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RESEARCH ARTICLE

Effect of Long Term Administration of Aspartame on the Parotid Salivary Glands of Male Albino Rats

*Mohamed El -Sakhawy¹, and ShereinSaeid²

1. Department of Cytology & Histology, Faculty of Vet. Med, Cairo University, Egypt.

2 Department of Pathology, Faculty of Vet. Med, Cairo University, Egypt.

Manuscript Info Abstract

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*Corresponding Author

ShereinSaeid

shereinpathology@staff.cu.
edu.eg

40 male albino rats were utilized in the current study. They were divided into two groups, 20 animals each. The first group was considered control group and daily received saline solution by stomach tube for 4 months. The second group was the experimental group and daily received 40mg/kg body weight of aspartame for the same period. Samples from the parotid salivary glands were fixed in 10% buffered neutral formalin and prepared routinely for paraffin sectioning and staining for histopathological and immunohistochemical investigations of proliferating cell nuclear antigen (PCNA). Aspartame treated animals revealed histopathological degenerative changes in the parotid salivary glands; the acinar cells showed numerous intracytoplasmic vacuoles and cellular destruction. The nuclei of the acinar cells revealed hyperchromatism, pleomorphism, and numerous mitotic figures. edema was recorded in between the acini and ducts. The ducts showed stagnant secretion and the blood vessels were congested. The connective tissue septa increased in thickness and infiltrated with chronic inflammatory cells. The immunorexpression of PCNA in the nuclei of the acinar cells was intense after 4 months daily administration of aspartame. To our knowledge, there is no paper in the available literature reporting the immunohistochemical expression of PCNA exclusively in parotid salivary glands. Therefore, the aim of our study is to study the expression of PCNA in the parotid salivary glands.

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1. Introduction

Aspartame (ASP) 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. Approximately 50% of aspartame molecule is phenylalanine, 40% is aspartic acid and 10% is methanol (Newsome, 1986). Puica et al., 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 (34

mg/kg bw; 100 mg/kg bw; 150 mg/kg bw) 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 bw 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 50.4 mg/kg bw.) 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 off spring showed marked histopathological changes. However, the reports of presumptive toxic effect of aspartame on salivary gland tissue, especially in human or mammals are scanty and mostly based on documentation of some clinical parameters like thirst sensation. Proliferating cell nuclear antigen (PCNA) was originally described in proliferating mammalian cells as a nuclear protein. The highly homologous nature of PCNA suggests that protein plays an essential role in DNA replication (**Nakane et al., 1989**). Recently, it is found to be necessary for proliferation with no relation to cyclin protein. Proliferating cell nuclear antigen (PCNA) was involved in the cellular cycle (**Itall et al., 1990**), and could be identified in replicating cells of both benign and malignant lesions. Higher expression of this marker had been shown in aggregative tumours (**Cardoso et al., 2000; Lazzarbo et al., 2000; and Tsuji et al., 1995**). This directed the present investigation, to study the effect of long-term administration of aspartame on the parotid salivary glands of male albino rats both histopathologically and immunohistochemically, as such study was lacking in the available literature.

2. Material and methods

2.1 Experimental-animals:

The study was carried out on forty (40) adult male albino rats weighing about 200-220 gram (gm); they were caged in the animal room in the faculty of Veterinary Medicine Cairo University throughout the experimental period (4 month). The animals were maintained on stock diet and kept under fixed appropriate conditions of housing and handling. Animals in each group were caged in separate cages. Pure aspartame (ASP) powder was Purchased from ADWIA.Co.Cairo,Egypt. Aspartame was dissolved in water and introduced to the esophagus using metallic stomach tube. The animals were classified into 2 groups.

2.2 Grouping of the experiment:

Group I (control): Composed of twenty male albino rats received daily saline solution orally during the experimental period.

Group II (Aspartame): Composed of twenty male albino rats received a daily oral dose of aspartame (40mg/kg body weight) for 4 months.

2.3 Obtaining-of-specimens-and-tissue-preparation:

Samples from The Parotid salivary glands were obtained at 2 and 4 months along the experimental period. Rats were sacrificed by cervical decapitation then the parotid salivary glands were carefully dissected out, fixed in neutral buffered formalin and prepared for histopathological and immunohistochemical study.

2.4 Histopathological examination:

Specimen from Parotid salivary glands were washed, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Sections of 5-6 μ m in thickness were cut out, deparaffinized and stained with Haematoxylin and Eosin (H&E) for examination under the light microscope. (**Bancroft et al., 1994**).

2.5 Immunohistochemical-examination:

Immunohistochemistry was performed on paraffin sections, and mounted on coated glass slides. Antigen was retrieved in citrate buffer (PH 6.0) microwave digestion (2 cycles of 12 minute each). Endogenous peroxidase was blocked with 0.05% hydrogen peroxide for 30 min. After incubation with a 1:20 dilution of normal horse serum, the slides were incubated overnight at 4°C with primary antibodies (Dako, 1: 50). Secondary antibodies associated with a streptavidin-biotin-peroxidase method were applied (Dako A/S). Diaminobenzidine was used as cromogen. All sections were counter-stained with haematoxylin. The sections were washed with phosphate buffered saline after each step. Negative controls were used using non-immune serum instead of the primary or secondary antibodies. The method used was outlined according to **Ramos-Vara (2005)**.

3. Results

Examination of H&E stained **control group** sections of the Parotid salivary glands revealed that, the gland consisted of secretory acini and ducts. These serous acini appeared round and had a narrow lumen. The acini were lined by pyramidal cells with apical acidophilic cytoplasm. Their nuclei were prominent, deeply stained, Spherical in shape and basally situated (**Figure 1**). The duct system presented intercalated, striated and excretory ducts. The intercalated ducts were hardly identified, as they were compressed between the acini. The striated ducts were lined by a single layer of columnar cells which showed well-defined outlines and central, rounded, darkly stained nuclei. The cytoplasm appeared eosinophilic and showed basal striation (**Figure 2**). Thick fibrous connective tissue was present between the lobes and lobules of the Parotid glands. Both acini and ducts revealed negative immune reaction to PCNA (**Figure 3**). Examination of H&E stained **aspartame group** sections of the Parotid salivary glands contributed that the daily administration of aspartame for **two months** caused multiple histopathological and immunohistochemical changes in the Parotid salivary glands; the serous acini appeared widely separated. Multiple large and small vacuoles were present intra-cellularly (**Figure 4**). The cell boundaries of the serous acini were ill-defined. Some elongated as well as pyknotic nuclei were observed. Some striated ducts showed granular cytoplasm and pyknotic nuclei. The connective tissue septa surrounding the lobes and lobules showed increase in the thickness (**Figure 5**), and was infiltrated by inflammatory cells. The blood vessels showed wide dilatation and were engorged with blood (**Figure 6**). The nuclei of both secretory acini and ducts revealed strong immune staining for PCNA (**Figure 7**). The cytoplasm of the acinar cells gave positive reaction. Examination of the parotid salivary glands **after four months** of daily administration of aspartame showed more numerous degenerative changes. Some acini revealed destruction (**Figure 8**) other acini showed increase in the intracytoplasmic vacuoles. These vacuoles varies in size from small to large ones occupying most of the cell (**Figure 9**). Large edematous spaces were present between the serous secretory acini (**Figure 10**). Increased mitotic figures were frequently detected in the serous acinar cells (**Figure 11**). Some cells presented enlarged hyperchromatic nuclei and others revealed binucleation (**Figure 12**). The striated duct cell showed degeneration with indistinct cell boundaries and basal striation and filled with stagnant secretion. The connective tissue septa appeared severely thick and infiltrated by chronic inflammatory cells (**Figure 13**). Inter-acinar edema was noticed. The nuclei of the acinar cells showed intense immunoreactivity for PCNA (**Figure 14**). The reaction was less expressed in the nuclei of ductal epithelial cells (**Figure 15**).

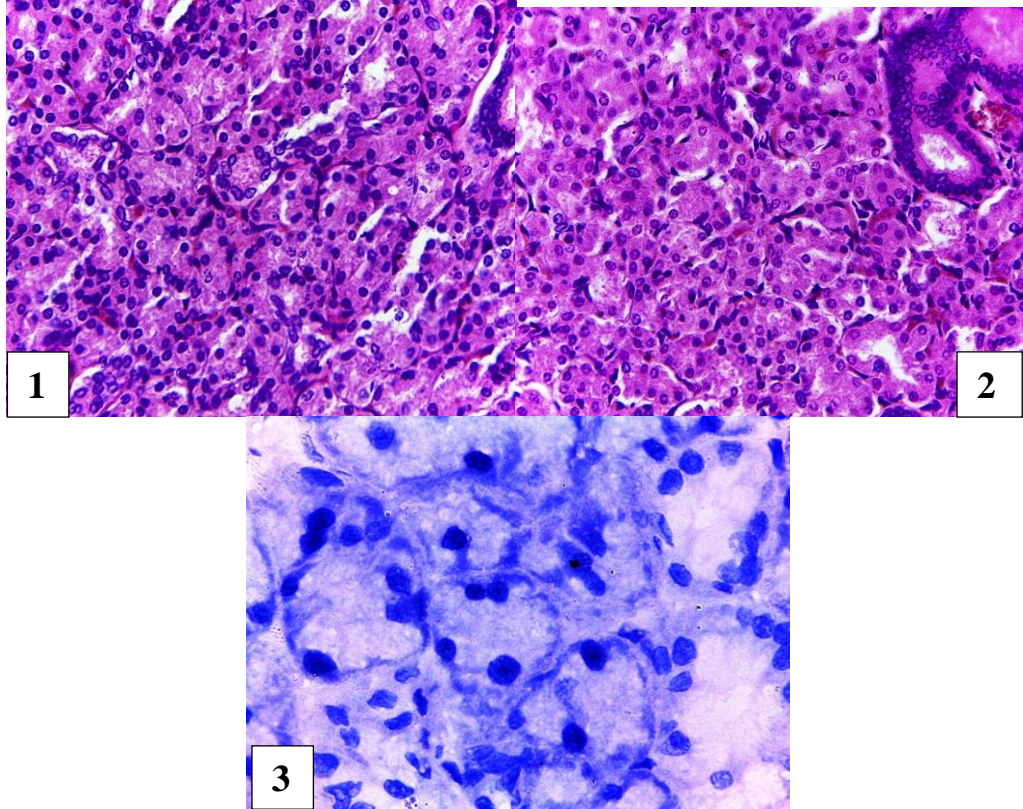


Figure 1: Photomicrograph of parotid salivary glands of albino rats from control group showing healthy acini (H&E X 200).

Figure 2: Photomicrograph of parotid salivary glands of albino rats from control group showing healthy striated ducts (H&E X 200).

Figure 3: Photomicrograph of parotid salivary glands of albino rats from control group showing negative immune reaction to PCNA (PCNA X 1000).

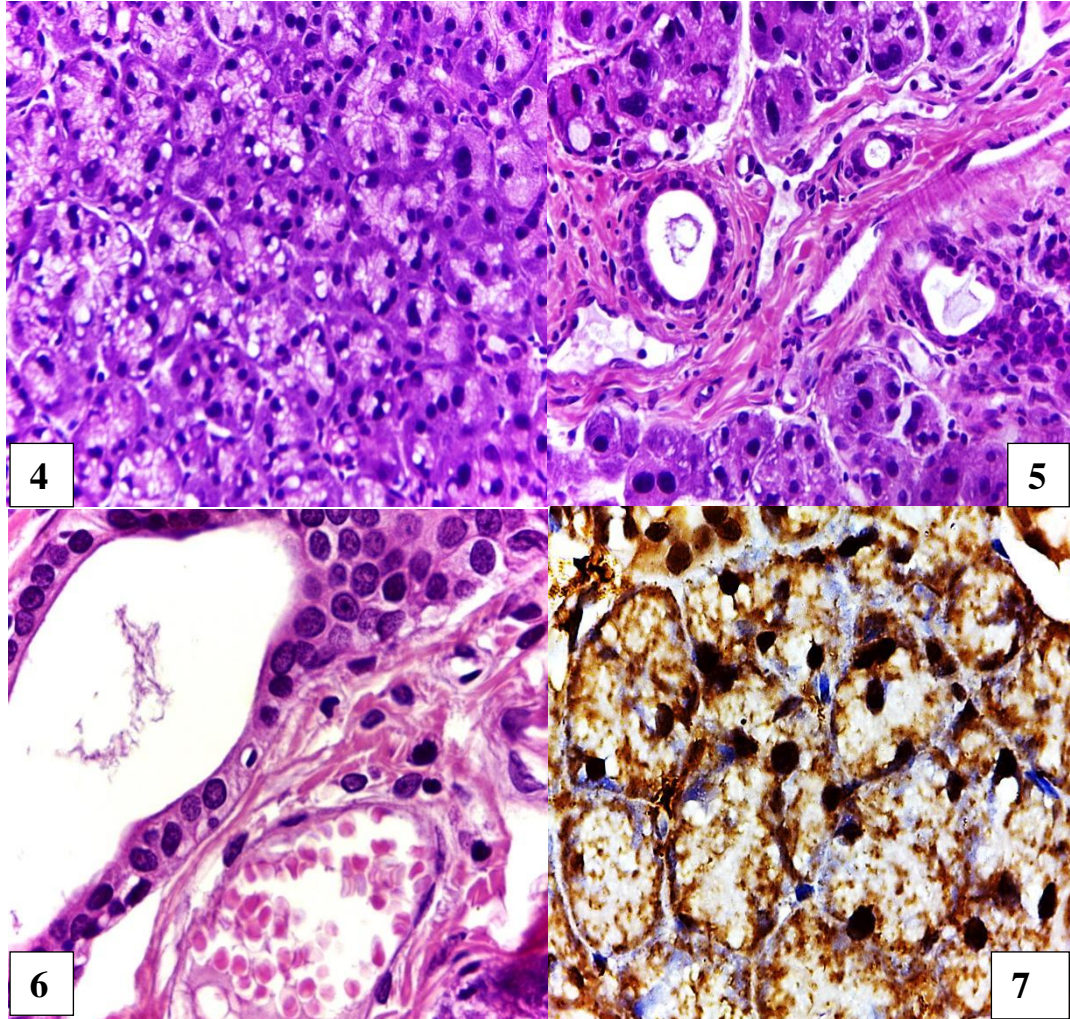


Figure 4: Photomicrograph of parotid salivary glands of albino rats from aspartame group after 2 months showing intra-cellular multiple large and small vacuoles, (H&E X 200).

Figure 5: Photomicrograph of parotid salivary glands of albino rats from aspartame group after 2 months showing increase in the thickness of the connective tissue septa, (H&E X 400).

Figure 6: Photomicrograph of parotid salivary glands of albino rats from aspartame group after 2 months showing dilatation and congestion in the blood vessels, (H&E X 400).

Figure 7: Photomicrograph of parotid salivary glands of albino rats from aspartame group after 2 months showing strong immune staining in the nuclei of both secretory acini, (PCNA X 1000).

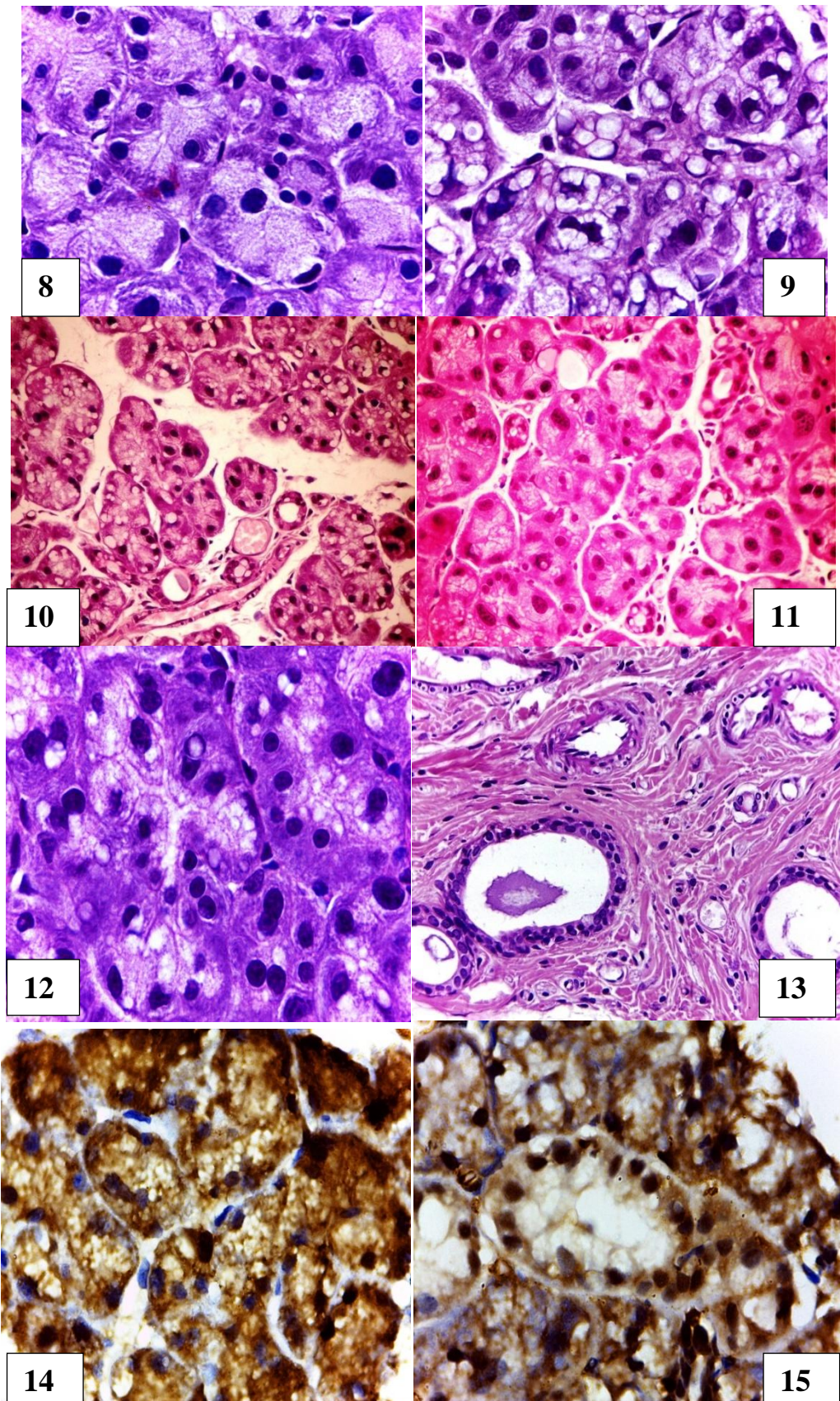


Figure 8: Photomicrograph of parotid salivary glands of albino rats from aspartame group after 4 months showing numerous degenerative changes with acinar destruction (H&E X 1000).

Figure 9: Photomicrograph of parotid salivary glands of albino rats from aspartame group after 4 months showing increase in the intracytoplasmic vacuoles that occupying most of the cell (H&E X 1000).

Figure 10: Photomicrograph of parotid salivary glands of albino rats from aspartame group after 4 months showing large edematous spaces between the serous secretory acini (H&E X 400).

Figure 11: Photomicrograph of parotid salivary glands of albino rats from aspartame group after 4 months showing increased mitotic figures in the serous acinar cells (H&E X 400).

Figure 12: Photomicrograph of parotid salivary glands of albino rats from aspartame group after 4 months showing enlarged hyperchromatic nuclei in the serous acinar cells (H&E X 1000).

Figure 13: Photomicrograph of parotid salivary glands of albino rats from aspartame group after 4 months showing severe thickening in the connective tissue septa with chronic inflammatory cells infiltration (H&E X 400).

Figure 14: Photomicrograph of parotid salivary glands of albino rats from aspartame group after 4 months showing intense immunoreactivity in the nuclei of the acinar cells (PCNA X 1000).

Figure 15: Photomicrograph of parotid salivary glands of albino rats from aspartame group after 4 months showing less intense immunoreactivity in the nuclei of ductal epithelial cells (PCNA X 1000).

Discussion:

In the present work, the effect of long periods of aspartame administration on the parotid salivary glands of albino rats was studied. The results of the present study showed that aspartame administration to rats resulted in many histopathological changes in the parotid salivary glands. The serous cells and some striated ductal epithelial cells revealed indistinct cell boundaries and intracytoplasmic vacuoles. The connective tissue septa showed increased in thickness, with chronic inflammatory cells infiltration. The blood vessels showed dilatation and congestion. Serous acini separation was noticed in aspartame group which might be attributed to edema. **Geokas (1984)** demonstrated edema in the human lungs after chronic administration of methanol. Also, **John (1994)**, noticed edema of the optic nerve as a result of formaldehyde administration. **Stegink et al., (1987a)** stated that methanol and formaldehyde were products of breakdown of aspartame. Also, **Kaplen (1987)** concluded that, edema was due to increased capillary hydrostatic pressure in case of congestion and due to increased capillary permeability as in inflammation. **Liesivuori (1991)** suggested that, the indistinct cell boundaries of serous cells might be attributed to the increased influx of calcium into the cells as a result of the action of methanol and aspartate. The present work showed that, the cytoplasm of the acinar and ductal epithelial cells appeared granular after aspartame administration. This agreed with the suggestion of **Woolf (2000)**, who stated that the first manifestation of cell injury or degeneration is cellular swelling due to accumulation of water and thus the cytoplasm of the cells appeared granular. Moreover, **Puica et al., (2008)** revealed degenerative changes of all cellular contents of the hypothalamic neurons of juvenile rabbits after aspartame administration. The presence of intracytoplasmic vacuoles in the acinar cells of the parotid gland of rats from aspartame group simulate the results of **Eells et al., (2000)** in the retinal pigment epithelium following methanol administration. **Omar (2009)** demonstrated vacuoles in the cytoplasm of pyramidal cells of the frontal cortex of rats after 8 weeks of aspartame administration. He concluded that, aspartame metabolism leads to generation of large number of free radical species as nitrogen and oxygen. These free radicals could damage cellular proteins. The excretory ducts of the parotid salivary glands of experimental rats showed stagnant secretion. **Steven and Lowe (1995)** concluded that, the stagnant secretion in the excretory ducts might be due to mitochondrial affections. This mitochondrial affection lead to depletion of ATP with failure of biosynthesis and membrane pumps and as a result, the cells had no energy for the process of secretion transport. The present Investigation revealed blood vessels dilatation and congestion from aspartame group. **Barua and Bal (1995)** stated that, formaldehyde liberated after aspartame ingestion lead to cytotoxicity to cultured endothelial cells. Moreover, **Stevens and Lowe (1995)** concluded that, blood vessels congestion might be a part of the inflammatory process to bring more blood to the area of inflammation. The present work showed signs of premalignancy as hyperchromatism of nuclei of serous cells of experimental animals, abnormal mitosis and pleomorphism. These changes in the nuclei of acinar cells were more obvious after 4 months of aspartame administration, which might be attributed to the cumulative harmful effect of aspartame (**Barua and Bal, 1995**). The proliferative activity of the parotid salivary glands was detected in the present work using an immunohistochemical staining of proliferating cell nuclear antigen (PCNA). The present study revealed that intense expression of PCNA was recorded in the nuclei of the acinar cells of the parotid salivary glands after 4 months of aspartame administration. This could be an indication for increasing proliferation rate, as an attempt to repair and renew the damaged cells. **Pusztai et al., (1993)** stated that, the accelerated proliferation might indicate an increased mutagenic risk on cells. **Itall et al., (1990)** concluded that PCNA which were involved in the

cellular cycle could be identified in replicating cells of both benign and malignant lesions. Aspartame might cause brain, uterine, ovarian, and pancreatic tumours (Blaylock (1994)). Moreover, Rigas et al., (1983) concluded that, due to the similarities between the pancreas and parotid salivary glands, it is possible that the same effect present in the salivary glands.

Conclusion:

From the existing study, long term administration of aspartame to male albino rats revealed obvious histopathological changes in the parotid salivary glands. Moreover, the proliferation rate of the acinar cells was increased. Therefore, we recommended that Aspartame administration must be restricted.

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