EGYPTIAN DENTAL JOURNAL

VOL. 64, 1275:1282, APRIL, 2018

I.S.S.N 0070-9484

ORAL MEDICINE, X-RAY, ORAL BIOLOGY AND ORAL PATHOLOGY

www.eda-egypt.org • Codex : 190/1804

HISTOLOGICAL CHANGES OF TONGUE PAPILLAE INDUCED BY THE ARTIFICIAL SWEETENER ASPARTAME AND THE PROTECTIVE EFFECT OF PLATELET-RICH PLASMA

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ABSTRACT

Aspartame (ASP) is one of the most controversial food additives found in various food products all over the world. Once ingested, ASP is metabolized to phenylalanine, aspartic acid, and methanol. The aims of the study are to evaluate the effects of ASP administration on the dorsal surface of rats' tongues and to investigate the possible protective effect of Platelet-rich plasma (PRP). Thirty adult male albino rats were used and divided into three equal groups. The control group received the vehicle only. ASP group received a dose of 250 mg/kg b wt for four weeks while the PRP group were treated as ASP group, in addition they received a single local injection with PRP in the right lateral border of the tongue. The most obvious changes were loss of normal conical appearance with hyperkeratosis of filiform papillae. The epithelial cells showed hyperplasia (acanthosis), cellular pleomorphism and nuclear hyperchromatism. Distorted and atrophied fungiform papilla with degeneration of epithelial cells and pyknotic nuclei were visible. In the lamina propria numerous dilated and congested blood vessels were obvious. SEM examination revealed disfigurement of tongue papillae. The fungiform papillae showed irregular wrinkled surface and ill-defined taste pore. PRP treatment almost restored the normal architecture of tongue papillae. Chronic aspartame ingestion could result in marked morphological alteration of dorsal surface of the tongue and PRP exerted a protective role against this effect.

KEYWORDS: Aspartame, platelet-rich plasma, tongue papillae, rat.

INTRODUCTION

Artificial non-nutritive sweeteners; also referred to as low caloric and alternative sweeteners; are considered as one of the food additives which are added to foods to sweeten its taste without increasing its caloric content [(1)]. Aspartame (ASP) (L-aspartyl-L-phenylalanine methyl ester) has been widely used as an artificial sweetener to reduce sugar consumption and to decrease caloric intake in persons on diet as well as in diabetic

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patients [2]. It is found in many products, including soft drinks, chewing gum, candy, yogurt, and some pharmaceutical products, such as vitamins and sugar free cough drops [3]

According to recommendations from FDA and the JECFA (Joint FAO/WHO Expert Committee on Food Additives), the acceptable daily intake of aspartame is 50 mg/ kg and 40 mg/kg per day, respectively [4,5]. Although, the Food and Drugs Administration and other advisory agencies have considered ASP to be safe in acceptable daily intake range, however some experimental and epidemiological studies have recently reported that ASP consumption at abuse doses results in some adverse effects such as obesity [6,7], nephrotoxicity [8], metabolic syndrome [9], cancer [10,11], and adverse neurobehavioral effects [12]. Immediately after ingestion, ASP is metabolized in the intestinal lumen and hydrolyzed into three constituents named as phenylalanine (50%), aspartic acid (40%) and methanol (10%) [13]. Most of the adverse effects of ASP were related to the generation of aspartame metabolites, particularly to methanol metabolites as formaldehyde, formate and a number of highly toxic derivatives. It is supposed that metabolites of ASP including methanol is accompanied by generation of superoxide anion and hydrogen peroxide in different organs as kidney [8], liver [14] and brain [15]. Experimental studies on animals have shown that ASP consumption was associated with degenerative changes in different tissues and organs including hypothalamic and anterior pituitary cells [16], frontal cortex [17], liver and kidney [18] and salivary glands [19]. Since, ASP has been widely used in many products and the everyday consumption of it is increasing, it is questionable whether the ingestion of ASP at abuse doses is still safe because it is possible that people might unintentionally take large dose of ASP. Therefore, more studies are recommended to prove or disapprove the safety of ASP.

Platelet-rich plasma (PRP) has grown as an attractive biologic instrument that is widely used recently in regenerative medicine. It is an autologous blood product composed of concentrated platelets and it stimulates the production and, accordingly, increase in the concentration of several growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β1, vascular endothelial growth factor (VEGF) insulin-like growth factor-1 (IGF-1). PRP plays a key role in angiogenesis and tissue regeneration by promoting cell migration, differentiation, proliferation and controlling physiological functions [20,21]. It also induces the secretion of proteins which can maximize the healing process at the cellular level [22]. It is also well documented that PRP improves the regeneration of different tissues and reduces scar formation, since it accelerates the maturity and regeneration of the epithelium of wound [23].

There are no previous reports on the effects of ASP ingestion on tongue mucosa. Accordingly, the aim of the current study was to evaluate, the possible toxic effect of long term ASP administration (8 weeks) on histological and scanning electron microscopic features of the mucosa of dorsal surface of tongue in rats and to investigate the possible protective effect of PRP to ASP-induced injury.

MATERIAL& METHODS

Animals

Thirty adult male albino rats (200-240 g b.w.) were obtained from Faculty of Medicine, Cairo University. They were fed standard food and tap water *ad libitum* and were kept under fixed laboratory conditions (temperature [25±2] lighting (12h light-dark cycle). The care and treatment of the animals was carried out in accordance with the guidelines of the National Institutes of Health for the care and use of laboratory animals (NIH Publication Number 85-23, Rev. 1985).

Chemicals

- Pure aspartame (ASP) powder was purchased from ADWIA Co. Cairo- Egypt and was dissolved in water to be given orally to rats.

PRP Preparation: A 3.5mL volume of autologous blood was drawn from 24 animals into vacuum tubes containing 10% sodium citrate. The PRP was prepared according to a double-centrifugation protocol [24].

Experimental design

Rats were randomly divided into three equal groups. The first group served as control and received the vehicle only orally throughout the whole experimental period. The second group (ASP group) received a daily dose of ASP (250 mg/kg b wt dissolved in distilled water). Animals of the third group (ASP+ PRP group) received a daily dose of ASP as the second group, in addition they received local injection with PRP in the right lateral border of the tongue (a single dose). Injection was done using a 1mL insulin syringe with a needle size 27 gauge × 1/2 inch (BD Nokor). The experimental period was 6 weeks. At the end of the experiment, all animals were sacrificed by cervical dislocation under light ether anesthesia and the tongue was carefully removed and immediately cut into two halves. Each half was prepared for either histological or scanning electron microscopic examinations.

RESULTS

Light microscopic results:

The dorsal surface of the tongue of the control group was covered by keratinized stratified squamous epithelium. The lamina propria was located beneath the epithelium and separated from it by basement membrane. The filiform papillae appeared as numerous thin, elongated, conical projections with their tips pointed backward toward the base of the tongue. They were covered by thick keratinized epithelium (Fig. 1a). The fungiform papillae appeared as dome- shape structure elevated above the surface and scattered in between the filiform (Fig. 1b).

H& E examination of the ASP group revealed abnormal morphology of the filiform and fungiform papillae. The filiform papillae showed loss of normal conical appearance with hyperkeratosis of their epithelial covering (Fig. 2a). There were epithelial hyperplasia (acanthosis), cellular pleomorphism and nuclear hyperchromatism (Fig. 2b). The fungiform papilla was distorted and atrophied. Some epithelial cells were degenerated with pyknotic nuclei. The cells of taste bud were irregular and separated from each other (Fig. 2c). In the lamina propria numerous dilated and congested blood vessels were obvious.

PRP treatment restored almost normal architecture of tongue papillae. The epithelial covering of the filiform papillae showed hyperplasia and hyperkeratosis (Fig. 3a). Some degenerated epithelial cells of the prickle cell layer were still present. The fungiform papilla and taste buds were closely resembled the control group (Fig. 3b).

Scanning Electron Microscopic results:

The filiform papillae of the control group appeared closely packed and regularly oriented in parallel rows with uniform keratinized tapered ends (Fig. 4a). The fungiform papilla was rounded with a smooth surface and well-defined taste pore located on the center (Fig. 4b). SEM examination of the ASP group showed disfigurement and areas of fusion of the filiform papillae. Some of these papillae were having keratinized blunt ends. The interpapillary areas were prominently rough (Fig. 5a). The fungiform papillae of the same group were distorted with irregular wrinkled surface and ill-defined taste pore. Some fungiform papilla showed irregular depressed top surface (Fig. 5b).

In the PRP group, the filiform and fungiform papillae were nearly normal resembling the control group. The filiform papillae showed more regular orientation compared to ASP group. They were having tapered ends and normal interpapillary distances (Fig. 6a). The fungiform papilla was regular with well-defined outlines and well defined central taste pore. The surface appeared smooth, but the top surfaces were still depressed (Fig. 6b).

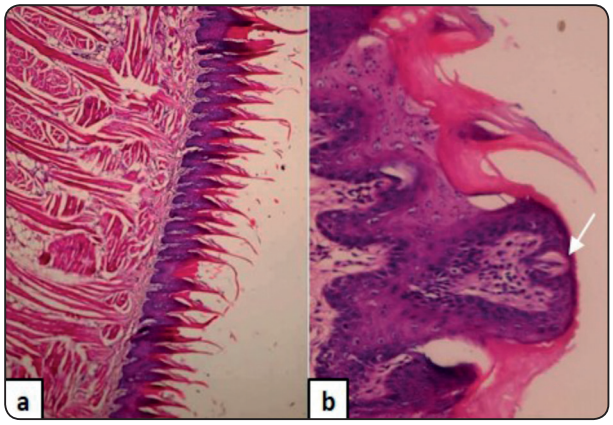

Fig. (1) Photomicrograph of the dorsal surface of the tongue of the control group showing (a): normal architecture of filiform papillae, keratinized stratified squamous epithelial covering and thin underlying lamina propria. The tongue muscle fibers are running in different directions underneath the papillae (b): Fungiform papilla is covered by keratinized stratified squamous epithelium and a well-defined barrel-shaped taste bud is present on its dorsal surface (arrow). (H&E a x100 and b x200).

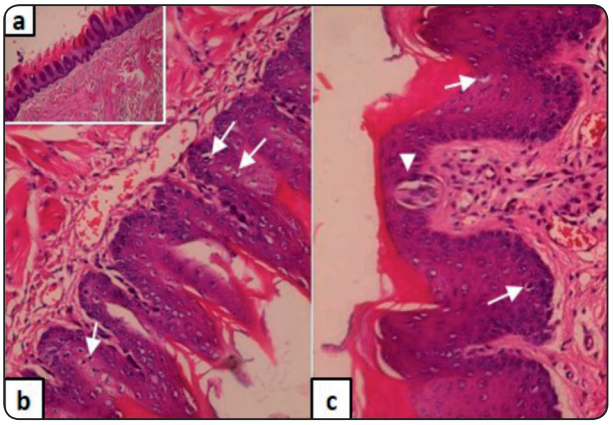

Fig. (2) Photomicrograph of the dorsal surface of the tongue of ASP group showing (a): ill defined filiform papillae with epithelial hyperkeratosis. (b): The epithelial cells of filiform papillae show cellular pleomorphism and nuclear hyperchromatism. Some epithelial cells appears degenerated with pyknotic nuclei (arrows). The lamina propria is infiltrated with inflammatory cells. (c): ill defined fungiform papilla with epithelial hyperplasia. Some epithelial cells appear degenerated with pyknotic nuclei (arrows). The taste bud shows irregularly arranged and separated cells (arrow head). The blood vessels of the lamina propria appear dilated and congested (H&E a x100 and b& c x200).

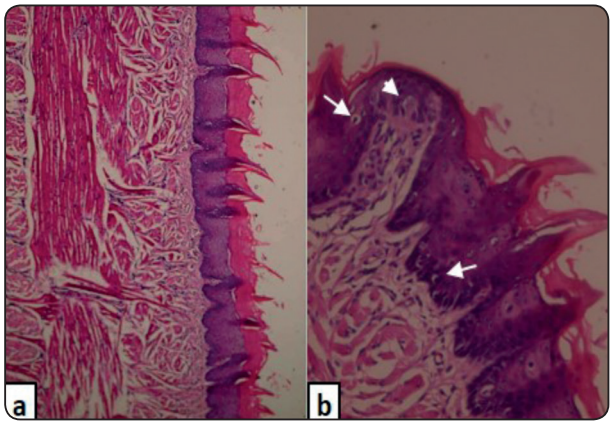

Fig. (3) Photomicrograph of the dorsal surface of rat tongue of PRP group showing (a): almost normal histological appearance of filiform papillae and fairly normal connective tissue papillae. Epithelial hyperplasia and hyperkeratosis are observed. (b): The fungiform papilla appear nearly normal with few degenerated epithelial cells having pyknotic nuclei (arrow). The cells of the taste bud show almost regular arrangement (arrow head) (H&E a x100 and b x200).

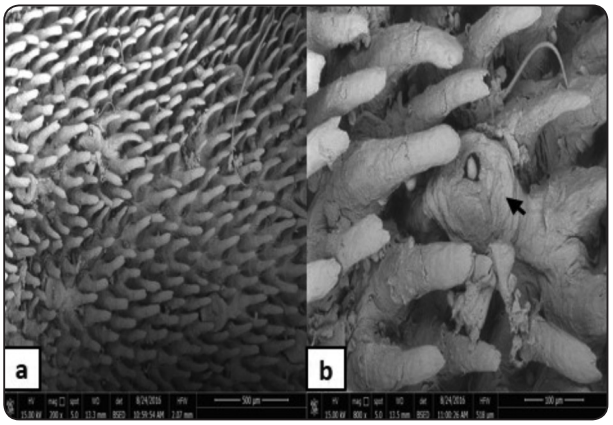

Fig. (4) Scanning electron micrographs of the control rat tongue showing (a): regular parallel rows of long conical filiform papillae with tapering ends. The papillae show uniform arrangement with antero-posterior inclination. (b): Mushroom-like fungiform papillae are interposed in between the numerous filiform ones (arrow). A well defined taste orifice can be seen on its top surface

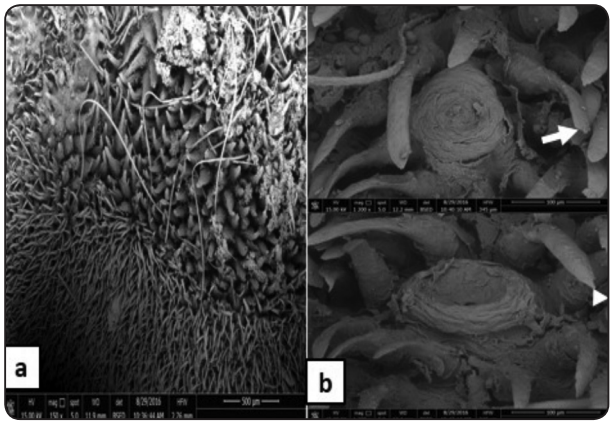

Fig. (5) Scanning electron micrographs of ASP rats' tongues. (a) The filiform papillae show disorganized orientation and inclination. Numerous grouping of bacteria can be seen on the surface of filiform papillae. Note the area of fusion of the papillae (asterisk) (b) Some filiform papillae show keratinized blunt ends (arrow) while other papillae exhibit pointed ends with constricted keratin covering (arrow head). The interpapillary areas were rough and prominent. The fungiform papilla appears distorted with wrinkled keratinized epithelial covering and ill defined taste pore while others appear with depressed top surface.

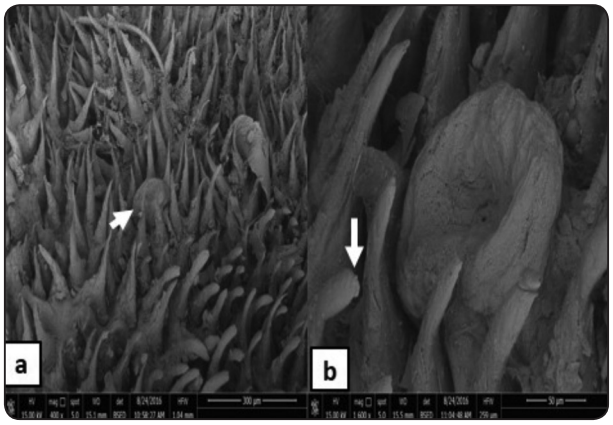

Fig. (6) Scanning electron micrographs of the PRP rats' tongues showing (a): partial restoration of normal appearance of filiform papillae with scattered fungiform papillae (arrow). The filiform papillae appear nearly normal and more regular with tapering ends and normal interpapillary distance. (b): almost normal fungiform papilla with depressed surface and regular smooth epithelial covering. Some filiform papillae still appear blunt (arrow).

DISCUSSION

The present study confirms that daily oral administration of aspartame at a dose of 250 mg/kg for four weeks led to marked alterations in the dorsal surface of the tongue in rats. Although ASP is widely used all over the world in many products, however there was lack of awareness among consumers about its potential toxic effects [25]. Several experimental researches have confirmed the negative effects of ASP consumption on different tissues and organs such as kidney [8], liver [14,26], cerebral cortex [27] and salivary glands [28,29].

In the present work, using light microscopy and SEM revealed obvious degenerative morphological changes of the tongue mucosa subsequent to ASP administered orally to rats. The filiform and fungiform papillae were disorganized and the epithelial cells showed histopathological changes as cellular pleomorphism, nuclear hyperchromatism. In addition degenerated epithelial cells with hyperchromatic nuclei were present. The reported histological results of the ASP group were previously reported by Omar 2009 who revealed marked cytoplasmic vacuolization with pyknotic nuclei in some pyramidal cells of the frontal cortex after ingestion of ASP at a dose of 250 mg/kg/day for 8 weeks [30]. The authors also reported the presence of other pyramidal cells with darkly stained nuclei and surrounded by halos. This observation was similar to some epithelial cells of tongue papillae in the present study. In addition, comparable signs of pre malignancy of the acinar cells in the submandibular glands in rats were reported by Mohammed et al. [28] after ASP administered at a dose of 40 mg/kg/day for four months.

SEM examination confirmed the atrophic changes of the tongue papillae in the form of loss of regular orientation and keratinization of the filiform papillae, rough prominent interpapillary ridges, wrinkled keratinized epithelial covering and ill-defined taste pore of the fungiform papillae.

These atrophic changes of the tongue papillae of ASP group could be attributed to the oxidative stress induced by ASP. Aspartame is hydrolyzed in the body to form phenylalanine (50%), aspartic acid (40%), and methanol (10%) and further breakdown of these products including formaldehyde and formic acid. The toxic effects of ASP was attributed to the toxic actions of methanol and its metabolic product formaldehyde, which was found to be more toxic than methanol [31]. Previous researches have confirmed the release of large number of free radicle species due to methanol and aspartic acid production during ASP metabolism [32]. These free radicles resulted in inflammation and injury to the cells and might be the cause of atrophy of tongue papillae in the present study. The increased free radicles and the consequent increased oxidative damage to the cellular proteins and mitochondrial DNA could induce apoptotic cell death in the brain following long term ASP administration [32]. Also, chronic treatment with high dose of ASP (80 mg/kg/day for 90 days) produced formaldehyde from methanol metabolism. Formaldehyde was found to form adducts with protein and nucleic acids in rat hepatocytes [33] and induce cell death in rat thymocyte [31].

In the current study, PRP treatment were able to restore the normal architecture of tongue papillae after ASP treatment. The improved histological and SEM features detected here were in agreement with the results of a recent study by Elsaadany et al. [34] who concluded the positive effects of PRP treatment in preventing or minimizing the epithelial atrophy of the dorsal surface of the tongue after radiotherapy. The positive effect of PRP observed here could be explained in the light of many previous studies that confirmed the effectiveness of growth factors derived platelets to enhance cell proliferation, differentiation, chemotaxis, angiogenesis, and extracellular matrix synthesis involved in the healing of mucositis after irradiation [35,36]. Elsaadany et al. concluded a unique angiogenic character of PRP and attributed it to the platelet functions and the coagulation factors. Also, it has been reported that platelets could store and release several angiogenic factors such as platelet-derived epidermal growth factor (PD- EGF), platelet-derived growth factor (PDGF), VEGF, basic fibroblast growth factor (bFGF), angiopoietins (Angs), and transforming growth factor beta (TGF-β) [36,37,38]. Furthermore, PRP was reported to increase the recruitment, proliferation and differentiation of the cells involved in the tissue regeneration [39].

In conclusion, the current study demonstrated detrimental effects of ASP on the dorsal surface of rats' tongues. Based on the results of the present research, PRP appeared to be protective against ASP induced changes. Science people consuming ASP products is increasing worldwide, warning the public against the hazard use of ASP and its possible serious injury is necessary. Further investigations are needed to explain and report the various toxic effects of ASP in different tissues and organs.

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