Kaempferol protects the tongue in experimentally induced diabetic rats: a histological and immunohistochemical study

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SUMMARY

Uncontrolled diabetes impairs the taste response. Kaempferol is known to possess antioxidant and anti-inflammatory activity and to protect various tissues against diabetes. Here, the potential protective role of kaempferol was investigated against the tongue complications induced by diabetes. Four experimental groups were established (n = 10 each), including control group; kaempferol group, rats received kaempferol orally at dose of 100 mg/kg /day; diabetic group, rats received intraperitoneal STZ (60 mg/kg, single dose) and diabetic with kaempferol group. After eight weeks, all animals were sacrificed, The tongues were dissected and processed for light microscopic examination using haematoxylin and eosin stain, Verhoeff-Van Giesson's stain and immunohistochemical staining for Bcl-2 and PCNA and for SEM examination. Serum blood glucose, TNF-α and IL-6 were measured. Morphometric and statistical analyses were performed. The diabetic with kaempferol group showed preserved histological and morphological structure of filiform and fungiform papillae. Area percentage of collagen fibers was significantly reduced. Immunohistochemical findings revealed significantly increased Bcl-2 surface area and increased PCNA immunopositive cells as compared with the diabetic group. Also, this group revealed significant improvement of the serum levels of blood glucose, TNF-α and IL-6. These findings suggest that kaempferol attenuates histological diabetic-induced changes through its anti-inflammatory and anti-apoptotic effects, and modulates PCNA expression. Therefore, kaempferol can be used as adjuvant therapy in diabetic tongue.

Key words: Diabetes – Kaempferol – Tongue – Bcl-2 – PCNA

INTRODUCTION

The tongue is composed of several tissues, including epithelial, neural, and connective tissues, and also contains taste buds, which are located in the lingual papillae. The function of the taste buds is to detect sweet, bitter, and other chemical stimuli (Mistretta and Kumari, 2017).

It is known that patients with uncontrolled diabetes suffer from taste alterations. This abnormality in the taste sensation of these patients affects their choice of nutrients, leading to more consumption of sweet-tasting foods that aggravate hyperglycemia (De Carli et al., 2018)

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Diabetes mellitus is an irreversible condition. It is a slowly developing but progressive state, and its progression takes several years (Lindstrom et al., 2018). Therefore, the ultimate aim of health care is to try to prevent diabetes or at least delay its development, which is much better than treating the disease after it occurs (Gothai et al., 2016).

Recently, there has been increased interest in the use of medications from natural sources in the management of medical conditions such as diabetes (Gothai et al., 2016). Over the past decade, oxidative stress has been claimed as a leading cause in the development of diabetic complications (Ali and Agha, 2009). Natural antioxidant products have gained much attention for use as medicines and food supplements. Antioxidant compounds have shown high effectiveness due to several pharmacological properties (Taghvaei and Jafari, 2015). Potent antioxidant properties can be obtained from flavonoids, which can suppress the inflammatory cytokines and increase the cell sensitivity to insulin (Rendeiro et al., 2012).

Kaempferol, a naturally occurring flavonoid, is found in tea, tomato, broccoli, grapefruit, nuts and other plant sources (Yoshida et al., 2008). The pharmacological properties of kaempferol include antioxidant, anti-inflammatory, and anticancer activities (Park et al., 2009). Several studies have proven that intake of kaempferol protects various tissues against diabetes such as kidney (Sharma et al., 2019), heart (Chen et al., 2018) and pancreas (Zhang and Liu, 2011). The purpose of this study is to demonstrate the protective role of kaempferol against the tongue complications induced by diabetes.

MATERIAL AND METHODS

Experimental animals

Forty male albino rats (12 weeks old, weighing between 150 and 250 g) were used. The rats were locally bred at the animal house of the Research Center and Bilharzial Research Unit, Faculty of Medicine, Ain Shams University. The rats were treated in accordance with relevant guidelines and regulations approved by the animal Committee of Ain Shams University.

The rats were left two weeks for acclimatization, and all efforts were made to minimize animal suffering. The rats were housed in stainless-steel cages, five rats per cage. The animals were fed on standard laboratory chow and allowed free access to water in an air-conditioned room with a 12-hour-light/12-hour-dark cycle and at a temperature of 25 °C.

Induction of diabetes

After 12 hours of fasting, the animals of model groups received a single 60 mg/kg intraperitoneal injection of Streptozotocin (STZ) dissolved in 0.1mol/L sodium citrate buffer, PH 4.5. The animals were allowed to drink 5% glucose solution to overcome STZ-induced hypoglycemia. After 72 hours, animals with blood glucose levels greater than 200 mg/dL were considered diabetic (Faheem and Askary 2017).

Drug and chemicals: STZ and kaempferol were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Experimental design

The rats were divided into four groups (n=10):

- Control group (given citrate buffer once by intraperitoneal injection).
- Kaempferol group**:** rats received kaempferol dissolved in 5% dimethylsulfoxide orally at a dose of 100 mg/kg body weight/day for eight weeks (Al-Numair et al., 2015).
- Diabetic group: included STZ diabetic rats.
- Diabetic with kaempferol group: it included STZ diabetic rats that received kaempferol immediately after being diagnosed as diabetic at the same dose, route of administration and duration as the kaempferol group.

Sampling Blood

Samples were obtained from the veins of animals' tails under gentle ether anesthesia every two weeks, and then centrifuged for 15 minutes. The separated serum was used for the detection of serum glucose. At the end of the experiment, blood samples were collected and serum tumor necrosis factor (TNF-α)

and IL-6 were measured by ELISA kits (RayBiotech, Inc., GA, USA), according to the manufacturer's instructions. The animals were sacrificed by high dose of ether anesthesia. From each animal, the tongue was removed, and the anterior two-thirds of the tongue were dissected. Then, each specimen was cut longitudinally into two halves. Half of them were fixed in 10% formaldehyde and processed for the preparation of paraffin sections (5-µm). The sections were stained by H&E stain and Verhoeff-Van Giesson's stain (Bancroft and Layton 2013). The other halves of the specimens were washed by phosphate-buffered saline (PBS), and fixed in glutaraldehyde solution. The specimens were processed for examination using a Philips Scanning Electron Microscope (XL30; Philips, Amsterdam, the Netherlands) at the Scanning EM Unit of the Anatomy Department, Faculty of Medicine, Ain Shams University (Duro et al., 2012).

Immunohistochemical study for Bcl-2 and PCNA

Using poly-L-lysine-coated slides, tongue sections were prepared and heated in an oven for twenty five minutes at 60ºC. After heating, sections were deparaffinized in xylene and rehydrated in graded alcohol. Sodium citrate buffer of concentration 10 mM was heated till boiling in a microwave for antigen retrieval. Succinctly, 0.03% hydrogen peroxide sodium azide was used to block the endogenous peroxidase for five minutes followed by washing the tissue sections carefully using wash buffer, and then half of the specimens were incubated with antiapoptotic protein Bcl-2 biotinylated primary antibodies, and the other halves were incubated with anti- PCNA primary antibody for fifteen minutes. Diaminobenzidine substrate chromagen was applied to the sections and incubated for eight minutes, followed by careful wash and hematoxylin counterstaining were applied. Positive cells expressing PCNA were identified by brown nuclei, while Bcl-2 was demonstrated brown cytoplasmic staining (Buchwalow and Bocker, 2010).

Quantitative morphometric study

Five sections were randomly chosen from each group. Ten fields per section were taken from high-power images (400x), and the Image J software program was used to detect:

1. Area percentage occupied by Bcl-2 immunopositive cells.
2. Percentage of PCNA immunopositive cells.
3. Area percentage occupied by collagen fibers using Verhoeff-Van Giesson's stain.

Statistical analysis

The results were represented as mean ± Standard Deviation (±SD). All statistical comparisons between the four studied groups were analyzed via the one-way ANOVA, followed by post hoc test for multiple comparisons. All statistical analyses were performed using SPSS software (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Serum blood glucose, TNF-α and IL-6 levels

The diabetic rats had significantly ($P < 0.05$) elevated serum blood glucose levels as compared with control rats. Meanwhile, the diabetic with kaempferol group had significantly ($P < 0.05$) reduced the glucose levels when compared with the diabetic group (Fig. 1A). Treatment of the diabetic rats with kaempferol significantly decreased ($P < 0.05$) serum TNF-α and IL-6 levels as compared with the diabetic group. However, diabetic rats treated with kaempferol showed significantly higher levels ($P < 0.05$) when compared with control and kaempferol groups (Fig. 1B).

Light microscopic results

Histological examination of tongues of the control group showed the usual tongue architecture. The dorsal surface of the tongue was covered with keratinized stratified squamous epithelium with normal lingual papillae and regularly arranged skeletal muscle fibers (Fig. 2A). No observable differences between the control and kaempferol groups were detected (Fig. 2B). However, the diabetic group showed variable histological changes. The dorsal surface showed thickened keratin layer, attenuated lingual papillae and irregularly arranged muscles with

congested blood vessels in-between (Fig. 2C). On the other hand, the dorsal surface of the diabetic with kaempferol group showed improvement with regular arrangement of the covering epithelium and a thinner keratin layer than the diabetic group. Indeed, the skeletal muscle fibers were regularly arranged (Fig. 2D).

In the control group, filiform papillae were elongated, conical in shape, and had a definitive connective tissue core. Fungiform papillae were localized between filiform ones, showing a broad flat surface, a solitary well defined intraepithelial taste bud, relatively thinner keratin layer and richly vascular stroma (Fig. 3A). Kaempferol group showed no observable difference from findings presented in the control group (Fig. 3B). However, the diabetic group showed variable histological changes. The dorsal surface showed hyperkeratosis, attenuated papilla with inflammatory cells infiltration and congested blood vessels in the lamina propria**.** Most filiform papillae showed flattening of their tips with reduced connective tissue core. Moreover, fungiform papillae were apparently shorter with a thicker keratin layer than the control group. Furthermore,

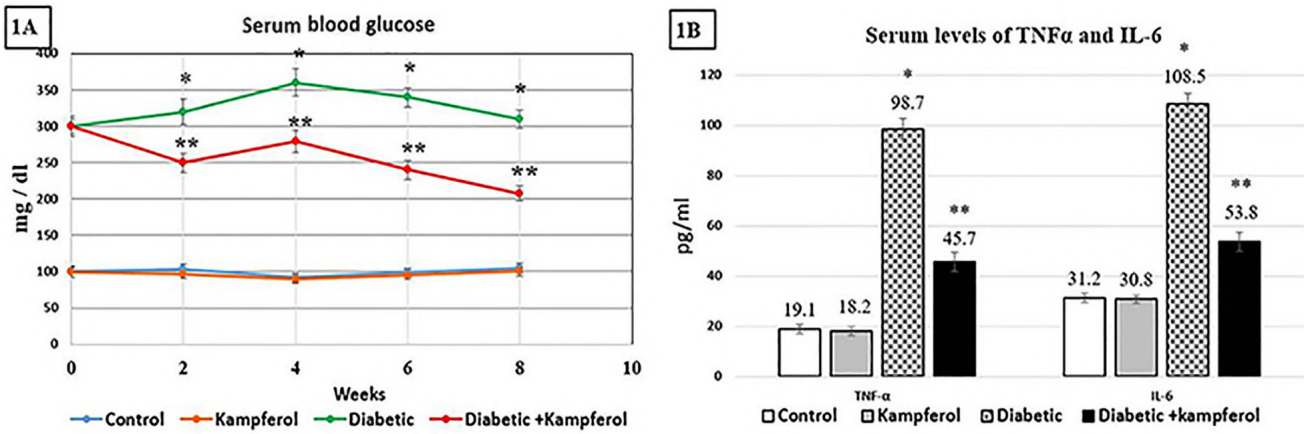

Fig. 1.- A. Means±SD of serum blood glucose levels (mg/dl) at 2nd, 4th, 6th and 8th week. B. Means ± standard deviations of serum TNF-α and serum IL -6 (pg/ml) in the studied groups. P < 0.05 (*) vs other groups, (**) vs control and kaempferol groups.

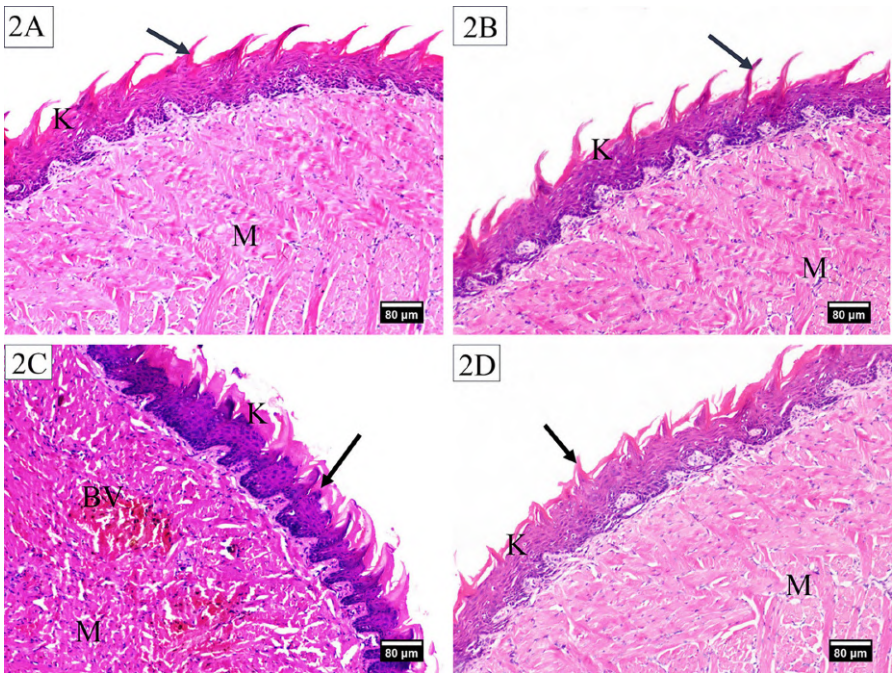

Fig. 2.- Photomicrographs of the dorsal surface of the tongue stained by H&E. A and B. Control and kaempferol groups showing keratinized epithelium (K), lingual papillae (arrow) and regularly arranged skeletal muscle fibers (M). C. Diabetic group showing thickened keratin layer (K), attenuated papillae (arrow), irregularly arranged muscle fibers (M) and congested blood vessels inbetween the muscles (BV). D. Diabetic with kaempferol group showing keratinized epithelium (K), conserved lingual papillae (arrow) and regular arrangement of skeletal muscle fibers (M).

the taste buds of the diabetic group showed variable alterations in the form of a marginal arrangement of the cells with a halo centre (Fig. 3C). In addition, other histological alterations were detected in this group, such as separation of the covering keratin, reduced connective tissue core, shallow epithelial ridges, and variable signs of vacuolar degeneration, pyknotic nuclei, hyperchromatic nuclei and crescent like nuclei in keratinocytes (Fig. 3D). On the other hand, the dorsal surface of the anterior two-thirds of the tongue of the diabetic with kaempferol group, showed prominent lingual papillae with conserved underlying connective tissue. Most filiform papillae of this group showed a preserved thread like shape with pointed ends and thin smooth keratin (Fig. 3E). The fungiform papilla of this group closely resembled those of the control group. Their taste buds appeared barrel-shaped with apparently normal cells (Fig. 3F).

In both control and kaempferol groups, the trench of the circumvallate papillae was generally covered with non-keratinized stratified squamous epithelium with normal taste buds (Fig. 4A, B). However, the trench of the diabetic group was diminished with disrupted epithelial covering. Many taste buds were vacuolated. Areas of diffuse leucocytic infiltration were detected in the lamina propria (Fig. 4C). Meanwhile, the trench of the circumvallate papillae of the diabetic with kaempferol group were apparently normal and covered with non-keratinized stratified squamous epithelium. Numerous pale, regularly arranged taste buds were observed in its lateral walls. Each taste bud is formed of cluster of pale-staining cells which opens into the surface by means of a taste pore (Fig. 4D).

Van Giesson-stained sections of both control and kaempferol groups showed dense, positively stained collagen fibers in the lamina propria of the ventral surface (Fig. 5A, B), while the ventral surface of the diabetic group showed faint, dispersed collagen fibers in the lamina propria (Fig. 5C). Meanwhile, the diabetic with kaempferol group showed regularly arranged, positively stained collagen fibers (Fig. 4D). Statistical comparison of the collagen fibers area percentage in Van Giesson stained sections revealed that the collagen area percentage of the diabetic with kaempferol group was significantly ($P < 0.05$) increased as compared with the diabetic group (Table 1).

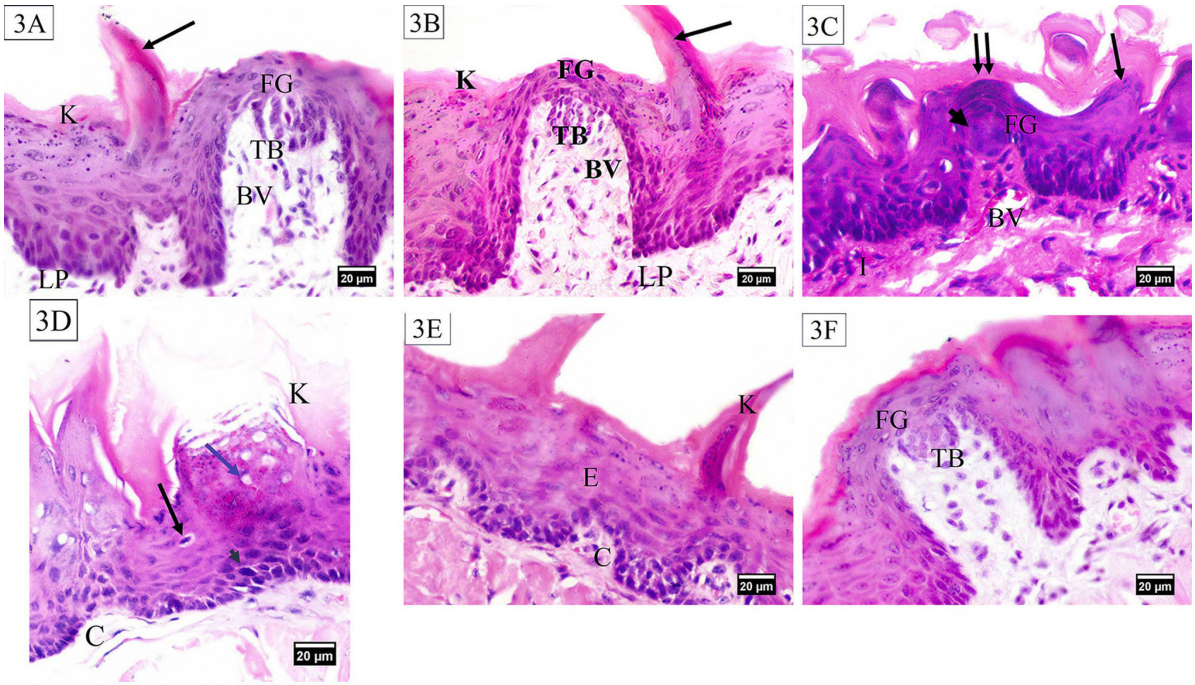

Fig. 3.- Photomicrographs of the dorsal surface of the tongue stained by H&E. A and B. Control and kaempferol groups showing keratinized epithelium (K), conical filiform papilla (arrow), fungiform papilla (FG) with taste bud (TB), lamina propria (LP) and blood vessels (BV). C, D. Diabetic group showing C. Filiform papilla with flattened tips (arrow), fungiform papillae (FG) with thickened keratin layer (double arrows), taste bud with peripheral margination of the cells and empty centre (arrow head), congested blood vessels (BV) and inflammatory cells infiltration (I). D. Vacuolated cytoplasm and pyknotic nuclei (black arrow), crescent-like nuclei (blue arrow), hyperchromatic nuclei (arrow head), separated keratin (K) and reduced connective tissue core (C). E, F. Diabetic with kaempferol group showing: E. Surface epithelium (E), filiform papilla with thin keratin layer (K) and conserved connective tissue papilla (C). F. Fungiform papilla (FG) and its taste bud (TB).

Bcl-2 and PCNA immunostaining results

Immunohistochemical staining for the antiapoptotic marker Bcl-2 showed moderately positive cytoplasmic reaction in the basal and suprabasal layers of the tongue epithelium of the control group (Fig. 6A). Similarly, the kaempferol group disclosed similar results (Fig. 6B). On the other hand, the diabetic group revealed negative to mild cytoplasmic reaction in the tongue epithelium (Fig. 6C). Meanwhile, the diabetic with kaempferol group showed a moderate positive cytoplasmic reaction in the tongue epithelium (Fig. 6D). Statistical analysis revealed significantly increased area percentage of Bcl2 positive cells *(P*

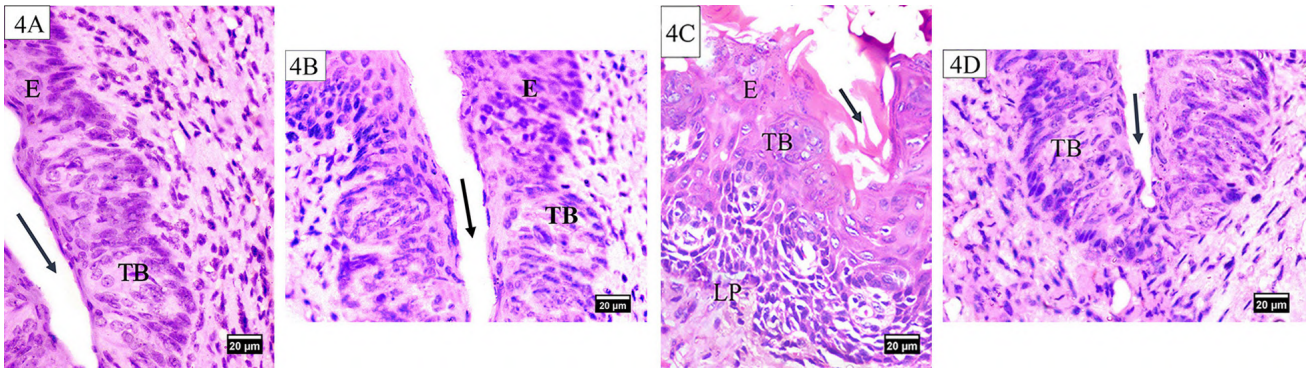

Fig. 4.- Photomicrographs of the dorsal surface of the tongue stained by H&E. A and B. Control and kaempferol groups showing a trench of a circumvallate papillae (arrow) covered with non-keratinized stratified squamous epithelium (E). Note the taste buds (TB) on the trench. C. Diabetic group showing disrupted epithelial covering of circumvallate papilla (E), vacuolated taste bud (TB) and diminished trench of the circumvallate papillae (arrow). Notice diffuse leucocytic infiltration in the lamina propria (LP). D. Diabetic with kaempferol group showing a trench of the circumvallate papillae (arrow) with regularly arranged taste buds (TB).

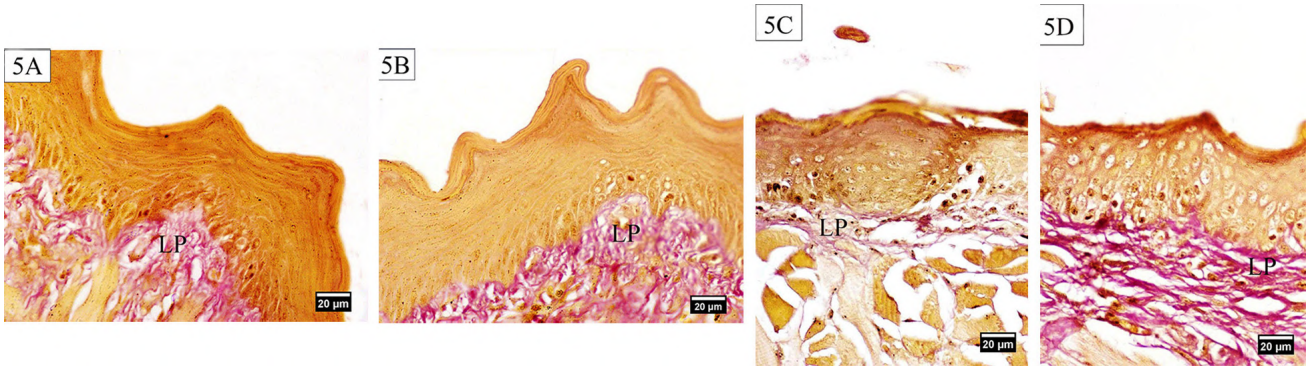

Fig. 5.- Photomicrographs of the ventral surface of the tongue stained by Van Gieson Stain. A and B. Control and kaempferol groups showing dense positively stained collagen fibers into the lamina propria (LP). C. Diabetic group showing faint dispersed collagen fibers into the lamina propria (LP). D. Diabetic with kaempferol group showing dense positively stained collagen fibers into the lamina propria (LP).

Table 1. Comparison between the studied groups regarding the morphometric data

Parameter	Control group	Kaempferol group	Diabetic group	Diabetic with kaempferol group
Mean area percent of collagen fibers	16.04 ± 0.95	15.85 ± 0.89	8.4 ^{a,b} ± 2	13.1 ^{a,b,c} ± 1.03
Mean area percent of Bcl2 immunopositive cells	38.6 ± 3.3	38.9 ± 2.6	9.3 ^{a,b} ± 2.8	28.3 ^{a,b,c} ± 3.2
percent of PCNA immunopositive cells	85 ± 3.7	83 ^{a} ± 3.8	32 ^{a,b} ± 4.5	68 ^{a,b,c} ± 4.1

Data are expressed as mean ± SD. $p > 0.05$: no significant difference, $p < 0.05$: significant difference.
a Significantly different from the control group
b Significantly different from the kaempferol group
c Significant different from the diabetic group

< 0.05) in the diabetic with kaempferol group as compared with the diabetic group (Table 1).

PCNA immunohistochemical staining showed PCNA positive cells in the basal and para-basal cells of the tongue epithelium (Fig. 7A). Indeed, kaempferol group revealed identical findings (Fig. 7B). However, the diabetic group showed few PCNA positive nuclei (Fig. 7C). Furthermore, the diabetic with kaempferol group showed PCNA positive nuclei mainly in the basal cell layer of the tongue epithelium (Fig. 7D). Statistical analysis revealed significantly increased PCNA positive cell percentage *(P < 0.05)* in the diabetic with kaempferol group as compared with diabetic group (Table 1).

Scanning electron microscopic results

The dorsal surface of the tongue of the control group was covered by many filiform papillae. They were elongated, conical and lightly curved papillae with intact pointed tips. Fungiform papillae were seen intermittently between the filiform ones. They were broad and had a flattened, smooth nearly rounded upper portion with an apparent central taste pore. Scanty rough keratinized cells were seen on fungiform and at the basal part of the surrounding filiform papillae (Fig. 8A). Examination of tongue sections of the kaempferol group showed no observable difference from the results of the control group (Fig. 8B). On the other hand, the tongue papillae of the diabetic group showed a noticeable atrophy. Filiform papillae were short, discrete and disarranged in various directions. The apical parts of some papillae were bifurcated. Meanwhile, other papillae showed detached upper portions. Furthermore, some filiform papillae showed an irregular heavily keratinized base (Fig. 8C). Some fungiform papillae were shorter than the control and had a rough nearly rounded upper portion, with a wrinkled, keratinized epithelial covering. Upper portions with an indistinct taste pore were observed (Fig. 8D). In the diabetic with kaempferol group, the tongue papillae appeared orderly arranged. Filiform papillae revealed a regular distribution and inclination. Most of their

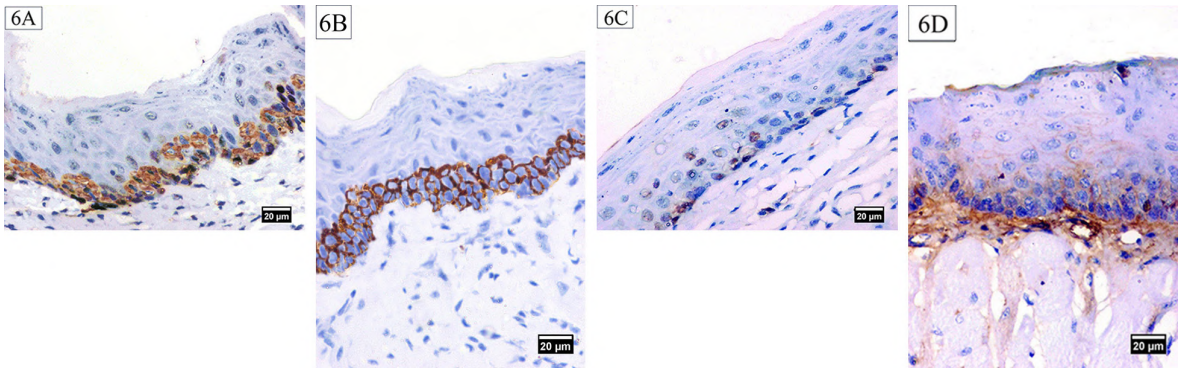

Fig. 6.- Immunohistochemical staining for the Bcl-2 of the ventral surface of the tongue. A and B. Control and kaempferol groups showing moderate positive cytoplasmic reaction in tongue epithelium. C. Diabetic group showing mild cytoplasmic reaction in tongue epithelium. D. Diabetic with kaempferol group showing moderate positive cytoplasmic reaction in tongue epithelium.

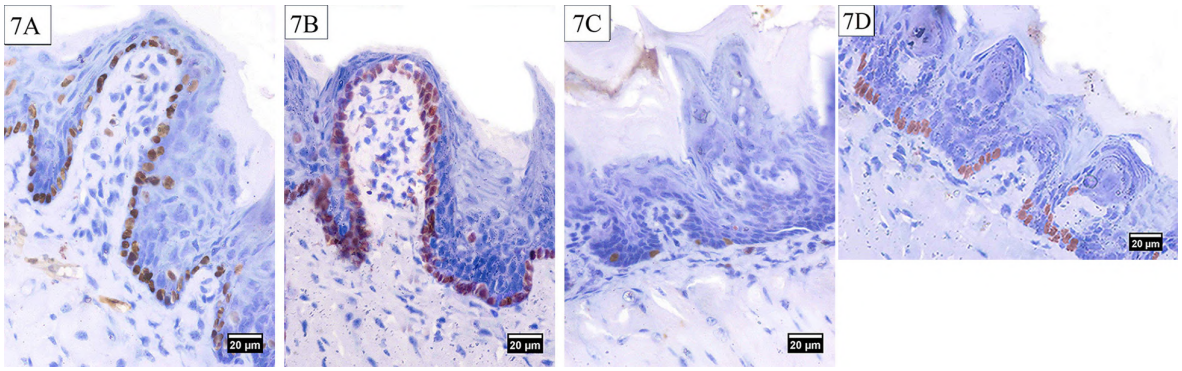

Fig. 7.- Immunohistochemical staining for the PCNA of the dorsal surface of the tongue. A and B. Control and kaempferol groups showing PCNA positive nuclei in the basal cell and para-basal cells of the tongue epithelium. C. Diabetic group showing few PCNA positive nuclei. D. Diabetic with kaempferol group showing PCNA positive nuclei mainly in the basal cell layer of the tongue epithelium.

tips were tapering and directed to one side (Fig. 8E). Fungiform papillae were located in between them with an orderly arranged epithelial covering and a clear organized gustatory pore (Fig. 8F).

DISCUSSION

Flavonoids are antioxidants that are found abundantly in nature in a variety of plants. Among these natural flavonoids, kaempferol is a member of the flavonol subclass widely distributed in many edible plants. Kaempferol has a potent superoxide scavenger activity, antioxidant and anti-inflammatory effects in vitro and in vivo (Du et al., 2018).

In the present study, treatment of the diabetic rats with kaempferol significantly improved the alteration of glucose levels. Al-Numair et al. (2015) concluded that administration of kaempferol to the diabetic rats adjusted plasma glucose and insulin near to normal levels. Consistently, another study showed that oral administration of kaempferol improved glucose homeostasis through reducing hepatic gluconeogenesis and decreasing glycogenolysis in streptozotocin-induced diabetic mice (Alkhalidy et al., 2018).

In the diabetic group of the present study, SEM examination revealed that filiform papillae appeared short, discrete and disarranged in various directions, while fungiform papillae were atrophied with indistinct taste pores. That atrophy of the tongue could be attributed to chronic inflammation which leads to alterations in microvasculature and innervation due to DM. Moreover, the atrophic changes might be related to the decreased cell proliferation (Mohsen et al., 2019), and to the increased apoptosis (Soleymaninejad et al., 2017; Hamza et al., 2018).

In addition, the circumvallate papilla of the diabetic group showed disrupted epithelial covering with diminished trench and many vacuolated taste buds. Pai et al. (2007) contributed the decreased taste buds per circumvallate papilla in the diabetic rats to the significantly reduced taste cells innervation. Taste impairment in diabetes could include a defect of the taste receptors, peripheral neuropathy and microangiopathy (Perros et al., 1996). Kishore et al. (2018) indicated that kaempferol can be considered as a natural antidiabetic agent and can be used as an adjuvant therapy to diabetic neuropathic pain.

Our results showed that treatment of diabetic rats with kaempferol significantly reduced serum proinflammatory cytokines TNFα and IL6 levels as compared with the diabetic group. Hyperglycemia induces the expression

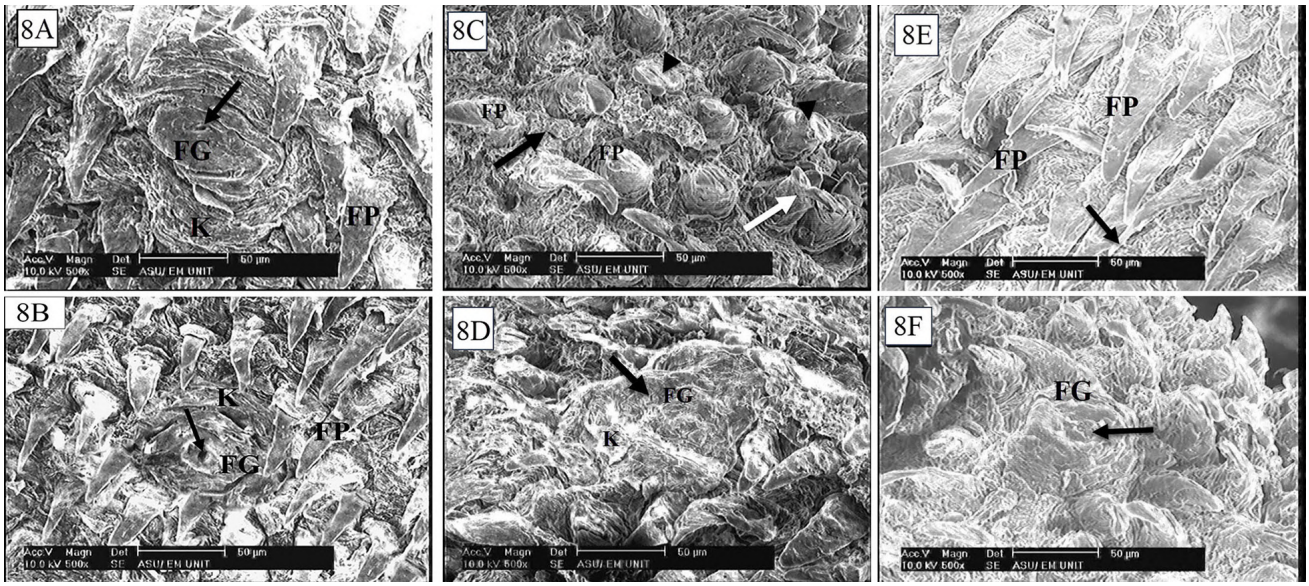

Fig. 8.- Scanning electron micrographs of the the dorsal surface of the tongue. A and B. Control and kaempferol groups showing filiform papillae (FP) and fungiform papilla (FG) with an apparent taste pore (arrow). Notice few rough keratinized cells seen on fungiform (K). C, D. Diabetic group showing C. Irregularly arranged filiform papillae (FP), bifurcated papilla (white arrow) and highly keratinized interpapillary ridges (black arrow). Notice complete loss of the upper portions of some papilla (arrow head). D. Atrophied fungiform papilla (FG) with wrinkled keratin (K) and indistinct taste pore (arrow). (E, F) Diabetic with kaempferol group showing E. Filiform papillae with regular distribution and inclination (FP). Notice the tapering ends of most of papillae (arrow). F. Fungiform papilla (FG) with a well-defined regular gustatory pore (arrow).

of pro-inflammatory cytokines including TNFα and IL-6 which in turn exacerbates oxidative stress (Fuentes-Antrás et al., 2014). Kaempferol reduced hyperglycemia-induced TNF-α and IL-6 expression in heart tissues of STZ-induced diabetic mice. Moreover, kaempferol prevented macrophage infiltration (Chen et al., 2018). Kaempferol significantly preserved oxidant-antioxidant status, attenuating inflammation and apoptosis by inhibiting the mitogen-activated protein kinase (MAPK) pathways, which are responsible for several inflammatory mediators' synthesis such as TNFα and IL-6 and oxidative damage in diabetic rats (Suchal et al., 2017).

In addition, the observed atrophic changes in lingual papillae in our SEM results could be attributed to the observed vacuolar degeneration and nuclear apoptosis. Treatment of diabetic rats with kaempferol ameliorated the vacuolation and nuclear apoptosis in lingual papillae, and this was confirmed by increased Bcl-2 immunopositive cells. These vacuolations were observed in previous studies of diabetes effect on several tissues (Zickri et al., 2012; Faheem and Askary 2017). Sivitz and Yorek (2010) explained these vacuolations on the basis of glucose-induced mitochondrial dysfunction, accumulated reactive oxygen species and oxidative stress. Wu et al. (2020) reported the ability of kaempferol to translocate Bcl-2 to the mitochondria and improve mitochondrial functions as oxidative phosphorylation, ATP production and increasing mitochondrial respiration. Kaempferol treatment improved cell survival, repressed cellular death and improved Bcl-2 expression in pancreatic β-cells with chronic hyperglycemia (Zhang and Liu, 2011).

Suchal et al. (2017), stated that kaempferol inhibited apoptosis by reducing Bax expression and caspase3, decreasing the pro-apoptotic proteins and augmenting the expression of Bcl2. Kaempferol also ameliorated the apoptotic changes of osteoblastic cells as proven by the increased Bcl-2 expression (Adhikary et al., 2018). In contrast, another study suggested that kaempferol induced apoptosis by increasing the Bax/Bcl 2 ratio; this cytotoxic action was more evident in leukemia cells than in normal leukocytes (Moradzadeh et al., 2018).

In the present study, the diabetic rats treated with kaempferol revealed significant improvement of collagen surface area in Van Giesson-stained sections as compared with the diabetic group. Diabetes impedes the integrity of dermal collagen (Argyropoulos et al., 2016). Desta et al. (2010), reported that the number of apoptotic fibroblasts and neutrophils increased while the number of normal fibroblasts and the connective tissue volume decreased in diabetic gingival connective tissue. Dag et al. (2014) explained collagen changes on the basis of hyperglycemia which reduced fibroblast proliferation and altered collagen. Ozay et al. (2019) concluded that kaempferol regulated collagen formation and collagen fibrils by elevating the hydroxyproline levels.

In the current study, the statistical results of the diabetic group revealed significant reduction in the mean number of PCNA positive cells indicating reduced epithelial cell proliferation. Similar findings were observed by Mohsen et al. (2019). Kaempferol treatment significantly enhanced the cell viability, elevated the protein levels of PCNA and Cyclin D1, which are the key cell proliferation marker molecules (Nagy et al., 2019).

CONCLUSION

The results of the current work suggest that kaempferol prevented the histological and morphological diabetic induced tongue alterations. Moreover, kaempferol modulates inflammatory promarkers TNF-α, IL-6 and positively affects the antiapoptotic marker (Bcl-2) and the cell proliferation PCNA marker. Thus, our results showed that kaempferol can be used as an adjuvant natural therapy for tongue complications induced by diabetes.

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