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(Article)

The Possible Protective Role of Pumpkin Seed Oil in Improving Tongue Mucosal Damage Induced by Artificial Sweeteners Aspartame (Histological study)

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ABSTRACT

Aspartame is a low-calorie artificial sweetener commonly used in a variety of processed foods; accompanied by degenerative changes in different tissues. The present study designed to evaluate the possible protective role of pumpkin seed oil in improving tongue mucosal damage induced by artificial sweetener aspartame in albino rats histologically. Thirty adult male albino rats (180 -200 gm) divided randomly into three groups (n=10). Group I (Control): rats received 1 ml distilled water. Group II: rats received aspartame (250 mg/kg in distilled water) orally. Group III: rats received dose of aspartame as the group II, additionally topical application of pumpkin seed oil. After four weeks, rats were euthanized and tongues were dissected and cut longitudinally, for histological and ultrastructure microscopic examination. Histological examination revealed abnormalities in the morphology of tongue papillae at group II represented by hyperplasia (acanthosis), cellular pleomorphism, nuclear hyperchromatism, congested blood vessels, and brown positive immunoreaction of only some basal epithelial cells to Ki67. This confirmed by ultrastructure microscopic examination which revealed a disfigurement of tongue papillae. In contrast, minimal changes appeared at group III. Conclusion, aspartame induced marked morphological changes at the tongue's mucosa of albino rats. pumpkin seed oil has a protective role against these effects.

Keywords: Aspartame, pumpkin seed oil, tongue papillae

1. Introduction

The tongue's mucosa exhibits regional variations in its epithelial structure whereas its dorsal surface covered by keratinized stratified squamous epithelium. In contrast, the ventral surface of the tongue is lined with non-keratinized stratified squamous epithelium. Underlying both surfaces is a layer of dense connective tissue known as the lamina propria, which supports the epithelium and contains blood vessels, nerves, and immune cells. Tongue's dorsal surface exhibits a variety of lingual papillae giving it a rough surface appearance, each with distinct structures and functions.

Filiform papillae are the most abundant type of papillae on the tongue, covering most of the anterior two-thirds. Fungiform papillae are dispersed in between the filiform papillae, especially near its tip and sides, and circumvallate papillae which is a large, dome-shaped structures found in a V-shaped row immediately in front of the foramen cecum and sulcus terminalis. The health and appearance of these papillae, particularly the filiform type, can serve as indicators of systemic health conditions. Nutritional deficiencies, particularly in iron, folic acid, and B vitamins can lead to depapillation, where these papillae are lost, resulting in a smooth and possibly sore tongue surface. Other factors, including enzymatic disturbances, vascular insufficiencies, or medication toxicities, may also cause noticeable changes in these papillae [1].

Aspartame (ASP) is a low-calorie artificial sweetener; it is a methyl ester of a dipeptide composed of two amino acids: L-aspartic acid and L-phenylalanine. It’s commonly used in low-calorie or sugar-free products like soft drinks (diet sodas), chewing gum, candy, yogurt, and Pharmaceuticals (sugar-free cough drops, chewable vitamins, even some liquid medications). While, its consumption has been increasing daily due to its widespread use as a sugar substitute [2].

Acceptable Daily Intake (ADI) for aspartame at 50 mg/kg of body weight per day. [3]. Upon ingestion, (ASP) is rapidly hydrolyzed in the small intestine by digestive enzymes into its constituent components: phenylalanine (50%), aspartic acid (40%), and methanol (10%). These metabolites are then absorbed into the bloodstream and metabolized through natural pathways similar to those originating from other dietary sources [4]. The methanol produced is further oxidized in the liver to formaldehyde and then to formats. This metabolic process is associated with the generation of reactive oxygen species (ROS) containing oxygen, such as

superoxide anions and hydrogen peroxide, which can lead to oxidative stress, and potential cellular damage [5].

Furthermore, a significant dose at 250 mg/kg of (ASP) can lead to histopathological changes in kidney, liver, cerebral cortex, anterior pituitary cells, and salivary glands [6]. Also, it has been associated with notable morphological changes on oral tissues, particularly the dorsal surface of the tongue [7].

Pumpkin plants (Cucurbita spp.) belong to the Cucurbitaceae family and are widely cultivated in Asia. Their seeds are rich in various bioactive compounds, contains sterols, proteins, and peptides. Also, it has a high content of unsaturated fatty acids, such as linoleic (Omega-6) and oleic acids (Omega-9) and alpha-linolenic acid (Omega-3), which are essential for various bodily functions as healing, hormone synthesis, and help reduce inflammation. Additionally, they contain significant levels of essential minerals like magnesium, zinc, and iron, as well as vitamins such as vitamin E and various B vitamins, making them a nutritional powerhouse [8].

Regular consumption of pumpkin can help in prevention of skin diseases and eye disorders, reducing cell damage in the body. The antioxidants in pumpkin seeds, including carotenoids and tocopherols have been linked to cancer prevention by neutralizing the free radicals that can lead to cell damage. Also, pumpkin seeds and their oil has a benefit especially for blood glucose control and urinary tract health. So, incorporating pumpkin seeds and their oil into your diet can provide these health benefits, supporting overall well-being [9].

Accordingly, this study has been done to evaluate the possible protective role of (PSO) in improving the tongue mucosal damage in rats consuming artificial sweeteners, specifically (ASP) histologically.

2. Experimental or Materials and Methods (please choose either header)

Ethical Committee Consent:

The current study was granted approval from the Egyptian Russian University Institutional ethical committee with approval number: FD-ERU-REC-19.

Sample size calculations:

Sample size calculation was performed using G*Power version 3.1.9.2, [10]. University Kiel, Germany.

The effect size *f* was 0.76 (large) according to the previous studies with alpha (α) level of 0.05 and Beta (β) level of 0.05, i.e., power = 95%; the estimated sample size (n) should be 30 samples and will be divided equally into 3 groups as follows. (Table 1)

Table 1. Showed samples distributions at different groups

Descriptive	No. of samples
Control group	10
ASP group	10
ASP + PSO group	10
Total	30

Materials:

Pure aspartame: powder was purchased from ADWIA Co. Cairo- Egypt.

Pumpkin seed oil: a glass bottle contains 30 ml of oil in liquid form. El Captain Company.

Grouping of animals:

Thirty adult male albino rats weighting approximately 180 -200 gm were obtained from animal house.

Rats were housed under good ventilation and adequate diet at the Medical Experimental Research Center (MERC) of the Faculty of Pharmacy, Egyptian Russian University, and all procedures were conducted in accordance with the protocol approved by its ethical committee.

All rats divided randomly into three groups; 10 rats per group (n =10), as described below:

- Group I (Control group): rats received normal saline orally using oral needle gavage for four weeks.
- Group II (Aspartame group) (ASP): rats received ASP (250 mg/kg dissolved in distilled water) orally using oral needle gavage for four weeks.
- Group III (Aspartame + Pumpkin seed oil) (ASP + PSO): rats received a daily dose of ASP as the group II, in addition they received topical application of PSO daily, for four weeks [11].

At the termination of the experiment, rats were anesthetized using a combination of ketamine and xylazine due to their synergistic effect. Following anesthesia, the tongues were bisected along the midline. The anterior two-thirds of the right halves were processed for light microscopic examination, while the left halves were prepared for scanning electron microscopy (SEM).

Histological evaluation:

For Light Microscope (LM): after collection of tongue specimens, the right halves were fixed in 10% neutral-buffered formalin. This step preserves tissue architecture by cross-linking proteins, thereby preventing degradation. Following fixation, tissues were washed and dehydrated through a series of increasing ethanol concentrations to remove water content. The dehydrated tissues were then cleared using a solvent like xylene, which is miscible with both ethanol and paraffin wax, ensuring proper infiltration, and then embedded in paraffin wax, providing a solid medium to support the tissue during sectioning.

Subsequently, serial sections of 5µm thickness were cut from the paraffin-embedded tissues. Then, stained with hematoxylin and eosin (H&E) as routine stain differentiates cellular components, and Masson Trichrome histochemical stain for distinguishing collagen. After staining, the sections were examined under a light microscope to assess the histological features of the tongue tissues (L/M) [12].

For Immunohistochemistry: the process can be summarized as follows: deparaffinized in xylene, hydrated and washed with tap water. According to manufacturer instructions, antigen retrieval was performed. The slides were washed and incubated with ki 67 (Lab Vision, USA) antibody which used for detecting the proliferative activity in tissues. This was done overnight at room temperature after that phosphate buffer saline wash was done, incubated with biotinylated antibody for 30 minutes and then washed with phosphate buffer. After that, the peroxidase labeled streptavidin was applied then DAB chromogen was added for antibody staining brown. The samples were then allowed to react with hematoxylin stain, then dehydrated and mounted.

Three microscopic fields showing the highest immunopositivity were selected and photomicrographs by a digital camera (CS060.Olympus, Japan). Then, all the steps for image analysis were carried out using image analysis software (Image J. 1.41a, NIH, USA). The area fraction of the positive cells was calculated automatically. The collected data was tabulated in an excel sheet and statistically analyzed.

For Scanning Electron Microscope: Specimens from left halves of the tongues 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 tongue surface and prepared for scanning electron microscope study. Specimens were then examined and photographed with JEOL, JSM-53009 at magnification of 400x in SEM unit, Mycological center, Al-Azhar University [13].

Statistical analysis:

All data calculated, tabulated, and statistically analyzed using suitable statistical tests as follows. A normality test (Shapiro-Wilk) was done to check the normal distribution of the samples. Descriptive statistics were calculated in the form of Mean ± Standard deviation (SD). One way ANOVA (Analysis of variance) was used to compare between the groups under study. Bonferroni post hoc test was performed for pair wise comparisons among the groups. P value ≤ 0.05 is considered statistically significant. Statistical analysis was performed using the computer program SPSS software for windows version 26.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp) at significant levels <0.05 (P- Value).

3. Results and Discussion

Histological results:

Group I (negative control):

H&E-stained sections: Filiform papillae are the most numerous, elongated, conical structures with pointed tips, and arranged in parallel rows. Each filiform papilla consists of a connective tissue core that provides structural support and contains small blood vessels that nourish the epithelium. surrounded by a stratified squamous keratinized epithelium resting on a basement membrane with numerous epithelial ridges (Fig. 1a). Hence, fungiform papillae are mushroom-shaped structures and interspersed among the numerous filiform papillae primarily concentrated at the top and sides (Fig. 1b). Each fungiform papilla typically contains three to five taste buds contributing to the perception of the taste sensations. Beneath epithelium lies the lamina propria, which was very thin, contains small blood vessels. The skeletal muscle fibers were located at submucosal layer that arranged in multiple directions. The ventral surface is typically having thin keratinized stratified squamous epithelium which is thinner compared to the dorsal surface and appeared without lingual papillae (Fig. 1c).

Masson's trichrome staining: revealed normal distribution of regularly arranged collagen fibers in the CT core, lamina propria and as thin margins between muscle fibers suggests healthy tissue architecture (Fig. 2a).

Immunohistochemical staining: basal and parabasal epithelial cells have a brown positive immunoreaction of Ki67 at dorsal and ventral surface of the tongue that aligns with normal proliferative activity (Fig. 3a) and (Fig. 3b).

Group II (Aspartame ASP):

H& E-stained sections: at this group, consumption of aspartame (ASP) has been associated alterations in the morphology of filiform papillae, such as loss of their typical conical shape and hyperkeratosis (thickening of the epithelial covering). Additionally, there was epithelial hyperplasia (acanthosis), cellular pleomorphism, nuclear hyperchromatism, and separation of keratin layers. The fungiform papillae appeared distorted and atrophied, with degeneration of epithelial cells and pyknotic nuclei. The taste buds within these papillae showed irregular and disorganized cells. Furthermore, the lamina propria, a connective tissue layer beneath the epithelium, displayed numerous dilated and congested blood vessels (Fig. 1d). The ventral surface of the tongue has variations in epithelial thickness, separation of the keratin layer, and disrupted muscle fibers with interstitial spaces (Fig. 1e).

Masson's trichrome staining: presence of increased, disorganized, and wavy collagen fibers within connective tissue (CT) and between muscle fibers (Fig. 2b, c).

Immunohistochemical study: only some basal epithelial cells have a brown positive immunoreaction of Ki67 in both dorsal and ventral surfaces of the tongue (Fig. 3c, d).

Group III (ASP + PSO):

H& E-stained sections: much preservation of the histological features of the tongue surface and restoration of regular arrangement and shape of filiform papillae with normal fungiform papillae in between them, a few disfigured filiform papillae, and some pyknotic nuclei were observed. Some dilated congested blood vessels at lamina propria (Fig. 1f, g). The ventral surface had a thin keratin layer over the stratified epithelium, most muscle fibers beneath the epithelium appear normal (Fig. 1h).

Masson's trichrome staining: a moderate amount of collagen fibers within CT core and a normal distribution in the lamina propria among muscle fibers (Fig. 2d, e).

Immunohistochemical staining: basal and parabasal epithelial cells have a brown positive immunoreaction of Ki67 at the dorsal and ventral surfaces of the tongue (Fig.3e).

Scanning electron microscopic results:

Group I (negative control):

There is diversity in the morphology and arrangement of filiform over the entire surface of the tongue. It was closely packed, arranged in parallel rows with sharp conical projections

(Fig 4a). The fungiform papilla interposed in between the numerous filiform papillae which has a regular surface with a central taste pore (Fig. 4b).

Group II (Aspartame ASP):

At this group, the filiform papillae had an irregularly distribution of short filiform papillae in different directions with bifurcated tapering ends and numerous bacterial clusters invasion on its surface (Fig. 4c). The fungiform papillae showing irregular, wrinkled surfaces and poorly defined taste pores. (Fig. 4d).

Group III (ASP + PSO):

There was almost normal distribution of filiform papillae. However, few filiform papillae had serrated tips (Fig. 4e). The fungiform papilla was regular with well-defined outlines and well-defined central taste pore, but the top surfaces were still depressed (Fig. 4f).

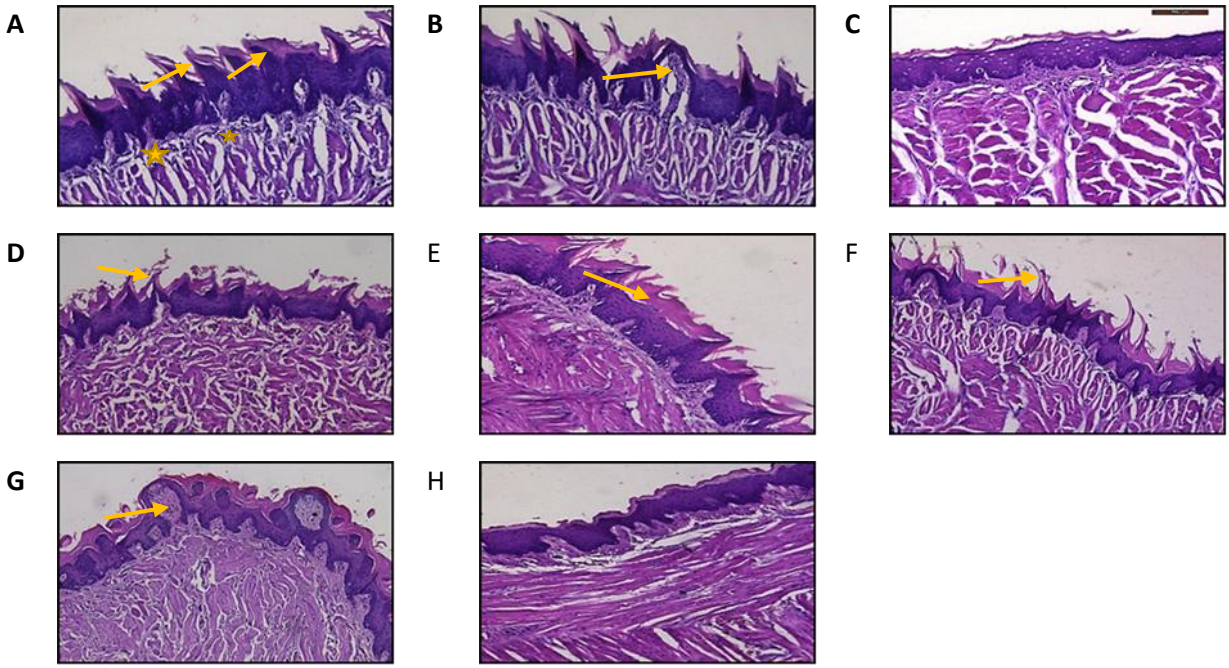

Figure 1. Photomicrographs of tongue mucosa of all examined groups showing: (H&E × 100), (**A**) Numerous, sharp conical filiform papillae covered by keratinized stratified squamous epithelium (yellow arrow). The lamina propria has a dense connective tissue fiber, and submucosal layer has muscle fibers arranged in more directions. (yellow star) (group I); (**B**) Well-formed fungiform papillae with a taste bud (yellow arow) (group I); **(C)** Normal appearance of ventral surface of the tongue which covered by stratified squamous epithelium with thin keratin layer (group I); **(D)** Shortening and thinning of the filiform papillae, separation of the keratin layer (yellow arrow) from the underlying epithelium (group II); **(E)** Both thinned and thickened stratified epithelium (yellow arrow) on the ventral tongue surface (group II); **(F)** Restoration of regular arrangement of filiform papillae (yellow arrow) (group III); **(G)** Regular distribution of fungiform papillae between the filiform ones with a taste bud (yellow arrow) (group III); **(H)** Almost normal appearance of ventral surface of the tongue (group III).

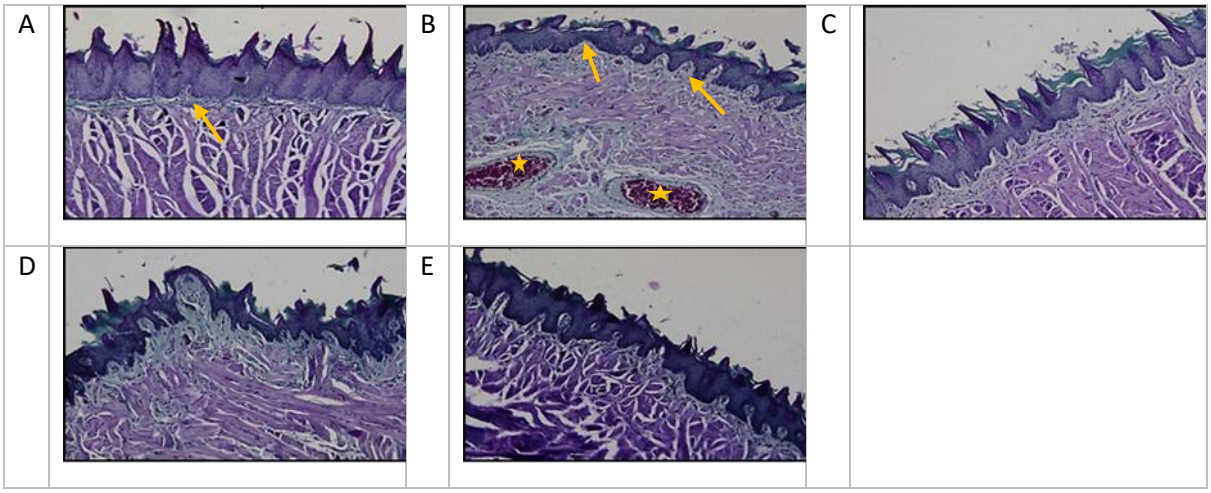

Figure 2. Photomicrographs of tongue mucosa of all examined groups showing: (Masson trichrome× 100), **(A)** Normal distribution of collagen fibers in lamina propria, and between muscle fiber (group I); **(B)** Disorganized, increased, and wavy collagen fibers in lamina propria and between muscle fibers (yellow arrow) and congested blood vessels (yellow star) (group II); **(C)** Excessive collagen fibers at the filiform papillae (group II); **(D)** A moderate amount of collagen fibers (group III); **(E)** Almost normal distribution of collagen fibers at the tongue surface (group III).

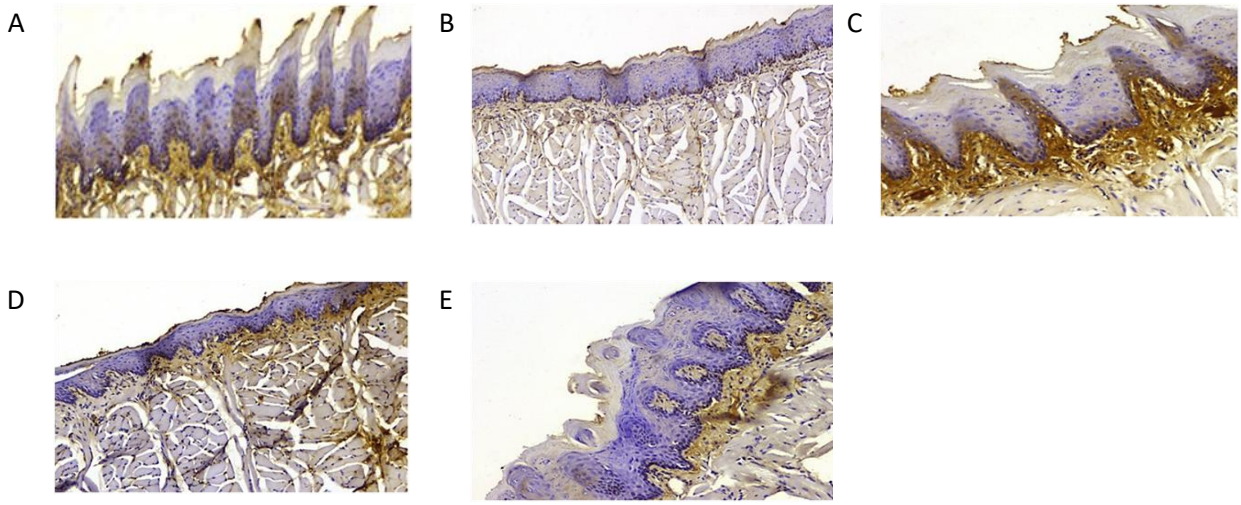

Figure 3. Photomicrographs of tongue mucosa of all examined groups showing: (Ki67 immunostaining × 200), **(A)** Brown positive immunoreaction of Ki67 in the basal and parabasal epithelial cells at tongue papillae (group I); **(B)** Brown positive immunoreaction of Ki67 in the basal and parabasal epithelial cells at ventral surface of tongue (group I); **(C)** Only some basal epithelial cells have a brown positive immunoreaction at tongue papillae (group II); **(D)** Only some basal epithelial cells have a brown positive immunoreaction at ventral surface of tongue (group II); **(E)** Many basal and parabasal epithelial cells have brown positive immunoreaction of Ki67 (group III).

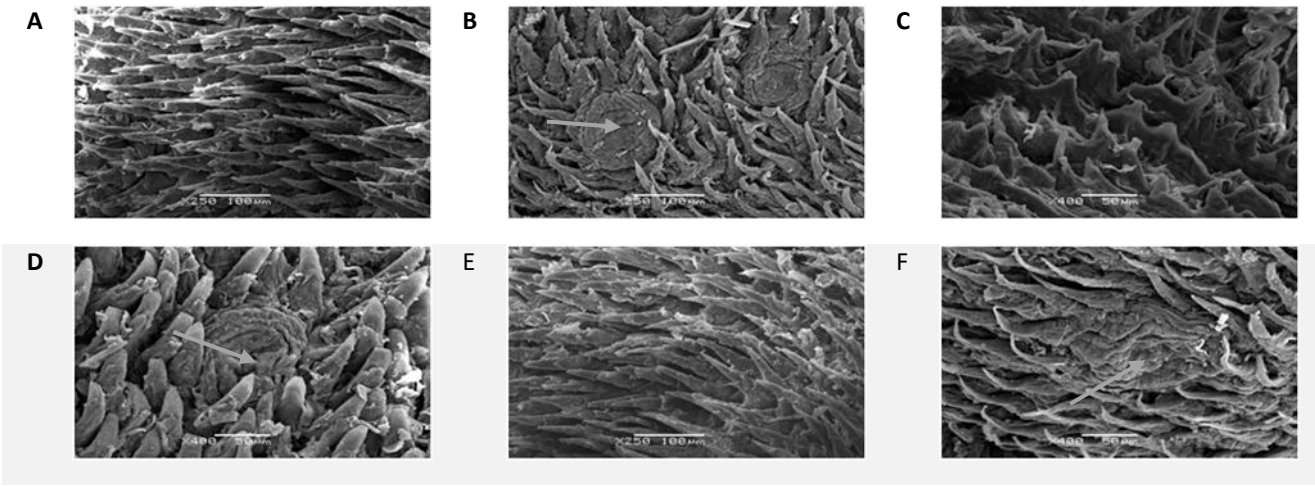

Figure 4. scanning electron microscope of the dorsal surface of the tongue from all examined groups showing, **(A)** Regular parallel rows of long conical filiform papillae with tapering ends and uniform orientation (group I); **(B)** Mushroom-like fungiform papillae are interposed in between the numerous filiform papillae, A well-defined regular taste pore at its center (arrow) (group I); **(C)** Irregularly distribution of short filiform papillae in different directions with bi-furcated tapering ends and numerous bacterial clusters can be seen on the surface of papillae. (group II); **(D)** Fungiform papillae with irregular surface, distorted and ill-defined taste pore (arrow) (group II); **(E)** Partial restoration of normal appearance and distribution of filiform papillae and some of them still appear blunt with serrated tips (group III); **(F)** Almost normal fungiform papilla with depressed surface, regular smooth epithelial covering and a well-de-fined regular taste pore (arrow) (group III).

Statistical results:

Mean and Standard deviation (SD), of Ki67 positive cells for different groups were presented in table (2) and figure (5)

The results showed that there are clearly significant differences between groups for Ki67 positive cells.

Using one-way ANOVAs ($F=56.15$, $P=0.001$) at a significant level $P< 0.05$. The pairwise comparison showed a significant difference between each group to another. The control group recorded the highest mean value (184.66±15.12) followed by group (135.44±10.06) while the ASP group was the lowest (91.75±4.09).

Table 2. Showed mean number of Ki67 expressions at epithelial cells and their standard deviation.

	Mean	SD	95% Confidence Interval for Mean		F test	P value
			Lower	Upper		
Control	184.66	15.12	147.11	222.21		
ASP	91.75	4.09	81.61	101.90	56.15	<0.001**
Asp+pum2	135.44	10.06	110.46	160.42		
Bonferroni post hoc						
	mean difference	**P value**				
control vs ASP	92.91	<0.001**				
control vs Asp+pum2	49.22	0.004**				
ASP vs ASP	43.68	0.008**				
**** and different letters mean significant difference at P<0.05**						

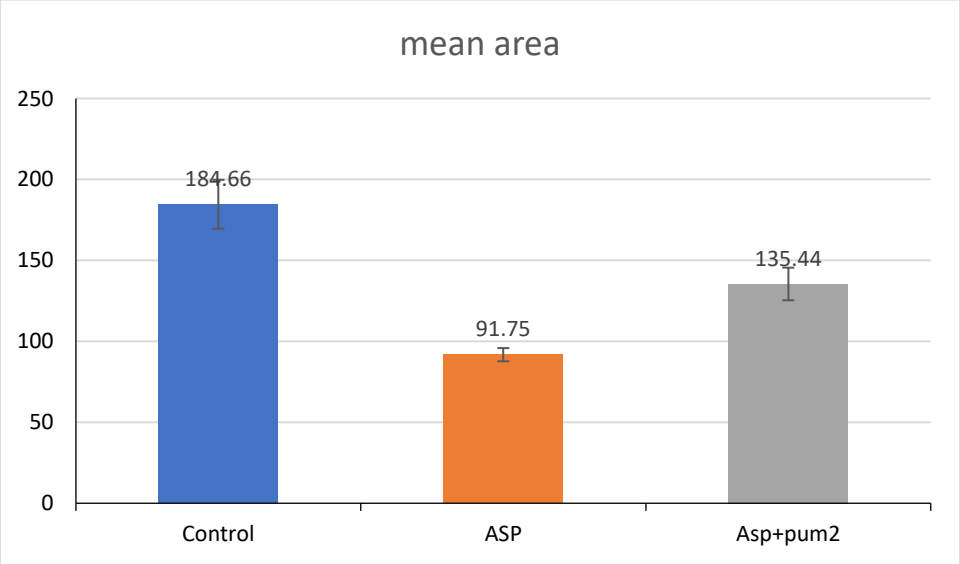

Figure 5. Expression of (ki 67) for different group.

Pumpkin seeds, are nutrient-dense and offer numerous health benefits. They are rich in proteins, healthy fats, fibers, vitamins (such as E, K, and B vitamins), and minerals like

magnesium, zinc, and iron. These components have anti-inflammatory and antioxidant properties that support various aspects of health [9]. Accordingly, this study has been done to evaluate the possible protective role of pumpkin seed oil in improving the tongue mucosal damage induced by artificial sweeteners aspartame using different histological methods.

The tongue's dorsal surface is densely covered with various papillae, notably the filiform papillae, which play a crucial role in reflecting overall health. These papillae exhibit high metabolic activity, making them particularly sensitive to systemic changes. Consequently, any nutritional deficiencies, or enzymatic disturbances can lead to their atrophy. Additionally, certain medications—including antibiotics, chemotherapeutic agents, and treatments for conditions like diabetes and cancer—as well as exposure to chemical or metal toxins, have been associated with lingual atrophy [14]. Subsequently, tongue was selected at this study.

Histological results of our study revealed that (ASP) administration in albino rats leads to significant morphological alterations in the tongue's dorsal surface, particularly affecting the filiform and fungiform papillae. These adverse structural alterations in the tongue can manifested as distortion of filiform and fungiform papillae, focal loss of papillae, decreased epithelial thickness, disrupted muscle fibers, increased collagen deposition, and decrease in the ki 67-immunoreaction of the epithelium.

At our study, histological examination of group II showed that (ASP) ingestion can lead to significant morphological alterations on the tongue's mucosal tissue. Notably, these changes include hyperkeratosis of filiform papillae, epithelial hyperplasia (acanthosis), cellular pleomorphism, and nuclear hyperchromatism. Additionally, distorted and atrophied fungiform papillae with degenerated epithelial cells exhibiting pyknotic nuclei have been observed. The lamina propria often shows numerous dilated and congested blood vessels. This finding was agreed with the observation of Zahran et al., [7] who investigated the effects of (ASP) on the dorsal surface of rats' tongue and evaluated the potential protective role of platelet-rich plasma (PRP).

Furthermore, SEM examination revealed an irregularly distribution of short filiform papillae in different directions with bifurcated tapering ends, and the fungiform papillae showed irregular wrinkled surfaces with poorly defined taste pores. These findings were in accordance with Azez et al., [15] who demonstrated the toxic effect of (ASP) on renal and hepatic tissues and found more their toxicity by raising the dose.

Concerning the atrophic changes of the tongue papillae occurred potentially due to oxidative stress induced by (ASP). Upon ingestion, (ASP) is metabolized into phenylalanine, aspartic acid, and methanol. Methanol is further broken down into formaldehyde and formic

acid, and compounds associated with toxicity. The oxidative stress resulting from these metabolites may lead to inflammation and cellular injury, contributing to the atrophy of tongue papillae as explained by Ashok, Sheeladevi [16]. These free radicles resulted in inflammation and damage of cells and might be the cause of lingual papillary atrophy at this study. The increased free radicles and the consequent increased oxidative damage to the cellular proteins and mitochondrial DNA could induce apoptotic cell death following prolonged (ASP) intake.

The inflammatory response involves various immune and vascular changes, including vasodilation, increased vascular permeability, and release of proinflammatory cytokines. Therefore, inflammatory cell infiltration and dilated congested blood vessels observed at group II can indeed result from the production of reactive oxygen species (ROS) and proinflammatory cytokines which increases the vascular permeability and promote the inflammation [17].

In concern with the disrupted muscle fibers with interstitial spaces which were noticed at group II of (ASP) treated. These findings were somehow relevant to the results of Farag, and Mehanny, in 2020 [18] who detected the degeneration of tongue muscle fibers following zoledronic acid (a nitrogen-containing bisphosphonate, or N-BP) treatment, attributed to the activation of nuclear factor kappa-B (NF-κB) signaling pathways due to oxidative stress and proinflammatory cytokines. Their findings indicated that such degeneration is associated with skeletal muscle loss and degradation as mentioned by Thoma, Lightfoot [19].

Masson's trichrome-stained sections from group II treated with (ASP) showed a presence of increased, disorganized, and wavy collagen fibers within connective tissue (CT) and between muscle fibers, in comparison to group I. Such changes explained that the (ASP) may induce a significant alteration that associated with fibrosis, and irregular deposition of collagen fibers in tissues. These findings were in accordance with studies done by Farag and Mehanny, [18] who found that disorganization of collagen fibers and an increase in collagen area percentage in skeletal muscle are associated with fibroblasts replacing degenerated muscle fibers and inflammation-induced cytokines promoting fibrosis.

Pumpkin seed oil has a protective role against (ASP) induced tongue mucosal damage, primarily due to its rich composition of antioxidants, vitamins, and anti-inflammatory compounds [20]. (PSO) has a tocopherol (vitamin E), fatty acids, and phytosterols, which contribute to its potential in supporting epithelial proliferation and wound healing. These constituents synergistically enhance the regenerative capacity of the oil, facilitating full re-epithelialization and promoting fibroblast migration by providing essential components for the connective tissue matrix as mentioned by Bardaa et al., [21].

Furthermore, (PSO) has been shown to significantly reduce lipid peroxidation and enhance antioxidant enzyme activity. These effects are primarily attributed to their high content of various amounts of bioactive compounds including (vitamin E), vitamin A, iron, beta-carotene, and unsaturated fatty acids (ω-6 and ω-9) as stated by Galaly et al., [22]. These components contribute to PSO's ability to protect the polyunsaturated fatty acids in cell membrane phospholipids from oxidative damage, acting as potent peroxyl radical scavengers. This protective action helps in maintain the integrity of biological cell membranes against harmful free radicals, neutralizes the elevated (ROS), and prevents DNA oxidative damage as explained by Syed et al., [23].

The histological characteristics of the tongue surface have been preserved at group III in this study, and the filiform papillae's regular shape and arrangement, together with the usual distribution of fungiform papillae between them, have been restored. SEM findings can support and enhance observations made with LM. These results were consistent with previous research done by Kassab et al., [24] who found that orlistat administration led to significant structural alterations in the tongue's filiform and fungiform papillae. However, concurrent treatment with PSO has been shown to attenuate the adverse effects of certain medications on tongue mucosa with much preservation to the tongue's mucosa due to its antioxidant effect, as well as its renewed abilities.

Also, in many respects to Oh et al., [25] who indicated that pumpkin seed extract has antioxidant properties and may help reduces the adverse effects associated with menopause, including issues related to oxidative stress, body weight, serum lipids, cardiovascular diseases, osteoporosis, and thermogenesis.

Regarding the immunohistochemical results, we selected the Ki67 which is a nuclear protein and considered as a proliferation index of cell proliferation. At group I (negative control), basal and parabasal epithelial cells have a brown positive immunoreaction of Ki67 at dorsal surface and ventral surfaces of the tongue represented the normal proliferative activity. While group II (ASP) revealed only some basal epithelial cells which have a brown positive immunoreaction of Ki67 in both dorsal and ventral surfaces of the tongue These findings were in accordance with Shalaby, Kashef [26] and Essawy et al., [27] results. Also, in agreement with Barbosa et al., [28] who explained that (ASP) decreased the proliferation rate of tongue mucosa which appeared as decrease in number of Ki67 positive cells. Group III, revealed a brown positive immunoreaction for Ki67 in both basal and parabasal epithelial cells in comparing to group II. This result coincided with previous studies done by Ahmed et al., [29].

4. Conclusions

The current study showed the adverse effects of aspartame ASP on the tongue's mucosal surface (PSO) may mitigate these effects due to its antioxidant, anti-inflammatory, and regenerative properties. Therefore, (PSO) could be a promising protective agent for people consuming (ASP) products to minimize its adverse effect on the tongue's mucosa.

Recommendations:

This is an experimental study requires further investigation to determine its clinical applications and optimal use.

- **Conflict of Interest**

The authors have no conflicts of interest related to this study.

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