The Possible Modulatory Effect of Vitamin E Administration on Submandibular Salivary Gland of Albino Rats Receiving Fat Rich Diet: A Histological and Ultrastructural Study

Original Article

Mona B. Morsy[1], Marwa M. Abd Elhameed[2,3] and Iman Fathy[3]

[1]*Department of Oral Biology, Field of Dentistry, Dentistry Program, Galala University, Egypt*

[2]*Department of Oral Biology, Faculty of Oral and Dental Medicine, Egyptian Russian University*

[3]*Department of Oral Biology, Faculty of Dentistry, Ain Shams University*

ABSTRACT

Introduction: The impact of a fat-rich diet on oral health could be deteriorative. A well-known fat-soluble antioxidant is Vitamin E. Few research has investigated its impact in preserving oral and para-oral tissues.

Objective: This study was established to determine how vitamin E intake affected the histological modulation of fatty degeneration in the submandibular salivary gland in Albino rats.

Materials and Methods: Three groups were created out of 24 male albino rats: The control group, the Fat Rich Diet (FRD) group and the Vitamin E (Vit. E) group. Twelve weeks were spent conducting the experiment. Subsequently, Submandibular salivary gland samples were prepared for light and electron microscopic analysis. Morphometric data were collected for statistical analysis regarding the acini's diameter, excretory's lumen, and striated ducts.

Results: Regular vitamin E administration with the selected dosage in this study had a partial protective effect. Signs of degeneration, increased percent of area fraction of vacuolation, and decreased diameter of the acini were noticed in the FRD group. The Vit. E group experienced a reduction in those findings. There was no statistically discernible difference between the Vit. E group and the Control group regarding these parameters, but marked stricture of the striated duct lumen was noticed in Vit. E group, although that stricture was not noticed in the excretory duct lumen.

Conclusion: It appears that vitamin E in the examined dose (as antioxidants) ameliorate the Submandibular salivary gland but is not completely protective. A fat-rich diet is considered a regular lifestyle nowadays. This study presents hopeful prophylactic advice as administering Vitamin E can reduce the side effects of this addictive prevalent dietary habit.

Received: 26 January 2023, **Accepted:** 07 May 2023

Key Words: Antioxidant; degeneration; eating behavior; tocopherol.

Corresponding Author: Mona Bahaa Morsy, MSc, Department of Oral Biology, Field of Dentistry, Dentistry Program, Galala University, Egypt, **Tel.**: +20 12 2647 0068, **E-mail:** monarchmonabahaa@gmail.com

ISSN: 1110-0559, Vol. 47, No. 2

INTRODUCTION

A sedentary lifestyle and a fat-rich diet are widely affecting our lives, so individuals are highly subjected to metabolic diseases[1,2].

As reported, lipids have been linked to the development of insulin resistance and peripheral tissue dysfunction[3,4]. An inflammatory immune reaction that causes swelling, inflammation, and severe pain in the mouth, even when speaking and eating, could be triggered by fat accumulation[3].

It was stated that the histological Changes affecting the salivary gland might increase the incidence of oral diseases[5,6]. The salivary glands in humans are either major or minor glands that secrete the digestive saliva that aids in taste, speaking, and mastication, as well as hydrating the mouth. The parotid, submandibular (SMG), and sublingual glands are three pairs that constitute the major salivary glands[3,7].

Corn and sunflower oils have recently gained popularity. These oils contain large amounts of linoleic acid, a crucial polyunsaturated fatty acid (PUFA), which makes them susceptible to lipid peroxidation, and possible risk factors for free radical sensitivity even though they decrease cholesterol synthesis and levels[8,9].

It was established in a prior work that the fat-soluble vitamin D protected against lipid peroxidation[10]. An antioxidant that is fat-soluble but not enzymatic is vitamin E[11].

 DOI: 10.21608/ejh.2023.188090.1844

There has been numerous medical research on vitamin E, but more needs to be addressed on how vitamin E affects the submandibular salivary gland when combined with a diet high in fat. In this investigation, we sought to test the hypothesis that, in rats, fat-rich diet intake could be modulated by vitamin E, a well-known anti-inflammatory and antioxidant. Our study also sought to assess the possible deterioration of SMG.

MATERIALS AND METHODS

Drugs

Capsules of vitamin E ®1000mg (purchased from Pharco pharmaceuticals, Alexandria, Egypt) were cut and emptied into separate clean containers. According to a previous study designed by Magdy *et al*., (2016) [12], Vitamin E (50 mg/kg body weight) was dissolved in Sesame oil to prepare a suspension containing 67 mg of vitamin E in 1 mL. The proper vitamin dosage was given orally to the rats once daily, based on their body weight[13].

Experimental Animals

The ethics committee at Ain Shams University Faculty of Dentistry (FDASU-REC) approved the experimental work with authorisation number (FDASU-Rec IM122102) and offered ethical principles for the investigation. The sample size was determined using the same experimental approach as a prior study by El Serwi *et al*., (2021)[10].

Twenty-four adult male albino rats six to eight weeks of age in the Medical Centre for Research, Faculty of Medicine, Ain Shams University weighing between 150 and 200 grams were kept in cages made of metal mesh with a maximum number of five per cage, under a controlled temperature and dark-light cycle following the institution requirements of Ethical Committee, Faculty of Dentistry, Ain Shams University. (FDASU-REC).

Experimental design

Eight rats each ascribed to one of the following groups: Control group, fat-rich diet (FRD) group and Vit. E group. A typical, nutritious diet was used to maintain the Control group. To cause fatty, dysfunctional salivary glands, the FRD group was kept on a diet that included corn oil with polyunsaturated fatty acids (PUFA). The diet contained 21.4% fat, 17.5% protein, 50% carbohydrates, and 3.5% fibre. The required percentages were measured using a graded beaker; the used materials were all ground to resemble a paste. For each 50 ml of the carbohydrate used: 21.4 oil was added, 17.5 proteinous material was added, and 3.5 fibres were added. The Vit. E group consumed a fat-rich diet with the same percentage as the FRD group; in addition to Vitamin E, vitamin E dissolved in sesame oil was given to the rats early in the morning before giving them the fat-rich diet. The appropriate dose of a fat-rich diet was calculated according to body weight with five gm.\100-gram body weight\ per day, and after finished, they were given access to a regular meal. El Serwi *et al*., (2021)[10] stated in a previous study that the meals and treatments were continued for 12 weeks.

A limitation we faced at the beginning of the study is that when administering the required daily dosage of oil to the rats by oral gavage, the rats showed a high mortality rate. Accordingly, the required dose was added to their diet according to the percentage mentioned above until the daily dosage was finished then a normal diet was given to them for the rest of the day[14].

Preparation of Histopathological sample

The experimental animal investigation ended with the anaesthetic overdose given to the animals. The submandibular salivary gland was then meticulously sectioned into two sections for use in the histopathological microscopy and transmission electron microscopy (TEM) analyses that would follow. Salivary gland samples were preserved for 12 hours at room temperature in 10% neutral buffered formalin. Following fixation, specimens were dehydrated in a series of progressively stronger alcohols, washed in two xylene changes and then embedded in molten paraffin. At the Faculty of Medicine, Ain Shams University, the pathology department, a rotary microtome was used to cut slices of a 5-m thickness. Placed on clean slides. Salivary slices were stained with hematoxylin and eosin H&E for histological analysis[15,16].

Electron microscopic study

Glutaraldehyde 3% was used to fix the samples for two hours in 0.1 M sodium cacodylate buffer (pH 7.0), washed in the same buffer, and then 1% osmium tetroxide was used for post-fixation of the samples for two hours at room temperature. Ethanol dilution, ranging from 10% to 90%, and absolute ethanol were used for the dehydration of the samples. Finally, the samples were immersed in pure resin through a graded series of injections of epoxy resin and acetone. Copper grids were used to gather extremely thin pieces. After that, sections were thrice stained with lead citrate and uranyl acetate. Stained sections were seen under TEM equipped with a JEOL JEM 1010 (70 kV voltage) at the Regional Center For Mycology And Biotechnology(RCMB), Al-Azhar University[17,18].

Digital analysis and morphometric analysis

The morphometric study for H&E results was performed using a computed image analysis system (Leica Quin software 500, Germany) in the Faculty of Dental Medicine for Girls, Oral and Dental Pathology Department, Al-Azhar University. a gadget linked to the microscope that includes a colour video camera, a colour monitor, and a hard drive from HP personal computer. The image analyser was automatically calibrated to translate pixels, the image analyser application's measuring units, into precise micrometre measurements to assess the percent of area fraction of vacuolation and the diameter of acini and ductal lumen for the striated and excretory ducts[19,20].

For H&E digital analysis, the recorded Measurements were exported as an Excel file.

STATISTICS

Statistical software for Social Science (SPSS 15.0 for Windows; SPSS Inc., Chicago) was used to revise, code, tabulate, and input the obtained data. One-way ANOVA was performed, and the groups' means ± SD were compared using a one-way ANOVA post hoc multiple comparison test. The *p-value* threshold for each analysis was set at 0.05, with a 95% confidence range.

RESULTS

Results from light microscopy

The serous acini, connective tissue stroma, duct system, and granular convoluted tubules had normal histological architecture in the control group (Figures 1 A,B). In line with expectations, the FRD group showed a reduction in the diameter of the acini along with the absence of the typical spherical outline, darkly stained nuclei, and Cytoplasmic vacuolisation was also visible in the cellular component of nearly all the sections (Figure 1C). The striated ductal lumen showed marked protrusions, microbuds and irregularities of the luminal membrane, degeneration was obvious in some areas of the cells lining the lumen wall (Figure 1D). In addition, the excretory duct cells showed signs of degeneration, epithelial hyperplasia, and desquamation into the ductal lumen, but there were no apparent irregularities of the luminal membrane.

There were areas of fibrosis and hyalinisation surrounding the excretory ducts of the FRD group (Figure 2E). These signs were apparent in some sections of the Vit. E group but to a lesser extent, and they were not apparent in other sections.

In Vit. E group, acinar architecture and diameter were restored. Deeply stained nuclei were also seen, with varying degrees of minor cytoplasmic vacuolisation. The excretory ductal cells were significantly recovered, and the acinar architecture was significantly improved, although the microbuds and irregularities of the luminal wall of the striated ducts was still found in this group. Additionally, there was no evidence of ductal lumen cellular lining degeneration (Figures 2 F,G).

Results from electron microscopy

Serous acinar cells with a vesicular nucleus and secretory granules with different electron densities were seen in the control group and had a consistent morphology (Figures 3 A,B). We noticed substantial cell organelle degeneration and ultrastructural alterations in the FRD group, swollen mitochondria, lipid droplet deposition, marked vacuolisation, dilated strands of rough endoplasmic reticulum and pyknotic nuclei with irregular outline. Secretory granules looked to merge with one another (Figures 4 C,C*). The striated duct in this group showed irregular lumen, loss of basal enfoldings, and swollen mitochondria and (Figures 5 D,E). Serous acinar cells with vesicular nuclei and immature bright, dense serous granules were present in Vit. E group, this was evident in some cells, while other cells showed secretory granules that appeared fused to each other. The striated duct showed vesicular nuclei and basal enfoldings with parallel mitochondria radiating in between them (Figures 6 F,G).

Statistical results

Signs of degeneration, increased area fraction of vacuolation and decreased diameter of the acini were noticed in FRD. There was a statistically significant difference (SSD) in the area fraction of vacuolisation and diameter of the acini between the FRD and the negative control group. In contrast, those parameters showed improvement in the Vit. E group and there was no significant difference between the Vit. E group and the control group (Table 1, Bar Charts 1,2).

On the other hand, there was marked narrowing of the lumen of the striated ducts of both the FRD group and the Vit. E group with statistically significant difference between these two groups in relation to the control group, although that stricture was not noticed in the lumen of the excretory duct (Table 1, Bar Charts 3;4).

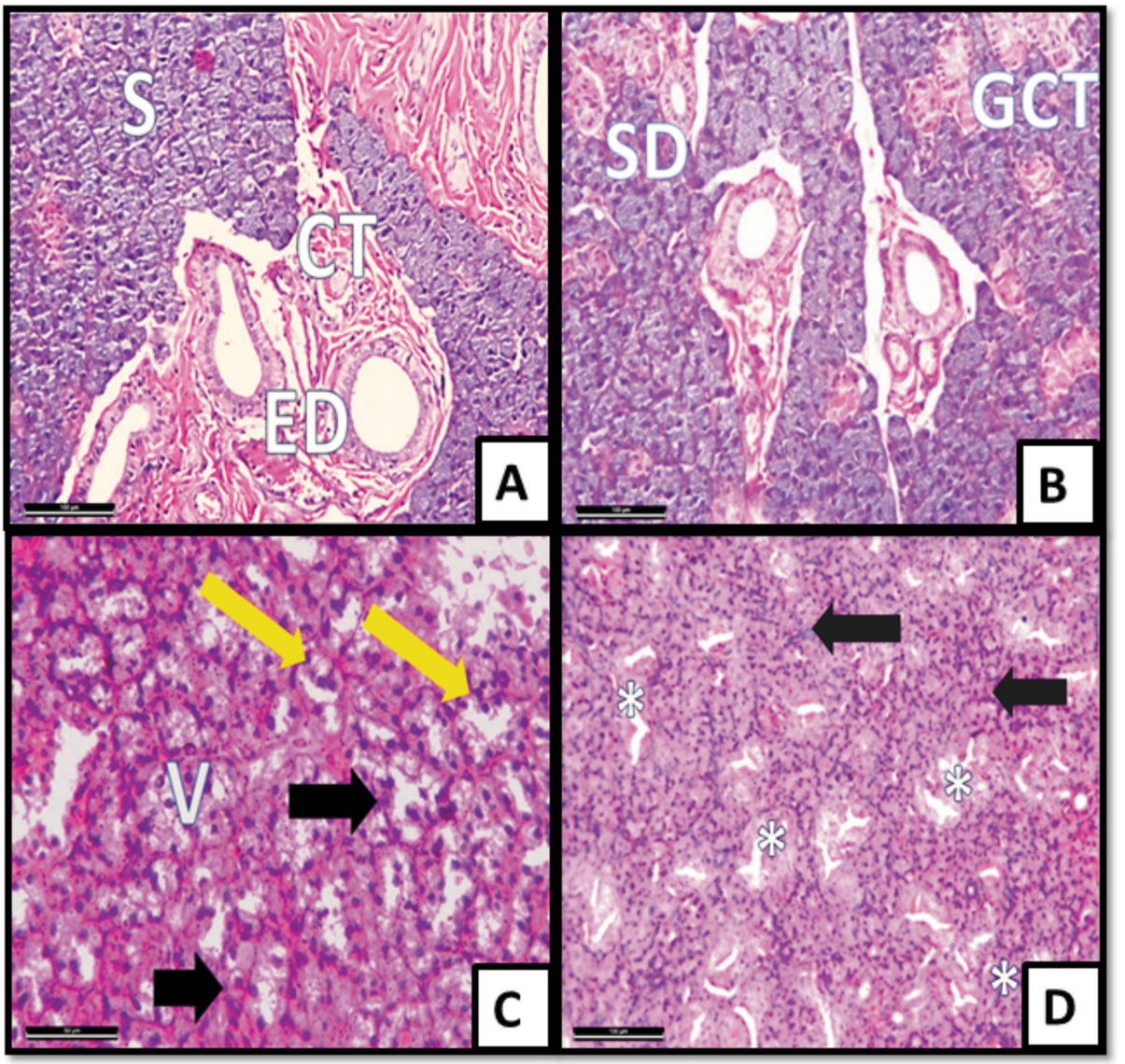


Fig. 1: A histological picture of the H&E-stained SMG: (Panel A): The control group shows serous acini (S) with basal spherical nuclei. The intralobular excretory duct (ED) is clearly encircled by connective tissue stroma. (H&E, Org.mag×200). (Panel B): the striated duct (SD) is surrounded by thin inter-acinar connective tissue (CT). Granular convoluted tubules (GCTs) show an acidophilic cytoplasm. (H&E, Org.mag×200). (Panel C): FRD group exhibits vacuolated acini (V), loss of the typical circular outlines (black arrow), pyknotic darkly stained nuclei (yellow arrow). (H&E, Org.mag×400). (Panel D): Note the considerable stricture of the duct system with apparent signs of cell lining degeneration (*) and infiltration of inflammatory cells (black arrow). (H&E, Org. mag×200).

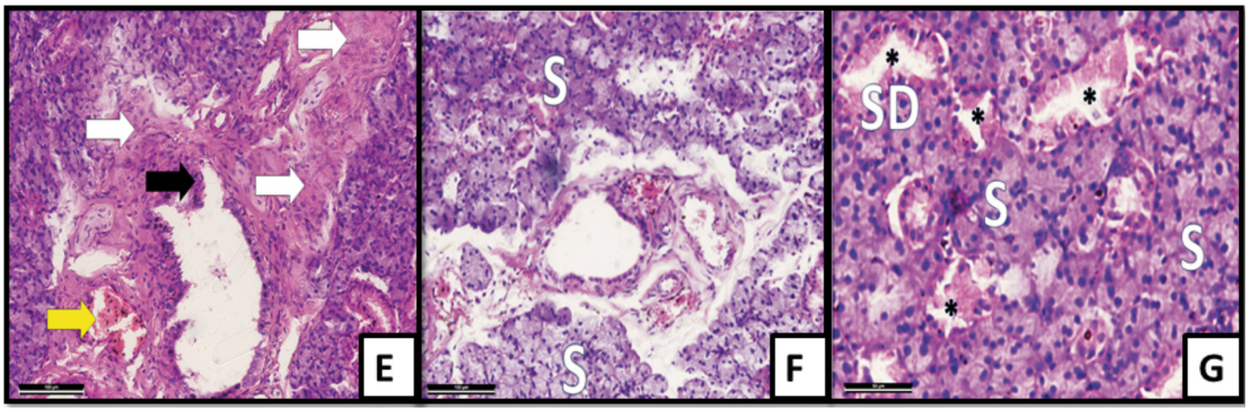


Fig. 2: A histological picture of the H&E-stained SMG: (Panel E): FRD group showing fibrosis and hyaline degeneration (white arrow) surrounding excretory duct with defective cell lining, apparent disruption, loss of pseudostratification (black arrow) and extravasated BV (yellow arrow). (H&E, Org.mag×400). (Panel F): Vit. E group demonstrates restored acinar architecture with obviously stained nuclei (S). (H&E, Org.mag×200). (Panel G): Note that the striated duct (SD)shows stricture of its lumen with less evidence of cell lining degeneration. (H&E, Org.mag×400).

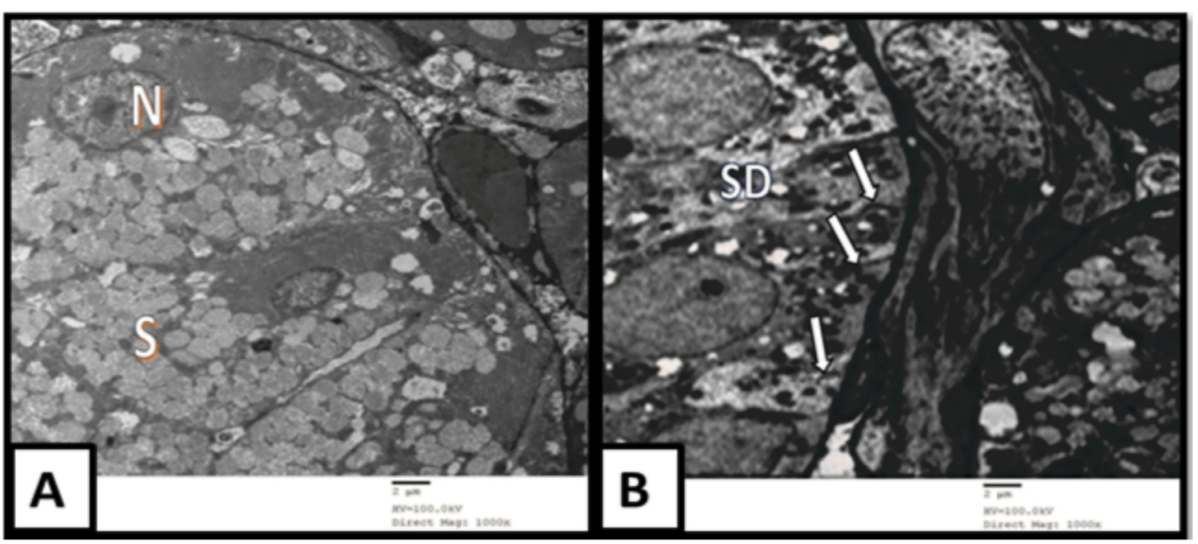


Fig. 3: Electron micrograph of SMG serous acini: (Panel A): The Control group displays cells of serous acini with a normal shape, vesicular nucleus (N), and secretory granules with different electron densities (S). (x1000). (Panel B): Note that the striated duct (SD)shows basal enfoldings and parallel radiating mitochondria in-between(arrows). (x1000).

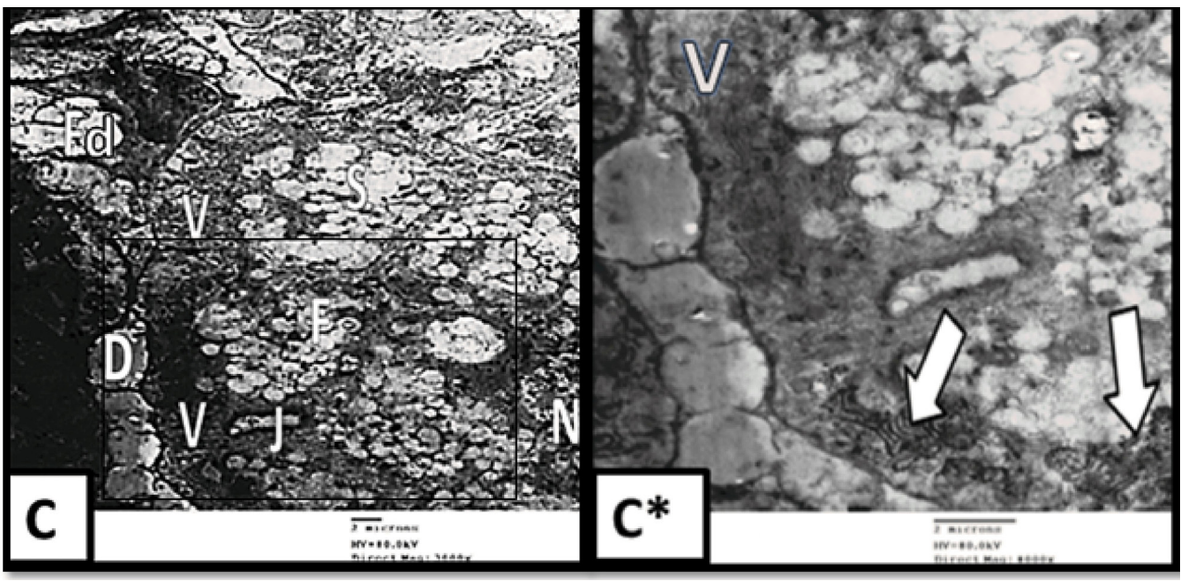


Fig. 4: Electron micrograph SMG serous acini (Panel C): FRD group displays cells of the serous acini with apical secretory granule(S) fused with each other (F)and pyknotic nucleus(N), fat droplets are obvious (Fd), detachment of basement membrane(D), defective intercellular junction(J)and multiple vacuoles (V). (x3000). (Panel C*): Magnification for the inset in (Panel C) showing dilated rER (white arrows) and cytoplasmic vacuolisation (V). (x8000).

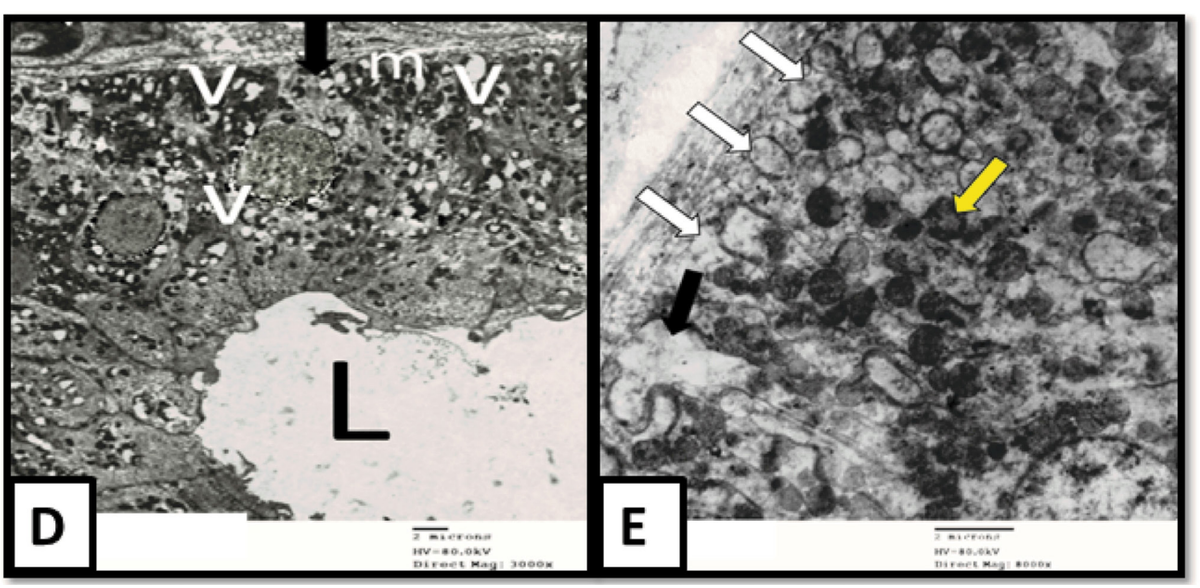


Fig. 5: (Panel D): FRD group showing striated duct (SD) cells with marked vacuolization (V), loss of basal striations (black arrow), basal mitochondria(m), and irregular lumen wall with micro buds (L). (x3000). (Panel E): Magnification for the basal part of SD cell showing swollen mitochondria (yellow arrow), vacuolization (black arrow), loss of basal striation (white arrow). (x8000).

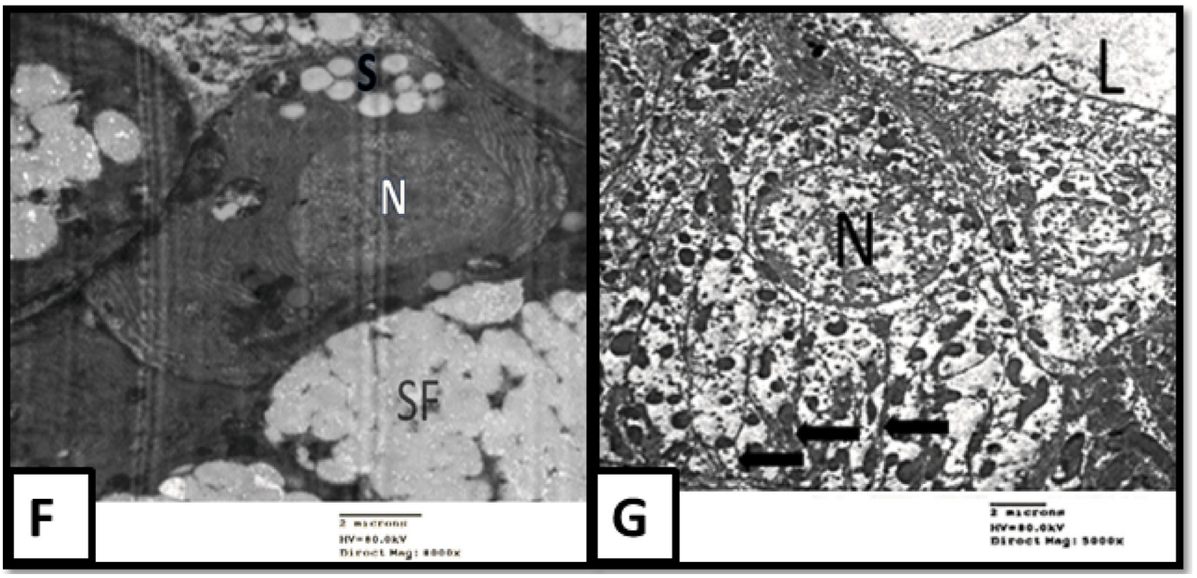
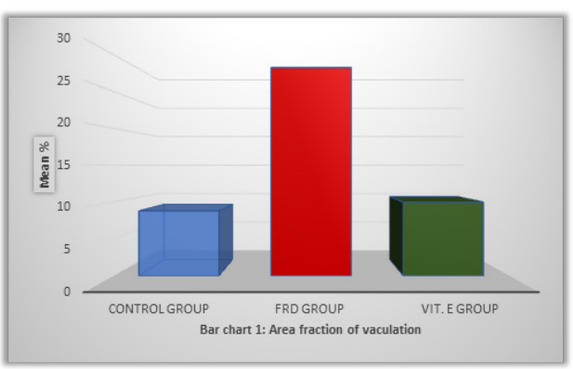


Fig. 6: Electron micrograph of SMG serous acini: (Panel F): Vit. E group showing serous acinar cells, vesicular nuclei (N), and certain areas show apical immature light, dense serous granules (S), other areas show fused secretory granules (SF). (x8000). (Panel G): SD cell showing vesicular nucleus (N), basal enfoldings (arrows) with parallel radiating mitochondria in -between and irregular lumen outline (L). (x 5000).

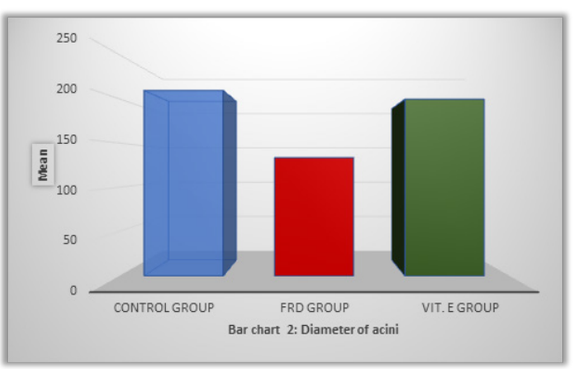
Table 1: Area fraction of vacuolation, diameter of acini, diameter of lumen of striated and excretory ducts in all groups (H&E)

	Control gp	FRD gp	Vit. E gp
Area fraction of vacuolation%	8.8 ± 1.9	28.2 ± 4.3*	10.0 ± 2.8
Diameter of acini	209.0 ± 65.0	133.4± 25.7*	199.3 ± 27.2
Diameter of striated duct lumen	171.5 ± 96.2	75.9 ± 55.5*	84.6 ± 53.9*
Diameter of excretory duct lumen	520.8± 510.6	797.6± 496.1	585.4 ± 238.3

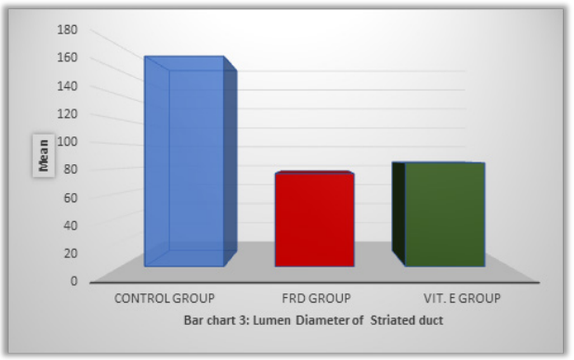
Values are displayed as means with standard deviations ± SD
*: statistically significant when compared to the equivalent value in the control group (P<0.05)



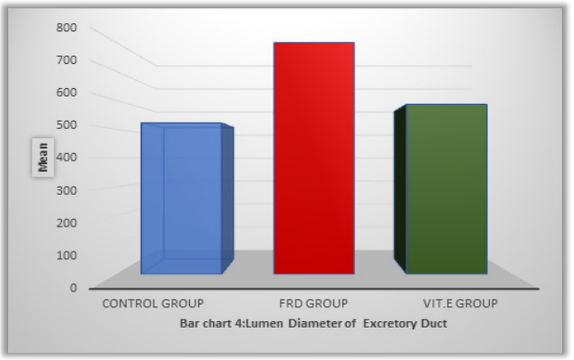
Bar chart 1: Representing comparison between groups regarding area fraction of vacuolation



Bar chart 2: Representing comparison between groups regarding diameter of acini



Bar chart 3: Representing comparison between groups regarding lumen diameter of Striated duct



Bar chart 4: Representing comparison between groups regarding lumen diameter of Excretory duct

DISCUSSION

The SMG was selected for the current investigation because in humans, it is the second-largest of the three major salivary glands and because it generates the majority of saliva (about 70%) when not stimulated; subsequently, its functional impairment could contribute largely to the development of xerostomia and this condition leads to oral health impairment such as dental caries, candidiasis, and mucosal complications[21].

Male rats were chosen over female rats to avoid any possible modulatory effect of estrogen on the effect of a fat-rich diet and lipid accumulation[22].

In the current study, the FRD group showed vacuolisation, areas of degeneration, and atrophy of the acinar element.

FRD Group showed excessive vacuolisation, degeneration, and atrophy of the acinar element. Some areas showed hyaline degeneration, and the duct system showed stagnant secretion, cellular element degeneration, and normal architecture loss. These degenerative signs are consistence with Al-Serwi etal. (2021)[10], who stated that degenerative signs were obvious in serous acinar cells due to chronic exposure to fat rich diet.

It is worth mentioning that these degenerative signs are also consistent with a study conducted by Kandeel *et al*.,(2022)[23] who stated that the high-fat diet group showed significantly disturbed architecture, inflammatory cellular infiltrations, acinar cells with cytoplasmic vacuoles, darkly stained nuclei beside vacuolated ducts. They also reported decreased proliferating cell nuclear antigen reaction (PCNA), decreased beta-cell lymphoma 2(Bcl-2), and lost E-cadherin expression in duct cells.

A remarkable finding that was observed in our study was the increased density of protrusions and micro buds of the luminal membrane of FRD Group and Vit. E Group; it was mainly noticed among the SD and GCT and absent for the ED. The luminal wall showed irregularity and pointed edges, causing a narrowing of the lumen. These findings are consistence with what Roa *et al*. (2018)[24] reported in their study of the fatty diet effect on SMG to find the increase in the density of micro folds and protrusions throughout the luminal membranes. These findings could be suggested to be a stricture in the duct lumen.

Furthermore, fibrosis and hyalinization with signs of chronic inflammation were also present in the CT stroma surrounding the ED, which revealed loss of Pseudostratified appearance in the FRD Group, wide lumen, degenerated epithelial lining, and stagnation of secretion. In the FRD Group in the current study, the CT surrounding the EDs also revealed areas of dilated and congested BVs. These findings are consistence with histopathologic features described by Marcus *et al*., (2021)[25] regarding ductal stenosis.

Long-term inflammatory alterations in the ductal system and parenchyma frequently accompany stenoses in salivary glands. The result is a decrease in salivary flow, an ascending infection of the duct, and the development of mucous or fibrinous plaques, strictures, or stenoses[26].

A novel classification for ductal stenosis was published by Koch *et al*. (2017)[26] according to various criteria, including the location of the stenosis in the duct system, whether it is distal, proximal or intraparenchymal; in addition to location, length of the stenotic area, grade of luminal narrowing and tissue quality within the stenotic area. The classification system ranges from inflammatory (type I) to purely fibrotic (type III).

It is worth mentioning that the signs of fibrosis, hyalinization, and chronic inflammatory cells noticed in the FRD group were less obvious in Vit. E group, they were apparent to a lesser extent in some sections and not in others.

Fibrosis, hyalinization, and chronic inflammatory cells were observed in the connective tissue stroma of the FRD group. These signs were apparent in some sections of Vit. E group, but to a lesser extent.

It was addressed in previous studies on pancreatic tissue that chronic fatty diet administration aid in the transition of fatty degenerative signs to fibrosis[27].The

pancreas and the salivary glands share histological, functional, physiological, and anatomical similarities, and their progenitor is very similar, including their role in repair and regeneration[28]. This suggests that the fibrosis and hyalinization viewed in the light microscopic results of our study originated in response to fatty degeneration that may have occurred as a temporary stage.

Regeneration and fibrosis are both significantly triggered by tissue injury and inflammation. Through the recruitment and activation of numerous different cells of innate and adaptive immune systems, tissue injury affects the kind and polarity of inflammation[29].

High morbidity and mortality rates can result from fibrotic remodeling in numerous organs, affecting organ function. Adaptive and innate immunity both play a role in fibrogenesis. According to Zhang *et al*. (2020)[30], T-cell subsets exhibit profibrotic and anti-fibrotic activities that are tissue- and disease-specific.

The lipid droplets detected within the glandular element of our study are consistence with Matczuk *et al*. (2016)[31], who stated that chronic fat-rich diet results in hyperglycemic blood levels, which can contribute to the accumulation of lipid droplets in the cytoplasm of many non-adipose tissues, including salivary glands. The observed lipid droplets could be attributed to fatty degeneration, as stated by Selim (2013)[32] in a previous study investigating the effect of chronic use of PUFA on pancreatic tissue that reported fatty degeneration.

The fused atypical secretory granules detected in the electron microscopic results of our study are consistent with the results detected by a previous study[10]. The fused secretory granules were investigated in another previous study. CDC42 is a key regulator of intracellular vesicles formation. This molecule impacts the formation and maturation of secretory vesicles. Trans Golgi network generates the secretory granules. CDC42 depletion increases the number of secretory granules with altered structure due to accelerated budding and fission and subsequently the vesicular plasma membrane is defective[33]. In the light of the fused granules detected in our study; we suggest that a fat rich diet harms CDC42 and further studies are required to reveal and correlate this effect.

The fused secretory granules were restored in some sections of the vit.E group in our study and immature secretory granules were observed. This result is in consistence with the result detected in a study investigating the effect of fat rich diet on the salivary glands[10]. In a previous study, it was stated that maturation of secretory granules involves several steps including fusion of newly formed granules, acidification, selective removal of plasma membrane protein, processing and aggregation of certain proteins from the vesicular plasma membrane[33]. This reveals that vitamin E in the prescribed dose had a modulatory effect on the formation of secretory granules formation , but the maturation process was defective.

In the Electron Microscopic results of our study, swollen mitochondria were obvious in the FRD group. The SD ducts showed loss of basal striation in the FRD group. The loss of basal striation noticed in the FRD group was absent in Vit. E group; basal striation was restored in this group, with mitochondria radiating between them. Although the irregular protrusions and thickening of the luminal wall were still obvious in the two groups.

Mitochondria are highly dynamic intracellular organisms; their ultrastructural morphology reflects their response to various physiological and pathological conditions. The dynamic nature of mitochondria is controlled by two counteracting processing, namely fusion and fission; an imbalance between these two processes and constant stresses insulting the cells and exceeding the cell's capacity can result in defective changes in mitochondrial morphology. This has been reported in many diseases, such as heart failure, diabetes, and nickel-induced hepatotoxicity[34, 35].

The mitochondrial membrane potential is altered under pathologic conditions, as stated by Sivagurunathan *et al*.,(2023)[36], in which the inner mitochondrial membrane loses its selective permeability, which results in increased permeability, subsequent mitochondrial swelling, defective mitochondrial function and release of cytochrome C activating the downstream apoptosis signaling pathway and cell death; when the stress exceeds the cellular capacity; lower levels of ATP can lead to cell apoptosis with the release of cytochrome C and activation of caspases. Swelled mitochondria are a hallmark of damage and defective function. Mitochondrial dysfunction has been linked to a crucial role in cell apoptosis, inflammation, and oxidative stress[37,38]. This could histologically explain the degenerative signs detected in the FRD group and confirmed ultra-structurally.

The rER changes noticed ultrastructurally in the FRD group of our study correlate with altered rER function and signaling, as stated by Perrotta (2020)[37], and can contribute to cell injury and apoptosis with the subsequent impact of the pathophysiology of various human disorders .this could further explain the degenerative signs detected in the FRD group and highlights the negative effect of fat-rich diet on various intracellular organelles.

The deleterious histological and electron microscopic findings observed in the FRD group were markedly reduced in Vit. E group, except for the marked irregularity of the SD lumen that was noticed in the FRD and Vit. E group.

According to the Food and Agriculture Organization, the ratio of ω-6 to ω-3 in the diet should be either 5:1 or 10:1[39]. The ratio of ω-6 to ω-3 in corn oil is about 71.6:1[40]. Violating the ω-6: ω-3 ratio and increased consumption of ω-6 produce pro-inflammatory mediators and regulators, promoting inflammation and thrombosis and altering the immune response. As a result, the uneven ratio of ω-6: ω-3 in favor of ω-6 is very proinflammatory and prothrombotic,

which aids in developing atherosclerosis, obesity, and diabetes[41,42]. These reported data in the literature reveal the dark side of a PUFA-rich diet and explain the inflammatory and degenerative signs detected in our study.

To quantitively measure the effect of FRD and the modulatory effect of vitamin E supplementation, in the experimental dose of our study, on SMG; Morphometric and statistical analysis were performed, adding descriptive evidence to the histological findings. The morphometric digital image analysis supported the noticed histological findings where the fat rich diet caused decreased acinar diameter of the FRD group in comparison to the Control group (with a mean difference of 75.6 between the Control group and FRD group and a mean difference of 65.9 between Vit. E group and FRD group) denoting statistically significant difference (SSD) between this group and Control group and Vit. E group respectively. On the other hand, the acinar diameter was restored in the Vit. E group (with a mean difference of 9.7 between the Control and Vit. E groups), denoting no SSD between this group and the Control group.

Another profound effect of FRD; was the marked cellular vacuolisation that was markedly noticed in the FRD group than in the Control group and Vit. E group (with a mean difference of 19.4 percent area fraction of vacuolation between the FRD group and Control group and a mean difference of 18.2 percent of area fraction of vacuolation between the FRD group and Vit. E group) denoting SSD between this group, Control group and Vit. E group, respectively. The percent area fraction of vacuolisation of the Vit. E group showed no SSD compared to the Control group (with a mean difference of 1.2 percent of the area fraction of vacuolisation between the two groups). Glumac *et al*.,(2023)[43] Reported that vacuolisation and changes in cell shape and architecture are characteristic of the cell death pathway.

The lumen of the SD of the FRD group and Vit. E group showed marked stricture or narrowing (with a mean difference of 95.6 between the Control group and FRD group and 86.9 between the Control group and Vit. E group), denoting SSD between these two groups and Control group.

The lumen of the ED in both the FRD group and Vit. E group showed no SSD in relation to the Control group, although the diameter of the ED lumen in the FRD group was wider than the Control group and Vit. E group, this could be explained by the obvious degeneration of the cell lining of this group.

The morphometric analysis of SD in the FRD group and the vit. E group; in addition to the histological and electron microscopic results, suggests that a massive reaction occurred at the mitochondrial level and possibly the marked stricture observed histologically and confirmed by the morphometric and statistical analysis in the FRD group and the vit. E group was due to this reaction; the degenerative changes observed in the cellular lining of the striated ducts in the FRD group were not apparent in the vit. E group, suggesting that vitamin E had a protective effect to some extent in this group, although stricture was apparent. Moreover, the signs of degeneration of the lining cells were absent in the vit. E group and the basal striations in this group were retained; vitamin E played a significant role in partially protecting these cells from the deteriorating damage observed in the FRD group.

The findings detected in the SD in our study could be attributed to their rich content of mitochondria. It is generally known that the SD cells have a massive columnar form and display a mitochondrial compartment approximately three times the amount of cell volume as intercalated duct cells (16.5%)[44].

The results can be further explained by Zalaweska *et al*.,(2019)[45] who stated that a high-fat diet regimen increases the salivary gland ceramide composition, intensifying the oxidative damage to proteins and lipids, which results in inflammation and apoptosis of submandibular gland mitochondria, furthermore they reported increased Bax concentration and TNF-α. It is worth mentioning that Matczuk *et al*.,(2016)[31] reported that fat-rich diet administration resulted in a decreased mass of SMG with elevated levels of MDA, a hallmark of lipid peroxidation.

The moderate protective effect observed in our study could be attributed to other factors, such as the solvent itself or the concentration of vitamin E, as reported by Barouh *et al*., (2022)[46], who stated that The solvent system itself and the concentration of the vitamin E are two factors that influence how it behaves in oil systems.

Górnicka *et al*.,(2019)[47] Stated that alpha-tocopherol accumulates in all examined organs proving that supplementation with vitamin E increases its content in different tissues protecting them from cellular stress and inflammatory changes due to its anti-inflammatory and antioxidant properties.

There is a limitation in this study that could be addressed in future research. This study focused on histological and ultrastructural results; blood tests or tissue cultures were not in the scope of the study protocol. Measurement of the lipid profile, lipid peroxidation, and antioxidant enzymes is essential to further explain the histological and ultrastructural results.

A description of the effect of fat rich diet administration on the duct system and CT stroma, in addition to morphometric analysis of the lumen diameter and acinar diameter in response to fat-rich diet administration, has not, to our knowledge, been described previously. Thus, this study provides a detailed description of the histologic and ultrastructural effects of FRD administration on SMG.

Our study confirmed that regular daily intake of vitamin E in the dosage mentioned above has a partially protective effect on SMG, proved by reduced degenerative signs in the Vit E group than FRD group.

CONCLUSION

Regular Vitamin E administration with the selected dosage in this study had a partial protective effect against the effect of fat rich diet ameliorating the SMG; we recommend implementation of Vitamin E with appropriate dosage in our daily dietary habits, as it could have a possible prophylactic effect against fatty degeneration. Further studies are recommended with longer durations and higher doses of Vitamin E using different solvents. The authors will consider measuring lipid peroxidation, antioxidant enzymes, and lipid profile in their future research investigating the effect of fat rich diet on the salivary glands.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Koloverou E, Panagiotakos DB, Pitsavos C, Chrysohoou C, Georgousopoulou EN, Grekas A, *et al*. Adherence to Mediterranean diet and 10-year incidence (2002-2012) of diabetes: correlations with inflammatory and oxidative stress biomarkers in the ATTICA cohort study: Mediterranean Diet and Diabetes. Diabetes Metab Res Rev.(2016) 32(1):73–81.
2. Raut SK, Khullar M. Oxidative stress in metabolic diseases: current scenario and therapeutic relevance. Mol Cell Biochem. (2023) 478(1):185–96.
3. Amano O, Mizobe K, Bando Y, Sakiyama K. Anatomy and Histology of Rodent and Human Major Salivary Glands—Overview of the Japan Salivary Gland Society-Sponsored Workshop—. Acta Histochem Cytochem. (2012)45(5):241–50.
4. Banerjee A, Das D, Paul R, Roy S, Bhattacharjee A, Prasad SK, *et al*. Altered composition of high -lipid diet may generate reactive oxygen species by disturbing the balance of antioxidant and free radicals. Journal of Basic and Clinical Physiology and Pharmacology. (2020)31(3).
5. Park JE, Pichiah PBT, Cha YS. Vitamin D and Metabolic Diseases: Growing Roles of Vitamin D. JOMES. (2018)27(4):223–32.
6. Saito I. Pathology of salivary gland dysfunction and restoration of function. Pathology International. (2021)71(5):304–15.
7. Chibly AM, Aure MH, Patel VN, Hoffman MP. Salivary gland function, development, and regeneration. Physiological Reviews. (2022) 102(3):1495 – 1552.
8. Ramadan MF, Afify Amer MM, El -Saadany SS, Abd El -Fatah El -Masry R, El - Said Awad A. Changes in Lipid Profile by Vegetable Oil Blends Rich in Polyunsaturated Fatty Acids in Rats with Hypercholesterolemia. Food sci technol int. (2009) 15(2):119 – 130.
9. Ksas B, Havaux M. Determination of ROS -induced lipid peroxidation by HPLC - based quantification of hydroxy polyunsaturated fatty acids. In Reactive Oxygen Species in Plants: Methods and Protocols. New York, NY: Springer US. (2022) pp: 181 -189
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(4): e06932.
11. Hefnawy HTM, Ramadan MF. Protective effects of Lactuca sativa ethanolic extract on carbon tetrachloride induced oxidative damage in rats. Asian Pacific Journal of Tropical Disease. (2013) 3(4):277–285.
12. Magdy BW, Mohamed FE, Amin AS, Rana SS. Ameliorative effect of antioxidants (vitamins C and E) against abamectin toxicity in liver, kidney and testis of male albino rats. The Journal of Basic & Applied Zoology. (2016) 77:69 – 82.
13. Bilbis, L. S., Muhammad, S. A., Saidu, Y., & Adamu, Y. Effect of Vitamins A, C, and E Supplementation in the Treatment of Metabolic Syndrome in Albino Rats. Biochemistry Research International. (2012) (1): 1–7.
14. Vento PJ, Swartz ME, Martin LB, Daniels D. Food intake in laboratory rats provided standard and fenbendazole -supplemented diets. J Am Assoc Lab Anim Sci. (2008) 47(6):46 –50.
15. Bancroft JD, Gamble M, editors. Theory and practice of histological techniques. 6. ed. Philadelphia, Pa: Churchill Livingstone, Elsevier (2008) pp: 725.
16. Bindhu P, Krishnapillai R, Thomas P, Jayanthi P. Facts in artifacts. J Oral Maxillofac Pathol. (2013) 17(3):397.
17. Abdel -Aziz MM, Yosri M, Amin BH. Control of imipenem resistant -Klebsiella pneumoniae pulmonary infection by oral treatment using a combination of mycosynthesized Ag -nanoparticles and imipenem. Journal of Radiation Research and Applied Sciences. (2017) 10(4):353 – 360.
18. Yosri M, Elaasser MM, Abdel -Aziz MM, Hassan MM, Alqhtani AH, Al -Gabri N, *et al*. Determination of Therapeutic and Safety Effects of Zygophyllum coccineum Extract in Induced Inflammation in Rats. Li WW, editor. BioMed Research International. (2022) 2022:1 –17.
19. Corby -Harris V, Snyder LA. Measuring Hypopharyngeal Gland Acinus Size in Honey Bee (Apis mellifera) Workers. JoVE. (2018) (139):58261.
20. Ali Ghanim al Okaili, Ban Ismael Sedeeq, Muhammed Ibraheem Hazeem. Histological changes of the submandibular Salirary gland of mice maintained on a liquid diet. College of Dentistry, Tikrit University. (2008) 14(3): 1 - 4 .

21. Möller I, Janta I, Backhaus M, Ohrndorf S, Bong DA, Martinoli C, *et al*. The 2017 EULAR standardised procedures for ultrasound imaging in rheumatology. Ann Rheum Dis. (2017) 76(12):1 – 6.
22. Escalante C, Mora S, Bolaños L. Hormone replacement therapy reduces lipid oxidation directly at the arterial wall: A possible link to estrogens' cardioprotective effect through atherosclerosis prevention. J Mid -life Health. (2017) 8(1):11 –16.
23. Kandeel S, M. Elwan walaa. Effect of high fat diet on the rat submandibular salivary glands with the possible protection by Gomisin A: Light and scanning electron microscopic study. Egyptian Journal of Histology. (2022)0(0):0–0.
24. Roa I, del Sol M. Obesity, salivary glands and oral pathology. Colombia Medica. (2018)280–287.
25. Marcus KS, Zhu VL, Eigsti RL, Hoffman HT. Fibrous Salivary Ductal Stricture: A Novel Histopathologic Correlate. Ear Nose Throat J. (2021) 100(10):NP429–431.
26. Koch M, Iro H. Extended and treatment-oriented classification of parotid duct stenosis: Parotid Duct Stenoses. The Laryngoscope. (2017)127(2):366–371.
27. Barrientos C, Pérez A, Vázquez J. Ameliorative Effects of Oral Glucosamine on Insulin Resistance and Pancreatic Tissue Damage in Experimental Wistar rats on a High-fat Diet. comp med. (2021)71(3):215–221.
28. Tiffon C. Defining Parallels between the Salivary Glands and Pancreas to Better Understand Pancreatic Carcinogenesis. Biomedicines. (2020)8(6):178.
29. Mack M. Inflammation and fibrosis. Matrix Biology. (2018)68–69:106–21.
30. Zhang M, Zhang S. T Cells in Fibrosis and Fibrotic Diseases. Front Immunol. (2020) 11:1142.
31. Matczuk J, Zalewska A, Łukaszuk B, Knaś M, Maciejczyk M, Garbowska M, *et al*. Insulin Resistance and Obesity Affect Lipid Profile in the Salivary Glands. Journal of Diabetes Research. (2016) 2016:1–9.
32. Selim SA. The effect of high-fat diet-induced obesity on the parotid gland of adult male albino rats: histological and immunohistochemical study. The Egyptian Journal of Histology. (2013)36(4):772–780.
33. Shitara A, Bleck CKE, Weigert R. Cdc42 controls secretory granules morphology in rodent salivary glands in *vivo*. Communicative & Integrative Biology. (2020) 13(1):22–6.
34. Duranova H, Valkova V, Knazicka Z, Olexikova L, Vasicek J. Mitochondria: A worthwhile object for ultrastructural qualitative characterization and quantification of cells at physiological and pathophysiological states using conventional transmission electron microscopy. Acta Histochemica. (2020) 122(8):151646.
35. Guo H, Wei L, Wang Y, Cui H, Deng H, Zhu Y, *et al*. Nickel induces hepatotoxicity by mitochondrial biogenesis, mitochondrial dynamics, and mitophagy dysfunction. Environmental Toxicology. (2023) tox.23758.
36. Sivagurunathan N, Gnanasekaran P, Calivarathan L. Mitochondrial Toxicant-Induced Neuronal Apoptosis in Parkinson's Disease: What We Know so Far. DNND. (2023) 13:1–13.
37. Perrotta I. The microscopic anatomy of endothelial cells in human atherosclerosis: Focus on ER and mitochondria. J Anat. (2020)237(6):1015–1025.
38. Quintana-Cabrera R, Scorrano L. Determinants and outcomes of mitochondrial dynamics. Molecular Cell. (2023)83(6):857–876.
39. Sheppard KW, Cheatham CL. Omega-6/omega-3 fatty acid intake of children and older adults in the U.S.: dietary intake in comparison to current dietary recommendations and the Healthy Eating Index. Lipids Health Dis. (2018) 17(1):43.
40. Almas Mukhametov , Moldir Yerbulekova , Gulsim Aitkhozhayeva , Gulim Tuyakova , Dina Dautkanova. Effects of ω-3 fatty acids and ratio of ω-3/ω-6 for health promotion and disease prevention. Food Science and Technology (2022) 42.
41. Innes JK, Calder PC. Marine Omega-3 (N-3) Fatty Acids for Cardiovascular Health: An Update for 2020. Int J Mol Sci. (2020)21(4):E1362.
42. Panezai J, van Dyke T. Polyunsaturated Fatty Acids and Their Immunomodulatory Actions in Periodontal Disease. Nutrients. (2023)15(4):821.
43. Glumac M, Čikeš Čulić V, Marinović-Terzić I, Radan M. Mechanism of cis-Nerolidol-Induced Bladder Carcinoma Cell Death. Cancers. (2023)15(3):981.
44. Cope GH. Stereological analysis of the duct system of the rabbit parotid gland. J Anat. (1978)126(Pt 3):591–604.
45. Zalewska A, Maciejczyk M, Szulimowska J, Imierska M, Błachnio-Zabielska A. High-Fat Diet Affects Ceramide Content, Disturbs Mitochondrial Redox Balance, and Induces Apoptosis in the Submandibular Glands of Mice. Biomolecules. (2019) 9(12):877.
46. Barouh N, Bourlieu-Lacanal C, Figueroa-Espinoza MC, Durand E, Villeneuve P. Tocopherols as antioxidants in lipid-based systems: The combination of chemical and physicochemical interactions determines their efficiency. Comp Rev Food Sci Food Safe. (2022)21(1):642–688.
47. Górnicka M, Ciecierska A, Hamulka J, Drywień ME, Frackiewicz J, Górnicki K, *et al*. α-Tocopherol Protects the Heart, Muscles, and Testes from Lipid Peroxidation in Growing Male Rats Subjected to Physical Efforts. Palmery M, editor. Oxidative Medicine and Cellular Longevity. (2019) 2019:1–13.

الملخص العربى

التأثير التعديلى المحتمل لفيتامين (هـ) على الغدد اللعابية تحت الفكية للفئران البيضاء المستقبلة لغذاء عالى الدهون: (دراسة بالمجهر الضوئى و الإلكترونى النافذ)

منى بهاء مرسى[١]، مروة محمد عبد الحميد[٢]، إيمان أحمد فتحى[٢]

قسم بيولوجيا الفم، كلية طب الأسنان، [١]جامعة الجلالة، [٢]جامعة عين شمس، مصر

المقدمة: من الممكن أن تتأثر صحة الفم بالسلب نتيجة تناول غذاء عالى الدهون. فيتامين (هـ) من مضادات الأكسدة التى تذوب فى الدهون والتى تم إثبات فاعليتها وتأثيرها على مختلف أنسجة الجسم بالعديد من الدراسات.

الهدف من الدراسة: دراسة التأثير التعديلى المحتمل لفيتامين(هـ) (كمضاد معروف للأكسدة)دعلى الغدد اللعابية تحت الفكية للفئران البيضاء المستقبلة لغذاء عالى الدهون و تقييم آلية التنكس المحتمل عن طريق الميكروسكوب الضوئي والميكروسكوب الإلكتروني النافذ.

مواد وطرق البحث: تم فصل ٢٤ فأرا ذكرا بالغاً إلى ثلاث مجموعات: المجموعة الضابطة، و مجموعة النظام عالى الدهون، ومجموعة الغذاء عالى الدهون بالإضافة إلى فيتامين (هـ). بعد انقضاء المدة الزمنية للتجربة (١٢ إسبوع) تم أخذ عينات الغدد اللعابية تحت الفكية من جميع المجموعات وجهزت للفحص بالمجهر الضوئي والإلكتروني. كما تم جمع البيانات المورفومترية من أجل التحليل الإحصائى فيما يتعلق بقطر الحويصلات اللعابية وتجويف القنوات المفرزة والمخططة.

النتائج: تناول فيتامين (هـ) بانتظام بالجرعة المختارة فى هذه الدراسة كان له تأثير وقائى جزئى، بالنسبة لمجموعة الغذاء عالى الدهون وبمقارنتها بالمجموعة الضابطة فقد لوحظ إنخفاض فى قطر الحويصلات اللعابية مع زيادة فى المساحة النسبية للفراغات السيتوبلازمية للخلايا و تضيق لتجويف الأقنية المخططة كما لوحظ إنحطاط خلايا القنوات المفرزة و لم يكن هناك تضيق واضح فى تجويفها. وبالنسبة لمجموعة فيتامين (هـ) فقد لوحظ إستعادة بنية وقطر الحويصلات اللعابية مع نقص فى المساحة النسبية للفراغات السيتوبلازمية وعلى الرغم من ذلك ، إلا أن تضيق القنوات المخططة كان لا يزال واضحًا في هذه المجموعة مع عدم وجود علامات انحطاط في البطانة الخلوية لتجويف الأقنية وتم استعادة خلايا الأقنية المفرزة ولم يكن فيها أى تضيق ملحوظ.

الخلاصة: كان لإعطاء فيتامين (هـ) بانتظام بالجرعة المحددة في هذه الدراسة تأثير وقائي جزئي ضد الأكسدة الدهنية ، و يمكن أن يكون لتطبيق فيتامين (هـ) بالجرعات المناسبة في عاداتنا الغذائية اليومية تأثير وقائي محتمل ضد التنكس الدهني.

الأهمية السريرية: حيث أن النظام الغذائى الغنى بالدهون أصبح أسلوب حياة فى الوقت الحالى ، تقدم هذه الدراسة نصيحة وقائية مأمولة لإعطاء فيتامين (هـ) بشكل منتظم لتقليل الآثار الجانبية لهذه العادة الغذائية السائدة.