Effect of Aspartame on Submandibular Salivary Glands of Adult Male Albino Rats

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Abstract: This study was carried out to evaluate the effect of administration of aspartame in two doses (40mg/kg and 80mg/kg body weight) on the submandibular salivary glands of male albino rats. Ninety adult male rats weighing (200-250g) were used in this study. Animals in each group were caged in separate cages in the animal house at the Faculty of Dentistry, Minia University. Pure aspartame (APM) powder was purchased from ADWIA Co., Cairo, Egypt, The animals were divided into 3 groups: **Group I:** composed of 30 rats. They were daily administrated distilled water by means of gastric tube and considered control group. **Group II:** composed of 30 rats. They were daily administrated APM (40mg/kg) dissolved in distilled water for, 2, 4, and 6 months by means of gastric tube, and considered as experimental group 1. **Group III:** composed of 30 rats that were daily administrated APM (80mg/kg) dissolved in distilled water for 2, 4, and 6 months and considered as experimental group 2.Ten animals from each group were sacrificed after 2, 4 and 6 months under chloral hydrate anesthesia. Portions from the submandibular salivary gland were prepared histologically and stained with Haematoxylin & Eosin. The histological examination of submandibular salivary gland sections of rats after 2 months received low and high doses of aspartame revealed some histological changes in the gland parenchyma. Some serous acini appeared distended and most of them tended to be spaced with vacuolated cytoplasm and ill-defined cell boundaries. Nuclei were pushed toward basal portion and showed signs of hyperchromatism. Some acini presented division of nucleus without division of cytoplasm. Most blood capillaries appeared to be engorged with collected red blood corpuscles. In addition striated ducts appeared dilated while granular ducts showed stagnant eosinophilic secretion. Histological examination of the submandibular gland after 4 months revealed obvious morphological changes. The parenchyma of the submandibular gland lost its normal acinar architecture. Some acinar cells presented granular cytoplasm while others exhibited empty vacuoles. Signs of pre- malignancy such as nuclear hyperchromatism, pleomorphism and abnormal mitosis were evident. Submandibular gland sections after 6 months revealed that the granular convoluted tubules became the most prominent feature of the gland on the expense of the serous acini. The submandibular glands presented atrophic changes. The serous acini in submandibular gland were atrophied.
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1. Introduction

Aspartame is an artificial sweetener used throughout the world in food and beverages. It is a methyl ester of a dipeptide composed of aspartic acid and phenylalanine (**Sweetman, 2002, FCC, 2003; Burdock, 2005).** The use of an artificial non-carbohydrate sweetener was all the time demanded by many around the world. Populations on diet who need a sweetener with lower calories than sugar, diabetics and others are examples of those consuming this agent. So, there is a rapid development in its consumption over years. Aspartame is manufactured by coupling of the amino acids L-phenylalanine methyl ester and L-aspartic acid to produce the dipeptide methyl ester **(Burdock, 1997).** Approximately 50% of aspartame molecule is phenylalanine, 40% is aspartic acid and 10% is methanol **(Newsome, 1986).**In 1974, The Food and Drug Administration (FDA) approved the use of aspartame as a sweetener in dry sugar substitutes, chewing gum, and dry base for beverages, instant coffee and tea **(Fed.Regist,1981a)**. In 1983, the FDA also approved the use of aspartame in carbonated beverages **(Fed. Regist, 1984)**.

Puica (2008) investigated the effect of administration of 2 mg/kg body weight (bw) of aspartame on hypothalamic and anterior pituitary cells ultrastructure in juvenile rabbits. Authors found that hypothalamus neurons presented pyknotic nuclei. On the other hand, cytoplasmic organelles were found to demonstrate degenerative features. **Omar (2009)** evaluated the effects of daily oral ingestion of 250 mg/kg body weight/day of aspartame on the structural and ultrastructural morphology of the frontal cortex of

rats for 8 weeks. Pyramidal cells of experimental group appeared to be darkly stained, vacuolated or irregular in shape with pyknotic or faint nuclei**.** **Mourad & Noor (2011)** investigated the effect of daily oral ingestion of 40 mg/kg of body weight of ASP on the oxidative stress in rat cerebral cortex in 2, 4 and 6 weeks period. **Humphries and Pretorius (2007)** determined the effect of administration of different doses of ASP (34mg/kg bwt; 100 mg/kg bwt; 150 mg/kg bwt) on the histological morphology of liver and kidney of rabbits. They found that the cytoplasm appeared to be granular with lace like appearance appeared more spaced and broken with more transparent areas compared to controls. **Mourad (2011)** examined the effect of daily oral administration of 40 mg/kg bwt of ASP for 2, 4 and 6 weeks in renal tissue. **AbdElfatah** *et al.* **(2012)** evaluated the effect of aspartame intake (single daily oral dose of 50 mg/kg bwt) on the histological and genetic structure in liver and bone marrow of mother albino rats and their off spring during whole gestation period and for nine weeks after delivery. Results revealed that treated animals and their offspring showed marked histopathological changes. However, the reports of presumptive toxic effect of aspartame on salivary gland tissue, especially in human or mammals werescanty.

The injurious effect of aspartame on the histological behavior of body tissues have promoted the present investigation which aimed to evaluate the effect of long term administration of aspartame for six months on the structural features of the submandibular salivary glands of adult male albino rats.

2. Materials and Methods

Animals: 90 adult male rats weighing (200-250g) were used in this study. All experiments were carried out in accordance with the research protocols established by **Institute of Laboratory Animal Research (1996).** Animals in each group were caged in separate cages in the animal house at the Faculty of Dentistry, Minia University.

- **Chemicals:**

Pure aspartame (APM) powder was purchased from ADWIA Co., Cairo, Egypt.

- **Experimental design:**

The animals were divided into 3 groups:

- **Group I:** composed of 30 rats. They were daily administrated orally distilled water by means of gastric tube. This group was considered as control group during 6 month period.10 rats were sacrificed at the end of 2, 4 and 6months.
- **Group II:** composed of 30 rats. They were daily administrated orally APM (40mg/kg) dissolved in distilled water for 2, 4, and 6 months. (Experimental group 1).
- **Group III:** composed of 30 rats that were daily administrated orally APM (80mg/kg) dissolved in distilled water for 2, 4, and 6 months (Experimental group 2).

- 10 animals from each group were sacrificed after 2, 4 and 6 months under chloral hydrate anesthesia.

Portions from the submandibular salivary glands were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections 5-7 micrometers thick were stained with Haematoxylin and Eosin stain **(Drury &Wallington, 1980).**

3. Results and discussion

Aspartame is an unstable substance, a fact which contributes to its toxicity. It is made up of three chemicals which are the amino acids aspartic acid and phenylalanine and methyl ester. After oral ingestion, aspartame is hydrolyzed, either within the lumen of the gastro-intestinal tract, or within the mucosal cells lining the inside of the GI-tract. The products that result from these reactions are methanol and the amino acids aspartic acid and Phenylalanine. The amount of aspartame that enters the bloodstream is not detectable. Following aspartame consumption, the concentrations of its metabolites are increased in the blood stream **(Stegink, 1988).**

The parenchyma of the submandibular gland was morphologically composed of secretory end pieces, collecting ducts and granular convoluted tubules. The secretory end pieces or the acini were found to be composed of pyramidal shaped serous cells with round or ovoid deeply basophilic nuclei in the basal one third of the acinarcells.

The striated ducts were found to be consisted of low columnar cells with centrally placed open faced nuclei. The granular convoluted tubules were found to be larger than striated ducts and composed of pyramidal shaped cells with numerous deeply acidophilic granules located apically and large open faced nuclei basally (Fig.1).

The histological examination of submandibular salivary gland sections of rats after 2 months of consumption of low and high doses of aspartame revealed some histological changes in the gland parenchyma. Some serous acini appeared distended and most of them tended to be spaced with vacuolated cytoplasm and ill-defined cell boundaries (Fig.2). Nuclei were pushed toward basal portion and showed signs of hyperchromatism. Some acini presented division of nucleus without division of cytoplasm. Most blood capillaries appeared to be engorged with collected red blood corpuscles. In addition striated ducts appeared dilated while granular ducts showed stagnant eosinophilic coagulum. (Fig.3).

Our histological findings revealed more aggravated changes in experimental groups treated with the over dose of aspartame (80mg/kg body weight). These findings could be explained by **Hathway, (2000)** who stated that cellular response to injurious stimuli depends on the type of injury, its duration, and its severity. Thus, low doses of toxins or a brief duration of ischemia may lead to reversible cell injury, whereas larger toxin doses or longer ischemic intervals may result in irreversible injury and cell death.

Our results regarding spaced serous acini could be attributed to edema. This result is supported by **Barceloux *et al.* (2002)** who reported that formic acid specifically targets the optic disc and retrolaminar section of the optic nerve, causing optic disc edema. Formic acid is one of the breakdown products of aspartame as reported by **(Stegink, 1987)**.

The current work results regarding cytoplasmic vacuolization came in accordance with **Abdallah, (2002)** who found slight hydropic degeneration in liver hepatocytes after long term administration of 100 mg/kg body weight of aspartame for 14 weeks.The results were also in line with **Osfor and Elias, (2003)** who stated that examination of liver sections of adult male rats treated with aspartame for six and twelve weeks revealed cloudy swelling of hepatocytes compared to the controls. The present work revealed that most blood capillaries were engorged with collected R.B.Cs and some of them were completely obliterated by thrombus, a finding that could result in ischemia. **Kobb *et al.* (1996)** mentioned thatlack of oxygen dramatically increases the need for anaerobic glycolysis to maintain intracellular ATP reserves. The total protein synthesis is decreased while induction of glucose-regulated proteins is increased. If these changes are inadequate to prevent ATP depletion, membrane ion pumps fail and membrane integrity is lost. (**Henics and Wheatley, 1999).**

Meanwhile, **Frey and Olson (2003)** reported that cellular swelling is the first manifestation of cell injury where small clear cytoplasmic vacuoles could be detected which represent distended and pinched off segments of endoplasmic reticulum (hydropic or vacuolar degeneration).

The ill-defined cell boundaries detected in the current work came in accordance with **Choudhary and Devi (2014)** who found cellular disruption in the spleen of adult male albino rats treated with 40mg/kg body weight of aspartame for 90 days.

Division of nucleus without division of cytoplasm detected in our experimental groups of submandibular gland came in accordance with **Humphries and Pretorius (2007)** who found increase in the percentage of binuclear hepatocytes in the liver of rabbits after treatment with aspartame for 37 days. Authors stated that mononuclear cells undergone endomitosis when nuclear volume and DNA content have been approximately doubled. They attributed these numerous mitotic figures to attempts of the cells to repair following injury.

Dilated striated ducts and stagnant secretion of granular tubules detected in our histological findings could be explained by **Starkov and Wallace (2002)** who claimed that dilatation and stagnant secretion in the lumen of some ducts could be presumably due to the mitochondrial damage that might led to ATP depletion with subsequent failure of biosynthesis and membrane pumps. Thus, the cells had no energy for the process of transport of secretions resulting in ductal dilatation and congestion.

Histological examination of the submandibular gland sections after 4 months revealed obvious morphological changes. The parenchyma of the submandibular gland lost its normal acinar architecture. Some acinar cells presented granular cytoplasm while others exhibited empty vacuoles (fig.4). Signs of pre- malignancy such as nuclear hyperchromatism, pleomorphism and abnormal mitosis were evident.

Our finding regarding loss of the typical parenchymal architecture could be explained by **Abdin (1981)** who mentioned that hydropic degeneration is a disturbance in the metabolism of the cell resulting in morphologic abnormalities. Thus, the cells could lose their normal architectural pattern of arrangement. The blood vessels were engorged with blood (Fig.5).

Granular appearance of cytoplasm could be explained by **Robbins *et al.*, (1984)** who had reported that vacuoles represent a cellular defense mechanism against toxic substances, in which these substances were aggregated in the vacuoles, thus preventing their interference with cellular metabolism giving the cytoplasm its granular appearance.

The premalignant changes detected in our histological sections might be explained by **Humphries *et al.* (2008)** who mentioned that methanol in the body is not metabolized within enterocytes and rapidly enters portal circulation to be oxidized in the liver to formaldehyde and diketopiperazine (a carcinogen). Authors stated that formaldehyde adducts accumulate in the tissues, in both proteins and nucleic acids. The formed adducts of the metabolic poisons alter both mitochondrial DNA and nucleic DNA. Thus, methanol and formaldehyde are known to be carcinogenic and mutagenic. The damaged DNA could cause the cell to function inadequately or have an unbalanced homoeostasis.

After 6 months of consumption of aspartame, the submandibular gland exhibited deleterious

morphological alterations in the parenchyma of the gland. The granular convoluted tubules became the most prominent feature of the gland on the expense of the atrophic serous acini (Fig.6).

The serous acini appeared shrunken and presented variable morphological patterns. The acinar cells exhibited disrupted cellular boundaries and foggy cytoplasm. Most nuclei exhibited hyperchromatism (Fig.7).

Granular convoluted tubules appeared to be completely destructed with complete loss of their normal architecture. The tubules showed loss of their cellular and basal boundaries. Cytoplasm in tubules appeared as one eosinophilic homogenous mass showing criteria of coagulative necrrosis (Fig.8).

All end capillaries were engorged with collected R.B.Cs). There was marked increase in the thickness of the interlobular connective tissue (Fig.9).

McKinnell and Rudnicki (2004) reported that atrophy is shrinkage in the size of the cell by the loss of its substance. They emphasized that atrophic cells may have diminished function, but they are not dead. Authors stated that atrophy could be resulted from diminished blood supply and inadequate nutrition. The fundamental cellular changes in atrophic cells were found to represent a retreat by the cell to have a smaller size at which survival is still possible. A new equilibrium was found to be achieved between cell size, and diminished blood supply, or nutrition.

Moreover, **Geokas (1984)** stated that these degenerative changes might demarcate a failure in the adaptive response of the cells when high dose of the toxin was given for a prolonged period of time.

The increase in area of granular convoluted tubules on the expense of serous acini was in line with **Chiou (1990**) who investigated the histological changes in the submandibular gland of adult male mice arising during the growth of sarcoma subcutaneous tumors after inoculation of the tumor cells. The author found that there was a slow increase in the relative cross-sectional area of the granular convoluted tubule (GCT) as the tumors grew with constant mean weight of the gland. In addition, the average number of granules per granular convoluted cell was found to be increased above the normal level. These results indicated that the growth of the sarcoma subcutaneous tumor caused morphological changes in the GCT and GCT cells, suggesting an alternation in the requirements of the secretions contained in the granules, such as the epidermal growth factor, during the growth of the tumor.

Thickened fibrous connective tissue septa observed after 6 months of our experiment can be explained by **Menezes *et al.* (2005)** who reported that all extensive injuries might be repaired with collagen fibers (scar) irrespective of its cause. Therefore, thickened connective tissue septa detected in our experiment could be due to increase in collagen fibers deposition induced by injury resultant after prolonged period of aspartame administration.

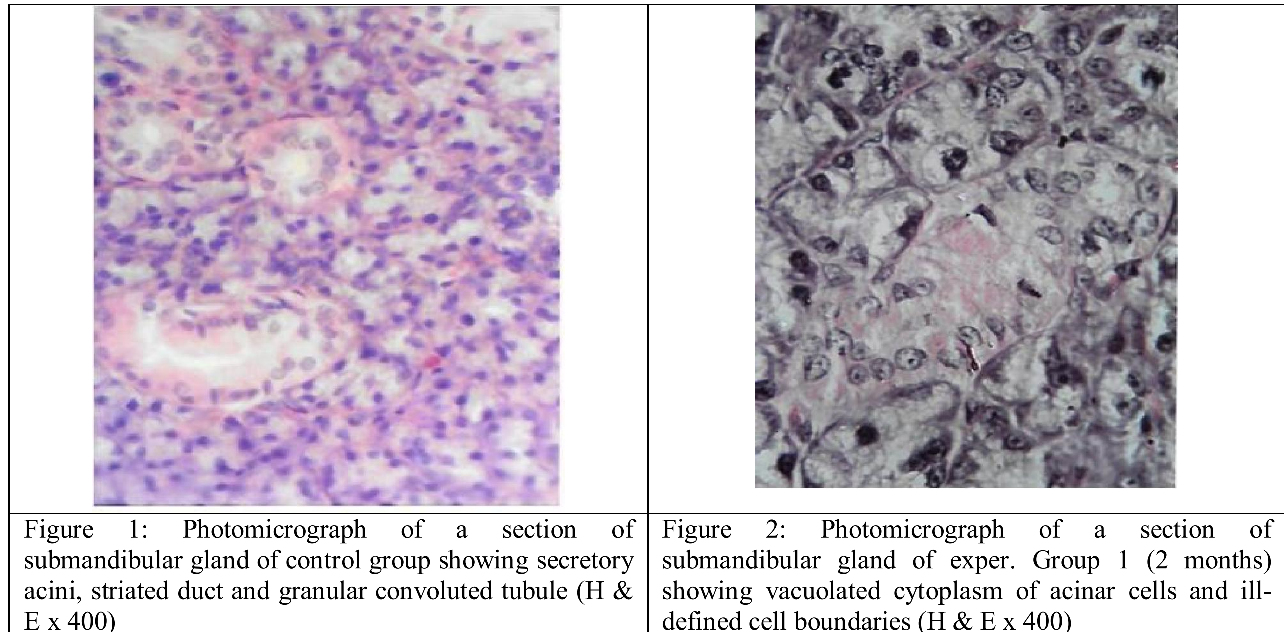

Figure 1: Photomicrograph of a section of submandibular gland of control group showing secretory acini, striated duct and granular convoluted tubule (H & E x 400)

Figure 2: Photomicrograph of a section of submandibular gland of exper. Group 1 (2 months) showing vacuolated cytoplasm of acinar cells and ill-defined cell boundaries (H & E x 400)

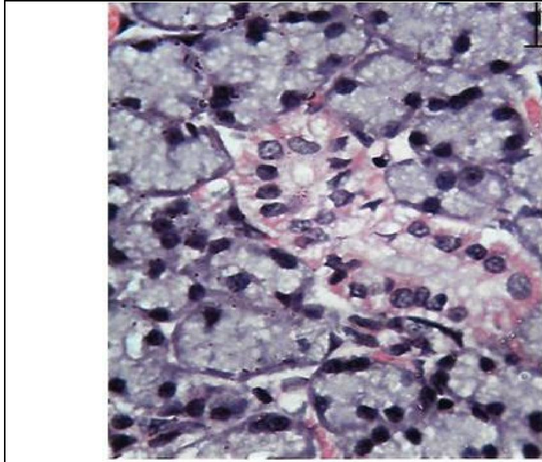

Figure 3: Photomicrograph of a section of submandibular gland of exper.group 2 (2 months) showing granular duct with stagnant eosinophilic secretion (H & E x 400)

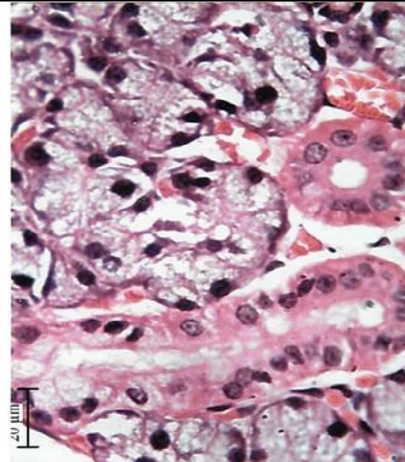

Figure 4: Photomicrograph of a section of submandibular gland of exper. group 2 (4 months) showing vacuolated acinar cells cytoplasm and hyperchromatism of nuclei (H & E x 400)

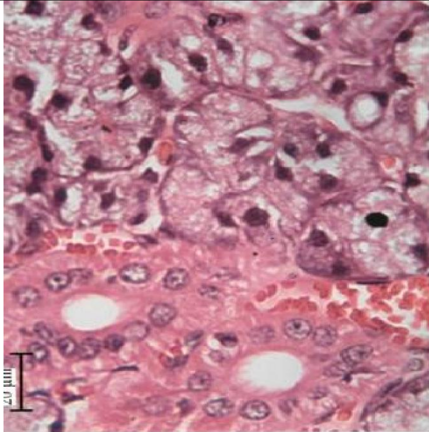

Figure 5: Photomicrograph of a section of submandibular gland of exper. group 2(4 months) showing blood vessels engorged with blood (H & E x 400)

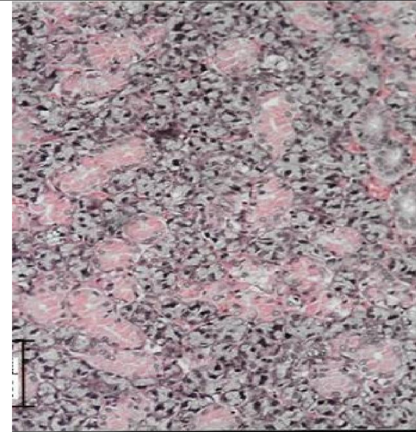

Figure 6: Photomicrograph of a section of submandibular gland of exper. group 1 (6 months) showing more prominent granular convoluted tubules (H & E x 400)

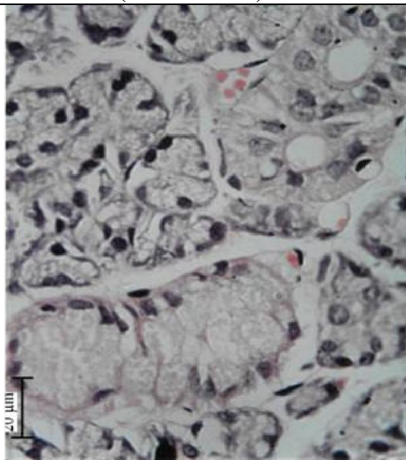

Figure 7: Photomicrograph of a section of submandibular gland of exper. group 1 (6 months) showing foggy appearance of acinar cells cytoplasm and hyperchromatism of nuclei (H &E x400)

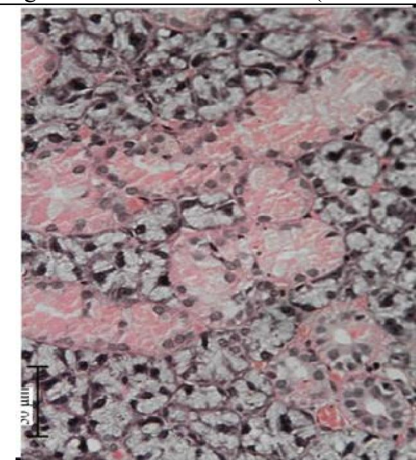

Figure 8: Photomicrograph of a section of submandibular gland of exper. group 1 (6 months) showing coagulative necrosis of epithelium of granular convoluted tubules (H & E x 400)

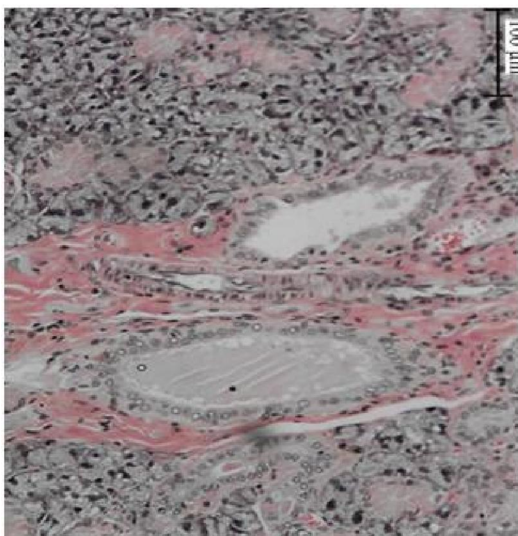

Figure 9: Photomicrograph of a section of submandibular gland of exper. group 2 (6 months) showing increase thickness of interlobular connective tissue (H & E x 400).

4. Conclusion:

It was concluded that consumption of aspartame induced mild toxic effects in the structure of the submandibular salivary glands in the first two months of intake. These toxic effects became more deleterious as the dose and period of aspartame consumption increased.

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