

Effects of Experimentally Induced Hyperthyroidism on Rat Tongue Mucosa: Histological and Ultrastructural Study

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Abstract: Background: Hyperthyroidism has been associated with a variety of abnormalities in different tissues of the body. There are limited data for the effect of hyperthyroidism on the histological and ultrastructure of tongue mucosa. **Aim:** To focus on the histological, immunohistochemical and ultrastructure changes of tongue mucosa as a consequence of experimentally induced hyperthyroidism. **Material and Methods:** Rats were divided into two equal groups; the control and hyperthyroid group. Thyroxin was administered to hyperthyroid group orally at a dose of 100 µg/kg for three weeks. Rats' tongue was processed for histological, immunohistochemical and ultrastructural assessment. **Results:** The tongue epithelium demonstrated histological changes such as detachment of keratin layer, irregular and distorted tongue papillae in some areas. The basal cell layer showed loss of polarization and sporadic apoptotic nuclei. The nuclei of spinous cells exhibited chromatin margination imparting "Orphan-Annie" appearance. The granular cells layer appeared to be enlarged with washed-out cytoplasm mimicking hydropic degeneration. Caspase-3 expression in the tongue epithelium of hyperthyroid group was increased. Ultrastructurally, there were marked alterations of the cellular components, nuclei and cell junctions. Disrupted desmosomes, irregular nuclei, chromatin margination of basal cells, fragmented nuclei of the spinous cells and decreased aggregation of tonofilaments and keratohyaline granules of the granular cells were particularly detected. **Conclusion:** Experimental hyperthyroidism induced several degenerative changes of tongue mucous membrane and the taste buds in rats. [Gihan S Hassan and Dalia H Zahran. **Effects of Experimentally Induced Hyperthyroidism on Rat Tongue Mucosa: Histological and Ultrastructural Study.** *Life Sci J* 2019;16(1):127-134]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). <http://www.lifesciencesite.com>. 16. doi:10.7537/marslsj160119.16.

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1. Introduction:

Thyroid hormones (THs) have wide range of effects on multiorgan systems of the human body. They are responsible for control of normal functions of nearly all tissues with prominent effects on thermogenesis, lipogenesis and oxygen consumption (1). Thyroid disorders are among the most common metabolic disorders worldwide. Furthermore, dietary iodine deficiency, which is a major determinant of thyroid pathology remains a public health problem in developing countries(2). It has been confirmed that THs have initiated their action via binding to specific thyroid hormones receptors (TR). Two major (TR) isoforms have been identified, TR α and TR β , and they mediate the biologic actions of THs via transcriptional regulation (2). Previous, studies have strongly suggested the existence and expression of TR in different human tissues including brain, liver, kidney, pancreas, heart, lung, placenta and skeletal muscle (3).

Hyperthyroidism, or thyrotoxicosis, is a clinical condition that occurs due to over secretion of (THs) in blood, mainly triiodothyronine (T3) and Thyroxin (T4). It is characterized by significant increase in metabolic rate that causes sweating, weight loss, increased appetite, tachycardia and irritability(4). Several investigations have reported that hyperthyroidism has many adverse effects on different

organs and systems. Hyperthyroidism induced changes in reproductive system such as changes of Luteinizing hormone (LH) and Follicle Stimulating Hormone (FSH) levels and also changes in steroids ratio in humans and animals(5,6). It has also been reported to induce degenerative changes in rats' adrenal cortex (7). Experimental thyrotoxicosis adversely affected the hepatocytes in rats as evidenced ultrastructurally. The resultant significant damage to the plasma membrane and intracellular components of hepatocytes and endothelial cells had a consequent adverse effect on liver functions(8).

Caspases (cysteinyln aspartate-specific proteases) are a family of important signaling molecules that comprises two distinct classes, the initiators and the effectors. The activation and inhibition of these classes are differentially regulated, even though they have general structural features. Activation of a series of caspases is a marker for cellular apoptosis (9). Caspase 3 is an effector caspase that is considered an important marker for the entry of cells into apoptotic signaling pathway. This protease is activated by the upstream caspase-8 and caspase-9 and therefore is considered as a novel marker for apoptosis (10)

The tongue is an anatomical organ that reflects the healthy status of the body. A number of systemic and local factors have been described to affect the

structure of tongue mucosa including medications, chemicals and hormonal disturbances (11–13). Clinically, patients with thyroid dysfunction suffered from burning mouth and/or metallic taste sensation (14,15). Also, an association between thyroid dysfunction and a significant decrease in salivary parameters such as flow rate and buffering capacity has been confirmed (16). Up to our knowledge, there are limited data about the effects of hyperthyroidism on the histological composition of different oral tissues, as most of the literature have concentrated on the clinical oral manifestations of thyroid dysfunction. We hypothesized that THs could have crucial effects on the structure of mucous membrane of the tongue. Therefore, the objective of the present work was to assess the histological, immunohistochemical and ultrastructural changes of the tongue mucosa of Albino rats as a consequence of experimentally induced hyper-thyroidism.

2. Material and Methods:

Animals:

The experiment was planned to be conducted on twenty adult male albino rats weighing approximately (200–240 gm). The animals were maintained under standard laboratory conditions at the Pharmacology Department, Faculty of Medicine, Tanta University. They were kept in metallic cages for three days acclimated period and had free access to standard rat diet and water *ad libitum*. All animal procedures were in accordance with the Guidelines for Care and Use of Laboratory Animals published by the Research Ethics Committee, Faculty of Dentistry, Tanta University. The experimental protocol was approved by the Research Ethics Committee, Faculty of Dentistry, Tanta university.

Experimental Protocol:

The rats were divided randomly into two equal groups: Group I (Control Group) received no treatment, while Group II (Hyperthyroid Group) received daily oral administration of thyroxin (T4) tablets (Eltroxin tablets, Glaxo Wellcome, Germany) dissolved in distilled water. The dose was 100 µg/kg for three weeks to induce hyperthyroidism(17).

Histological and Immunohistochemical studies

At the end of experimental period, rats were sacrificed under mild diethyl ether anesthesia. The tongues were dissected, excised and divided into halves. One half of each tongue was immediately fixed in 10% formaldehydes solution for 24 h and processed for routine technique of paraffin inclusion. Histological sections of 4 µm thickness were cut and stained with the Hematoxylin and Eosin (H & E) stain and examined under the Light Microscope (LM). Immunohistochemical staining was carried out to detect the expression of Caspase-3 as a marker for

apoptosis. The paraffin sections were cut into 4 µm thickness on positively charged slides. Sections were dewaxed and rehydrated through graded alcohol to distilled water. For antigen retrieval, sections were subjected to boiling for 10 min in 10 mmol/L citrate buffer (pH 6) in a microwave. Sections were then left to cool at room temperature for 20 min, then incubated with primary polyclonal antibodies to caspase-3 (Wuhan boster Biotechnology Co. LTD) at a dilution of 1:100 and incubated overnight at 4°C. Thereafter, slides were rinsed three times in TBS for 10 min each and incubated again with secondary antibody (En Vision detection Kit Peroxidase/DAB Rabbit/mouse, Dako Cytomation) according to the manufacturer's instructions, followed by rinsing three times in TBS for 10 min each. Sections were stained with 3,3'-diaminobenzidinetetrahydro-chloride (En Vision detection Kit Peroxidase/DAB Rabbit/mouse, Dako Cytomation) and counter-stained with Mayer's hematoxylin solution. Replacing the primary antibody by non-immune normal rabbit or mouse sera in one slide served as a negative control to assess non-specific staining.

Transmission Electron Microscopic (TEM) study

The other half of each dissected tongue was immediately cut into strips of 2 mm thickness and fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer adjusted to pH 7.4 followed by washing in 5% sucrose in 0.1 M cacodylate buffer. The specimens were then dehydrated using a graded series of alcohol followed by two washes in acetone. Then they were dried, mounted on stubs and coated with gold using a sputter coater. The processed specimens were analyzed at 25 kV accelerating voltage by scanning electron microscope (JSM 5600LV, Jeol, Tokyo, Japan) in Electron Microscopic Unit of Faculty of Medicine, Tanta University. Rat's body weight and food and water consumption were recorded at the beginning and in the end of experimental period.

3. Results:

Body weights and food and water intake:

At the end of experimental period, the body weights of rats on both groups were increased, however the weight gain of the hyperthyroid animals was less than the control. This was also associated with an increase in food and water consumption of the hyperthyroid rats. In addition, hyperthyroid rats showed signs of thyrotoxicosis such as irritability, hair loss, enlargement of thyroid gland causing swelling in their necks. The control group showed normal growth and activities.

Light Microscopic results

Normal control tongue tissue showed numerous, regularly-distributed tongue papillae covered with keratinized stratified squamous epithelium. The

epithelium comprised four layers: basal, spinous, granular, and corneum (Figure 1A). The basal cell layer consisted of cuboidal to low-columnar cells, vertically oriented on the basement membrane with rounded to ovoid, hyperchromatic nuclei. Spinous cell layer exhibited several rows of irregular, polygonal cells which gradually became flattened as they

approached the granular cell layer. The latter was formed of rows of cells that exhibited large, vesicular nuclei and basophilic, rounded, cytoplasmic keratohyalin granules. The underlying lamina propria was well vascularized and appeared to merge with subjacent tongue muscles without a clear line of demarcation (Figure 1B, C).

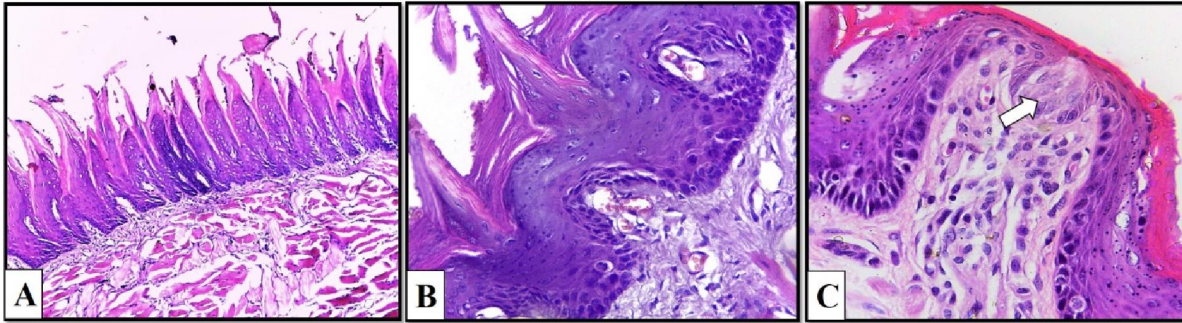


Figure (1): Photomicrograph of the dorsal surface of normal rat tongue illustrates; **A**: Numerous regularly oriented lingual papillae covered by keratinized stratified squamous epithelium. The underlying lamina propria has dense collagen fibers. The lingual muscle fibers run in different directions. **B**: Higher magnification of normal rat tongue papillae showing regular arrangement of basal cell layer, polygonal spinous cells, granular cells and superficial keratin layer. **C**: Fungiform papilla with Keratinized epithelial covering and intraepithelial taste bud on its upper surface (arrow). (H & E, original magnification A $\times 100$, B, C $\times 400$).

In the hyperthyroid group, tongue exhibited irregularly oriented filiform papillae and distorted fungiform papillae in some areas. Keratin layer was generally thin and detached from underlying epithelium and the epithelial ridges were few and blunt compared to the control (Figure 2A & B). The basal cell layer showed loss of their characteristic polarization in some areas and sporadic shrunken, apoptotic bodies in another (Figure 2B). The fungiform and circumvallate papillae were atrophied with very thin keratin layer on their surface and degenerated taste buds (Figure 2C & 3A & B). The most noticeable changes were observed in the nuclei of spinous and granular cell layers. The nuclei exhibited enlarged, round to ovoid outlines and

nuclear clearing due to peripheral margination of chromatin. In some cells, chromatin margination was complete imparting "Orphan-Annie" appearance of the nuclei. Other cells showed remnants of nuclear chromatin material with few residual nucleoli (Figure 3C). The blood vessels of the lamina propria were dilated with proliferating plump endothelial cells and homogenous eosinophilic substance. In superficial parts of the epithelium, granular cells layer appeared to be enlarged with washed-out cytoplasm mimicking hydropic degeneration. The underlying lamina propria showed proliferation of plump endothelial cells forming numerous, scattered, newly-formed blood capillaries, some of which were engorged with RBCs (Figure 2).

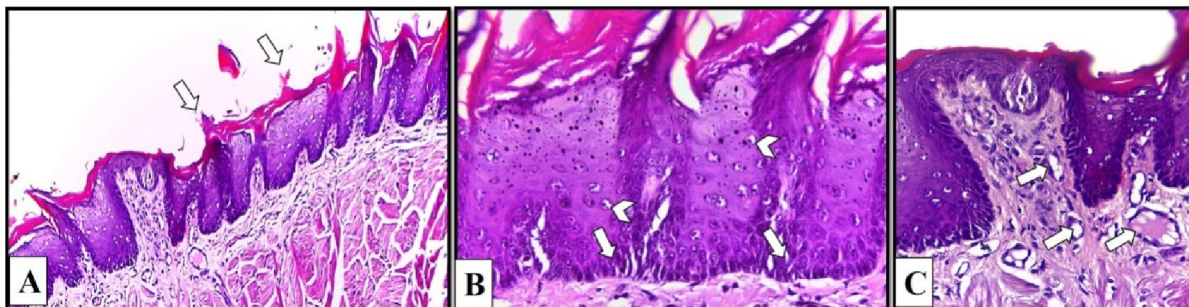


Figure (2): Photomicrograph of Hyperthyroid group illustrates; **A**: ill formed filiform papillae of the dorsal tongue. The keratin layer is disfigured and detached from the underlying epithelium (arrows). The epithelial ridges were few and blunt. **B**: Higher magnification shows loss of polarization of epithelial cells (arrows) and shrunken nuclei. The nuclei of the spinous cells show remnants of chromatin material with residual nucleoli (arrow heads). **C**: Atrophied Fungiform papilla with very thin keratin layer and degenerated intraepithelial taste bud. The CT of the lamina propria shows numerous, dilated capillaries with proliferating, plump endothelial cells (arrows). Notice, the dilated blood vessel of the lamina propria with homogenous eosinophilic material. (H & E, original magnification A $\times 100$; B, C $\times 400$).

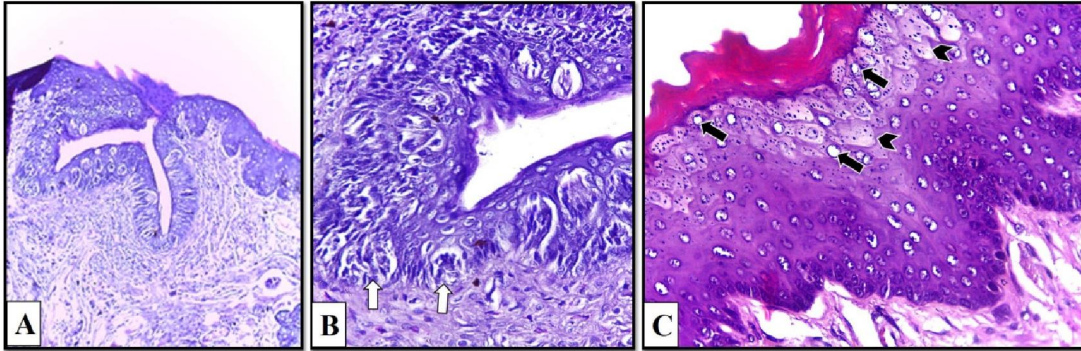


Figure (3): Photomicrograph of the circumvallate papilla of the hyperthyroid group illustrates; **A:** Atrophy of circumvallate papillae with distorted morphology. **B:** Higher magnification shows numerous taste buds present in the trench of circumvallate papilla (arrow), their cells are degenerated as well as the adjacent epithelial cells lining the trench. **C:** Ventral surface of the tongue showing numerous spinous cells with signs of vacuolar degeneration and nuclear changes of the altered spinous cells. Chromatin margination with nuclear clearing is observed imparting the so-called “orphan Annie eye” nuclei (arrows). The basal cell layer is inconspicuous in many areas. Granular cell layers are significantly swollen and exhibits a washed-out cytoplasm mimicking cells in hydropic degeneration (arrow heads). The lamina propria shows numerous vascular slits. (H & E, original magnification A $\times 100$; B, C $\times 400$).

Immunohistochemical results

In normal control tissue, most cells of all epithelial layers exhibited negative nuclear staining of caspase-3 except for few sporadic cells residing mainly in basal and parabasal region. Focal areas of

the spinous cell layer showed positive cytoplasmic staining (Figure 4).

The hyperthyroid group revealed positive immune reactions in the nuclei of epithelial cells in different layers. Cytoplasmic staining was minimally observed (Figure 5).

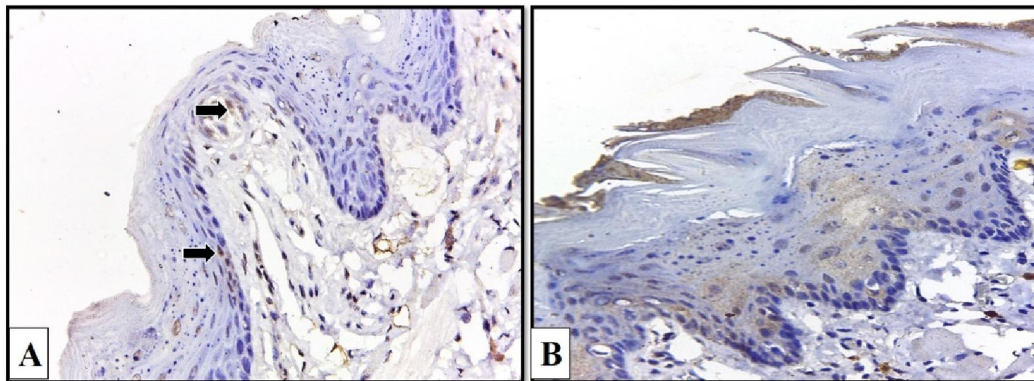


Figure (4): Photomicrograph of normal dorsal tongue epithelium illustrates; **A:** sporadic nuclear expression of caspase-3 (arrows). **B:** Focal areas in spinous and granular cell layers show faint cytoplasmic immunohistochemical expression (ABC, original magnification $\times 400$).

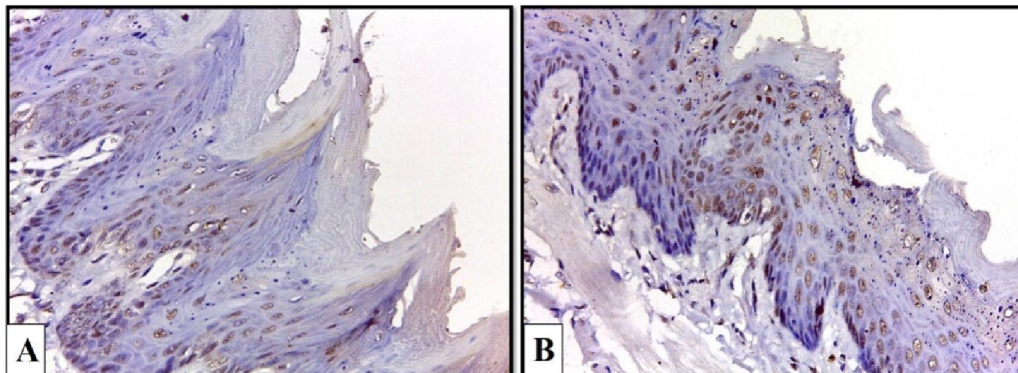


Figure (5): Photomicrograph of Hyperthyroid tongue epithelium illustrates; **A:** prominent nuclear immunostaining of caspase-3. **B:** Strong nuclear expression of caspase-3 is noticed within epithelial cells in almost all layers of tongue epithelium (ABC, original magnification $\times 400$).

TEM results

The ultrastructural observations of the normal tongue epithelium of the control group showed the basal cells had an oval nucleus and the usual cell organelles. The nucleus revealed normal chromatin distribution and regular nuclear membrane. Hemidesmosomes were present in contact with the basement membrane (Figure 6A). The spinous cells were polygonal and had oval nuclei. The lateral cell membranes were connected by desmosomes (Figure 6B). The granular cells were flattened and had keratohyaline granules (Figure 6C). The hyperthyroid tongue epithelium demonstrated various

ultrastructural abnormalities of the three epithelial cell layers. The basal cells presented obvious nuclear changes such as nuclear irregularities, invagination and chromatin margination. Mitochondria were round and swollen, whereas the hemidesmosomes were irregular and disrupted (Figure 7A). Nuclear fragmentation of the spinous cells was prominent as well as multiple cytoplasmic vacuolization and disrupted intercellular junctions (Figure 7B). The granular cells were characterized by decreased aggregation of tonofilaments and keratohyaline granules (Figure 7C).

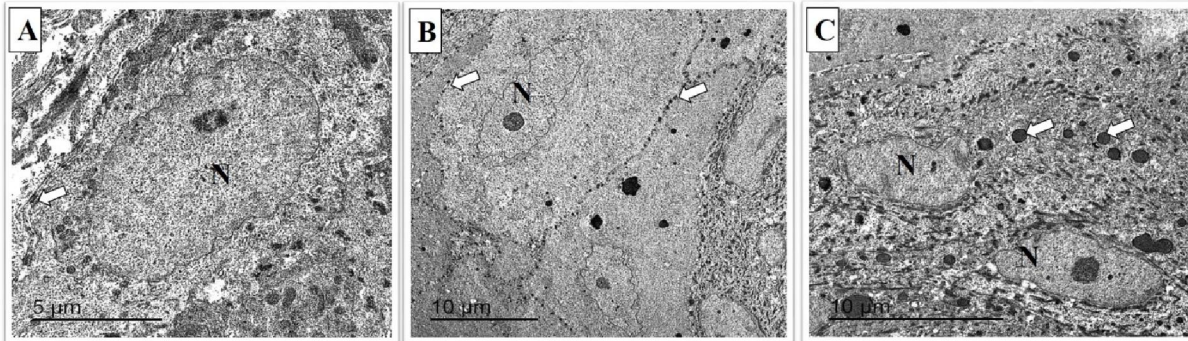


Figure (6): Electron micrograph of normal tongue epithelium of the control group illustrates; **A**: The basal cell having an oval nucleus (N) with normal chromatin distribution, prominent nucleolus, regular nuclear membrane and the usual cell organelles. Arrow shows hemidesmosomes in contact with the basement membrane. **B**: Spinous cells that are polygonal in shape with oval nucleus and prominent nucleolus. Adjacent cells are connected to each other via desmosomes (arrow). **C**: Flattened cells of the granular cell layer with oval nuclei (N) and keratohyalin granules (arrow). (A $\times 1200$ scale bar= 5 μm ; B $\times 500$ scale bar= 10 μm ; C $\times 800$ scale bar= 10 μm)

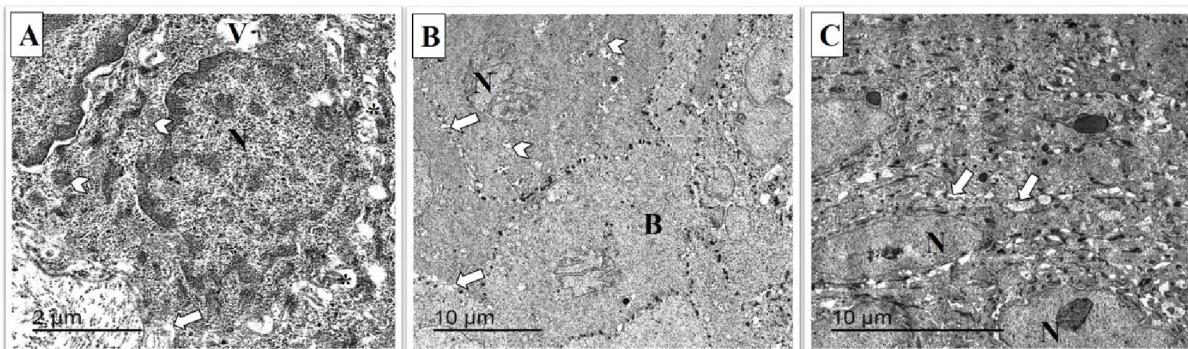


Figure (7): Electron micrograph of hyperthyroid tongue epithelium illustrates; **A**: The basal cells are irregular in shape and having irregular deformed nuclei (N) with chromatin margination. Multiple swollen mitochondria (arrow heads) and intracytoplasmic vacuoles (V) are seen. Intercellular junctions are deformed and the cells are widely separated (*). The basal lamina and hemidesmosomes appear irregular (arrow). **B**: Nuclear fragmentation of the spinous cells (N) with deformed intercellular junctions (arrows). Intracytoplasmic vacuoles are present (arrow head). **C**: Granular cell layers with decreased aggregation of tonofilaments and keratohyaline granules. Notice, the widened intercellular spaces (arrow). (A $\times 2500$ scale bar= 2 μm ; B $\times 500$ scale bar= 10 μm ; C $\times 800$ scale bar= 10 μm)

4. Discussion:

Thyroid gland is one of the most important glands that affects different tissues by its secreted

hormones. In view of the present study, it was evident that hyperthyroidism induced marked degenerative changes in the tongue mucosa of Albino rats.

Although hyperthyroidism has been associated with decreased body weight (18–20), this was not present in our study in spite of the observed decreased weight gain of the hyperthyroid rats compared to the control group. This could be explained by the short induction period of hyperthyroidism in this study, while the changes in the body weight of animals with thyroid dysfunction are usually associated with long induction period of the dysfunction. Previous reports explained the decreased body weight that occurred in hyperthyroid models by the reported effects of TH on growth of the body and organ development (19). Also, hyperthyroidism was associated with increased metabolic activities in the body, as well as decreased serum cholesterol level and increased lipid peroxidation level (20,21).

At the light microscopic level, examination of the tongue mucosa of hyperthyroid rats revealed various degenerative changes that were in accordance to previous reports conducted on different tissues of Albino rats as a result of increased level of THs and hyperthyroidism (7,22). An interesting finding of our study is the observation of nuclear clearing due to chromatin margination in the epithelial cells of hyperthyroid tongue. These so-called “Orphan-Annie eye”-like nuclei are closely reminiscent to the changes seen in cases of thyrotoxicosis as well as papillary thyroid carcinoma (23). These changes could be explained by the notion that one of the thyroid hormone receptors namely the TR α 1 receptor protein was consistently expressed in mitochondria and nuclei of rat tongue muscles (24). In our results, the striking degeneration of tongue taste buds were clearly evident. This observation is worth mentioning, given the previously reported effect of THs on the maturation and specialization of tongue papillae and taste perception. Clinically, an altered taste perception was documented in patients with thyroid dysfunction (15). The presence of engorged blood vessels of the lamina propria observed here was in agreement with same finding in rats’ ovarian stroma and the renal cortex (7,22). This vascular congestion might be attributed to the reported effect of the drug used in our study, Eltroxin, in producing direct load on the cardiovascular system (25).

Apoptosis plays an important role in different pathologic conditions and patients with thyrotoxicosis have acquired a morphological changes in their livers that was attributed to necrotic cell death (26). Previous studies have shown that immunohistochemical detection of Caspase-3 is a reliable method to identify apoptosis even before any morphological cellular changes of apoptosis occur (27). In the current study, apoptosis of the tongue epithelial cells has been investigated by detection of caspase-3 activation. Caspase-3 is one of the crucial

executional caspases that are responsible for cleaving various intracellular compartments in the context of apoptosis (28). The nuclear transport of active caspase-3 is considered a vital step for initiation of apoptosis (29). In our study, normal tongue tissue showed positive caspase-3 expression in the cytoplasm of some cells which best conforms to its pre-activated cytoplasmic form that characterizes cells not undergoing apoptosis. Furthermore, the sporadic nuclear staining in some basal and parabasal cells of normal tongue papillae coincides with the normal rate of physiologic remodeling in these layers (28). A significant increase in the activity of caspase-3 has been found in hyperthyroid group. This finding depicted the observation of a previous report suggested that THs act directly on mitochondria in rats hepatocytes, inducing liver dysfunction through alteration of mitochondrial structure and induction of a release of proteins that activates downstream of the caspase cascade, which eventually, leads to apoptosis (30).

At the ultrastructural level, a significant damage to the epithelial cells of tongue mucosa was present as evidenced by the presence of multiple cytoplasmic vacuoles, swollen mitochondria as well as disrupted hemidesmosomes and intercellular junctions. The most prominent effect was the nuclear changes of the epithelial cells such as chromatin margination, nuclear fragmentation and irregularities. These observations were consistent with a previous work that confirmed the destructive effects of increased THs levels on the plasma membrane and intracellular membrane organelles of rats’ hepatocytes which had a marked adverse effect on liver function (8,30). It was also concluded that the resultant destructive effects were aggravated depending on the duration of hyperthyroid state (8).

It is worth mentioning that the destructive changes of tongue mucosa of hyperthyroid animals observed in our study are not specific and could be identified due to wide range of different pathologic conditions of tongue mucosa e.g. chemicals as ethanol (12), chromium (11) and nicotine toxicity (31); drugs as Carbimazole (32) or hormonal disturbances (13). A common mechanism that explained such pathologic changes include the activation of lipid peroxidation and induction of oxidative stresses in different tissues and organs. It has been reported that THs play a significant role in ROS production since they have the capacity to accelerate the basal metabolic rate and change the respiratory rate in mitochondria (33). Experimentally, the elevated levels of THs or hyperthyroidism were involved in the regulation of oxidative mechanisms and induced cellular oxidative stress in neural tissues, uterus, testes and liver (19,30,33,34). Oxidative stress has been

linked with a thyroxin dependent increase in the activity of mitochondria and a concurrent electron leakage from the mitochondrial electron transport chain(35). Also, a correlation between hyperthyroidism and deterioration of free radicals and reduction of antioxidant system that activate lipid peroxidation and oxidative stresses has been reported (20,36). Destruction of cellular membrane can lead to cell death and to production of toxic and reactive aldehyde free radical metabolites (37). Thyroid dysfunction has been reported to induce lipid peroxidation in different tissues including the liver, kidneys, heart, uterus and testes(19,37).

The findings in the present study suggest that hyperthyroidism might have significant influences on the epithelium of tongue mucosa via morphologic changes in the epithelium of lingual papillae and taste buds. The increased THs was found to be involved in activating caspase cascade and lead to cellular apoptosis. This is the first study to indicate the possible relationship between hyperthyroidism and the tongue mucosa and taste buds. Further studies are recommended to gain insights into the specific underlying mechanism of the effects of THs on the tongue mucosa and taste buds, and to exclude the possibility that these alterations had occurred as a secondary systemic effect of increased THs levels.

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