Original Article

The Effect of Daidzein on Alendronate Induced Changes on the Lingual Mucosa of Rats: Biochemical, Histological, and Scanning Electron Microscopic Study

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ABSTRACT

Introduction: Alendronate (AL) is one of the bisphosphonate group that contains nitrogen. It has been used in the treatment of bone disorders but, with other organ side effects. Daidzein (DZ) is one of the isoflavones possessing an antioxidant, anti-inflammatory, and anti-apoptotic effects.

Aim of the Work: Was to investigate daidzein effect on alendronate induced changes on the lingual mucosa of rats through biochemical, histological and scanning electron microscopic evaluation.

Materials and Methods: 25 adult male albino rats; 3-5 months; 180- 220 gm were divided into; Control, AL group: AL (0.05 mg/kg), AL+DZLD: AL + DZ low dose (50 mg/kg), AL+ DZHD: AL + DZ high dose (100 mg/kg). Doses were given orally for twelve weeks, then tongue specimens were processed for the biochemical, light and scanning electron microscopic studies.

Results: AL group displayed a significant increase of tissue malondialdehyde, while a significant decrease of tissue superoxide dismutase. Besides, disorganized, ill-defined and distorted lingual papillae, vacuolated epithelial cell' cytoplasm with pyknotic nuclei, lost epithelial ridges, congested apparently dilated blood vessels, and significantly decreased mean height and width of the filiform and fungiform papillae by H&E. While, a significant decrease of the optical density of collagen fibers, and E-cadherin. Also, desquamated irregularly arranged atrophied filiform papillae with wide separation, and rough upper surface fungiform with ill-defined taste pores by scanning electron microscope. AL treated group by DZ showed dose dependent improvement of the previously mentioned findings.

Conclusion: Daidzein dose dependently ameliorated alendronate induced changes on the lingual mucosa of rats thru its antioxidant and anti-inflammatory actions.

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Key Words: Alendronate; biochemistry; daidzein; histopathology; scanning electron microscope.

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INTRODUCTION

Osteoporosis and other bone diseases are common health problems that needs intervention. One of the regimen that has been used for treating such problems was Bisphosphonates (BPs)[1,2]. The latter acts through inhibiting the osteoclasts activity thus, prevent bone resorption[3,4]. On the other hand, the prolonged intake of alendronate (A member of BPs) can induce systemic side effects especially gastrointestinal irritation with heartburn, sickness, nausea, vomiting, and abdominal aches[5]. It is also associated with muscle aches, decreased blood calcium levels, jaw osteonecrosis and nerve injury especially the inferior alveolar[6]. Furthermore, researches showed that AL induced esophageal mucosal alterations besides different histopathological changes of the submandibular gland with dry mouth. The dry mouth may affect the tongue that may ends with lingual ulcerations and taste affection. AL also could affect the lingual mucosa with surface injury and ulcerations due to its effect on the epithelial cell junctions (especially E-cadherin) and communication. E-cadherin (epithelial- cadherin), is a calcium-dependent cell to cell adhesion molecule. It is of great important role in the epithelial cell formation and behavior as well as maintenance of epithelial integrity. Its disruption could lead to cell barrier disruption and loss of cell integrity, ending with ulcerations, inflammations even cancers[3,7,8].

The polyphenolic substances such as soy isoflavones showed a chemical structure similar to the estrogen receptors (ERs) so are easily bind to them[9], and one of them is daidzein (DZ)[10]. The latter provides an effective actions against different pathologies especially that confined to estrogen regulation like hyperglycemia, bone diseases including osteoporosis, breast carcinoma, as well as complications of the cardiac and circulatory systems[11]. In addition, DZ possess an effective ER-independent pharmacological actions which are in the form of antioxidation, anti-inflammation agent, and anti-apoptosis[12,13].

In this regard and according to the previously mentioned facts, this study is to investigate the possible ameliorative

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effect of DZ on AL- induced lingual mucosal alterations in adult male albino rats using biochemical, light and scanning electron microscopic methods.

MATERIALS AND METHODS

Animals and experimental groups

The present study included twenty-five male albino rats that aged from 3 to 5 months old and weighing from180 to 220 gm. Rats were gotten from the animal house of Tanta University and were placed at their cages with sanitary environmental circumstances (25° C, humidity 65%). Rats were allowed to have a free access to food and water. The animal care guiding principle is put in agreement with that of Tanta University Committee, Institution of Research Ethics; approval code No. 36264PR420,11,2023.

Rats were divided arbitrarily into;

- Control group: 10 rats that were further subdivided into; subgroup A (5 rats): rats kept without any treatments, and subgroup B (5 rats): rats received normal saline in a dose and duration correspondent to alendronate & daidzein respectively.
- Alendronate group (AL): five rats received AL (Fosamax, Merck, US A) at a dose of 0.05 mg/kg dissolved in 3 ml normal saline. 30 minutes before animal breakfast once/week for 12 weeks according to Papamitsou *et al*. (2020)[8].
- Alendronate +Daidzein low dose group (AL+DZLD): 5 rats received AL as in previous group and a low dose of DZ (50 mg/kg) once/day starting from the 5th week of the experiment by an oral gavage according to Durgun & Aşır, (2023) and Goel & Chaudhary, (2020)[13,14].
- Alendronate+Daidzein high dose group (AL+ DZHD): 5 rats received AL 5 rats received AL, same dose route and duration as in Alendronate group and high dose of DZ (100 mg/kg) once/day starting from the 5th week of the experiment by an oral gavage according to Durgun & Aşır, (2023) and Goel & Chaudhary, (2020)[13,14].

By the last experimental day, rats were anesthetized by pentobarbital (60 mg/kg) injected intraperitoneally. Then, part of the tongue specimens was taken and homogenized with homogenate centrifuged with the supernatant stored at -30 oC, to be used for the assessment of malondialdehyde and superoxide dismutase tissue levels[9]. Whereas, the other ones were undergoing further processing for evaluation by light and scanning electron microscope.

Assessment of tissue malondialdehyde (MDA; a marker for lipid peroxidation) and superoxide dismutase (SOD; an oxidative stress marker)

- Tissue MDA (Biodiagnostic, Cairo, Egypt) measured as µmol/g protein in relation to Bayramoglu *et al*., (2022)[15].
- Tissue (SOD) (Biodiagnostic, Cairo, Egypt) measured as U/mg protein according to Masre *et al*., (2019)[16].

Light microscopic evaluation[17]

Lingual specimens were fixed in 10% formal saline followed by dehydration, clearance and embedding and 5 microns' sections were obtained and stained with hematoxylin & Eosin (H&E) and Masson's Trichrome.

Immunohistochemical evaluation of E-cadherin (Epithelial- cadherin)[18]

The deparaffinized sections were rehydrated and subjected to antigen retrieval solution followed by the addition of 10% normal goat serum for protein blocking. Then, the addition of primary antibody; E-cadherin (Santa Cruz Biotechnology, USA); 1:100 with the sections left at 4°C overnight in a humid chamber. Followed by the addition of the secondary antibody (Vector Labs, Peterborough, United Kingdom); 1:100. Afterwards, 3, 3′-diaminobenzidine chromogen was added, then Mayer's hematoxylin and examination by a light microscope (Olympus, Japan).

The positive results were brownish membranous reaction, While, the negative control was obtained by a routine processing without the addition of the primary antibody. Additionally, the mammary gland was considered to be the positive control.

Scanning electron microscopic assessment (SEM)[19]

The specimens were fixed in 0.25% glutaraldehyde with 0.25% paraformaldehyde. Then, post-fixation in 1% osmium tetroxide. Afterwards, specimens were embedded in agarose, and sectioned. Eventually, specimens were dehydrated, dried and coated with platinum, and finally examined by the scanning electron microscope (JEOL). At the Electron Microscopy Unit of Faculty of Medicine, Tanta University, Egypt.

Morphometric assessment

The software program (Image J) (1.47 v) (USA) was used. By which ten non-overlapping fields from each of the five dissimilar images (x 200) of each experimental group were used to evaluate the followings:

- The mean height and width of both filiform and fungiform papillae were measured by µm; at H&E stained sections. By which, the height was measured starting from the tip to the papillae base, while the width was measured by measuring the base diameter at the widest points that was extended from one external epithelial wall to the other. These were in accordance to Takahashi *et al*. (2019)[20].
- The optical density of the collagen fibers at Masson's trichrome stained sections.
- The optical density of the E-cadherin immunohistochemically stained surface area.

Analysis of the statistical results

The collected data were expressed using mean ± standard deviation (SD). Then, the Graph Pad in Stat Software (Inc., USA) was used to compare between the different experimental groups by the student t-test. Finally, results were considered statistically significant when **P value** ≤ 0.05.

RESULTS

Tissue levels of MDA and SOD

There was a significant increase in the tissue levels of MDA of AL group in comparison to the control group. While, a significant decrease at groups AL+DZLD and AL+DZHD when compared to AL group with the best significance was AL+DZHD (Figure 1).

Regarding SOD tissue levels, a significant decrease was recorded at AL group while significant increase was noticed at groups AL+DZLD and AL+DZHD when compared to AL group (Figure 1).

H&E

The examined control group exposed abundant regularly arranged filiform papillae at the dorsal surface of the anterior two thirds (2/3) of the tongue. They appeared conical with tapering tips with the fungiform papillae scattered in-between them. Fungiform papillae were mushroom in shape with its characteristic taste buds on its upper surface. The lingual papillae contained thin connective tissue core with blood vessels and keratinized stratified squamous epithelial cover; resting on a basement membrane with multiple epithelial ridges. Furthermore, the epithelium with basal cell, spinosum cell, granulosum cell, and superficial corneum layers were distinguished (Figures 2A,B).

Alendronate group (AL) discovered ill-defined papillae with disorganization and distortion even lost at focal areas. Additionally, there were vacuolated cytoplasm and dark nuclei of covered epithelial cells. Also, straight basement membrane beside lost epithelial ridges at some parts. Regarding the lamina propria, congested apparently dilated blood vessels were encountered (Figure 2C).

Alendronate+Daidzein low dose group (AL+DZLD) discovered partial restoration with some disorganization and distortion of the lingual papillae, besides cytoplasmic vacuolations and dark pyknotic nuclei of some of the lining epithelial cells (Figure 2D).

As regards Alendronate+Daidzein high dose group (AL+DZHD), a nearly normal lingual papilla with its covering epithelium, nearly normal blood vessels and, basement membrane epithelial ridges were encountered (Figure 2E).

With reference to the mean height and width of the filiform and fungiform papillae; there was a significant decrease at AL group as compared to control. While, a significant increase at groups AL+DZLD and AL+DZHD dose dependently when compared to AL group with the AL+DZHD superiority (Figure 3).

Masson' Trichrome

The control group displayed lingual papillae with strong positive staining affinity of collagen fibers (Figure 4A). Conversely, AL group showed weak positive staining (Figure 4B). On the other hand, the AL+DZLD group revealed moderate positive staining (Figure 4C), and AL+DZHD group exposed a strong positive staining affinity of collagen fibers like control (Figure 4D).

Considering the optical density of collagen fibers; a significant decrease was noticed at AL group when compared to the control group. While, a significant increase was recorded at groups AL+DZLD and AL+DZHD when compared to AL group (Figure 5).

Immunohistochemistry E-cadherin

Concerning the negative control, there was no E-cadherin immunohistochemical reaction (Figure 6A). While, the examined sections of the control group revealed strong positive membranous immunohistochemical reaction for E-cadherin of the epithelial cells covering the filiform and fungiform papillae (Figure 6B). Conversely, faint even lost membranous reaction appeared at AL group (Figure 6C). Seeing the AL+DZLD group; a moderate positive E-cadherin membranous reaction was seen (Figure 6D). Oppositely, AL+DZHD group revealed a strong positive membranous immunohistochemical reaction for E-cadherin similar to the control group (Figure 6E).

About the optical density of E-cadherin-stained surface area; a significant decrease was noticed at AL group in comparison to the control group. Conversely, a significant increase was verified at groups AL+DZLD and AL+DZHD as compared to AL group with the best was AL+DZHD group (Figure 7).

Scanning electron microscopic results

The control group presented conical, regularly arranged and elongated filiform lingual papillae with intact tapering ends that were pointed into the same direction. Also, few fungiform papillae found scattered in-between the filiform ones. They were shorter, broader than the filiform with the taste pores seen in the centers of their upper surfaces (Figure 8A). AL group showed irregularly arranged filiform papillae with desquamation, atrophy, and wide separation. Besides, fungiform papillae with rough upper surface and ill-defined taste pores (Figure 8B). AL+DZLD group showed mild improvement. This was in the form of fewer desquamated filiform papillae, while the fungiform papillae showed rough surface (Figure 8C). AL+DZHD group revealed elongated regularly arranged filiform papillae besides short broad fungiform one, with taste pores at their upper surfaces; a picture like control (Figure 8D).

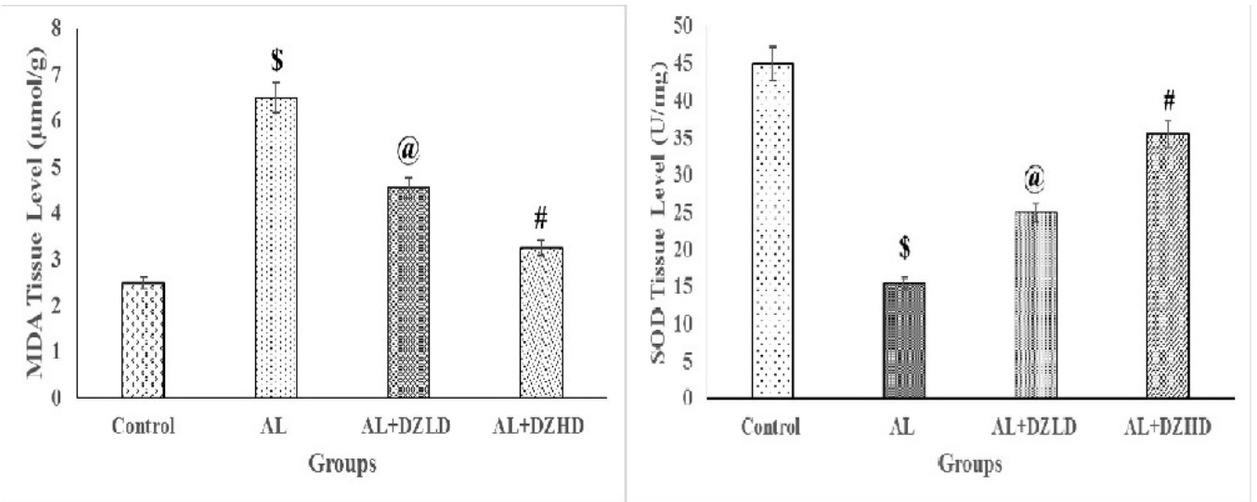

Fig. 1: Tongue tissue levels of SOD (U/mg) & MDA. Values were expressed as means ± standard deviation. $; AL group compared to control. @; AL+DZLD group compared to AL group. #; AL+DZHD group compared to AL group.

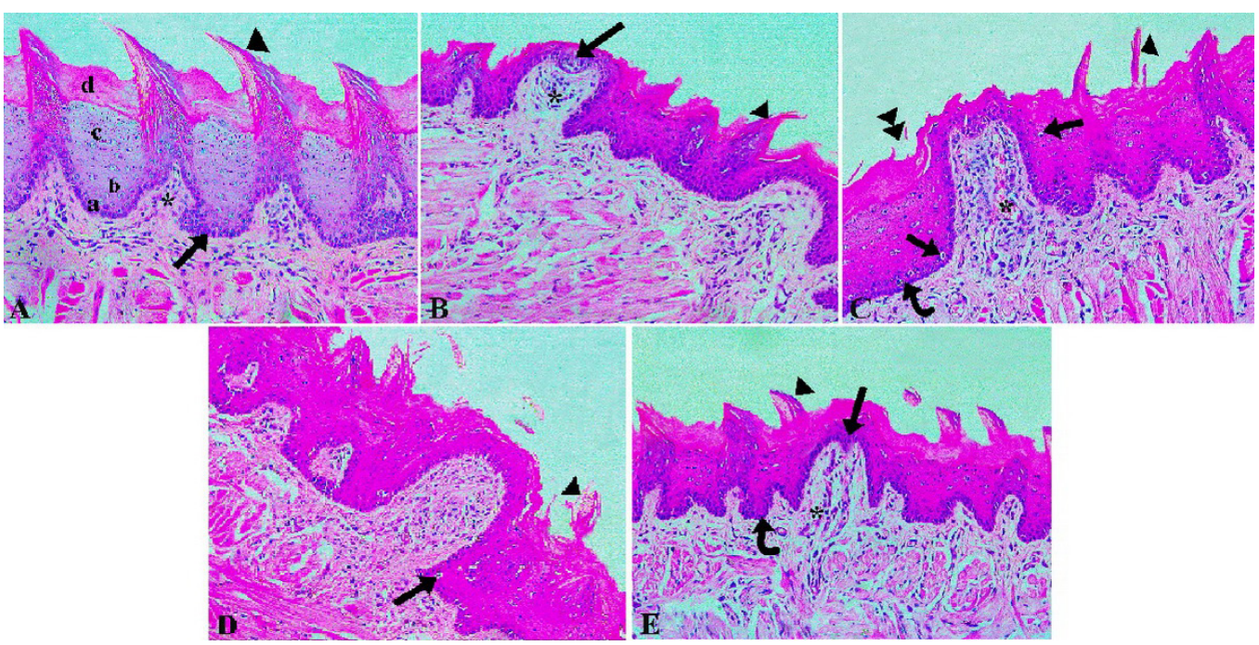

Fig. 2: A) Control group showing mucosa covering the dorsal surface of the anterior two thirds (2/3) of the tongue with numerous regularly arranged filiform papillae that were conical in shape with tapering tips (►), keratinized stratified squamous epithelium with basal cell layer (a), spinosum cell layer (b), granulosum cell layer (c), and superficial corneum layer (d), basement membrane with multiple epithelial ridges (→), and a thin connective tissue core containing normal blood vessels (*). B) Control group showing fungiform papillae scattered in-between the filiform (►). It was mushroom in shape with taste buds on its upper surface (→), and connective tissue core containing normal blood vessels (*). C) Alendronate group (AL) showing disorganized, distorted and ill-defined lingual papillae (►), focal areas with lost papillae (►►), vacuolated cytoplasm and dark pyknotic nuclei of the covering epithelial cells (→), straight basement membrane at some parts with loss of its epithelial ridges (curved arrow) and congested apparently dilated blood vessels of the lamina propria (*). D) Alendronate+Daidzein low dose group (AL+DZ LD) showing some disorganized, distorted lingual papillae (►), and cytoplasmic vacuolations with pyknosis of some epithelial cells (→). E) Alendronate+Daidzein high dose (AL+DZ HD) showing nearly normal filiform (►) and fungiform papillae with its covering epithelium (→), and nearly normal blood vessels at its lamina propria core (*). Notice: nearly normal basement membrane epithelial ridges (curved arrow). (H&E x 200).

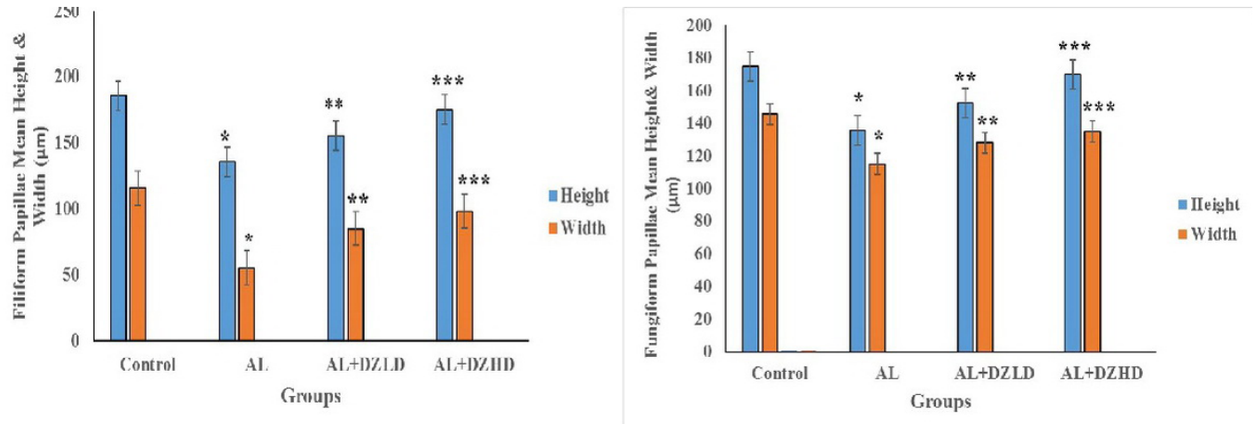

Fig. 3: The mean height and width of filiform &fungiform papillae of the dorsal surface of the tongue. Values were expressed as means ± standard deviation. *; AL group compared to control. **; AL+DZLD group compared to AL group. ***; AL+DZHD group compared to AL group.

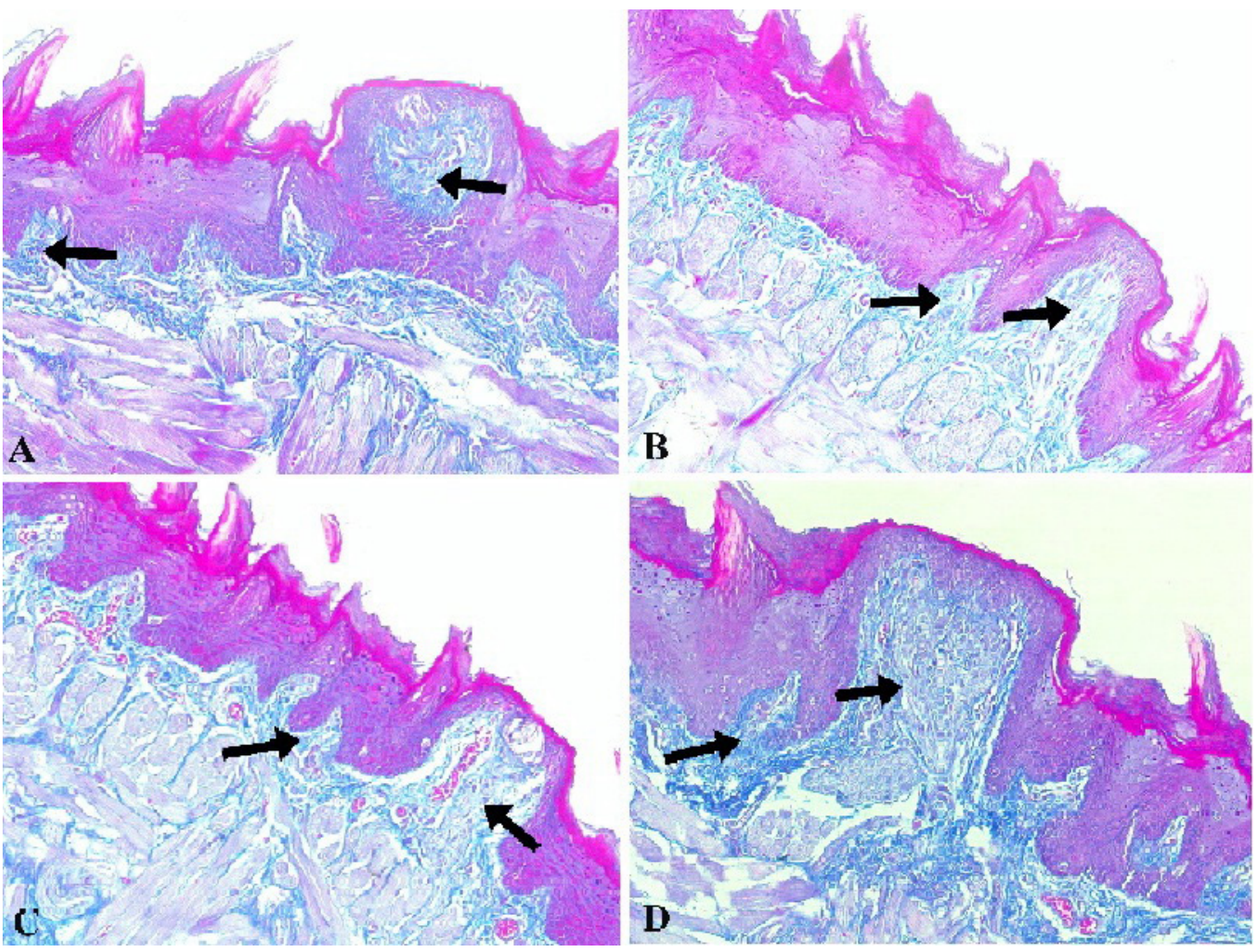

Fig. 4: A) Control group: apparently strong positive staining affinity of collagen fibers in the lamina propria of papillae of the dorsal surface of the anterior two thirds of the tongue (→). B) AL group: apparently weak positive staining affinity of collagen fibers (→). C) AL+DZLD: moderate positive staining affinity of collagen fibers (→). D) AL+DZHD: apparently strong positive staining affinity of collagen fibers in the lamina propria of the papillae (→). (Masson' Trichrome x 200).

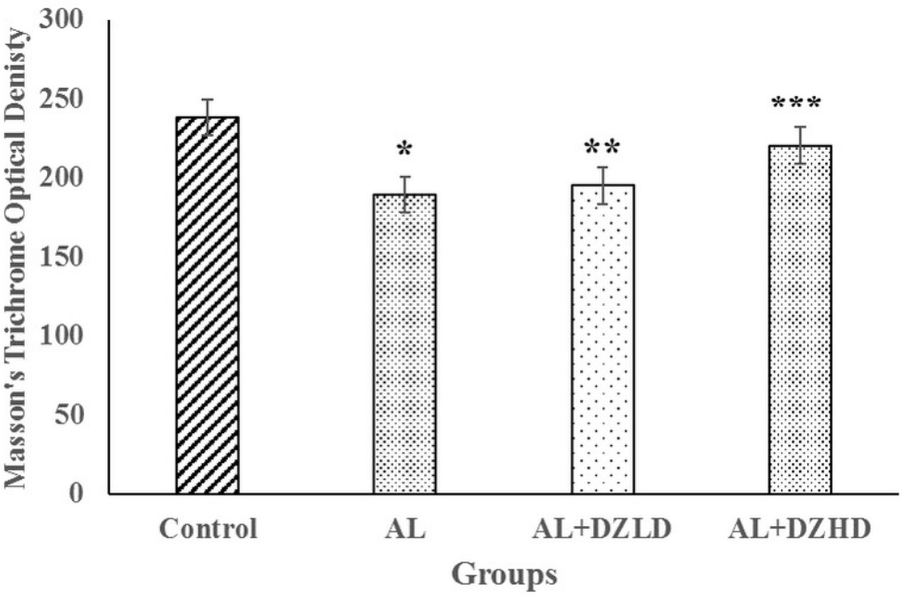

Fig. 5: The optical density of collagen fibers at Masson's trichrome stained sections. Values were expressed as means ± standard deviation. *; AL group compared to control. **; AL+DZLD group compared to AL group. ***; AL+DZHD group compared to AL group.

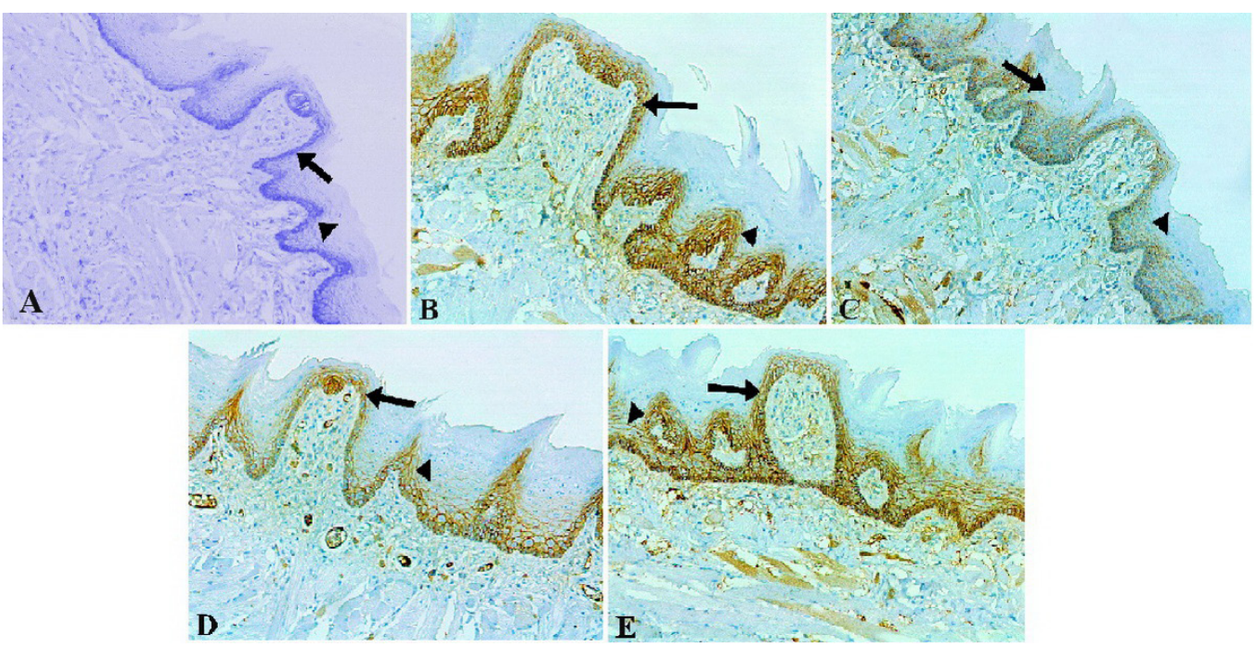

Fig. 6: A) Negative control: No E-cadherin immunohistochemical reaction of the epithelial cells covering the filiform (►) and fungiform papillae of the dorsal surface of the tongue (→). B) control group: strong positive membranous immunohistochemical reaction for E-cadherin of the epithelial cells covering the filiform (►) and fungiform papillae (→) of the dorsal surface of the tongue, C) AL group: faint (►) even lost (→) membranous reaction. D) AL+DZLD group: moderate positive E-cadherin membranous reaction of both filiform (►) and fungiform papillae (→). E) AL+DZHD group: strong positive membranous immunohistochemical reaction for E-cadherin of the epithelial cells covering the filiform (►) and fungiform (→) papillae of the dorsal surface of the tongue. (E-cadherin immunohistochemistry x 200).

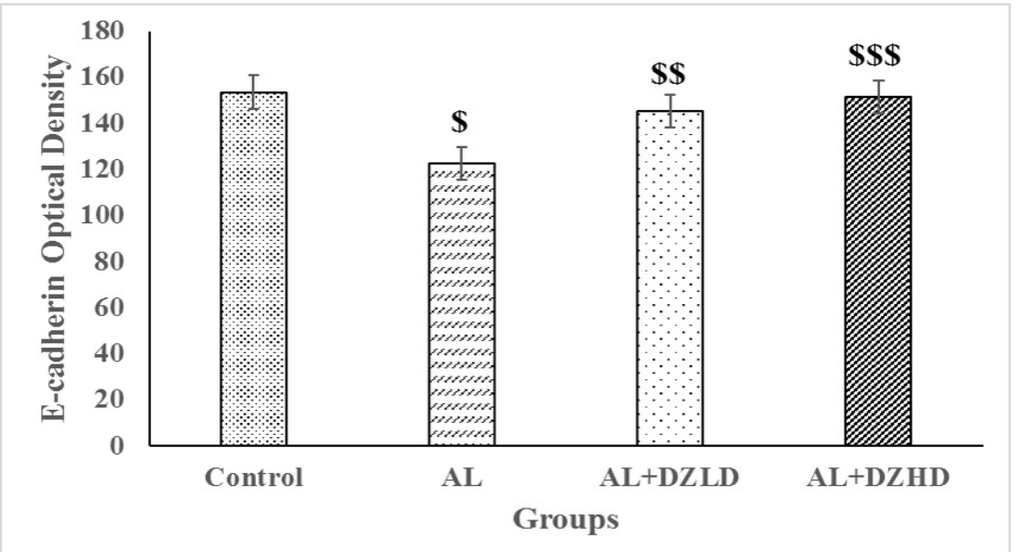

Fig. 7: The optical density of E-cadherin stained surface area. Values were expressed as means ± standard deviation. $; AL group compared to control. $$; AL+DZLD group compared to AL group. $$$; AL+DZHD group compared to AL group.

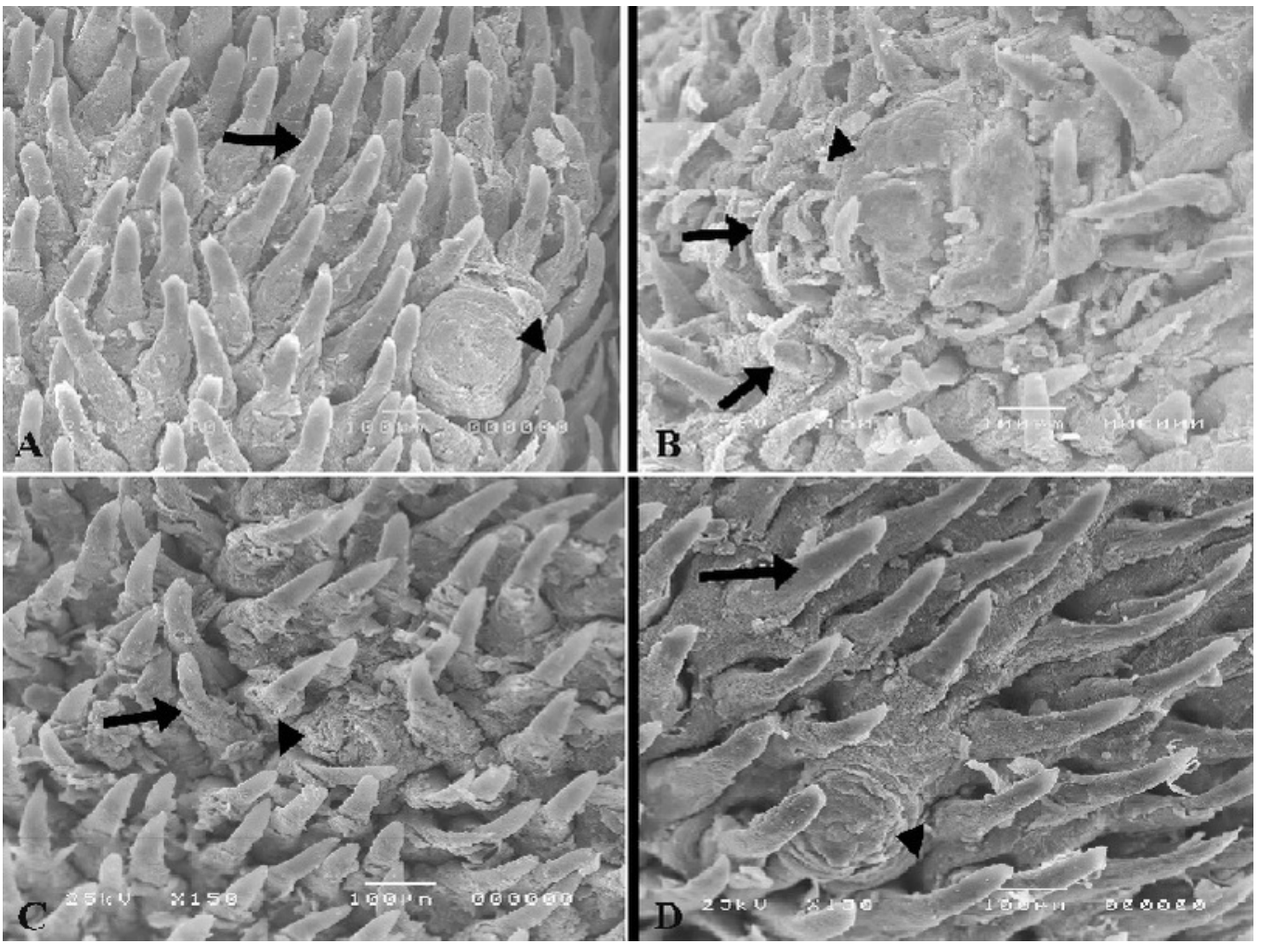

Fig. 8: Control group: many regularly arranged and elongated filiform lingual papillae, conical in shape with intact tapering ends pointed into the same direction (→), and few fungiform lingual papillae short, broad with taste pores in the centers of their upper surfaces (►) scattered in-between the filiform papillae. B) AL group: desquamated irregularly arranged atrophied filiform papillae with wide separation (→), and fungiform papillae with rough upper surface and ill-defined taste pores (►). C) AL+DZLD group: few desquamated filiform papillae (→), and fungiform papillae with rough surface (►). D) AL+DZHD: elongated regularly arranged filiform papillae (→), and short broad fungiform papillae with taste pores at their upper surfaces (►). (Scanning electron microscope x 100).

DISCUSSION

The adverse effects of AL which is a member of Bisphosphonates had been studied by many researchers with the net results were secondary hyperparathyroidism, jaw osteonecrosis, besides GIT mucosal irritations[6]. So, the need for adjuvant therapy with natural products carry the upper hand to avoid systemic complications that accompanied with AL treatment.

Dietary isoflavones are very important to boost one's health and improve life quality. It helps to decrease the age-related health issues especially that includes heart, and bone. One of these isoflavones is DZ. It is very powerful because of its an antioxidant, anti-inflammatory, antiapoptotic, cardioprotective, neuroprotective, anti-aging benefits[12].

At the current study, H&E-stained sections of AL group discovered disorganized and distorted lingual papillae with ill-defined filiform papillae even lost at some focal areas. The suppressed levels of farnesyl diphosphate synthase (FDPS) by AL; an enzyme found to be highly expressed in type II and sour sensitive type III taste buds' cells, also its downstream effects on the mevalonate (MVA) pathway which is necessary for the production of Geranylgeranyl Pyrophosphate (GGPP) and Farnesyl Pyrophosphate (FPP) could lead to some taste disorders[21,22]. The latter are considered to be metabolites needed for the prenylation of the small GTPases Rho and Rab. Consequently, well control of the intracellular vesicles and secretory activity trafficking[23].

In our study, AL group revealed cytoplasmic vacuolations in the covering epithelium of the lingual papillae. Research had shown that AL lowered the potential of the mitochondrial membrane, which produced reactive oxygen species (ROS) and free radicals that damaged cell membranes and eventually caused the energy-dependent Na+ and K+ ion pumps in the cell to malfunction and destroy intercellular connections[24,25].

Alendronate generated mucosal damage through two mechanisms: direct oxidative damage and indirect oxidative damage brought on by neutrophil infiltration. AL-induced activated and migrating neutrophils were a source of pro-inflammatory cytokines, primarily TNF-α and IL-1ß, which caused mucosal damage and seriously impaired cell membrane function[26]. Damage of the gastrointestinal tract mucosa was brought on by lipid peroxidation which was induced by ROS and glutathione depletion[27].

Moreover, Nitrogen-Containing bisphosphonates (N-BPs) significantly reduced the amount of coenzyme Q10 by blocking the mevalonate pathway and depriving cells of a crucial antioxidant and mitochondrial electron carrier. ROS were produced at a higher rate as a result, and mitochondrial respiratory activity was compromised[25].

Our statistical results recorded a significant increase of the levels of MDA and a significant decrease of the levels of SOD in AL group compared to control group. These biochemical results were in line with Silva *et al*. (2014) and Magierowski *et al*. (2016)[28,29] respectively. Lipid peroxidation and failure of anti-oxidative mechanisms were responsible for alendronate-evoked gastric lesions, evidenced by decreased mucosal glutathione (GSH), increased MDA, and decreased GPx-1 and SOD-2 mRNA expression[30].

Concerning the cells covering epithelium of the lingual mucosa; there were dark nuclei seen at AL group. That is might due to the mitochondrial dysfunction with cytochrome C release besides other pro-apoptotic proteins such as caspase-9 and -3 initiating apoptotic cell death[31,32]. Also, it was found that AL leads to Bax expression elevation (pro-apoptotic protein), besides Bcl-2 down regulation (anti-apoptotic protein)[33].

Regarding the lamina propria, the present work revealed congested apparently dilated blood vessels. AL augment histamine release by its action on the histamine-forming enzymes. So, causing hydrostatic pressure elevation with increased capillary permeability and tissue edema[34].

Regarding AL- Masson's Trichrome findings; a weak positive staining affinity of collagen fibers was seen. AL was found to block the MVA pathway that is involved in the antifibrotic action of AL[35]. Moreover, a study made by Tanner *et al*. (2021)[36] on the HFL-1 cells that was treated by BPs showed that TGF-β1 undergo downregulation with the decrease of myofibroblast transition, so decrease in the production of fibrotic proteins, and pro-fibrotic macrophage populations. This is also confirmed by Larson-Casey *et al*. (2021)[37] who reported the increased MVA pathway activity by AL with subsequent macrophages polarization.

As for the immunohistochemical results; it confirms the histopathological effects of AL on the lingual mucosa. As there was faint even lost membranous immunoreaction of E-cadherin. AL has a prominent inhibitory effect on the oral keratinocyte proliferation. It could disrupt the process of epithelial terminal differentiation as well as cytoskeletal derangement with subsequent prominent morphological and molecular abnormalities of the oral mucosa[38,39].

The naturally occurring isoflavone; DZ was found to ameliorate AL-induced histopathological manifestations of the lingual mucosa of the present work in a dose-dependent manner[11]. DZ is a phytoestrogen with an antioxidant, anti-inflammatory, antiapoptotic and anti-cancer effects[10,40]. The antioxidant property of DZ could be attributed to its transformation by the gut bacteria into O-desmethylangolensin (O-DMA); a powerful antioxidant[41].

In the current work; H&E-stained sections of DZLD group displayed partial restoration of the normal histological structure of the lingual mucosa. There was some disorganized, and distorted lingual papillae with cytoplasmic vacuolations and pyknosis of some epithelial cells. While, DZHD showed an ameliorative effect, with nearly normal lingual mucosal structure. This might be due

to the antioxidant property of DZ. It increase the levels of SOD and GSH enzymes through activating the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway[42].

In the present study, the levels of MDA were decreased, while the levels of SOD increased in AL+DZ treated groups dose dependently. Which come in agreement with Aras *et al*. (2015) and Al-Ganem *et al*. (2018)[43,44]. This could be attributed to the antioxidant and anti-inflammatory actions of DZ through the decreased synthesis of NF-κB p65 subunit with a decrease in the production of the proinflammatory cytokines like IL-6, TNF-α, and IL-10[45-47]. Several confirmatory studies were documented for the anti-inflammatory actions of DZ especially that made by Zheng *et al*. (2022) who proved that DZ increase neuronal regeneration after acute nerve injury thru inhibiting the MAPK pathway, also increasing the brain levels of GSH[14,48,49].

Also, DZ is helpful in the reduction of the apoptotic cell death rate. This could due to the upregulation of Bcl-2 protein while suppressing Bax expression[50]. In addition the anti-apoptotic activity of DZ might be due to the down regulation of caspase-3& 6 expression[13,51]. Moreover, DZ inhibits apoptosis progression through increased action on the cell Ki67; consequently, cell proliferation[13].

A nearly normal E-cadherin membranous immunohistochemical reaction was encountered at AL+DZHD group at the present work. This is in agreement with Chan *et al*. (2018) and Sun *et al*. (2022)[52,53]. The reduced inflammatory processes by DZ leads to the improvement of the lingual mucous layer due to high gene expression for mucin-2, besides epithelial barrier integrity with strengthening of the tight junction. The latter is due to the effect of DZ on the mRNA levels of tight junction proteins enhancing their expression[54,55].

Another theory that as DZ has an inhibitory effect on the expression of the proinflammatory cytokines like TNF-α and IFN-γ so, improves the cell survival and alleviates oxidative stress. These could affect the intracellular signaling for tight junction proteins with increased their expression, restoring the mucosal barrier's function[56,54]. This is confirmed by the action of DZ on the gastrointestinal barrier function in a model of DSS-induced acute colitis. It inhibited the collapse of tight junction proteins[57]. Similarly, it enhanced the expression of occludin and increased the expression of tight junction linked genes in the lipopolysaccharide-induced inflammation model[58,59].

CONCLUSION

In conclusion, the present research demonstrated the ameliorating effect of DZ on the lingual mucosal injury induced by alendronate in adult male albino rats. It was noticed that DZ ameliorated such injurious effects through its an antioxidant and anti-inflammatory actions and this amelioration is a dose dependent. Consequently, it is recommended to use Daidzein as a protective in patient which treated with Alendronate.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير الديدزين على التغييرات المستحثة بالأليندرونات في الغشاء المخاطي اللساني للجرذان: دراسة كيميائية حيوية ونسيجية ومجهرية إلكترونية ماسحة

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المقدمة: أليندرونات هو أحد مجموعة البايفوسفونيت التي تحتوي على النيتروجين. وقد تم استخدامه في علاج اضطرابات العظام ولكن مع آثار جانبية أخرى على الأعضاء. الديدزين هو أحد الأيسوفلافون الذي يمتلك تأثيرات مضادة للأكسدة ومضادة للإلتهابات ومضادة للموت المبرمج.

الهدف: هو دراسة تأثير الديدزين على التغيرات التي يسببها الأليندرونات على الغشاء المخاطي اللساني للفئران من خلال التقييم الكيميائي الحيوي والنسيجي الإلكتروني الماسح.

المواد والطرق: ٢٥ ذكراً بالغاً من الجرذان البيضاء؛ ٣-٥ أشهر؛ ١٨٠ - ٢٢٠ جرام تم تقسيمهم إلى؛ المجموعة الضابطة، مجموعة AL(: AL (٠,٠٥ مجم/كجم)، AL + DZ) :AL+DZLD جرعة منخفضة (٥٠ مجم/كجم)، AL + DZ) :AL+ DZHD جرعة عالية (١٠٠ مجم/كجم). تم إعطاء الجرعات عن طريق الفم لمدة اثني عشر أسبوعًا، ثم تمت معالجة عينات اللسان لإجراء الدراسات الكيميائية الحيوية و المجهرية الضوئية والإلكترونية الماسحة.

النتائج: أظهرت مجموعة AL ارتفاعا ذو دلالة إحصائية في المستوى النسيجى للمالونديالدهيد ، في حين إنخفض ذو دلالة إحصائية في المستوى النسيجى للديسموتاز فوق أكسيد. علاوة على ذلك يوجد حليمات لسانية غير منظمة وغير محددة ومشوهة، ووجود فجوات فى سيتوبلازم الخلايا الظهارية مع أنوية داكنة، وفقدان التلال الظهارية، وإحتقان وتوسع الأوعية الدموية ، وإنخفاض ذو دلالة إحصائية في متوسط إرتفاع وعرض الحليمات الخيطية والكمئية بواسطة الهيماتوكسيلين والإيوسين. بينما حدث انخفاض ذو دلالة إحصائية في الكثافة البصرية لألياف الكولاجين والكادهيرين. أيضًا، هناك حليمات خيطية ضامرة متقشرة ومرتبة بشكل غير منتظم مع تباعد واسع، وحليمات كمئية ذات سطح علوي خشن مع وجود مسام التذوق غير محددة بإستخدام المجهر الإلكتروني الماسح. أظهرت المجموعة المعالجة بواسطة DZ تحسنًا يعتمد على الجرعة للنتائج المذكورة سابقًا.

الإستنتاج: الديدزين يعتمد على الجرعة تعمل على تحسين التغيرات التي يسببها أليندرونات في الغشاء المخاطي اللساني الجرذان من خلال تأثيرها المضاد للأكسدة والمضاد للإلتهابات.