Experimental Study of Heat-Stress Induced Injury on the Dorsal Surface of Rat's Tongue

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Abstract: Heat stress was found to induce a serious of psychological and structural dysfunction in various organs and systems of the body. **Aim**: To characterize the effects of heat exposure on the structure of the dorsal surface of the rats' tongue. The effects were evaluated by histological, immunohistochemical and scanning electron microscopic examinations. **Material and Methods**: A model of heat stress was performed by exposing the rats to hot temperature of (39°C to 41°C) for a period of 5 days. The rats' tongue were excised and processed for histological, immunohistochemical and scanning electron microscopic examination. **Results**: H & E stained sections of heat stress group revealed clear distortion of the filiform papillae where the papillae appeared apparently short. Focal areas of flattened epithelial surface with complete loss of the lingual papillae were seen. The epithelial cells demonstrated signs of cellular degeneration, pyknosis and necrosis. A moderate to strong nuclear BAX expression was detected in tongue of rats exposed to heat indicating apoptosis. With Scanning Electron Microscopic examination, distortion of the filiform and fungiform papillae of the heat stress rats were noticed. The filiform papillae were irregularly oriented and most of them had desquamated keratinized epithelial covering. **Conclusion**: Heat exposure induced obvious morphological and structural alterations of the dorsal surface of rats' tongue.
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1.Introduction

A remarkable rapid changes in the average temperature of the Earth's surface has occurred in recent decades. This drastic change in climate certainly affect the human survival, human performance, well-being and undoubtedly their health. Therefore, an intimate relation between climate changes and human health is well recognized, in particular the recorded health problems that occurred in relation with heat waves in summer (1). Heat stress can induce a serious of psychological dysfunction and it is a risk factor for neurological, heart and respiratory disorders (2-4). Exposure to heat stress exposes the organs to anoxia and hypoxia (5). In addition, it has been reported that exposure to high temperature (40 degree Celsius) for thirty minutes could induce vascular lesions in various organs as adrenal gland, heart, lung, liver, pancreas and kidney (6,7).

Apoptosis is a form of cell death that occurs normally to balance the cell proliferation caused by mitotic division (8). Apoptosis might also happens in different experimental and clinical conditions. However, increased apoptosis results in organ atrophy, dysfunction or even failure that ends in many diseases (9). Qian et al. had shown that heat stress could trigger apoptosis and cell death of cardiomyocytes resulting in impairment of the cell physiological function (10). In addition, heat stress induced severe damage to the intestinal epithelial cells in rat with obvious deterioration of cell morphology (11).

BAX is a protein that belongs to BCL2, which is a family of proteins that regulates the intrinsic pathway of apoptosis. BCL2 proteins are classified into pro-apoptotic and pro-survival. The balance and location between these two types determine the fate of the cells (12). BAX functions as an apoptotic activator, and is activated during apoptosis via multiple conformational changes that are accompanied by mitochondrial intramembranous homo-oligomerization (13). Edlich et al. have proposed a regulatory mechanism of the pro-apoptotic BAX through constant retrotranslocation of BAX from the outer membrane of mitochondria to the cytosol (12). In different stress conditions such as heat stress and water deprivation, BAX undergoes translocation, leading to Cytochrome C release and subsequent activation of Caspase-3 and apoptosis (14).

Although previous reports have studied the physiological and functional alterations of heat stress on experimental animals, the research is less clear concerning the histological alterations of the tissues subsequent to exposure to such stress. This study was designed to characterize the effects of heat stress on the structure of the dorsal surface of the rats' tongue. The effects were evaluated by histological, immunohistochemical and scanning electron microscopic examinations.

2.Material and Methods:

Twelve male Albino rats (200-210 g) were purchased from the Pharmacology Department, Faculty of Pharmacy, Tanta University. Rats were acclimatized for one week before the start of the experiment. They were kept under 12-hour alternating light-dark cycle in a room maintained at 21-23°C and had free access to food and water.

Experimental Protocol

The study protocol was approved by the Research Ethics Committee, Faculty of Dentistry, Tanta University. All animal procedures were conducted according to the established animal welfare guidelines for the responsible use of animals in research as a part of scientific research ethics recommendation of Research Ethics Committee, Faculty of Dentistry, Tanta University. Animals were randomly divided into two equal groups. Group I, Control group (n=4) received dried food and water ad libitum, and were kept under controlled room temperature of 21-23°C. Group II, Heat exposure (n=8) was exposed to heat temperature (39°C to 41°C) for a period of 5 days and had dried food and water ad libitum. At the end of the experiment, all animals were sacrificed by cervical dislocation under light ether anesthesia and the tongue was carefully removed and processed for either histological, immunohistochemical and scanning electron microscopic examinations.

Histological and Immunohistochemical studies

The tongue was cut into two halves. One half was immediately fixed in buffered formaldehyde solution (10%) for 48h and processed for routine technique of paraffin inclusion Hematoxylin and Eosin Stain (H & E). Immunohistochemical staining was performed to detect BAX expression as an apoptotic marker. The Avidin-Biotin Complex (ABC) technique was performed using Primary antibody against BAX Ab-1 (Clone: 2D2/BAX, Mouse monoclonal, Thermo Fisher Scientific Anatomical Pathology. Fremont, CA, USA) and the ABC Universal Kit (Neomarkers, Fremont CA, USA) was used. The immunohistochemical procedures were performed according to the manufacturers' instructions. The deparaffinized sections associated with the kit were processed acting as positive controls whereas negative controls consisting of tissue sections on which primary antibody was replaced with non-immune serum. The sections were evaluated for BAX expression by assessing the site of staining (nuclear staining and/ or cytoplasmic staining) and the intensity of staining that was expressed as weak, moderate or strong staining.

Scanning Electron Microscopic (SEM) Study

The other half of rats' tongue were immediately fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4). The samples were treated with 8N hydrochloric acid at 60° for 30 minutes to remove mucus from the surface of the tongue and prepared for SEM examination. The dorsal surface of the tongue was examined and photographed with (JSM 5600LV, Jeol, Tokyo, Japan) in EM Unit of Faculty of Medicine, Tanta University.

3. Results:

Histological results

The dorsal tongue surface of the control group revealed evenly distributed numerous filiform papillae that appeared regular in size, shape and orientation. They are covered with thick keratinized stratified squamous epithelium (Figure 1). The dorsal tongue surface of the Heat stress group showed clear distortion of the filiform papillae where the papillae appeared apparently short and broad compared to the control group. Focal areas of flattened epithelial surface with complete loss of the lingual papillae were seen. The epithelial ridges were blunt and few. At higher magnifications, the epithelial cells demonstrated signs of cellular degeneration. The nuclei had chromatin remnants and residual nucleoli (Figure 2). The fungiform papillae showed distorted morphology. Pyknotic epithelial cells as well as degenerated necrotic cells were evident (Figure 3).

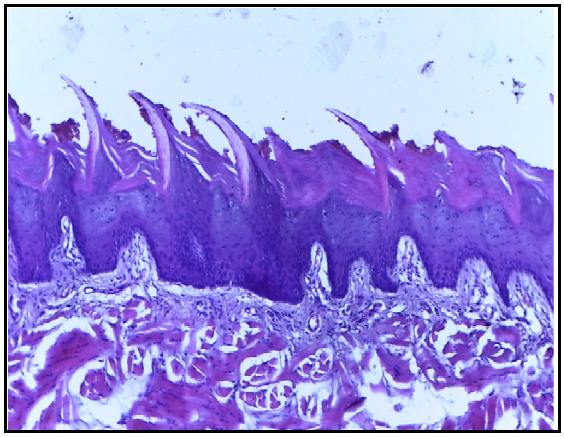

Figure (1): Photomicrograph of the control tongue illustrates the regularly oriented filiform papillae covered with thick keratinized stratified squamous epithelium. The underlying lamina propria is a connective tissue. The lingual muscle fibers run in different direction. (H & E; Mic. Mag. X200).

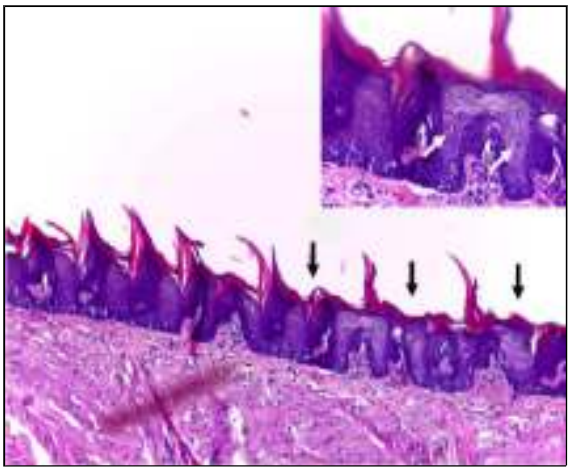

Figure (2): Photomicrograph of Heat stress tongue illustrates disfigurement and loss of height of the filiform papillae. Some areas show flattening of the surface with loss of the sharp conical projections of the filiform papillae (arrows). The epithelial ridges are few and blunt. *Inset*: Higher magnification shows degenerated epithelial cells with the nuclei having chromatin remnants and residual nucleoli (arrows). (H & E; Mic. Mag. X200; inset: x400)

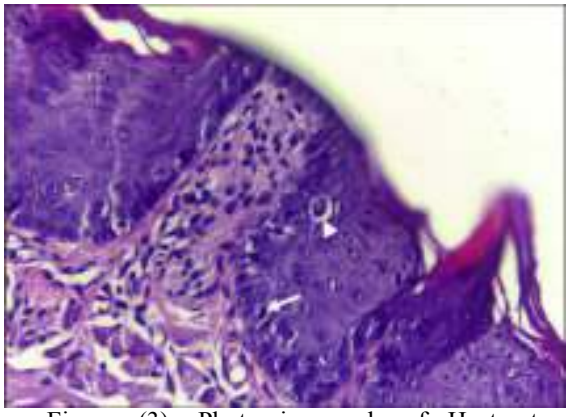

Figure (3): Photomicrograph of Heat stress tongue illustrates distortion of the fungiform papilla. Pyknotic epithelial cells (arrows) and degenerated necrotic cell are evident (arrow head). (H & E; Mic. Mag. X 400).

Immunohistochemical results

Immunohistochemical staining showed negative BAX expression of the epithelial cells of the control dorsal tongue (Figure 4). Whereas BAX expression in the Heat stress group showed positive nuclear expression throughout the epithelial cells. The reaction was mainly detected in basal and parabasal cells that displayed moderate to strong expression (Figure 5).

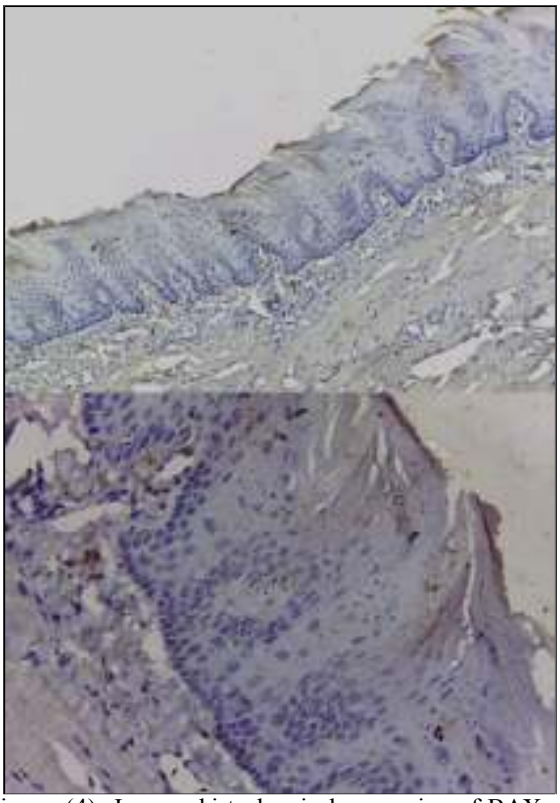

Figure (4): Immunohistochemical expression of BAX of normal dorsal tongue illustrates negative expression in the epithelial cells. (Immunohistochemistry; Mic. Mag. a X200, b x400)

SEM
results

The filiform papillae of the dorsal surface of the control group appeared closely packed, thread-like in shape and had regular orientation with normal interpapillary distances and intact tapered end keratinized covering (Figure 6). Fungiform papillae was observed sporadically in between the filiform papillae. They were dome-shape and has a flattened smooth upper surface. A well-defined centrally located taste pore was present on its upper surface surrounded by shallow indentation (Figure 7). In the Heat stress group the filiform papillae were distorted and irregularly oriented. Furthermore, most of papillae did not present the typical third-like appearance and had desquamated keratinized epithelial covering (Figure 8,9). The fungiform papilla was less prominent and distorted. It has irregular wrinkled upper surface and ill-defined taste pore (Figure 9).

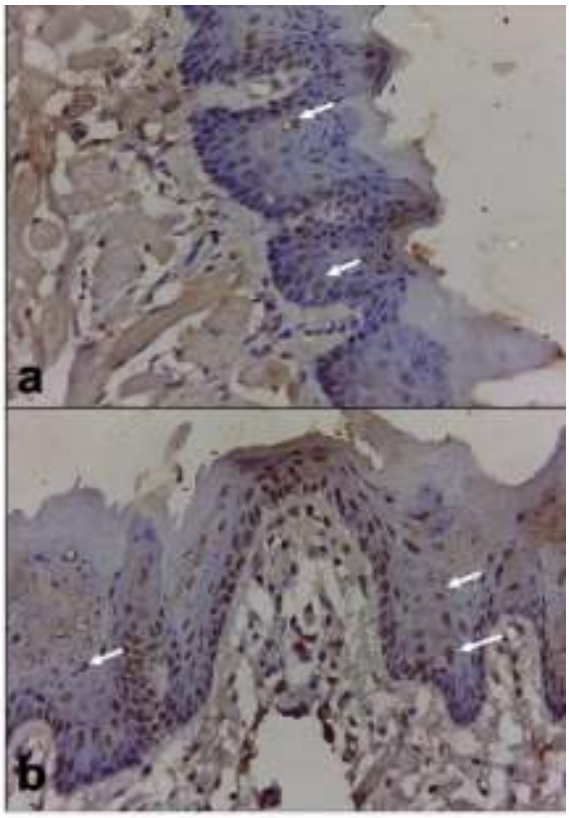

Figure (5): Immunohistochemical expression of BAX of Heat stress tongue illustrates positive nuclear expression in the epithelial cells (arrows). (Immunohistochemistry; Mic. Mag. a X200, b x400)

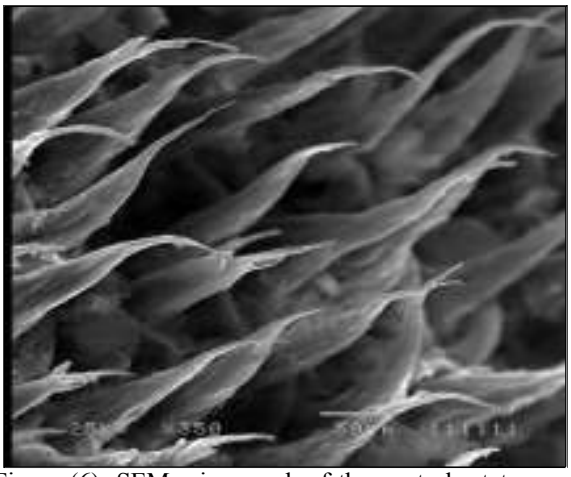

Figure (6): SEM micrograph of the control rat tongue illustrates the numerous, regular orientation of the long third-like filiform papillae with intact tapered end keratinized covering. (x 350).

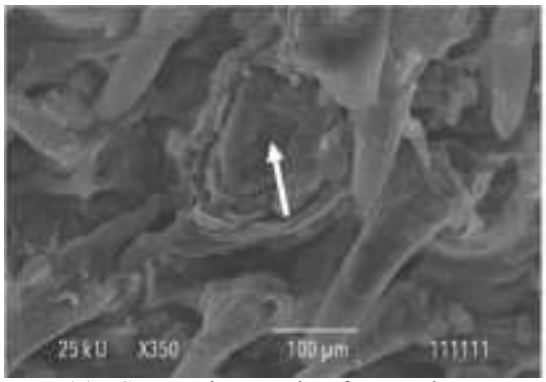

Figure (7): SEM micrograph of control rat tongue illustrates the dome shaped fungiform papilla in between the regularly directed filiform papillae. The taste pore is well defined on its upper surface (arrow). (x 200)

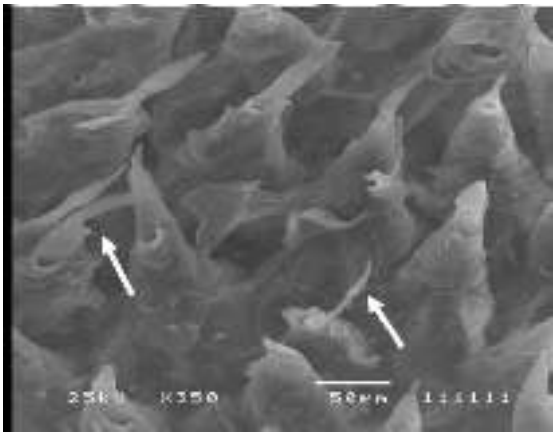

Figure (8): SEM micrograph of Heat Stress tongue illustrates irregularly oriented filiform papillae. Desquamation and disfigurement of filiform papillae are evident (arrows). (x350).

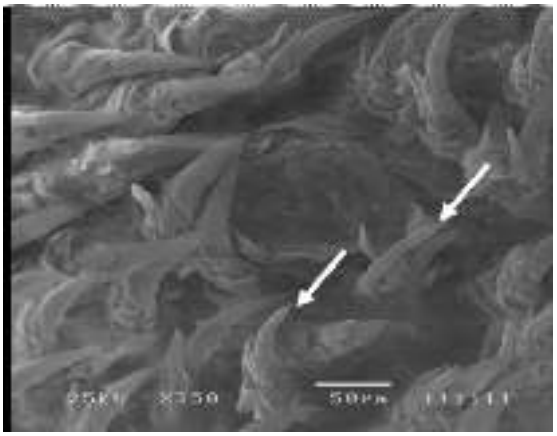

Figure (9): SEM micrograph of Heat Stress Tongue illustrates loss of the vertical orientation of the filiform papillae as well as bending of their tips (arrows). The fungiform papilla is distorted and the taste pore is ill-defined. (x350)

4.

Discussion:

The current study demonstrated the effects of heat exposure on rats' tongue following the protocol of heat stress model as suggested by Yadav et al. (15). The results of the study clearly demonstrated the alterations of the dorsal tongue surface as a result of heat stress. Previous reports had confirmed the deleterious effects of heat exposure on different tissues of experimental animals. In our study, at the light microscopic level heat exposure was found to induce marked changes of the tongue papillae such as distortion of the papillae, flattening of the surface associated with loss of the papillae. In addition obvious cellular changes of the epithelial cells such as cell degeneration and necrosis were detected. At the SEM level, the most obvious change was the desquamation of the filiform papillae and the disfigurement of the filiform and fungiform papillae. These observations were comparable to those reported on the epithelial cells of intestinal mucosa where heat injury as a result of hyperthermia induced profound damage to the epithelial cells of the small intestine. The authors observed that heat exposure at 40 °C to 42 °C for three consecutive days induced damage to the intestinal mucosa in rats, increase the intestinal permeability and caused injury to the immunological and biological intestinal barriers. They observed shortening of the height of the villi as well as sloughing of the epithelium of the basement membrane in the heat stressed tissue as compared to the control. Also vacuolization of the epithelial cells was reported (11). The exposure of rats to heat stress for 4 hours was reported to produce distortion of cell shape as swelling or shrunken cells, and chromatolysis in the dark neurons of parietal cerebral cortex (2). In addition, liver injury manifested as vacuolization of hepatocytes, necrosis, and inflammation occurred under hyperthermic challenge for two days only (7).

To explain the damage induced by heat stress, Hannah et al. concluded that heat stress produced a clear impressive rise in oxidative stress in rats' liver. The associated accumulation of ROS that occurred after heat exposure was associated with marked oxidative damage to the lipids and DNA 24 h following hyperthermia. The most pronounced effect among the oxidative damage as a result of heat stress was the higher level of lipid peroxidation (7). Peroxidation of the polyunsaturated fatty acids can change the permeability of the cell membrane and produce membrane leakage.

It is worth mentioning that, the results of the current study showed increased BAX expression in epithelial cells of the heat exposure group. In agreement with this finding, it was confirmed that heat exposure triggers apoptosis of germ cells and induce cell death leading to destruction of spermatogenesis (16). Also, heat stress related oxidative stresses was reported to induce apoptosis in the epithelial cells of the small intestine. Furthermore, heat stress for 4 h resulted in apoptosis and coexisting necrosis in cardiomyocytes. The authors confirmed that an important mechanism of cell injury induced by heat stress was suggested to be through mitochondria-mediated apoptosis pathway in which alteration of mitochondrial membrane permeability transition (MPT) is a key point to trigger apoptosis (10).

In conclusion, heat exposure was found to induce significant injury to the dorsal surface of rats' tongue. Therefore, it could be concluded that the elevations of the body temperature above normal range, that occurred in hot weather, might induce cellular damage and consequently organ dysfunction. This is especially of impact in today's world, where heat is a cause of great concern in different countries.

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