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Capsaicin induced histological and ultrastructural changes in the submandibular salivary gland of albino rats

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ABSTRACT
Capsaicin is a pungent principle of hot red pepper. It is used in spices, food additives and drugs. In the present work, twenty rats were divided into two groups: control and capsaicin groups, each consisting of ten rats. The capsaicin group daily received a capsaicin dose equivalent to 0.1 mg/kg body weight dissolved in 0.5 ml distilled water by oro-oesophageal tube while the control group daily received 0.5 ml distilled water. After twenty one days, the submandibular salivary glands of both sides were excised, processed and examined histologically and ultrastructurally. Histological results revealed presence of pure mucous acini in the submandibular salivary gland. Some granular convoluted tubules showed degeneration while the excretory ducts showed loss of pseudostratification with the appearance of some flattened lining cells. Concerning the ultrastructural findings, some acinar cells showed dilated rough endoplasmic reticulum, other presented ultrastructural features similar to mucous acini. Granular convoluted tubules cells showed some irregular, shrunken nuclei with condensed chromatin. Their secretory granules were less electron dense than the control and presented ill-defined and fused outlines. Some of the excretory duct lining cells showed apically displaced irregular nuclei. One to two rows of flattened epithelial cells were observed apical to the lining cells. Vacuolizations, mitochondrial swelling and loss of cristae were detected in cells of some acini, granular convoluted tubules and excretory ducts. Most intercalated and striated duct cells showed ultrastructural features similar to that of control group. However, the basal part of some striated duct cells presented variable grades of mitochondrial affection.

From the present work, it could be concluded that chronic capsaicin intake was associated with noticeable histological and ultrastructural changes in acini, granular convoluted tubules and excretory ducts of the SMSG in albino rats.

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1. Introduction
Natural flavors are widely used in various foods, cosmetic and pharmaceutical products. These kinds of additives are applied as colors, preservatives, aroma and tasting agents. The large-scale use of certain food flavors requires accumulation of data on these substances [17]. Capsaicin is a pungent principle of hot red pepper. It is used in spices, food additives and drugs [19]. Red pepper (Capsicum frutescens L.) is widely used as a spice for flavoring foods, particularly in South- East Asian and Latin-American countries and one of its major active ingredients is capsaicin [1,4]. It is thought that when red pepper is consumed in excessive amounts, it leads to “gastric ulcers” in view of its irritant and likely acid secreting nature. However, investigations carried out recently by [16] on gastric ulcers revealed that capsaicin is not the cause for ulcer formation but just a co-factor and it was found not to stimulate but on the contrary, to inhibit acid secretion. On the other hand, the same researchers reported that capsaicin stimulates alkali, mucus secretion and particularly gastric mucosal blood flow helping prevention and healing of ulcers. Capsaicin acts by stimulating afferent neurons in the stomach and signals for protection against injury causing agents [12], reported that Capsaicin in low concentration range (1–8 μg/mL, 100 mL) given by nasogastric tube before gastric injuries induced by ethanol or indomethacin could...
protect the stomach and this was attributed to stimulation of the sensory nerve endings. The effects of dietary capsaicin on the rat submandibular gland secretion were investigated by [9,10]. The researchers reported the induction of cystatin S substance in submandibular saliva and its contribution in enhancing ingestion of the capsaicin diet. Furthermore, it was suggested that dietary capsaicin could induce salivary cystatin either by stimulating the reflex arc involving the glossopharyngeal nerve [9] or by irritation of the oral mucosa [10].

Data on the effect of capsaicin on the submandibular salivary gland structure was scarce. So, the present study aimed to investigate the effect of capsaicin on the submandibular salivary gland of the albino rats histologically and ultrastructurally.

2. Material and methods

Twenty adult male albino rats (weighing about 250 ± 20 g, each) were used in this study. Animals were recorded in “The Medical Research Center”, Faculty of Medicine, Ain Shams University and were housed in wire mesh caged cages. They were fed certified pelleted diet and tap water ad libitum. Temperature and humidity conditions were controlled as possible on housing the animals during the experimental period. The capsaicin used in this study was purchased from Sigma chemical co., St. Louis, Mo, USA. The animals were divided into two groups: Control and capsaicin groups. The control group consisted of ten rats that received 0.5 ml distilled water daily by oro-oesophageal tube. The capsaicin group consisted of ten rats that received a daily capsaicin dose equivalent to 0.1 mg/kg body weight [8] (which is equivalent to the average consumption dose for capsaicin in people of Thailand) dissolved in 0.5 ml distilled water by oro-oesophageal tube. After twenty one days, all rats were killed by cervical dislocation, and the submandibular salivary gland of both sides were excised. Both glands of each animal were processed, one for light microscopic examination and the other for transmission electron microscopic examination.

2.1. For light microscopic study

The glands were fixed in 10% neutral buffered formalin for 24 h, washed, dehydrated and cleared to be embedded in paraffin. Then, sections (4–5 microns thickness) were sliced and stained by hematoxylin and eosin (H & E) for light microscopic examination.

2.2. For transmission electron microscopic study

The glands were sliced into fragments and primarily fixed in buffered formaldehyde–glutaraldehyde solution overnight at 4 °C, post fixed in buffered 1% osmium tetroxide solution for 1.5 h. The tissue samples were dehydrated in ascending concentrations of ethanol, and embedded in low viscosity resin (spur). Semi-thin sections were obtained, stained with toluidine blue and examined under light microscope to choose areas of interest. Ultra-thin sections were cut, stained with uranyl acetate and lead citrate to be examined by transmission electron microscope (TEM) in the electron microscopic unit of the Veterinary Hospital of Armed Forces.

3. Results

3.1. Light microscopic results

3.1.1. Control group

Examination of H&E stained sections of the SMSG of control rats revealed that the gland is predominantly formed of seromucous acini, granular convoluted tubules (GCTs), intercalated, striated, and excretory ducts, and finally connective tissue stroma. The acini appeared more or less spherical in shape and consisted of pyramidal cells having a moderately basophilic cytoplasm and rounded basally situated nuclei (Fig. 1A). The intercalated ducts were hardly encountered, they were lined by short cuboidal cells having basophilic cytoplasm and centrally placed large rounded nuclei (Fig. 1A). The granular convoluted tubules (GCTs) were lined with tall columnar cells having large rounded basally situated nuclei, and apical eosinophilic granules (Fig. 1B). The striated ducts were lined by columnar cells with centrally placed rounded nuclei and intensely eosinophilic cytoplasm with basal striations (Fig. 1A). The excretory ducts were lined by pseudostratified columnar epithelium and were surrounded by fibrous connective tissue stroma usually accompanied by variable sized blood vessels (Fig. 1C).

3.1.2. Capsaicin group

Histological sections of the capsaicin group glands presented acinar cells having morphological outline and staining almost identical to those of the control group (Fig. 1D). In other areas, some acini appeared smaller in size with darkly stained nuclei, while other acini presented variable sized cytoplasmic vacuoles (Fig. 1E). And still some acini presented histological features resembling mucous acini (Fig. 1D). GCTs showed two different histological patterns, few of them were normal with regular granular content and basally situated nuclei (Fig. 1D). However, most GCTs showed degeneration and ill-defined cells outline, increased eosinophilia and clumping of granular content were also detected (Fig. 1E). The intercalated and striated ducts presented almost normal features (Fig. 1D). The excretory ducts tended to present an irregular outline, their epithelial lining were frequently seen projecting towards the lumen. In most areas of these ducts, there was loss of pseudostratification with appearance of some flattened cells. The epithelial lining cells showed deformed and apically displaced nuclei. Areas of hydropic degeneration within the epithelial lining of these ducts were sometimes detected (Fig. 1F).

3.2. Electron microscopic results

3.2.1. Control group

Ultrastructural examination of the control SMSG revealed that the acinar end pieces appeared to be formed of pyramidal cells with spheroidal nuclei having prominent nucleoli with peripheral chromatin distribution (open faced nucleus). These cells showed parallel arrays of rough endoplasmic reticulum (RER) in their basal part, few oval mitochondria and Golgi complex. Membrane bounded secretory granules of variable sizes and electron densities occupied the cytoplasm lateral and apical to the nucleus (Fig. 2A). The intercalated ducts were lined by cuboidal cells with large centrally placed nuclei and rounded lumen. Few cisternae of RER, scattered mitochondria, few secretory granules were sometimes encountered. The GCTs were lined by tall columnar cells in which the apical two thirds were occupied by numerous well circumscribed membrane bounded electrondense secretory granules of variable size. The basal part of these cells contained rounded electron lucent nuclei surrounded by numerous mitochondria that were arranged near the basal plasma membranes and few cisternae of RER (Fig. 2B). Cells of the striated ducts were tall columnar with large or rounded centrally placed nuclei surrounded by few RER and Golgi complex. The basal part of these cells presented deep infoldings of the plasma membrane that were almost parallel to the long axis of the cell. The cytoplasmic processes resulting from the basal infoldings contained numerous rod shape mitochondria (Fig. 2C). The excretory ducts were lined by pseudostratified epithelium consisting of tall columnar cells and basal cells that didn’t reach the lumen. The columnar cells presented open faced
nuclei, abundant mitochondria, free ribosomes and lysosomes while the RER was sparse. These cells also showed numerous microvilli on their luminal surface while their lateral surfaces presented interdigitations with adjacent cells and numerous desmosomal junctions. On the other hand, the basal cells showed basally situated ovoid nuclei and scattered mitochondria (Fig. 2D). The connective tissue component consisted of fibroblasts, macrophages, lymphocytes and an extracellular matrix of collagen fibers. Blood vessels lined by endothelial cells, surrounded by pericytes and filled with electrondense RBCs were also observed.

3.2.2. Capsaicin group

Many acini presented normal ultrastructural features. However, some acinar cells presented different patterns of change, as some of the nuclei were centrally located with irregular outline and clumping of chromatin material. In such cells RER presented variable grades of affection ranging from luminal dilatation to discontinuity, fragmentation as well as apical dislocation. The secretory granules showed different electron densities with ill-defined outlines (Fig. 2E). Large cytoplasmic vacuoles and dilated mitochondria and RER were detected in other acinar cells (Fig. 2F). Meanwhile, other acinar cells showed mucous appearance with basally situated nuclei. The mucous acini were formed by cells filled with large sized electrondense granules with a network of thin cytoplasmic strands in between. (Fig. 2G). Ultrastructural examination of GCTs showed some cells with irregular nuclear outline surrounded by cytoplasmic vacuoles. Other nuclei presented noticeable shrinkage with condensed chromatin. Different electron densities secretory granules were seen, many presented ill-defined and fused outlines. In both cases, most granules were less electrondense than the control (Fig. 2H). Swollen mitochondria in GCTs with loss of cristae were frequently encountered. Most intercalated and striated duct cells showed ultrastructural features similar to that of control group. However, the basal part of some striated duct cells presented variable grades of mitochondrial affection such as dilatation, loss of cristae and even rupture (Fig. 2I). Some excretory duct lining cells showed vacuoles and apically displaced nucleus with shrunken and irregular outline (Fig. 2J). Other cells presented cytoplasmic vacuoles and irregularly outlined nucleus (Fig. 2K). Apical to the lining cells, one to two rows of flattened epithelial cells were observed (Fig. 2K). These flattened cells presented irregular nuclear outline, mitochondrial swelling. Loss of desmosomal junctions between the cells was also noticed (Fig. 2L). Apart from few detected dilated endothelial lined blood vessels congested with electrondense RBCs, the connective tissue component of the gland did not show remarkable changes.

4. Discussion

People of different cultures around the world have been using...
Fig. 2. (A, B, C, D, E) Electron micrographs of the control SMSG showing (A): acinar cell having parallel arrays of well-organized rER cisternae (R) in the basal portion of the cell and open faced nucleus (N) (X 10000). (B): GCT cell with electron-dense secretory granules (G) and basally situated open faced nucleus (N) (X 4000). (C): striated duct presenting round nuclei and basally situated mitochondria (arrows) (X 4000). (D): excretory duct tall columnar cells having open faced nuclei and mitochondria (M). Note interdigitations between adjacent cells (arrows) (X 4000). (E, F, G, H, I, J, K, L): Electron micrographs of the SMSG of the capsaicin group showing (E): acinar cell presenting centrally placed nucleus with clumped chromatin (N). Note the dilated, fragmented rER basally (R) and its apical displacement (arrows) (X 3000). (F): acinar cell with irregular nuclear outline and clumped chromatin (N), dilated rER (R), swollen mitochondria (M) and large cytoplasmic vacuoles (V) (X 8000). (G): acinar cell filled with electron-lucent granules (M) and basally situated nucleus (N) (X 2000). (H): GCT cells presenting shrunken and irregular nuclear outline (N) surrounded by cytoplasmic vacuole (V). Note the different electron densities of secretory granules and their fused outlines (G) (X 4000). (I): striated duct cells with dilated and ruptured mitochondria (arrows) (X 15000). (J): excretory duct cells with large cytoplasmic vacuoles (V) and apically situated nuclei (arrows) (X 2000). (K): excretory duct cells having irregular nuclear outline (N) and cytoplasmic vacuoles (V). Note the two rows of flat epithelial cells (arrows) (X 4000). (L): excretory duct cells presenting mitochondrial swelling (M), irregular nuclear outline (N) and loss of desmosomal junction (arrows) (X 10000).
herbs and spices to flavor their daily foods. With the increasing worldwide interest in using herbs and spices, such as capsaicin, in medicinal treatment, there is a vital need to assess any possible adverse effects of such spices on different tissues of the body [15]. Therefore, the present study aimed to investigate the effect of capsaicin on the histology of the submandibular salivary gland. In a previous study [9], reported that when mixing different concentrations of capsaicin with food for seven days, the daily food intake of rats decreased after two days. Therefore, in the present study it was preferred to administer the dose via oro-oesophageal tube, instead of adding it to food, to ensure an accurate standardized dose given to each individual rat during the experimental period. In many countries, the consumption of spices is part of the individual daily diet so it becomes more like a chronic intake, thus in the present study, capsaicin was administrated for twenty one days. Examination of H&E stained sections of the capsaicin group revealed large acinar cytoplasmic vacuoles. These histological results may be interpreted by the findings of [20] who suggested that vacuoles are a result of accumulation of lipid droplets, which come from utilized fatty acids as a result of decreased cellular activity. On the other hand [18], attributed the vacuolization to damage of the mitochondria that are very vulnerable to noxious agents and when damaged, the cellular metabolism fails and sodium ions enter the cell. This osmotic effect causes the breakdown of large macromolecules within the damaged cell leading to the appearance of cytoplasmic vacuoles. Taking into consideration the transmission electron micrographs of the present work that frequently presented mitochondrial affection, so the [18] explanation seems most likely to apply to such vacuolizations.

In the present work, it is worth noting that some acini of the capsaicin group showed a similar pattern to mucous acini with basally situated nuclei and foamy cytoplasm in both light and TEM. This could be due to the transformation of some seromucous acini into pure mucous. This assumption comes in agreement with a study conducted by Ref. [13] who reported that capsaicin and chili increased the secretion of mucus from the stomach. The authors suggested that this increase of mucus production upon exposure to capsaicin is the reason for its gastroprotective effect. Therefore, it could also be presumed here that a protective mechanism may be mediated by the mucous transformation of some acini in the submandibular salivary gland.

In the present study, H&E stained sections of the capsaicin group showed GCTs with ill-defined cell outlines, increased eosinophilia and clumping of granular content. The TEM examination of GCTs also revealed that the secretory granules were ill-defined, fused with less electron densities than the control. Normally, GCTs of rat submandibular salivary gland are known to secrete many growth factors [7]. Moreover [11], reported presence of digestive enzymes, antimicrobial substances and mucins synthesized and secreted by the GCTs cells. Based on previous studies reporting the induction of a cystatin S substance in the saliva of rats consuming dietary capsaicin [9,10] and on studies reporting the immunohistochemical detection of cystatin within GCT cells of submandibular glands of rats treated with isoproterenol (the sympathetic beta-agonist) [3], it could be predicted that histological and ultrastructural changes found in GCTs cells of the present work could be associated with biochemical changes in the secretory product of such cells. In 2002, Katsukawa et al. further investigated the physiological role of the salivary cystatin S, they reported that these proteins contributed to enhanced ingestion of the capsaicin diet. The same researchers also suggested that induction of salivary cystatin S substance may be triggered by irritation of the oral mucosa caused by capsaicin.

Transmission electron micrographs of the capsaicin group presented ill-defined secretory granules with fused outline in GCT cells. In such cells, the mitochondria were frequently swollen with loss of cristae. The accumulation of these granules appeared to be due to the inhibition of exocytosis, as described by Ref. [6]. Knowing that, ATP is essential for granule priming prior to exocytosis [5]. Additionally [18] noticed that mitochondrial affection led to depletion of adenosine triphosphatase (ATP) leading to failure of membrane pumps with subsequent no energy for secretion.

By examination of transmission electron micrographs, variable grades of mitochondrial affection were detected primarily in cells of the striated ducts and to a lesser extent in acini, GCTs and excretory ducts. This foregoing observation comes in agreement with [2] who stated that capsaicin was found to inhibit the respiratory response of rat liver mitochondria. Also, it was found to depress the 2, 4-dinitrophenol (DNP) activated adenosine triphosphatase (ATPase) activity of rat liver mitochondria. These previous studies indicated that capsaicin has profound effect on the energy linked functions of isolated mitochondria [14], reported mitochondrial swelling with rarified matrix and disorganized cristae as an immediate effect of capsaicin on duodenal absorptive cells following its intraduodenal and intra gastric administration. The same investigators also reported dilatation of endoplasmic reticulum and Golgi complex. They suggested that these cellular alterations reflected altered function and physiology of the absorptive cells. Such findings also come in agreement with ultrastructural results of the present study showing dilated RER in acinar cells of the capsaicin group.

H&E examined sections of the capsaicin group revealed excretory ducts with irregular outline and apparent loss of pseudotratification. Many epithelial cells appeared flattened with deformed and apically displaced nuclei. This was confirmed ultrastructurally as excretory duct lining cells demonstrated shrunked and apically displaced nuclei with irregular outline. In some areas, there were one to two rows of flattened epithelial cells that presented loss of desmosomal junctions.

5. Conclusions

From the present study, it could be concluded that chronic intake of capsaicin was associated with noticeable histological changes in acini, GCTs and excretory ducts of the SMSG. Finally, assessment of the flow rate, consistency as well as the chemical composition of the saliva produced by salivary glands following exposure to capsaicin may open the field for further researches.

References


