

## POSSIBLE PROTECTIVE EFFECT OF FISH OIL ON PAROTID SALIVARY GLANDS IN ADULT MALE ALBINO RATS WITH PTU - INDUCED HYPOTHYROIDISM (HISTOLOGICAL AND ULTRASTRUCTURAL STUDY)

Nihal T. Elkazzaz\*  and Sara A. Hamza\* 

### ABSTRACT

**Introduction:** Propylthiouracil is used to treat hyperthyroidism and to induce the hypothyroidism in experimental animals. Hypothyroidism has a negative impact on many body organs. However, few studies were done to evaluate the effect of hypothyroidism on parotid glands. Fish oil has an important antioxidant and anti-inflammatory properties.

**Aim:** This study aimed to investigate the effect of Propylthiouracil induced hypothyroidism on the histological and ultrastructure features of parotid glands of rats and the possible prophylactic effect of fish oil.

**Materials and Methods:** 30 adult male albino rats were divided equally into 3 groups. Control group (A), hypothyroidism induced group (B) and hypothyroidism induced + fish oil group (C). Hypothyroidism was induced in group (B) and (C) using 10 mg / kg body weight Propylthiouracil. For group (C), fish oil was given with Propylthiouracil as 0.5ml/100gm body weight daily for 28 days. At the end of the study the parotid glands were harvested and prepared for histological and ultrastructure examination.

**Results:** In group (B) the acinar cells illustrated signs of cytotoxicity including cytoplasmic vacuolations, separation between acini, dilated ducts with abnormal epithelial lining. Moreover, the ultrastructure results revealed shrunken nuclei, swelling in the rough endoplasmic reticulum, and scattered apoptotic bodies. Relative improvements in these features have been noticed in the fish oil treated group (group C).

**Conclusion:** Hypothyroidism represents an important risk factor for parotid salivary gland dysfunction. Fish oil may have significant protective capacity not only to improve salivary glands structure, but also to help in prophylaxis against hypothyroidism.

**KEYWORDS:** Propylthiouracil (PTU), Hypothyroidism, Parotid salivary glands, Fish oil.

\* Lecturer of Oral Biology Department, Faculty of Dentistry, Alexandria University, Egypt.

## INTRODUCTION

Thyroid gland is responsible for the production, storage and release of the hormones thyroxine (T4), triiodothyronine (T3) and calcitonin, thus it is considered as the body's "metabolic thermostat" <sup>(1)</sup>. Thyroxine (T4) and triiodothyronine (T3) hormones are crucial for physiological functions of almost all tissues of the body. They affect body temperature, heartbeat, and calorie due to their major role in controlling the body's energy metabolism. They are also essential in the balance of the cardiovascular system with significant effects on homeostasis <sup>(2)</sup>. Moreover, these hormones can affect widely the regulation of oxidative metabolism and emotional stability. Besides, they control proteins, lipids, carbohydrates metabolism and have antioxidant enzyme activities. Thyroid hormones influence tissues by synthesis of new proteins through the facilitation of DNA transcription. Thyroid hormones are essential for epithelial differentiation and in regulating the sensitivity of the autonomic stimulation of salivary glands <sup>(3)</sup>.

Thyroid dysfunction incidence among individuals is increasing worldwide. It approximately represents 30–40% of the endocrine patients; thus, it is considered one of the main endocrine disorders. Thyroid disorder prevalence among adult people in Egypt reached 29.3%, among of which 44.4% was reported as clinical hypothyroidism <sup>(4)</sup>. Hypothyroidism, also known as underactive thyroid disease, is a congenital or acquired endocrine disorder of the thyroid gland. It is either the result of damage in the mechanisms controlling the formation of thyroid hormones or as a result of complications during the treatment of hyperthyroidism <sup>(2)</sup>. Hypothyroid state is a complex hormonal disorder with many implications. It causes general metabolic disturbance, besides, it also has serious intellectual and behavioral abnormalities that affects the patient's daily functioning leading to additional stress and depression. Hypothyroidism increases levels of total cholesterol, low density lipoproteins and apolipoprotein B <sup>(5)</sup>.

Propylthiouracil (PTU) is a thiouracil-derived drug. It decreases thyroid hormones amount formed by the thyroid gland, thus it is used as an effective treatment for hyperthyroidism. PTU inhibits thyroid hormones synthesis by interfering with conversion of T4 to T3 <sup>(6)</sup>. Hypothyroidism is the most frequent consequence of the interaction of a large variety of drugs, environmental pollutants and industrial chemicals with the thyroid gland. The thyroid toxicity caused by PTU is similar to that produced by most environmental toxicants and drugs, thus it has been used widely to induce hypothyroidism experimental animals <sup>(6,7)</sup>.

Salivary glands produce and secrete saliva, which protects and maintains the integrity of the oral mucosa. Saliva has many host defense functions such as homeostatic processes, antimicrobial activity, control of demineralization/remineralization of teeth, lubrication and a buffering capacity for the maintenance of optimal oral pH and tooth integrity <sup>(8,9)</sup>. Major salivary glands are formed of three pairs of glands; parotid, submandibular, and sublingual glands; the three of which are responsible for the secretion of 90% of the total saliva. Parotid glands are the largest major glands which histologically contain serous acini, that secrete aqueous saliva, rich in protein <sup>(10)</sup>.

Fish oil is a polyunsaturated fatty acids (PUFAs) rich compound which is mainly represented by eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that influence multiple functions in the body mainly; blood pressure, blood clotting, modulation of inflammatory response, and correct development and functioning of brain and nervous systems. Epidemiological studies reveal its positive effects. It has been reported that populations ingesting PUFAs in large amounts, mainly found in fish oil, the risk of neurodegenerative disorders such as Alzheimer's disease is reduced. Also, the reduced incidence of acute myocardial infarction and chronic inflammatory diseases such as rheumatoid arthritis, ulcerative colitis, psoriasis, among

other inflammatory diseases has been noted<sup>(11)</sup>. As an important antioxidant, PUFAs regulate the antioxidant signaling pathways when incorporated in the cell membranes. Moreover, as mitochondrial membranes of eukaryotic cells have a high DHA content, this indicates that DHA is an essential phospholipid for adenosine triphosphate (ATP) synthesis by oxidative phosphorylation<sup>(12)</sup>.

The effect of fish oil and thyroid hormone on liver function has been widely studied. The use of fish oil in the treatment of hyperlipidemia and obesity has been attributed to its high potentiality in stimulating the effect of fatty acid oxidation and its strong inhibitory effect on hepatic lipogenesis and triglyceride synthesis, which lead to decrease in plasma triglycerides. It has also been proved that it modulates the hepatic metabolism of cholesterol, resulting in an important hypocholesterolemic effect. Similarly, thyroid hormone increases cholesterol metabolism and thus promotes lipogenesis and oxidation of fatty acid<sup>(13)</sup>. Thyroid hormones and omega-3 are fundamental for normal functions of the brain. Cognitive impairments have been reported in recent studies in hypothyroidism which was due to different mechanisms including: oxidative stress, reduction of neurotransmitters as serotonin, structural changes and up-regulation of Cav1.2 protein. Omega-3 improved cognitive deterioration associated with hypothyroidism through several mechanisms as down-regulation of Cav1.2 protein and modulation of serotonin neurotransmitters. Thus, omega-3 could be used as an adjuvant neuroprotective factor against hypothyroidism-induced cognitive impairment.<sup>(14)</sup>

Although thyroid diseases are one of the most common endocrine disorders worldwide, however, limited literature and research on the effects of thyroid gland on salivary glands disorder have been published<sup>(9)</sup>. Thus, this study aimed at focusing on the effect of PTU induced hypothyroidism on parotid gland and the possible prophylactic effect of fish oil.

## MATERIALS AND METHODS

### Animal housing

The study was conducted in accordance with the ethical guidelines of research on experimental animals at Faculty of Dentistry, Alexandria University (IRB NO:00010556 - IORG 0008839). Thirty adult male albino rats (3:6 months age) (200-250 g initial body weight) were included in the study. Rats were housed in cleaned and ventilated cages with constant controlled climate (at Institute of Medical Research, Alexandria University, Egypt). All animals received filtered tap water regular diet and water ad libitum.

### Sample size calculation

Sample size was calculated using Power Analysis and Sample Size Software (PASS 2020) "NCSS, LLC. Kaysville, Utah, USA, [ncss.com/software/pass](http://ncss.com/software/pass)". A minimal total hypothesized sample size of 30 adult male albino rats (3:6 months age) (200-250 g initial body weight) (10 per group) is needed to investigate the effect of PTU induced hypothyroidism on the histological and ultrastructure features of parotid glands of rats and the possible prophylactic effect of fish oil with an assumption of obtaining an effect size of 20%, with 0.05 probability of type I error and power of 80% using Chi-square test<sup>(15,16)</sup>.

### Chemicals

#### 1. Propylthiouracil (PTU)

PTU was purchased from Sigma Aldrich company\*. It was in the form of a white powder and it was dissolved in saline.

#### 2. Fish Oil

Fish oil was purchased from Sigma Aldrich company\*.

---

\* Sigma - Aldrich Chemical Company (St. Louis, MO, USA)

### Random allocation

Rats were randomly divided by computer-assisted software into 3 equal groups; Group A [Controls, (n=10)]: Received 10 ml/kg distilled water daily by gavage for twenty eight days (17), Group B [Hypothyroidism induced (n=10)]: Received 10 mg / kg body weight PTU (Sigma Aldrich) dissolved in 0.3 ml saline daily by oral gavage for twenty eight days (17) and Group C [Hypothyroidism induced+ fish oil (n=10) ]: Received 10 mg / kg body weight PTU (Sigma Aldrich) dissolved in 0.3 ml saline together with 0.5ml/100gm body weight fish oil daily by oral gavage for twenty eight day (18). Fish oil contains 20-31% omega-3 fatty acids as triglycerides, was used in this experiment. A random allocation was done using computer-generated random sequence of numbers to assign treatment status to reduce the possibility of error in which all personnel who performed the tests were unaware of the treatment assignment.

### Body weight and serological analysis

Body weight was recorded twice during the study period; after first week of induction and just before euthanization. While, blood samples were collected from para-orbital sinus puncture at the end of the experiment just before euthanization. The levels of T3 and T4 were measured in the sera of rats <sup>(19)</sup>.

After euthanization, the right and left parotid salivary glands were harvested and prepared for histological and ultrastructural examinations. Finally, any animal disposals were burned.

### Histological evaluation

The right parotid glands were prepared for light microscopic examinations to assess any histological changes in the parotid gland tissues among the different groups. Samples were fixed in 10% formalin neutral buffer, embedded in paraffin blocks according to the standard procedure, sectioned into 5 µm sections and stained by hematoxylin and eosin stain (H&E) <sup>(20)</sup>.

### Ultrastructural evaluation

The left parotid glands were cut into small pieces about 1 mm<sup>3</sup>, immersed in 2.5% glutaraldehyde in phosphate buffer to be prepared for transmission electron microscopic examination (TEM, Jeol 100 CX, Tokyo, Japan) at Faculty of Science, Alexandria University, Egypt <sup>(21)</sup> to observe the detailed ultrastructural description of the parotid gland tissues among the different groups.

### STATISTICAL ANALYSIS

The body weight and serological results were statistically analyzed using IBM SPSS software with version 20.0. ANOVA test in order to compare the 3 study groups for normally distributed quantitative variables <sup>(22)</sup>.

## RESULTS

### 1. Body weight results

The body weight of the rats was measured after the first week of induction and just before euthanization. The body weight statistical analysis showed significant difference in the body weight in the PTU induced hypothyroidism group and PTU induced hypothyroidism with fish oil groups compared to the control group. However no significant difference was detected between the PTU induced hypothyroidism group and PTU induced hypothyroidism with fish oil groups (Table 1).

### 2. Laboratory results

Blood samples were collected at the end of the experiment just before euthanization. The levels of T3 and T4 were measured in the sera of rats. The serological analysis revealed a significant difference in the levels of T3 and T4 in hypothyroid group in comparison to the control and PTU induced hypothyroidism with fish oil groups (Table 2,3).

TABLE (1) Comparison between the three studied groups according to body weight

Body weight	Control (n=7)	PTU (n=7)	PTU + Fish Oil (n=7)	H	p
<b>Week 1</b>					
Min. – Max.	240.0 – 250.0	175.0 – 220.0	176.0 – 240.0		
Mean ± SD.	246.14 ± 3.98	187.43 ± 16.33	194.43 ± 21.29	13.786*	0.001*
Median (IQR)	245.0(244.0–250.0)	182.0(176.0–191.50)	186.0 (185.50–194.0)		
<b>Sig. bet. grps</b>	$p_1 < 0.001^*$ , $p_2 = 0.006^*$ , $p_3 = 0.449$				
<b>Week 4</b>					
Min. – Max.	245.0 – 260.0	168.0 – 228.0	181.0 – 260.0		
Mean ± SD.	252.29 ± 5.82	193.0 ± 19.51	201.57 ± 26.73	10.425*	0.005*
Median (IQR)	250.0 (248.50–257.0)	197.0(180.50–198.50)	196.0(187.50–199.50)		
<b>Sig. bet. groups</b>	$p_1 = 0.002^*$ , $p_2 = 0.014^*$ , $p_3 = 0.560$				

IQR: Inter quartile range

SD: Standard deviation

H: H for **Kruskal Wallis test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**p: p value for comparing between **the three studied groups** $p_1$ : p value for comparing between **Control** and **PTU** $p_2$ : p value for comparing between **Control** and **PTU + Fish Oil** $p_3$ : p value for comparing between **PTU** and **PTU + Fish Oil**\*: Statistically significant at  $p \leq 0.05$ 

TABLE (2): Comparison between the three studied groups according to T3

T3	Control (n=7)	PTU (n=7)	PTU + Fish Oil (n=7)	F	p
<b>Before euthanization</b>					
Min. – Max.	75.0 – 87.0	46.0 – 67.0	69.0 – 80.0		
Mean ± SD.	79.14 ± 4.18	58.43 ± 7.48	75.29 ± 4.31	27.70*	<0.001*
Median (IQR)	79.0 (76.0–80.50)	59.0 (54.50–64.0)	75.0 (72.50–79.0)		
<b>Sig. bet. groups</b>	$p_1 < 0.001^*$ , $p_2 = 0.412$ , $p_3 < 0.001^*$				

IQR: Inter quartile range SD: Standard deviation

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)

p: p value for comparing between **the three studied groups** $p_1$ : p value for comparing between **Control** and **PTU** $p_2$ : p value for comparing between **Control** and **PTU + Fish Oil** $p_3$ : p value for comparing between **PTU** and **PTU + Fish Oil**\*: Statistically significant at  $p \leq 0.05$

TABLE (3): Comparison between the three studied groups according to T4

T4	Control (n=7)	PTU (n=7)	PTU + Fish Oil (n=7)	F	p
<b>Before euthanization</b>					
Min. – Max.	4.90 – 6.90	2.78 – 3.77	5.98 – 7.11		
Mean ± SD.	5.89 ± 0.73	3.26 ± 0.34	6.53 ± 0.36	81.273*	<0.001*
Median (IQR)	5.90 (5.40–6.35)	3.18 (3.04–3.49)	6.55 (6.35–6.70)		
<b>Sig. bet. groups</b>	$p_1 < 0.001^*$ , $p_2 = 0.070$ , $p_3 < 0.001^*$				

*IQR*: Inter quartile range

*SD*: Standard deviation

**F**: F for ANOVA test, *Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)*

*p*: *p* value for comparing between the three studied groups

$p_1$ : *p* value for comparing between Control and PTU

$p_2$ : *p* value for comparing between Control and PTU + Fish Oil

$p_3$ : *p* value for comparing between PTU and PTU + Fish Oil

\*: Statistically significant at  $p \leq 0.05$

### 3. Histological results

#### Group A (controls)

H & E sections from control group parotid glands showed the gland acini were formed of deeply stained pyramidal shaped cells that possess secretory granules in cytoplasm apical part. The nuclei revealed normal circular pattern at the center or near the base of the cells. The intercalated ducts showed normal sized lumen and normal histological cellular features with cuboidal cells and centrally located nuclei, while the secretory striated ducts appeared larger with wider lumen than the intercalated ducts and lined by a single layer of columnar cells with basal striations (**Fig. 1A**). Moreover, the excretory ducts located in between the lobules, appeared large with wide lumen and lined by pseudostratified columnar epithelium (**Fig. 1B**). Normal appearance of the blood vessels in close proximity to the secretory striated and excretory ducts (**Fig. 1A,1B**).

#### Group B (Hypothyroidism induced)

The cells of the serous acini showed, atrophic changes as the affected acini became small, separated and lost its normal architecture and their lining cells had vacuolated cytoplasm (**Fig. 1C**).

The intercalated and secretory striated ducts showed degenerative irregular epithelial lining accompanied with cytoplasmic vacuolizations and dilated lumen with loss of the basal striation of the secretory striated ducts (**Fig. 1D**). Also the excretory ducts exhibited degeneration of the epithelial lining with dilatation of the lumen and were surrounded by connective tissue fibrosis (**Fig. 1E**). Moreover the blood vessels in close proximity to the secretory striated and excretory ducts appeared engorged with red blood corpuscles (RBCs) (**Fig. 1D,1E**).

#### Group C (Hypothyroidism induced + Fish Oil)

The serous acini showed well preserved architecture. The acinar cells revealed normal rounded nuclei, however, the cytoplasm exhibited few vacuolizations (**Fig. 1F**). The intercalated and secretory striated ducts showed regular lining cells with normal nuclei and well preserved basal striations of the secretory striated ducts, but with slight widening of the lumen (**Fig. 1G**). Moreover, the excretory ducts showed relatively normal epithelial lining and surrounded by connective tissue stroma (**Fig. 1H**). The blood vessels in close proximity to the excretory ducts appeared normal with red blood corpuscles (RBCs).

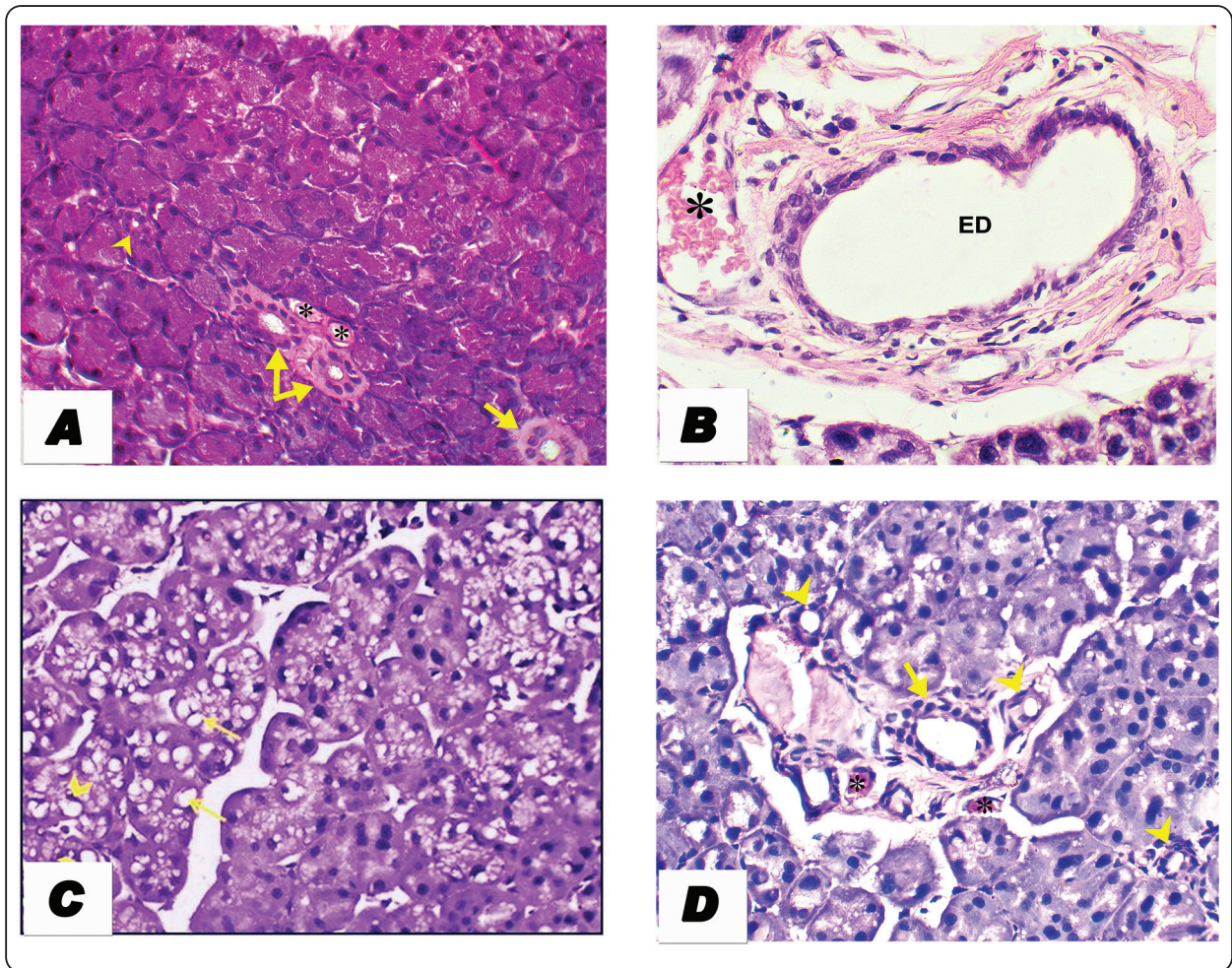


Fig. (1) Light micrograph (A) [control group] showing normal architecture of the serous acini where the acinar cells are pyramidal in shape with deeply stained cytoplasm and rounded basal nuclei surrounding a narrow lumen together with regular architecture of the intercalated duct (arrow head) and secretory striated ducts with normal epithelial lining, rounded nuclei and well defined basal striations and regular sized lumen (arrows). Note normal blood vessels (\*) in close proximity to the secretory striated ducts (X400 H&E). (B) [control group] showing normal shape of the excretory duct (ED) located in between the lobules, appeared large with wide lumen and lined by pseudostratified columnar epithelium. Note normal blood vessels (\*) in close proximity to the excretory duct (X400 H&E). (C) [hypothyroidism induced group] showing loss of the normal structural features of the acini, separation between the acini and extensive cytoplasmic vacuolations (arrows). Most of the nuclei appear pyknotic or apoptotic (arrow heads) (X400 H&E). (D) [hypothyroidism induced group] showing irregular shaped acini, atrophic vacuolated cytoplasm. The secretory striated duct (arrow) and the intercalated ducts (arrow heads) showed dilatation of the lumen with irregular epithelial lining. Note the blood vessels engorged with RBCs (\*) in close proximity to the secretory striated ducts (X400 H&E).

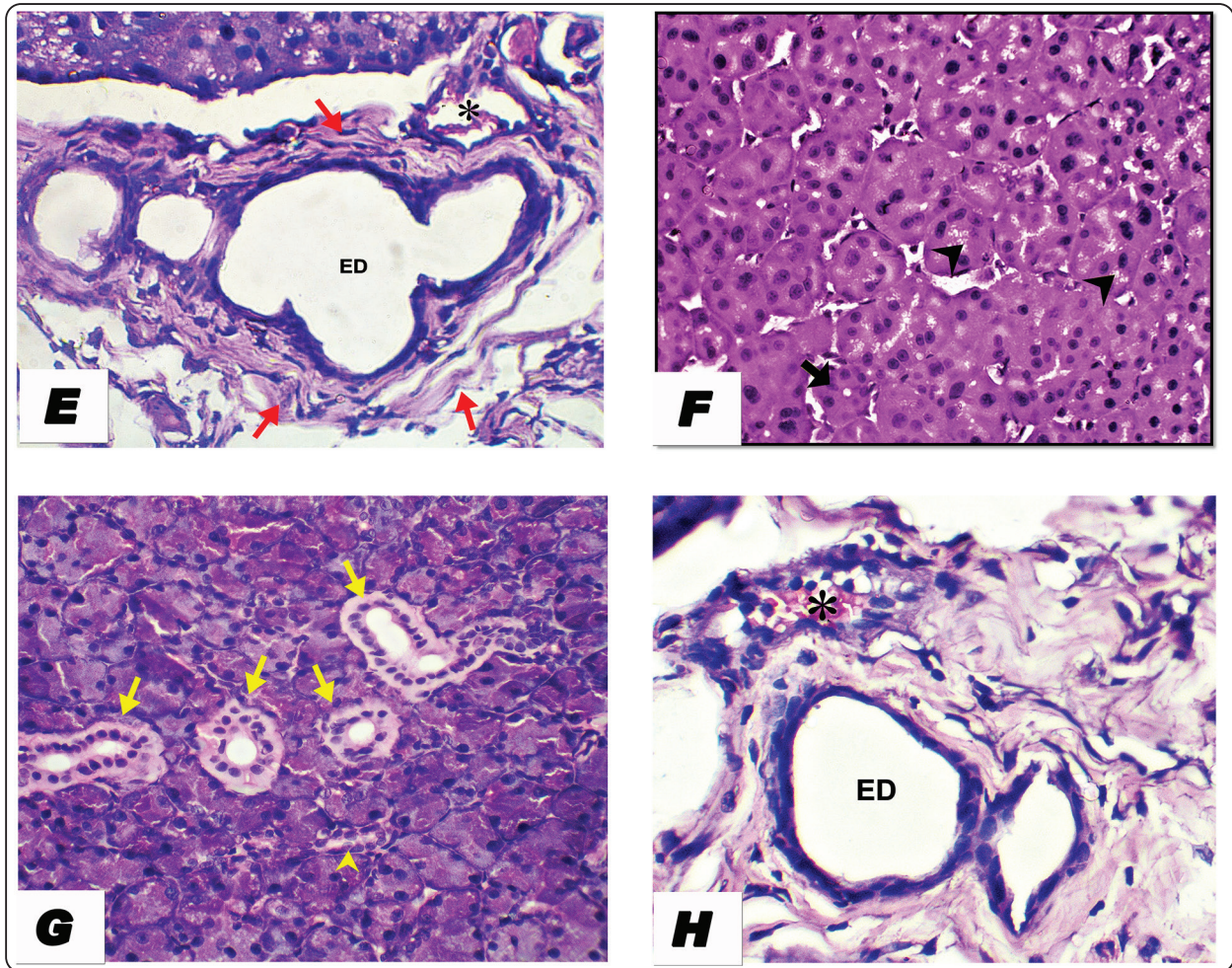


Fig. (1) **(E)** [hypothyroidism induced group] exhibiting abnormal excretory ducts (ED) with degeneration and thinning of the epithelial lining and dilatation of the lumen and surrounded by connective tissue fibrosis (arrows) Note: blood vessel with irregular endothelial lining (\*)(X400 H&E). **(F)**[hypothyroidism induced + fish oil group] showing preservation of the acinar architecture and their cell boundaries. The acinar cells appeared normal in shape with spherical nuclei (arrow). Note: slight cytoplasmic vacuolation can be seen (arrow heads) (X400 H&E). **(G)** [hypothyroidism induced + fish oil group] showing apparent preservation of the epithelial lining of the secretory striated ducts and its basal striations (arrows). Intercalated duct showed preserved epithelial lining with relative widening of the lumen (arrow head). (X400 H&E). **(H)** [hypothyroidism induced + fish oil group] showing relative regular epithelial lining of the excretory duct (ED) with a relatively normal lumen size within the connective tissue stroma. Note the blood vessels engorged with RBCs in close relation to the duct (\*)(X400 H&E).



### Ultrastructural results

The observations of the transmission electron microscope of the parotid salivary glands showed differences among the different groups. However, all specimens of the same group showed similar results.

#### Group A (Control)

The serous acinar cells of the gland showed, normal euchromatic nuclei. The cytoplasm accommodated abundant amount of well-organized rough endoplasmic reticulum. Also they contained numerous well organized golgi complex, numerous mitochondrial figures and plenty spherical secretory granules with different degrees of electron density (**Fig. 2A**). The Intercalated duct revealed normal cell lining with normal euchromatic nuclei, normal electron dense secretory granules, normal mitochondria, and narrow lumen (**Fig. 2B**).

#### Group B (Hypothyroidism induced)

The serous acinar cells exhibited numerous

dilated rough endoplasmic reticulum, apoptotic nuclei, and apoptotic bodies. The cytoplasm showed extensive degeneration with multiple vacuolations. (**Fig. 2C**). The cellular lining of the intercalated ducts exhibited degenerative changes which included altered mitochondria, vacuolization of the cytoplasm and widening of the lumen (**Fig. 2D**).

#### Group C (Hypothyroidism induced + Fish Oil)

The cells of the serous acini showed, normal nuclei with normal figures of chromatin distribution and numerous secretory granules with intermediate degree of electron density. The cytoplasm exhibited, few vacuolizations, slight dilatation of the rough endoplasmic reticulum with normal mitochondria and well developed golgi complex (**Fig. 2E**). The intercalated duct showed regular epithelial lining with normal nuclei, few scattered secretory granules were noticed, with well-developed golgi apparatus. However, some degree of lumen widening was observed (**Fig. 2F**).

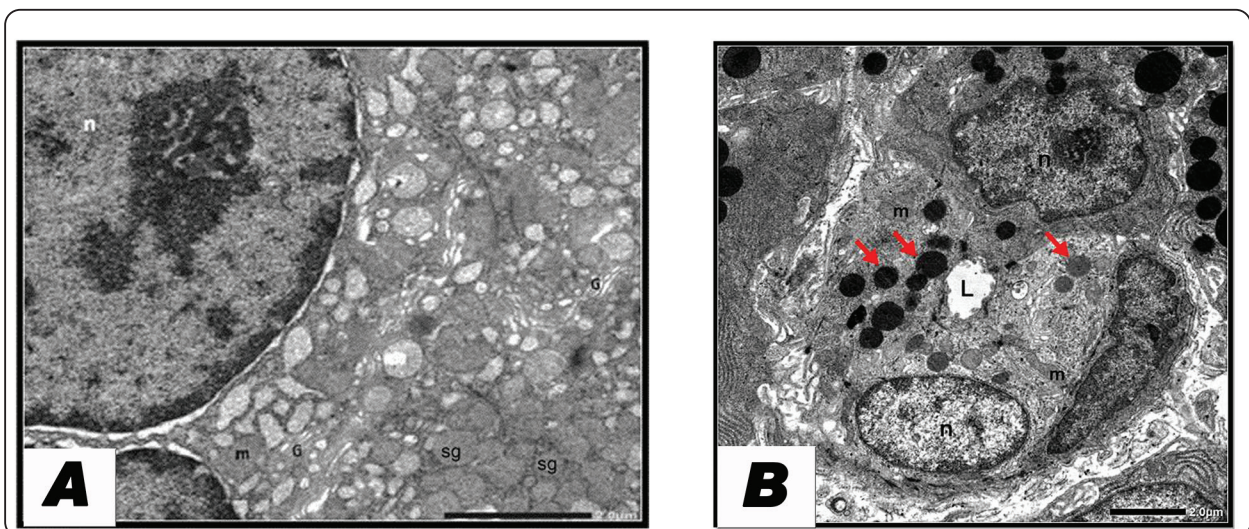


Fig. (2) Transmission electron micrograph (A) [control group] showing normal nucleus (n), abundant amount of secretory granules (sg), well developed golgi complex (G) and numerous mitochondria (m) in the cytoplasm of the secretory serous cell (X 4000). (B) [control group] showing intercalated duct with normal epithelial lining and narrow lumen (L). Normal euchromatic nuclei (n) and some secretory granules (arrows). Note multiple normal mitochondria (m) (X 2000).

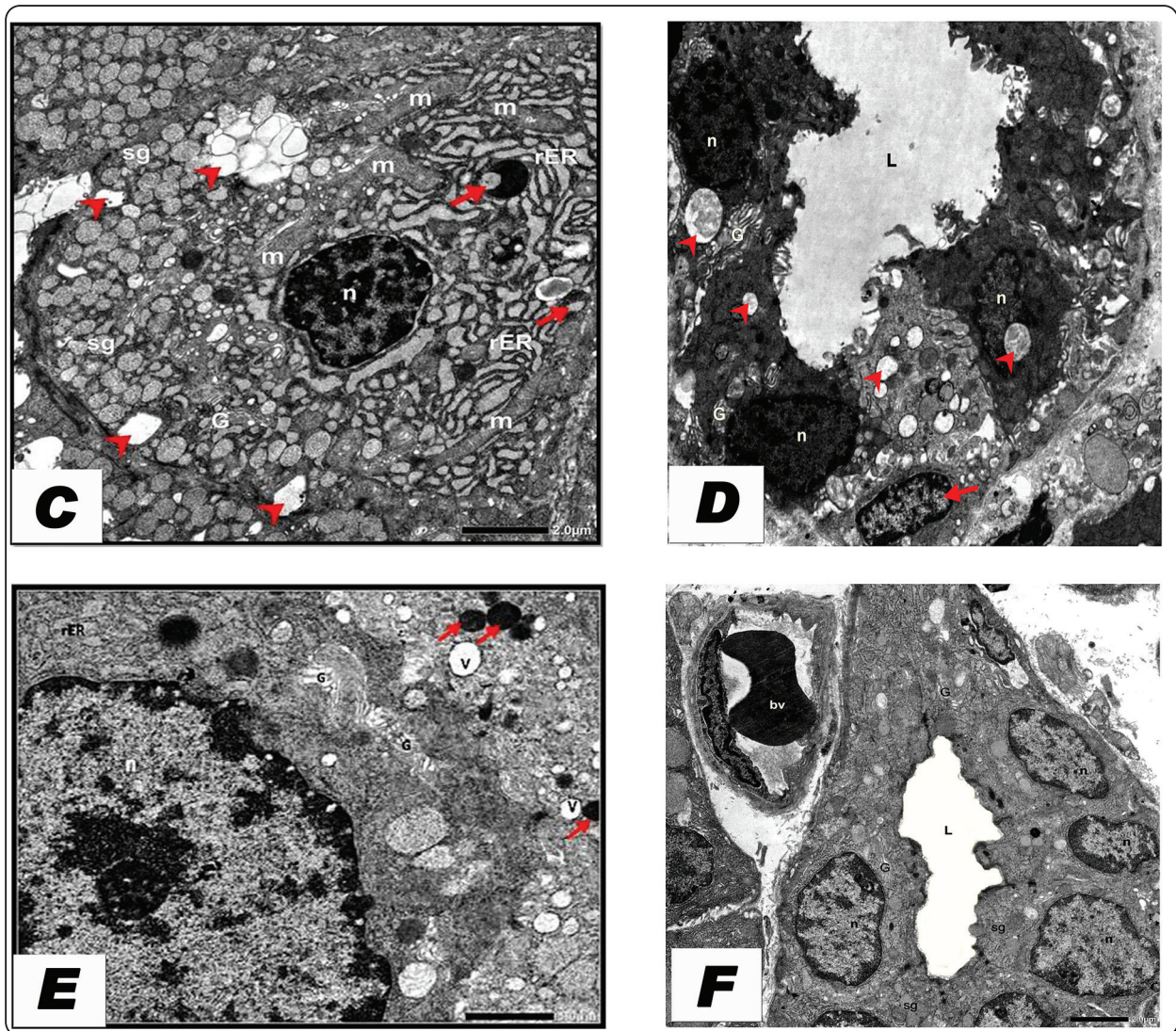


Fig. (2) (C) [hypothyroidism induced group] showing acinar cells with irregular dilatation of the rough endoplasmic reticulum (rER), phagocytosed apoptotic bodies (red arrows), irregular heterochromatic pyknotic nucleus (n), degenerated mitochondria (m), extensive vacuolation of the cytoplasm (arrow heads) (X 3000). (D) [hypothyroidism induced group] showing intercalated duct with degeneration of the epithelial lining together and widening of the lumen (L). Small pyknotic nuclei (n) with multiple cytoplasmic vacuolations (arrow heads) can be seen, together with some widening in the golgi apparatus (G). Note myoepithelial cell (red arrow) (X 2000). (E) [hypothyroidism induced + fish oil group] showing serous acinar cells with normal large regular nucleus and well developed nucleolus (n), slight dilatation of the rough endoplasmic reticulum (rER), well developed Golgi complex (G) and few vacuolation (v) of the cytoplasm. Note some electron dense secretory granules (arrows) (X 5000). (F) [hypothyroidism induced + fish oil group] showing normal epithelial lining of the intercalated duct with normal nuclei (n), well developed golgi complex (G). Some widening in the lumen was seen (L). Note: prominent blood vessel (bv). (X 2000).

## DISCUSSION

Several histological studies have been performed on salivary glands, but few studies have evaluated the effect of hypothyroidism on salivary glands. Thus, the present study was designed to assess the effect of experimentally induced hypothyroidism on the adult rat salivary glands and assess the possible ameliorating effect of fish oil. In the present study, PTU was used to induce hypothyroidism in rats, which was serologically monitored. This drug was used in previous studies to induce an animal model of hypothyroidism<sup>(23)</sup>. It was reported that PTU blocks the production of newly secreted thyroid hormone in the thyroid gland. Moreover, it works peripherally by inhibiting the conversion of T4 to T3. In the present study, blood tests revealed significant decrease in the levels of T3 and T4 in hypothyroid group. This was concomitant with what was mentioned previously which revealed that PTU affected the existing thyroid hormones stored in the thyroid gland or circulating in the blood<sup>(24)</sup>. These findings were also in accordance with Shibutani et al.<sup>(25)</sup>.

Meanwhile treatment with fish oil along with PTU significantly increased T3 and T4 levels compared with those of the group receiving PTU alone. These results were in agreement with a study by Sharif et al.<sup>(26)</sup> that was conducted on rabbits and with that of Shariatifar et al.<sup>(27)</sup> which was conducted on mice. The significant improvement in T3 and T4 hormones showed in the fish oil group in this study, was also supported by the findings of Lachowicz K et al.<sup>(28)</sup>. This previous study found that polyunsaturated n-3 USFAs consumption stimulates the thyroid peroxidase activity. Thus, fish oil administration could improve the TH action. The positive preventive effect of fish oil on hypothyroid state has been explained previously. It has been documented that omega-3 (polyunsaturated fatty acid (PUFA), including EPA) regulates the thyroid cell function via two main pathways: by regulating the signal transduction pathway

through the manipulation of the composition of membrane fatty acid; and rapid, direct modulation of gene transcription<sup>(29)</sup>. Moreover, in related several experiments, in animals, it has been shown that administration of eicosapentaenoic acid was responsible for inhibiting the decrease in plasma T3 and T4 concentrations and increase in plasma TSH concentration in the hypothyroid state.<sup>(30)</sup>

In the current study, the body weight of the rats was measured after the first week of induction and just before euthanization. The body weight statistical analysis showed a significant decrease in the body weight in the PTU induced hypothyroidism group compared to the control group. These results are in coincidence with L. Armada-Dias et al.<sup>(31)</sup>. There is a well-established association of salivary function with common illnesses such as diabetes, oral submucous fibrosis, and asthma. Specifically, salivary flow and volume usually depends on humoral agents including thyroid hormones<sup>(32)</sup>.

Light microscopic results of the current study showed that group B, PTU induced hypothyroidism had the capability to provoke a toxic effect on the cells of the acini as well as the ducts of the gland. These were seen by the presence of different degrees of degeneration affecting most of the cells. The cells of the acini demonstrated, loss of their normal architecture with apoptotic nuclei. The cytoplasm revealed extensive degeneration and vacuolization. The intercalated, secretory striated and excretory ducts showed degeneration in their epithelial lining and expansion of the lumen with loss of the basal striations of the secretory striated ducts. Moreover the secretory striated and excretory ducts were surrounded with connective tissue fibrosis and blood vessels engorged with RBCs in close proximity to the ducts. These results were in agreement with de Jesus et al.<sup>(33)</sup> who reported that hypothyroidism promoted shrinkage and atrophy of the lobular architecture as well as degenerative changes in the duct system of the submandibular salivary glands. These results were also in coincidence with

a study by Mohammed Ali et al. <sup>(34)</sup>, who studied the pancreatic acinar cells of the hypothyroid rats that showed pyknotic nuclei, and vacuolation of the cytoplasm with few secretory granules.

In the current study, these histological changes in the parotid glands may be due to the side effects of hypothyroidism on the metabolic systems within the cell as the serous cells of the parotid gland are specifically affected by hypothyroidism. This could implement the functional relationship between salivary and thyroid glands. Rodriguez et al. <sup>(35)</sup> found that the salivary glands have a high metabolic rate thus, they need a large blood flow to produce saliva and in a hypothyroidism condition, the energy supply commits the composition, synthesis, and secretion of saliva. Furthermore, studies show that thyroid hormones have influence on tissues, because they facilitate DNA transcription resulting in new protein synthesis. It means that in a hypothyroidism, significant changes occur in the structure of salivary glands <sup>(36)</sup>.

Fish oil treated group (C), revealed noticeable preventive effect on the histological structure of salivary glands. Cells of the acini and duct system showed a relatively normal structure compared to the PTU treated group (B). The antioxidant effect of fish oil has a great impact on its beneficial influence on parotid gland <sup>(37)</sup>. According to Usch et al. <sup>(38)</sup> parotid gland responded positively to treatment with antioxidants showing increased amylase activity. The increased  $\alpha$ -amylase activity in parotid gland could be related to adenylate-cyclase activity stimulation, particularly in the groups that received the fish oil supplementation that showed a more expressive increase of amylase activity <sup>(39)</sup>. Also, the anti-inflammatory effect of omega 3 PUFA was due to its inhibitory effect on the proinflammatory mediators such as prostaglandin (PGE2) and leukotriene B4 (LB4), this explains its beneficial preventive effect on histological structure of parotid gland <sup>(39)</sup>.

As an important antioxidant, fish oil

administration in the hyperlipidemic experimental rats has shown a significant improvement in hepatic oxidative stress due to omega 3 fatty acids effect. This has been attributed to the assembly of omega-3 fatty acids in membrane lipids and lipoproteins to make the double bonds less available for free radical attack and stimulation of antioxidant enzymes. Thus, omega-3 found in fish oil has the potentiality to up regulate gene expressions of antioxidant enzymes and down regulate genes associated with production of reactive oxygen species <sup>(40)</sup>. That's why fish oil was chosen in the present study.

In the present study, electron microscopic examination of the parotid glands in group B, demonstrated that PTU induced hypothyroidism have a potential to induce cytotoxic effects on the acinar cells as well as the duct system. These were revealed by the variable degrees of degenerative changes involving most of the cells. The acinar cells showed, apoptotic nuclei with heterogenic chromatin, as well as apoptotic bodies and numerous dilated rough endoplasmic reticulum and degenerated mitochondria. The cytoplasm showed a dramatic degeneration and vacuolization and it contained many disorganized figures of golgi complex. The intercalated ducts had degenerated epithelial lining with altered mitochondria, vacuolization of the cytoplasm and widening of the lumen. These structural changes were consistent with Yang et al. <sup>(41)</sup>, who found similar changes in hippocampus of hypothyroid rats. Abo-Elghait et al. <sup>(42)</sup> explained these results by the direct effect of thyroid hormone at the transcription level by binding to nuclear receptors leading to inhibition of incorporation of labeled amino acids into proteins and decrease in RNA content in the nervous tissue of hypothyroid young animals. Also, Koromilas et al. <sup>(43)</sup> found that thyroid hormones have a vital role in regulating the mitochondrial function in different tissues. Furthermore, mitochondria seemed to participate in hypothyroid-induced apoptotic phenomena that takes place in hippocampal neurons of developing rats. In addition, Bhanja and Chainy <sup>(44)</sup>

illustrated that PTU induced hypothyroidism has an oxidative stress effect in rat cerebellum that resulted in tissue destruction and apoptosis.

In current study, administration of fish oil showed a protective effect against most of the degenerative changes that occurred in the parotid gland. In fish oil treated group, significant improvement was obviously noticed in the different cell organelles in addition to increased vascularity. These results coincided with previous studies that showed that omega 3 fatty acids improved the blood flow and consequently cell metabolism. Also, these results were interpreted further by the protective effect of fish oil to the cells from damage by inhibiting the release of arachidonic acid from the cell membrane<sup>(45)</sup>.

## CONCLUSION

Fish oil plays a critical role in prophylaxis against the toxic effect of hypothyroidism on parotid glands. Thus, it should be considered as an acceptable approach for most patients with risk of hypothyroidism.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## FUNDING

No specific funding was received for this work.

## RECOMMENDATIONS

According to the results of the present study we can recommend:

1. The use of fish oil as prophylactic agent to avoid the toxic effects of hypothyroidism on the parotid salivary glands.
2. Further histological studies could be performed using different doses of fish oil with longer experimental period.

3. More studies should be conducted on the effect of hypothyroidism on other oral tissues.

## REFERENCE

1. Hassan IZ. Effect of experimentally induced hypothyroidism on the parotid gland of adult male albino rats and the possible role of thyroid hormone supplementation. *Br J Sci.* 2016;14:1.
2. Hwang JH, Jung HW, Kang SY, Kang AN, Ma JN, Meng XL, et al. Therapeutic effects of acupuncture with MOK, a polyherbal medicine, on PTU-induced hypothyroidism in rats. *Exp Ther Med.* 2018;16:310-20.
3. Hashem H, Saad S. Comparative study of the effect of experimentally induced hyperthyroidism and hypothyroidism on the parotid gland in adult male albino rats. *Egypt J Histol.* 2020;43:791-807.
4. Rashad NM, Samir GM. Prevalence, risks, and comorbidity of thyroid dysfunction: a cross-sectional epidemiological study. *Egypt J Intern Med.* 2019;31:635-41.
5. Hayat NQ. Effect of hypothyroidism on the histology of sublingual salivary gland in adult albino rats. *J Rawalpindi Med Coll.* 2017;21:395-9.
6. Alkalby JM, Alzerjawi SJ. Effect of propylthiouracil-induced hypothyroidism on reproductive efficiency of adult male rats. *Bas J Vet Res.* 2013;12:113-21.
7. Singh S, Panda V, S S, Dande P. Protective effect of a polyherbal bioactive fraction in propylthiouracil-induced thyroid toxicity in rats by modulation of the hypothalamic-pituitary-thyroid and hypothalamic-pituitary-adrenal axes. *Toxicol Rep.* 2020;7:730-42.
8. Jung JH, Lee CH, Son SH, Jeong JH, Jeong SY, Lee SW, et al. High Prevalence of Thyroid Disease and Role of Salivary Gland Scintigraphy in Patients with Xerostomia. *Nucl Med Mol Imaging.* 2017;51:169-77.
9. Naik MM, Vassandacoumara V. Qualitative and quantitative salivary changes and subjective oral dryness among patients with thyroid dysfunction. *Indian J Dent Res.* 2018;29:16-21.
10. De Paula F, Teshima THN, Hsieh R, Souza MM, Nico MMS, Lourenco SV. Overview of Human Salivary Glands: Highlights of Morphology and Developing Processes. *Anat Rec (Hoboken).* 2017;300:1180-8.
11. Leite MF, Lima AM, Kang SJ, Santos MT, Otton R. Effect of astaxanthin and fish oil on enzymatic antioxidant system and  $\alpha$ -amylase activity of salivary glands from rats. *Braz J Oral Sci.* 2014;13:58-63.

12. Oppedisano F, Macrì R, Gliozzi M, Musolino V, Carresi C, Maiuolo J, et al. The Anti-Inflammatory and Antioxidant Properties of n-3 PUFAs: Their Role in Cardiovascular Protection. *Biomedicines*. 2020;8:306.
13. Souza LL, Nunes MO, Paula GS, Cordeiro A, Penha-Pinto V, Neto JF, et al. Effects of dietary fish oil on thyroid hormone signaling in the liver. *J Nutr Biochem*. 2010;21:935-40.
14. Abd Allah ES, Gomaa AM, Sayed MM. The effect of omega-3 on cognition in hypothyroid adult male rats. *Acta Physiol Hung*. 2014;101:362-76.
15. Grimes DA, Schulz KF. Determining sample size and power in clinical trials: the forgotten essential. *Semin Reprod Endocrinol*. 1996;14:125-31.
16. Alkalby JM, Alzerjawi SJ. Effect of propylthiouracil-induced hypothyroidism on reproductive efficiency of adult male rats. *Bas J Vet Res*. 2013;12:113-21.
17. Hwang JH, Jung HW, Kang SY, Kang AN, Ma JN, Meng XL, et al. Therapeutic effects of acupuncture with MOK, a polyherbal medicine, on PTU-induced hypothyroidism in rats. *Exp Ther Med*. 2018;16:310-20.
18. Raghu Nadhanan RR, Skinner J, Chung R, Su YW, Howe PR, Xian CJ. Supplementation with fish oil and genistein, individually or in combination, protects bone against the adverse effects of methotrexate chemotherapy in rats. *PLoS One*. 2013;12:e71592.
19. Mark AS, Steven HW, Craig LF. *The laboratory rat*. 2<sup>nd</sup> ed. Amsterdam, Boston: El Sevier; 2006. p. 129.
20. Taatjes DT, Mossman BT. *Cell imaging techniques: methods and protocols*. Totowa, New Jersey: Human Press; 2006. pp. 216-9.
21. Woods A, Stirling J. *Electron Microscopy: The preparative techniques*. In: Bancroft J, Gamble M, (eds). *Theory and Practice of Histological Techniques*. 5<sup>th</sup>ed. New York, Edinburgh, London: Churchill Livingstone; 2002. pp. 682-700.
22. Kirkpatrick LA, Feeney BC. *A simple guide to IBM SPSS statistics for version 20.0*. Student ed. Belmont, Calif.: Wadsworth, Cengage Learning; 2013.
23. Faddladdeen K, Ali SS, Bahshwan S, Ayuob N. Thymoquinone preserves pancreatic islets structure through up-regulation of pancreatic  $\beta$ -catenin in hypothyroid rats. *Diabetes Metab Syndr Obes*. 2021;14:2913-24.
24. *Drugs and Lactation Database (Lactmed)*. Bethesda (MD): National Library of Medicine (US); 2006.
25. Shibusaki M, Woo GH, Fujimoto H, Saegusa Y, Takahashi M, Inoue K, et al. Assessment of developmental effects of hypothyroidism in rats from in utero and lactation exposure to anti-thyroid agents. *Reprod Toxicol*. 2009;28:297-307.
26. Sharif SH, Elmahdi BM, Ali Mohammed AM, Mohammed AH. The effects of *Nigella sativa* L. ethanolic extract on thyroid function in normal and alloxan induced diabetic rats. *Thyroid Res Pract*. 2012;9:48-54.
27. Shariatifar A, Riazi M, Ebnolelm M, Jahromy MH. Effects of *Nigella sativa* L. seed extract on fatigue, blood biochemical parameters and thyroid function in male mice. *Chinese Med*. 2014;5:16-21.
28. Lachowicz K, Koszela-Piotrowska I, Rosolowska-Huszez D. Thyroid hormone metabolism may depend on dietary fat. *J Anim Feed Sci*. 2008;17:110-9.
29. Qazzaz ME, Alfahad M, Alassaf FA, Abed MN, Jasim MH, Thanoon IA. Effects of omega-3 on thyroid function tests in healthy volunteers. *Sys Rev Pharm*. 2020;11:10-4.
30. Makino M, Oda N, Miura N, Imamura S, Yamamoto K, Kato T, et al. Effect of eicosapentaenoic acid ethyl ester on hypothyroid function. *J Endocrinol*. 2001;171:259-65.
31. Armada-Dias L, Carvalho JJ, Breitenbach MM, Franci CR, Moura EG. Is the infertility in hypothyroidism mainly due to ovarian or pituitary functional changes?. *Braz J Med Biol Res*. 2001;34:1209-15.
32. Naik MM, Vassandacoumara V. Qualitative and quantitative salivary changes and subjective oral dryness among patients with thyroid dysfunction. *Indian J Dent Res*. 2018;29:16-21.
33. De Jesus VC, Beanes G, Paraguassú GM, Ramalho LM, Pinheiro AL, Ramalho MJ, et al. Influence of laser photobiomodulation (GaAlAs) on salivary flow rate and histomorphometry of the submandibular glands of hypothyroid rats. *Lasers Med Sci*. 2015;30:1275-80.
34. Mohammed Ali MH, Saleh HA, Farghaly LM. Role of thyroxin versus Brewer's yeast supplementation in amelioration of pancreatic alterations induced by hypothyroidism in adult male albino rats. *Egypt J Histol*. 2013;36:918-30.
35. Rodriguez TT, Dantas VTA, Ramalho MJP. Participation of nitric oxide synthase and cyclooxygenase-2 in the salivary secretion of hypothyroid endotoxemic rats. *Rev Odonto Ciênc*. 2009;24:383-8.
36. De Jesus VC, Beanes G, Paraguassú GM, Ramalho LM, Pinheiro AL, Ramalho MJ, et al. Influence of laser photobiomodulation (GaAlAs) on salivary flow rate and histomorphometry of the submandibular glands of hypothyroid rats. *Lasers Med Sci*. 2015;30:1275-80.

37. Leite MF, de Lima AM, Sun Kang SJ, dos Santos MT, Otton R. Effect of astaxanthin and fish oil on enzymatic antioxidant system and  $\alpha$ -amylase activity of salivary glands from rats. *Braz J Oral Sci.* 2014;13:58-63.
38. Usch L, Sterin-Borda L, Borda E. Difference in the regulatory mechanism of amylase release by rat parotid and submandibular glands. *Arch Oral Biol.* 2002;47:717-22.
39. James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. *Am J Clin Nutr.* 2000;71:343-8.
40. Yang H, Zha X, Cai Y, Wang F, Wu Z, Wu B, et al. Impacts of thyroxine combined with donepezil on hippocampal ultrastructures and expressions of synaptotagmin-1 and SNAP-25 in adult rats with hypothyroidism. *Int J ClinExp Med.* 2015;8:17922-31.
41. Abdel-moneim AD, Mahmoud B, Mahmoud R. The beneficial effects of fish oil supplementation on hyperlipidemic and hypothyroid albino rats. *Asian J Pharm Clin Res.* 2015;8:328-33.
42. Abou-Elghait AT, Rateb A, Mahmoud FY, Galal O. Effect of experimentally induced hypothyroidism during pregnancy and lactation on the retina of juvenile and adult albino rats and the possible role of thyroid hormone supplementation. *Egypt J Histol.* 2011;34:28-45.
43. Koromilas C, Liapi C, Schulpis KH, Kalafatakis K, Zarros A, Tsakiris S. Structural and functional alterations in the hippocampus due to hypothyroidism. *Metab Brain Dis.* 2010;25:339-54.
44. Bhanja S, Chainy GBN. PTU-induced hypothyroidism modulates antioxidant defence status in the developing cerebellum. *Int J Dev Neurosci.* 2010;28:251-62.
45. Ajayer A, El-Baz D, Bashir M. Impacts of omega-3 fatty acids on the age related changes in the submandibular salivary glands of albino old rats. *Nat Sci.* 2015;13:22-8.