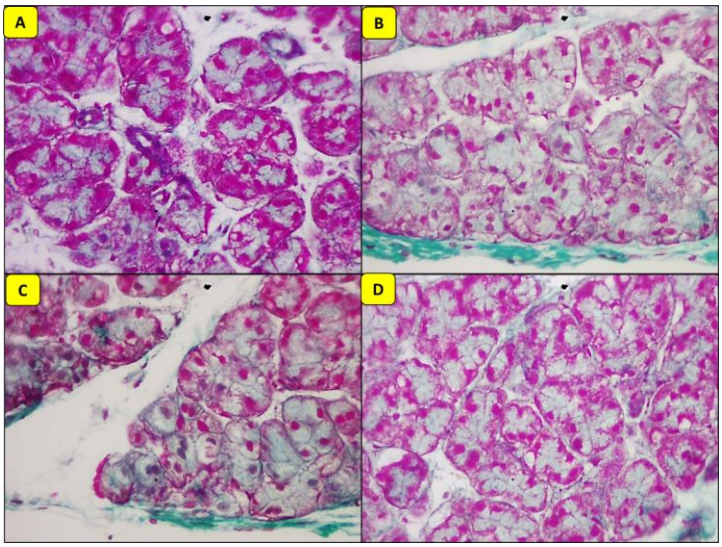


Figure 2: sections of sublingual salivary gland showing: Increased collagen fibers deposition in the sublingual salivary gland tissue of diabetic animals (B) compared to control group (A). Vildagliptin treatment has shown decreased collagen fibers deposition in the sublingual salivary gland tissue (C). The combination therapy demonstrated decreased collagen fibers deposition in the sublingual salivary gland tissue (D) compared to the individual treatments (X400, Masson trichrome).

Apoptotic changes: Increased markers of apoptosis (anticaspase-3 expression) were found in the sublingual salivary gland tissue of diabetic animals compared to control group. Vildagliptin treatment have shown anti-apoptotic effects in other tissues and are expected to reduce cell death in the salivary glands. The combination therapy demonstrated superior anti-apoptotic effects compared to the individual treatments in the form of decreased immunohistochemical expression of Caspase-3.

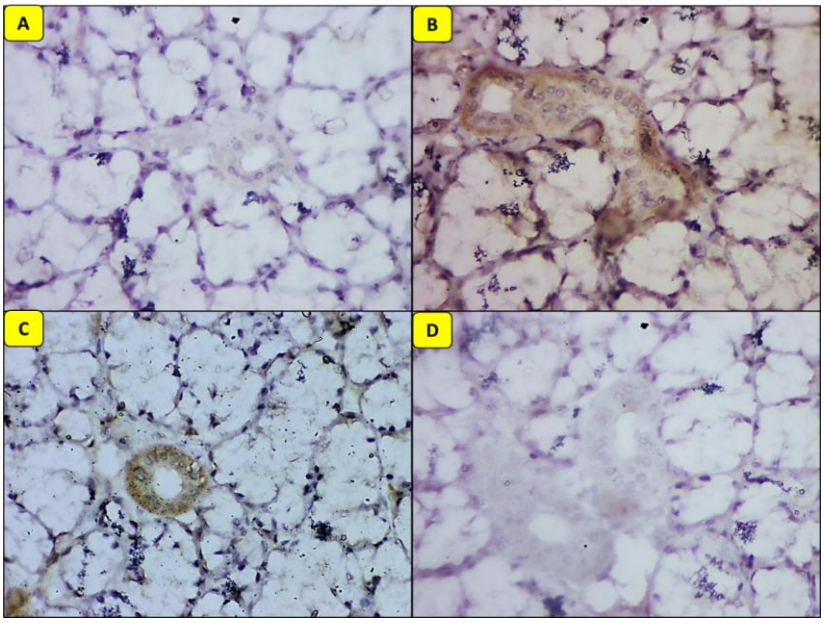


Figure 3: sections of sublingual salivary gland showing: Increased immunexpression of anticaspase-3 deposition in the sublingual salivary gland tissue of diabetic animals (B) compared to control group (A). Vildagliptin treatment has shown decreased immunexpression of anticaspase-3 deposition in the sublingual salivary gland tissue (C). The combination therapy demonstrated decreased immunexpression of anticaspase-3 deposition in the sublingual salivary gland tissue (D) compared to the individual treatments (X400, anticaspase-3 immune stain).

DISCUSSION

The present study aimed to investigate the therapeutic potential of a novel combination therapy, involving vildagliptin and omega-3 fatty acids, for protecting mucous salivary glands from diabetes-induced damage.

The glycemic data: The metabolic and glycemic data in this study indicated that the STZ-induced diabetic model was successfully established in the form of a significant increase in the serum blood glucose level and a fall in insulin levels compared to the control group, this was in agreements to previous studies [19, 22].

Structural changes: The induction of hyperglycaemia in the present study was accompanied by structural changes in the salivary glands as shown by histopathological analysis of the sublingual gland of diabetic rats revealed significant morphological abnormalities in the sublingual gland, including acinar cell atrophy, inflammation, fibrosis, and impaired epithelial structure. In agreements to our results, several studies demonstrated that the diabetic group's salivary gland parenchyma experiences gland atrophy, which is typified by degraded acini, a dilated duct system, and a duct-like structure with a preponderance of distinct fat cells and fibrous tissue compartments [6, 23].

Along similarity to our results, the development of diabetes was linked to changes in the morphology of the submandibular gland (one of the main salivary glands); as Fouani [24], which revealed that early-onset hyperglycemia led to structural abnormalities in the intralobular duct, shrinkage in the diabetic SMG, and vacuolization that verified their damage. This demonstrates that xerostomia is associated with D.M.

Mechanism of diabetic induced changes: Long-term hyperglycaemia, which causes metabolic problems that hinder glandular activity. Higher glucose levels result in the production of advanced glycation end products, which disrupt regular cellular processes and harm tissue [25].

Moreover, chronic hyperglycaemia, which triggers several processes that result in an excessive formation of reactive oxygen species (ROS), is the primary cause of diabetes complications. Diabetes is also linked to a decrease in total antioxidant capacity, which is caused by both increased ROS production and alteration of endogenous antioxidant enzyme activity. In aerobic organisms, events result in the development of chronic oxidative stress [26].

Inflammatory changes: In our study, the alterations in serum inflammatory markers were found in the diabetic group in comparison to the control group. The diabetes group had significantly higher blood levels of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6), suggesting a marked increase in inflammatory properties.

According to earlier research on the parotid and submandibular glands of diabetic rats, the levels of these markers were considerably higher ($p=0.0001$) in the diabetic group than in the control group, which is consistent with our findings [25].

Proinflammatory chemicals are released and intracellular signaling occurs as a result of advanced glycation end products (AGEs) interacting with their receptors. As a result, it is believed that DM patients have a higher inflammatory response and generate more cytokines linked to inflammation [27].

Oxidative stress: In this study, the diabetic group showed significant elevations in the tissue levels of oxidative markers (MDA and NO) and a significant decrease in the tissue levels of the antioxidant markers (GSH and SOD). Additionally, the diabetic group showed a significant decrease in the serum levels of total antioxidant capacity (TAC) compared to the control group, indicating a significant decrease in oxidative stability and **showing** how oxidative stress contributes to the development of diabetic-induced effects, (suggesting oxidative damage in this gland).

Our findings are consistent with other research on the parotid and submandibular glands of diabetic rats, which showed decreased reduced glutathione (GSH) concentration and glutathione peroxidase (GPx) activity, along with increased lipid peroxidation, protein oxidation, and SOD. [28-30].

The oxidation of polyunsaturated fatty acids by malondialdehyde, a consequence of lipid peroxidation, causes oxidative stress and the generation of very ROS, which are directly in charge of oxidative damage to cellular macromolecules [31]. The initial indication of oxidative damage via OS is thought to be lipid peroxidation since the cell membrane is exposed to free radicals before the other cellular components undergo oxidative modification [26].

A previous study indicated that increased production of ROS and a decrease in total antioxidant capacity are two mechanisms by which hyperglycemia induces oxidative stress. These alterations cause amino acid residues to oxidize, which in turn alters their structure and activity before ultimately causing the cells to lose their biological functions [32].

Apoptosis: Our research revealed that STZ-induced diabetes mellitus was linked to elevated salivary gland caspase-3, indicating that diabetes mellitus enhanced salivary gland apoptotic signaling.

This was consistent with earlier findings showing that hyperglycemia triggered apoptotic pathways. They linked this increase of caspase-3 to oxidative stress brought on by hyperglycaemia, which damages macromolecules and sets off apoptosis [33, 34].

Fibrosis: Our study revealed that there was increased collagen fiber deposition in the sublingual salivary gland tissue of diabetic animals compared to control group denoting fibrosis.

In agreement to our results, in a previous study showed a considerable increase in the area percentage of collagen fibers in the salivary glands of a diabetic group relative to the control group, confirming the presence of fibrotic alterations indicated by strong collagen deposition in diabetic group [35].

Chronic inflammation and tissue damage are frequently associated with fibrosis, and its rise in the untreated diabetic group highlights the severity of salivary gland changes [36].

From the above findings, D.M was found to have deterious effects on the sublingual salivary gland through various mechanisms; thus, treatment of D.M could ameliorate those pathological effects.

Vildagliptin, as a monotherapy: In this study, vildagliptin, as a monotherapy, effectively mitigated hyperglycemia and associated structural effects. The enhancement of the structural effects by vildagliptin could be explained by its antioxidative effects as shown in our results.

In agreement to our results, studies on vildagliptin and other DPP-4 inhibitors have consistently shown their ability to reduce oxidative stress and inflammation in different tissues of diabetic animal models [37-39].

Similarly, a previous study revealed that vvildagliptin may function as a radical scavenger in salivary glands, as evidenced by the significant decrease in mitochondrial ROS generation that resulted from vildagliptin medication [37].

In the present study, Vildagliptin treatment has shown to decrease immune expression of anticaspase-3 deposition which supports the antiapoptotic effects of vildagliptin on the sublingual salivary gland tissue compared to the diabetic group. The anti-inflammatory and antifibrotic effects of vildagliptin on the sublingual salivary gland tissue have also been demonstrated by the reduction of inflammatory markers and collagen deposition in the salivary gland tissues of treated diabetic rats when compared to the diabetes group.

Similar to our results, in other animal models of diabetes, DPP-4 inhibitors lower oxidative stress production, avoid mitochondrial dysfunction, enhance

endothelial function, and lessen oxidative stress and vascular inflammation [37, 40].

Vildagliptin in combination with omega 3: The most critical aspect of the current study is the comparison of monotherapy versus combination therapy. The combined therapy showed the most substantial histological preservation, with glands more closely resembling those of the healthy control group. The histopathological results further reinforce these findings. The severe acinar atrophy and inflammation seen in the untreated diabetic rats were significantly ameliorated in the combination therapy group, indicating that the biochemical protection translated into preserved tissue structure and function. This is a critical finding, as maintaining the morphological integrity of salivary glands is essential for preserving salivary secretion and overall oral health.

The improvement in the structural changes in the combination group could be related to reduced apoptosis due to mitochondrial protection via omega-3's, anti-apoptotic caspase-3 modulation.

The significant enhancement of the pathological diabetic changes in the sublingual salivary gland could be explained by the additive synergistic role of Omega-3, which aids in the body's detoxification and effectively combats free radicals. The tissues and cells are therefore shielded from oxidative stress and are able to perform at their best [41].

The beneficiary use of Omega-3 in this experimental study was in agreement with a previous clinical study on 84 healthy participants, where they found that giving an Omega-3 supplement for 14 weeks in a randomized, active-controlled interventional research study was found to improve their inflammatory biomarkers and sleep quality [42].

From the above findings, the results suggested that while both monotherapies offer some protective benefits, combination therapy provides a superior and synergistic effect. These findings hold significant implications for managing diabetic complications and represent a novel therapeutic strategy for treating salivary gland dysfunction. By elucidating the anti-oxidative and anti-apoptotic pathways through which the Vildagliptin-Omega-3 combination protects sublingual salivary glands, we provide the essential *in vivo* evidence required to transition toward clinical trials.

CONCLUSION

In conclusion, this study indicated that combination of vildagliptin and omega-3 provides a new and efficient treatment approach for shielding mucous salivary glands from hazardous effects induced by diabetes. The advantages of a multi-targeted strategy are demonstrated by the synergistic effects on inflammation, oxidative stress, and tissue morphology that have been reported.

Abbreviations: Analysis of variance (ANOVA); Diabetes mellitus (DM); Dipeptidyl peptidase-4 (DPP-4); docosahexaenoic acid (DHA); Eicosapentaenoic Acid (EPA); Glutathione (GSH); Interleukins (IL); Malondialdehyde (MDA); Nitric Oxide (NO); polyunsaturated fatty acids (PUFAs); Streptozotocin (STZ); Superoxide Dismutase (SOD); Total Antioxidant Capacity (TAC); Tumor Necrosis Factor (TNF); Standard Deviation (SD).

Competing Interests: We have no conflicts to declare.

Author Contributions: Conceptualization: A.G., L.A., O.R., R.T., M.A., O.A., A.A., A.D., A.E., A.M., M.D., N.M., F.A., I.A., M.E., N.O., A.A., and A.A. Methodology: A.G., L.A., O.R., R.T., M.A., O.A., A.A., A.D., A.E., A.M., M.D., N.M., F.A., I.A., M.E., N.O., A.A., A.A. Formal analysis: A.G., L.A., O.R., R.T., M.A., O.A., A.A., A.D., A.E., A.M., M.D., N.M., F.A., I.A., M.E., N.O., A.A., and A.A. data curation: A.G., L.A., O.R., R.T., M.A., O.A., A.A., A.D., A.E., A.M., M.D., N.M., F.A., I.A., M.E., N.O., A.A., and A.A writing original draft preparation: A.G., L.A., O.R., R.T., M.A., O.A., A.A.,

A.D., A.E., A.M., M.D., N.M., F.A., I.A., M.E., N.O., A.A., and A.A. Writing review and editing: A.G., L.A., O.R., R.T., M.A., O.A., A.A., A.D., A.E., A.M., M.D., N.M., F.A., I.A., M.E., N.O., A.A., A.A. visualization: A.G., L.A., O.R., R.T., M.A., O.A., A.A., A.D., A.E., A.M., M.D., N.M., F.A., I.A., M.E., N.O., A.A., and A.A. Supervision: A.G., L.A., O.R., project administration: O.R. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: AI was used only in generating the graphical abstract.

Funding: No funding.

Limitations of the study: While the STZ-induced diabetic rat model is well-established, it is not without limitations. Further studies are needed to precisely identify the molecular pathways underlying the observed synergistic effect. Furthermore, extrapolating animal findings directly to human applications requires caution. Variables such as species, sex, age, and individual responses can influence the outcome. Also, an Omega-3-only group was not included due to ethical and pharmacological considerations, the significant improvement seen in the combination group compared to the Vildagliptin-only group clearly demonstrates the added value of Omega-3 in this therapeutic context.

References

1. Huang X, Wu Y, Ni Y, Xu H, He Y. Global, regional, and national burden of type 2 diabetes mellitus caused by high BMI from 1990 to 2021, and forecasts to 2045: analysis from the global burden of disease study 2021. *Frontiers in Public Health*. 2025; 13:1515797. DOI: https://doi.org/10.3389/fpubh.2025.1515797.
2. Ramadan OI, Ali LS, Abobaker RM, Flifel HM, Abdelmonsef AS, Abdelhay OM, et al. Relation between Common Carotid Intima-Media Thickness and Molecular Markers in Patients with Newly Diagnosed versus Established Diabetes Mellitus: A Retrospective Study. *Med J Islam Repub Iran*. 2025;1939(23):84. DOI: https://doi.org/10.47176/mjiri.39.84
3. Fu D, Shu X, Zhou G, Ji M, Liao G, Zou L. Connection between oral health and chronic diseases. *MedComm*. 2025;6(1):e70052. DOI: https://doi.org/10.1002/mco2.70052
4. Ramadan O, Taha R, Awad M, Abdelhay O, Ereba R, Younes A, et al. Combination of empagliflozin and curcumin ameliorates the induced diabetic nephropathy in male albino rats, through their synergistic anti-diabetic and antioxidative effects. *Bioactive Compounds in Health and Disease*. 2025;8(3):90-103. DOI: https://doi.org/10.31989/bchd.v8i3.1558
5. Wang M-Y, Hu Z-R, Wang L, Zeng X-X, Li X-K, Fei G-J, et al. BuZhong YiQi Formula Alleviates Diabetes-Caused Hyposalivation by Activating Salivary Secretion Pathway in the Parotid and Submandibular Glands of Rats. *Pharmaceuticals*. 2025;18(3):377. DOI: https://doi.org/10.3390/ph18030377
6. Alqahtani MS, Hassan SS. Immunohistochemical evaluation of the pathological effects of diabetes mellitus on the major salivary glands of albino rats. *European Journal of Dentistry*. 2023;17(02):485-91. DOI: https://doi.org/10.1055/s-0042-1749159
7. Matsuoka M, Soria SA, Pires JR, Sant'Ana ACP, Freire M. Natural and induced immune responses in oral cavity and saliva. *BMC immunology*. 2025;26(1):34. DOI: https://doi.org/10.1186/s12865-025-00713-8
8. Chakrabarti SK, Chattopadhyay D. The Link Between Immune Aging and Type 2 Diabetes: A Review of Mechanisms and Implications. 2025. 2025;10(3):185-204 DOI: https://doi.org/10.14218/ERHM.2025.00018
9. Elsaeed MY, Mehanna OM, Abd-Allah E-EE, Hassan MG, Ahmed WMS, Moustafa AEGA, et al. Combination Therapy with Enalapril and Paricalcitol Ameliorates Streptozotocin Diabetes-Induced Testicular Dysfunction in Rats via Mitigation of Inflammation, Apoptosis, and Oxidative Stress. *Pathophysiology*. 2023;30(4):567-85. DOI: https://doi.org/10.3390/pathophysiology30040041
10. Awad MM, Younis AM. Empagliflozin alleviates diabetic nephropathy in albino rats through its antioxidative and anti-inflammatory action. *Jordan Journal of Applied Science-Natural Science Series*. 2023;17:33-7. DOI: https://doi.org/10.35192/jjoas-n.v17i1.1813
11. Ezzat SK, Anter HM, Habotta OA, Esmaeil DA, Farag DA, Aman RM. Poly lactic-co-glycolic acid enhances the efficacy of the phytomedicine chrysin against cisplatin induced toxicity in submandibular salivary glands. *Scientific Reports*. 2025;15(1):10262. DOI: https://doi.org/10.1038/s41598-025-93112-3

12. Parthasarathi V, Kanagarajan H. Dipeptidyl Peptidase-4–A Comprehensive Review. *Current Enzyme Inhibition*. 2025. DOI: https://doi.org/10.2174/0115734080377297250711143404
13. Sedik AA, Esmat N, Khalil WK, El-Mosallamy A. Unravelling the mechanism by which vildagliptin and linagliptin inhibit pyroptosis in lung injury through the NLRP3 inflammatory pathway in type 1 diabetic rats. *Scientific Reports*. 2025;15(1):20292. DOI: https://doi.org/10.1038/s41598-025-07204-1
14. Martirosyan D. Functional food science and bioactive compounds. *Bioactive Compounds in Health and Disease*. 2025;8(6):218-29. DOI: https://doi.org/10.31989/bchd.v8i6.1667
15. Hao Y, Sabihi Ss. Neurobiological Synergy of Plant and Animal Sources of Omega-3 and Exercise in Aging: Implications for Molecular Signaling, Memory, Spatial Learning, and Brain Function. *Food Science & Nutrition*. 2025;13(9):e70866. DOI: https://doi.org/10.1002/fsn3.70866
16. Shan F, Xiong Y, Pai P, Liu M. Systemic immune inflammation mediates the association of serum omega-3 and omega-6 polyunsaturated fatty acids with biological aging: a national population-based study. *Aging Clinical and Experimental Research*. 2025;37(1):1-15. DOI: https://doi.org/10.1007/s40520-025-02964-2
17. Ramadan O, Abuamara T, Taha R, Awad M, Mohammed M, Omar N, et al. Alleviation of the arsenic induced hepatotoxicity in rats by ginger or omega-3: a histological and biochemical study. *Bioactive Compounds in Health and Disease*. 2024;7(4):221-32. DOI: https://doi.org/10.31989/bchd.v7i4.1339
18. Zhang Z, Liu Y, Feng W, Mao P, Yang J, Zhao Z, et al. Omega-3 polyunsaturated fatty acids protect against cisplatin-induced nephrotoxicity by activating the Nrf2 signaling pathway. *International Journal of Biological Macromolecules*. 2024;282:137457. DOI: https://doi.org/10.1016/j.ijbiomac.2024.137457
19. Bauer BM, Bhattacharya S, Bloom-Saldana E, Irimia-Dominguez JM, Fueger PT. Dose-dependent progression of multiple low-dose streptozotocin-induced diabetes in mice. *Physiological genomics*. 2023;55(9):381-91. DOI: https://doi.org/10.1152/physiolgenomics.00032.2023
20. Alrefaei GI, Almohaimeed HM, Algaidi SA, Almuhayawi MS, AbdAllah FM, Al-Abbas NS, et al. Cinnamon and ginger extracts attenuate diabetes-induced inflammatory testicular injury in rats and modulating SIRT1 expression. *Journal of Men's Health*. 2023;19(8):22-32. DOI: https://doi.org/10.22514/jomh.2023.067
21. Ramadan OI, Damanhory AA, Ezz-eldin E, Gomah TA, Mahmoud ME, Abd-allah FM, et al. A novel therapeutic combination of vildagliptin and agomelatine alleviates the nephropathy in streptozocin-induced diabetic rats: A structural & biochemical study. *Journal of Research in Pharmacy*. 2024;28(4):1316-30. DOI: http://dx.doi.org/10.29228/jrp.811
22. Alfahed RON, Aljafen WK, Alduraibi MS, Rahmani AH. Assessment of the anti-diabetic potential of resveratrol in streptozotocin-induced diabetic animal models through modulation of oxidative stress and inflammation. *Functional Foods in Health and Disease*. 2026;16(4):315-30. DOI: https://doi.org/10.31989/ffhd.v16i4.1952
23. Salem ZA, Kamel AHM, AbuBakr N. Salivary exosomes as a new therapy to ameliorate diabetes mellitus and combat xerostomia and submandibular salivary glands dysfunction in diabetic rats. *Journal of Molecular Histology*. 2021;52(3):467-77. DOI: https://doi.org/10.1007/s10735-020-09935-z
24. Fouani M, Basset CA, Jurjus AR, Leone LG, Tomasello G, Leone A. Salivary gland proteins alterations in the diabetic milieu. *Journal of molecular histology*. 2021;52(5):893-904. DOI: https://doi.org/10.1007/s10735-021-09999-5
25. Naguib GH, Kayal RA, Abd El-Aziz GS, Alnowaiser A, Mazhar J, Mira A, et al. Innovative dual therapy: Magnesium oxide nanoparticles and royal jelly for parotid gland protection in diabetic male rats. *International Journal of Nanomedicine*. 2025:10301-24. DOI: https://doi.org/10.2147/IJN.S522526
26. Knaś M, Maciejczyk M, Daniszewska I, Klimiuk A, Matczuk J, Kołodziej U, et al. Oxidative damage to the salivary glands of rats with streptozotocin-induced diabetes-temporal study: oxidative stress and diabetic salivary glands. *Journal of diabetes research*. 2016;2016(1):4583742. DOI: https://doi.org/10.1155/2016/4583742
27. Al Shehhi YI, Elemam NM, Alsaegh MA. The response of salivary proinflammatory biomarkers to tooth extraction in individuals with type II diabetes mellitus. *BMC Oral Health*. 2024;24(1):250. DOI: https://doi.org/10.1186/s12903-024-04006-1
28. Gomes MA, Manzano C, Alves TM, Fiais GA, Freitas RN, Mattera MSdLC, et al. Assessment of redox state and biochemical parameters of salivary glands in streptozotocin-induced diabetic male rats treated with mate tea (Ilex paraguariensis). *Archives of Oral Biology*. 2022;143:105551. DOI: https://doi.org/10.1016/j.archoralbio.2022.105551

29. Ghada E-G, Hamad L, Ayad M, Shredah M. Parotid and Sublingual Salivary gland as an Indicator of Antioxidant Efficacy in Experimentally Induced Diabetic Rats. *Khalij-Libya Journal of Dental and Medical Research*. 2024:297-305. DOI: https://doi.org/10.47705/kjdmr.248221
30. Almohaimeed HM, Albadawi EA, Mohammedsaleh ZM, Alghabban HM, Seleem HS, Ramadan OI, et al. Brain-derived Neurotropic factor (BDNF) mediates the protective effect of Cucurbita pepo L. on salivary glands of rats exposed to chronic stress evident by structural, biochemical and molecular study. *Journal of Applied Oral Science*. 2021;29:e20201080. DOI: https://doi.org/10.1590/1678-7757-2020-1080
31. Poljšak B, Jamnik P, Milisav I. The importance of multifaceted approach for accurate and comprehensive evaluation of oxidative stress status in biological systems. *Antioxidants*. 2025;14(9):1083. DOI: https://doi.org/10.3390/antiox14091083
32. Taha M, Mahmoud ME, Al-Kushi AG, Sarhan A, Abdelbagi O, Baokbah TA, et al. Anxiolytic and antidepressant like effects of Zamzam water in STZ-induced diabetic rats, targeting oxidative stress, neuroinflammation, BDNF/ERK/CREP pathway with modulation of hypothalamo-pituitary–adrenal axis. *Frontiers in Neuroscience*. 2023; 17:1265134. DOI: https://doi.org/10.3389/fnins.2023.1265134
33. Hussein SM. Effect of Aloe vera gel on the parotid glands of streptozotocin-induced diabetic albino rats: histological and immunohistochemical study. *Erbil Dental Journal (EDJ)*. 2022;5(1):67-75. DOI: https://doi.org/10.15218/edj.2022.9
34. Fikry H, Saleh LA, Mohammed OA, Doghish AS, Elsakka EG, Hashish AA, et al. Agmatine alleviates diabetic-induced hyposalivation in rats: A histological and biochemical study. *Life Sciences*. 2024; 359:123220. DOI: https://doi.org/10.1016/j.lfs.2024.123220
35. Boughdady WAE-AA, El-Shafei AA, Sabry MM. Possible Reparative Effect of Stromal Vascular Fraction on Parotid Gland Injury Following Streptozotocin Induced Diabetes Mellitus. Histological Study in Adult Male Rat Model. *Egyptian Journal of Histology*. 2025;48(3). DOI: https://doi.org/10.21608/ejh.2024.292854.2074
36. Tuleta I, Frangogiannis NG. Diabetic fibrosis. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*. 2021;1867(4):166044. DOI: https://doi.org/10.1016/j.bbadis.2020.166044
37. Ittichaicharoen J, Apaijai N, Tanajak P, Sa-Nguanmoo P, Chattipakorn N, Chattipakorn S. Dipeptidyl peptidase-4 inhibitor enhances restoration of salivary glands impaired by obese-insulin resistance. *Archives of Oral Biology*. 2018;85:148-53. DOI: https://doi.org/10.1016/j.archoralbio.2017.10.015
38. Kang WS, Jung WK, Park S-B, Kim HR, Kim J. Gemigliptin suppresses salivary dysfunction in streptozotocin-induced diabetic rats. *Biomedicine & Pharmacotherapy*. 2021; 137:111297. DOI: https://doi.org/10.1016/j.biopha.2021.111297
39. Mizukami H, Inaba W, Takahashi K, Kamata K, Tsuboi K, Yagihashi S. The effects of dipeptidyl-peptidase-IV inhibitor, vildagliptin, on the exocrine pancreas in spontaneously diabetic Goto-Kakizaki rats. *Pancreas*. 2013;42(5):786-94. DOI: https://doi.org/10.1097/MPA.0b013e318287c9b5
40. Kim HR, Jung WK, Kim J. Effect of glucagon-like peptide 1 on salivary gland hypofunction in diabetic db/db mice. *Journal of Biomedical and Translational Research*. 2023;24(4):139-50. DOI: https://doi.org/10.12729/jbtr.2023.24.4.139
41. Heshmati J, Morvaridzadeh M, Maroufizadeh S, Akbari A, Yavari M, Amirinejad A, et al. Omega-3 fatty acids supplementation and oxidative stress parameters: A systematic review and meta-analysis of clinical trials. *Pharmacological research*. 2019; 149:104462. DOI: https://doi.org/10.1016/j.phrs.2019.104462
42. Currie C, Myklebust TÅ, Bjerknes C, Framroze B. Utilising the omega-3 index to assess the relative bioimpact of a whole. *Functional Foods in Health and Disease*. 2026;16(3):247-67. DOI: https://doi.org/10.31989/ffhd.v16i3.1947