

## EVALUATION OF THE THERAPEUTIC POTENTIAL OF TAMARIND SEEDS (AQUEOUS EXTRACT) VERSUS ANTIDIABETIC DRUGS ON THE HISTOLOGICAL STRUCTURE OF LINGUAL PAPILLAE IN DIABETIC RATS

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### ABSTRACT

**Objective:** Diabetic patients suffer from several oral complications; thus there has been unending efforts in searching for treatment agents either in synthetic forms or from plant sources. The aim of the present study is to prove the efficacy of aqueous tamarind seeds extract in treatment of the complications that might occur in tongue papillae of high fat diet/streptozotocin (HF/STZ) type 2 diabetic rats relative to the conventional anti-diabetic drugs. **Design:** Thirty five adult male albino rats (200-220 gm) were selected for this study. The animals were randomly divided into five groups (seven rats each). Group I (Control –ve), Group II (Diabetic control): Type 2 diabetes was induced by 58% calories HF diet for 4 weeks followed by intraperitoneal administration of STZ (35 mg/kg). Two weeks after STZ injection, diabetic rats were treated with oral doses of antidiabetic drugs as follows: Group III: Metformin (500mg/1 ml distilled water/ kg/twice daily), Group IV: Forxiga (0.1 mg/1 ml distilled water/ kg/day) and Group V: Aqueous tamarind seeds extract (80 mg/0.5 ml distilled water/100 g/day) for 4 weeks. Blood glucose level was measured every week. At the end of the experiment, tongue specimens were dissected and processed for light and environmental scanning electron microscopic examination. **Results:** Histological and ultrastructural examination of Group II (Diabetic group) revealed signs of deterioration and degeneration among the filiform and fungiform papillae. After 4 weeks treatment minor to moderate improvement in the architecture of the papillae has been reported in Groups III and IV respectively. In comparison to Groups III and IV, oral administration of tamarind seeds extract (Group V) caused significant improvement in the histological structure of the lingual papillae, covering epithelium, their taste buds and the associated gustatory pore. **Conclusion:** The findings of the present study indicate that aqueous extract of tamarind seeds possessed significant anti-diabetic and anti-inflammatory activity for improvement of diabetes associated oral complications that may prove its beneficial potentiality in treatment of type 2 diabetes.

**KEYWORDS:** Type 2 diabetes, Metformin, Forxiga, Aqueous tamarind seeds extract, Rats' lingual papillae.

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## INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) comprises about 90% of diabetic people around the world <sup>(1)</sup>. It is characterized by peripheral insulin resistance and insufficient secretion of insulin by beta cells of the pancreas <sup>(2)</sup>. Diabetic patients are subjected to oral complications including mucositis and oral infections as well as oral mucosal dysfunction due to variations in salivary constituents and flow <sup>(3)</sup>. Obvious reasons regarding poverty, availability, and the need for adequate storage of insulin lead to endless efforts in searching for synthetic or natural alternatives for treatment of diabetes<sup>(4)</sup>.

Metformin is an antihyperglycemic drug recommended for treatment of newly diagnosed type 2 diabetic patients <sup>(5)</sup>. It regulates blood glucose levels by enhancing sensitivity of cells to insulin and reducing production of glucose by the liver. In addition, metformin decreases intestinal glucose absorption and improves utilization of glucose in peripheral tissues. Sudden release of metformin in the gastrointestinal tract results in abdominal discomfort, vomiting and diarrhea<sup>(6)</sup>. Moreover, metformin may cause increased risk of lactic acidosis, which limits its use among patients with T2DM <sup>(7)</sup>.

Sodium-glucose co-transporter 2 (SGLT2) inhibitors are recent therapeutic agents for treatment of T2DM patients. They decrease blood glucose levels by blocking the renal SGLT2 co-transporter, thus preventing glucose reabsorption in the renal proximal convoluted tubules <sup>(8)</sup>. SGLT2 inhibitors can be used as monotherapy or in combination with other hypoglycemic drugs <sup>(9)</sup>. Dapagliflozin (Forxiga®) is a SGLT2 inhibitor which is used for treatment of T2DM without an increase in hypoglycemia. Dapagliflozin decreases body weight and glycosylated haemoglobin resulting in lowered systolic blood pressure <sup>(10)</sup>.

Despite of the existence of therapeutic drugs which reduces diabetic complications, there has been increased awareness in the use of more safe

and efficient herbal treatment to inhibit the genesis of such complications <sup>(11)</sup>.

Tamarind (*Tamarindus indica*. L), a fruit plant belongs to the legume family. It grows in tropical and subtropical regions as India, equatorial Africa and Southeast Asia. In Egypt, tamarind was cultivated as early as 400 B.C <sup>(12,13)</sup>. *T. indica* pulp fruit is used in juices, as a flavor for food components <sup>(14)</sup> and its seeds have been used as a coffee substitute for a long time <sup>(15)</sup>. *T. indica* is rich in minerals as calcium, magnesium, potassium but shows smaller amounts of iron and vitamin A. Its carbohydrate content is high enough to provide great energy <sup>(16)</sup>. It acts as antimicrobial, antibiotic and antiallergic agent. It also has cosmetic application for skin aging and is used in wound healing. Furthermore, *T. indica* is used as a digestive, laxative, antiasthmatic, cardioprotective and hepatoprotective agent <sup>(17)</sup>.

Tamarind seeds extract was reported to have potent antidiabetic, hypolipidemic and anti-inflammatory activities in diabetic rats <sup>(17, 18)</sup>. Treatment of diabetes by *T. indica* extract showed significant increase in body weight and serum insulin level, reduction in blood glucose level with improvement of lipid profile as well as the liver and kidney functions <sup>(19)</sup>.

Our aim in the present study was to prove the efficacy of *T. indica* in treatment of the complications that might occur in tongue papillae of high fat diet/streptozotocin (HF/STZ) induced T2DM rats, and to compare its effects with the conventional antidiabetic drugs (metformin and forxiga) by using both light and environmental scanning electron microscopes.

## MATERIALS AND METHODS

### Animals:

Thirty five adult male albino rats of average weight 200-220gm were used in this study; which was approved by the ethics committee of Cairo

University. The animals were housed in labeled separate cages in the animal house of medical research center – Ain Shams University. Rats were kept under optimal experimental conditions and fed on adequate stable diet consisting of fresh vegetables, dried bread and tap water ad-libitum.

### Experimental design:

After one week acclimatization period, the animals were randomly divided into five groups (seven rats each) as follows:

**Group I (Control -ve):** The rats were intrapretonially injected by 0.25mL/kg body weight; a single dose of citrate buffer (pH4.4) <sup>(20)</sup>.

**Group II (Diabetic control):** Diabetes type 2 was induced by feeding the rats 58% calories high fat diet for 4 weeks (Table.1), then they were injected with a single dose of streptozotocin (STZ) 35 mg/1 ml (0.01 M; pH 4.5) citrate buffer/ kg, intraperitoneally <sup>(21)</sup>.

*\*One week after STZ injection; blood samples was collected via puncture of the tail vein for measurement of plasma glucose level using a glucometer. Diabetes was confirmed when the reading exceeded 300mg/dl <sup>(22)</sup>. Confirmation of diabetes was monitored weekly throughout the experimental period by measuring the blood glucose level.*

Similar to group II, diabetes type 2 was induced in groups III, IV and V. Two weeks after the STZ injection, diabetic rats were treated for 4 weeks; as follows:

**Group III (Metformin):** The rats were given metformin (Sigma, St. Louis, MO, USA) (500mg/1 ml distilled water/ kg/twice daily) via oral gavage <sup>(23)</sup>.

**Group IV (Forxiga):** The animals received forxiga (Sigma, St. Louis, MO, USA) (0.1 mg/1 ml distilled water/ kg/day) via oral gavage <sup>(24)</sup>.

**Group V (Tamarind):** The rats were given aqueous extract of Tamarind indica seeds (80 mg/0.5 ml distilled water/100 g/ day) via oral gavage <sup>(20)</sup>.

### Preparation of Aqueous Extract of Tamarind Indica Seeds

Fresh seeds of tamarind were cleaned and dried in an incubator for 2 days at 40° C. The dried seeds were crushed in an electrical grinder and then powdered. 60 gm of this powder was then mixed with 900 ml of solvent (methanol:water), stirred for 2h in a magnetic stirrer and filtered. The filtrate was dried and concentrated at rotary evaporator (Rotavapor®), then refrigerated until used. Thus, 60 gm of tamarind seeds powder yielded 37 gm of powdered extract with yield percentage =  $37/60 \times 100 = 61.6\%$  <sup>(20,25)</sup>. This procedure was carried out at Faculty of Pharmacy, Future University in Egypt.

TABLE (1) Composition of High fat diet <sup>(21)</sup>.

Ingredient	g/kg diet
Powdered normal pellet diet	365
fat	310
Casein	250
Minerals and vitamin	60
Cholesterol	10
dl-Methionine	03
Yeast powder	01
Sodium chloride	01

Ten weeks from the beginning of the experiment, animals of all groups were sacrificed by an intracardiac anesthetic overdose (*sodium thiopental 80 mg/kg*)

Each tongue specimen was immediately dissected into two halves:

*\*One half was processed for **Light Microscopic Examination***

*\*Other half was processed for **Environmental scanning electron Microscopic Examination (ESEM)***

### Histological procedures

#### Light microscopic examination:

The dissected tongue specimens were fixed immediately in 10% formaldehyde solution for

24 hours, dehydrated, cleared and embedded in paraffin. Serial sections of about  $5\mu\text{m}$  thickness were obtained. For routine investigation, sections were stained with haematoxylin and eosin <sup>(26)</sup>.

### Environmental scanning electron microscopic examination:

Specimens were fixed in buffered glutaraldehyde (2.5% glutaraldehyde in 0.1 Molar phosphate buffer) pH 7.2, for 2hrs. The samples were not treated with any preparation technique to maintain natural hydration and to avoid any changes of the papillary surface <sup>(27)</sup>. Each tongue was placed on a cooled holder  $2^{\circ}\text{C}$  temperature and saturated water vapor pressure of 708 Pa. The specimens were studied using SEM model Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses) and accelerating voltage 30 K.V at the Central Laboratories Sector -Egyptian Mineral Resources Authority.

## RESULTS

### Histological results

#### Group I (Control –ve):

##### a) Filiform papillae:

The normal architecture of tongue filiform papillae was observed. The papillae appeared evenly distributed, sharp, conical and regular in size and shape. The epithelial covering showed uniform arrangement of epithelial strata. Keratinized stratified squamous epithelium showed basal cells with closed face oval nuclei resting on a regular basement membrane. The prickle cell layer consisted of numerous polyhedral cells with plump nuclei. Cells of the granular layer appeared flattened with numerous basophilic keratohyaline granules. A well developed overlying keratinous layer was evident. Also, few clear cells in basal and parabasal layers were detected. The lamina propria showed normal fibrous and cellular elements with blood vessels (**Fig. 1**).

##### b) Fungiform papillae:

The fungiform papillae appeared mushroom like with a narrow base and smooth rounded top. The epithelial covering was stratified squamous epithelium with a keratin layer thinning gradually on approaching the surface of the papillae. Moreover, a single well defined barrel shaped taste bud was seen at the summit of the papilla. The underlying connective tissue revealed numerous interlacing collagen fibers with scattered basophilic fibroblasts and blood vessels (**Fig. 2**).

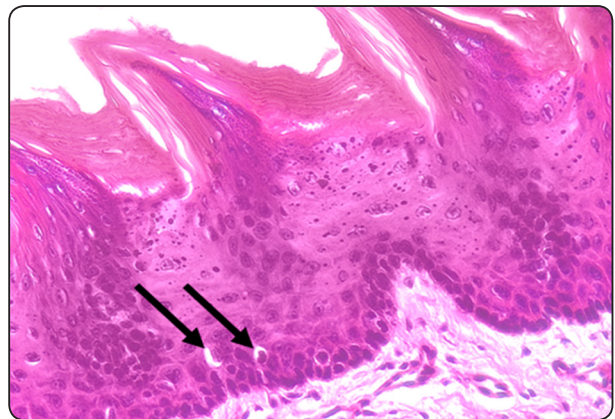


Fig. (1) Photomicrograph of the dorsal lingual surface of group I showing normal architecture of the filiform papillae with dispersed clear cells (arrows) (H&E, Orig. Mag.X 200).

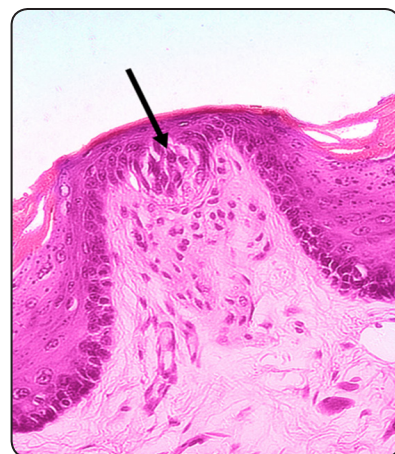


Fig. (2) Photomicrograph of the dorsal lingual surface of group I showing a uniform mushroom shaped fungiform papilla with a central intraepithelial taste bud (arrow) (H&E, Orig. Mag.X 200)

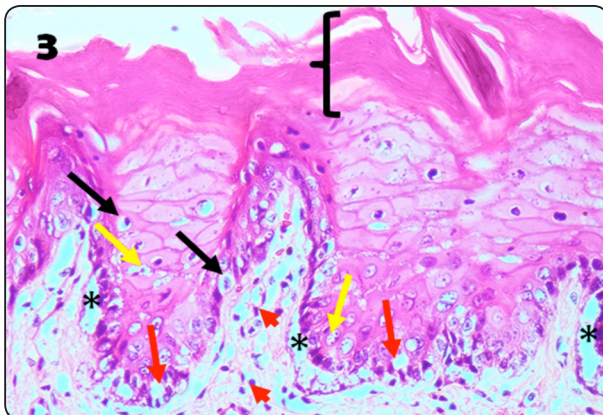


**Group II (Diabetic control):****a) Filiform papillae:**

In comparison to group I, filiform papillae revealed disfigurement in their normal architecture and some areas appeared with complete loss of the papillae. Flattened epithelial ridges and reduced connective tissue papillae were marked. Apparent increase in epithelial thickness was recognized with an irregular basement membrane. The epithelial cells showed marked difference in shape and size. The basal cell layer appeared disorganized with basilar hyperplasia, nuclear karyorrhexis and pyknosis. Many areas showed vacuolar degeneration of basal cells. The prickle cells were flattened with pale cytoplasm and large vesicular nuclei. Numerous vacuolated cells were observed while others exhibited binucleation. Numerous clear cells were also identified among the basal and parabasal cells. Cells of granular layer lacked their normal morphology with pale cytoplasm and few ill-distinct keratohyaline granules. The keratin layer showed hyperkeratosis with corrugated surface. The underlying lamina propria appeared disorganized with thin loosely arranged collagen fibers and wide degenerative areas. Dispersed few pale staining fibroblasts and intense inflammatory cells infiltrates were also demonstrated (Fig. 3)

**b) Fungiform papillae:**

The papillae of this group lost their regular



mushroom like appearance seen in the control group and presented marked signs of degeneration in the epithelial covering. Furthermore, the taste bud lacked its normal structure and its cells showed apparent degenerative changes with peripheral situation of the taste cells. Some of the papillae appeared with wide base, narrow rounded tops and degenerated bud; others assumed dome-shape with narrow pointed tip and complete absence of its taste bud. The underlying lamina propria displayed sparse collagen fibers, areas of matrix degeneration with congested thick walled blood vessels. Intense inflammatory cells infiltrates were also detected (Figs. 4&5).

**Group III (Metformin):****a) Filiform papillae:**

The filiform papillae began to regain their normal architecture when compared to group II. The ridges and connective tissue papillae were nearly developed. The epithelium appeared with considerable thickness and regular basement membrane. The basal cell layer exhibited mild basilar hyperplasia and hyperchromatic large nuclei. Prickle cell layer displayed few cells with binucleation or vesicular nuclei. Granular cells exhibited distinct keratohyaline granules but few cells appeared with vacuolation. Keratin layer was irregular of non-uniform thickness in certain areas. Few clear cells were identified throughout

Fig. 3: Photomicrograph of the dorsal lingual surface of group II showing distorted filiform papillae with disorganized basal cell layer, numerous clear cells (black arrows), nuclear karyorrhexis and pyknosis (yellow arrows), vacuolations (red arrows), hyperkeratosis and corrugated surface (bracket), disorganized lamina propria with wide degenerative areas (asterisks) and inflammatory cells infiltrates (arrow heads) (H&E, Orig. Mag.X 200)

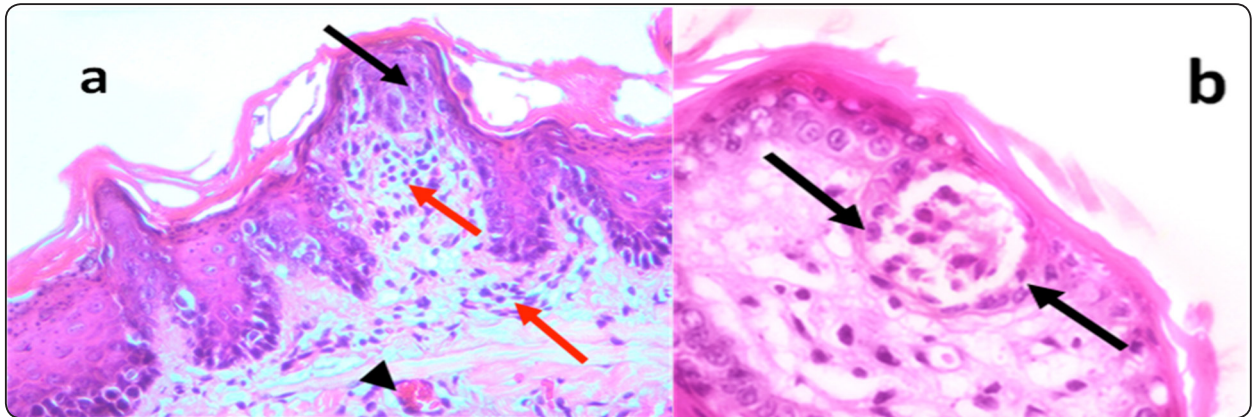


Fig. 4: a) Photomicrograph of the dorsal lingual surface of group II showing a distorted fungiform papilla, a taste bud with signs of degeneration (black arrow), lamina propria with wide degenerative areas, congested thick walled blood vessel (arrow head), intense inflammatory cells infiltrates (red arrows) (H&E, Orig. Mag.X 200)b) Higher magnification of a taste bud of fungiform papilla group II showing peripherally situated taste cells (arrows) (H&E, Orig. Mag.X 400)

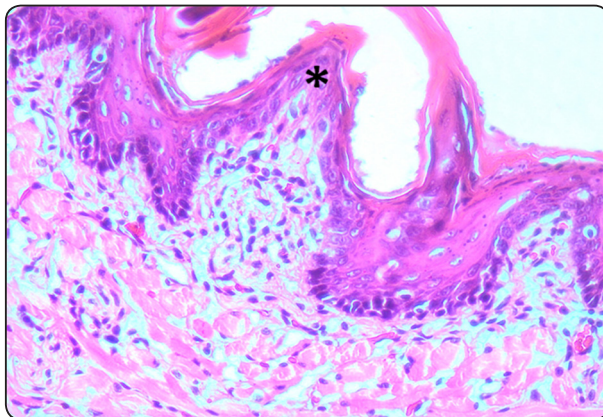


Fig. 5: Photomicrograph of the dorsal lingual surface of group II showing dome-shaped papilla with narrow pointed tip and complete absence of its taste bud (asterisk). (H&E, Orig. Mag.X 200).

the epithelial layers. The underlying lamina propria showed less degenerative areas, deeply stained basophilic fibroblasts and moderate population of inflammatory cells (**Fig. 6**).

**b) Fungiform papillae:**

In relation to group II, normal appearance of the papillae was nearly restored. The epithelial covering was almost normal with thin keratin layer. The underlying lamina propria revealed organized collagen fibers with areas of matrix degeneration. Dilated engorged blood vessels were also detected with numerous inflammatory cells (**Fig. 7**).

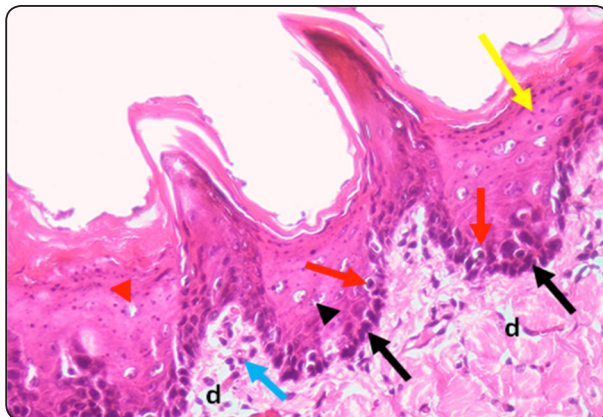


Fig. (6) Photomicrograph of the dorsal lingual surface of group III showing filiform papillae with mild basilar hyperplasia, hyperchromatic nuclei (black arrows), dispersed clear cells (red arrows), few vacuulations (red arrow head) and binucleation (black arrow head), distinct keratohyaline granules (yellow arrow), lamina propria with less degenerative areas (d) and moderate population of inflammatory cells (blue arrow) (H&E, Orig. Mag.X 200)

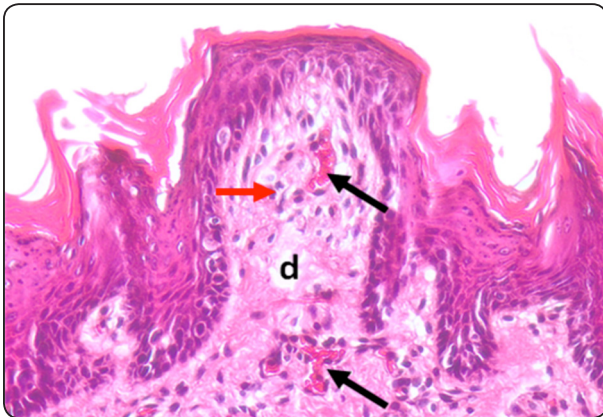


Fig. (7) Photomicrograph of the dorsal lingual surface of group III showing a uniform shaped fungiform papilla, less areas of degeneration (d), dilated engorged blood vessels (black arrows) and inflammatory cells in the lamina propria (red arrow) (H&E, Orig. Mag.X 200)

#### Group IV (*Forxiga*):

##### a) *Filiform papillae*:

The epithelial ridges and connective tissue papillae of the filiform papillae were well developed with regular distinct basement membrane. Basal cells showed normal nuclear size and shape, while few areas of basilar hyperplasia were detected. Prickle and granular cells appeared uniform with apparent increase of keratohyaline granules. Few vacuolated cells were also noticed. The overlying keratin restored the flame like appearance with moderate hyperkeratosis. Few clear cells were still recognized in basal and parabasal layers. Well-formed lamina propria displayed collagen fiber bundles as well as moderate infiltrates of inflammatory cells (Fig. 8).

##### a) *Fungiform papillae*:

The papillae almost exhibited its regular shape with a well developed taste bud at its top. However, few vacuolated taste cells were still detected. The papillary connective tissue core displayed inflammatory cells infiltrates, degenerative areas and few congested thin walled blood vessels (Fig. 9).

#### Group V (*Tamarind*):

##### a) *Filiform papillae*:

Well-structured flame like filiform papillae, epithelial covering and connective tissue core were nearly manifested similar to the control group. Only few scanty clear cells appeared within the epithelium. Also, few inflammatory cells were scattered in the lamina propria (Fig. 10).

##### b) *Fungiform papillae*:

The fungiform papillae in this group assumed mushroom-like appearance similar to those of the control group. Reduced signs of degeneration within the lamina propria were detected in the form of few dilated, lengthened and thin walled blood vessels as well as few areas of matrix degeneration (Fig. 11).

#### Scanning Electron Microscope

#### Group I (*Control -ve*):

##### a) *Filiform papillae*:

The tongue dorsal surface displayed numerous regularly orientated closely packed, thread or finger-like projections representing the filiform papillae. The papillae were arranged in parallel regular rows with uniform keratinized pointed tips directed towards the root of the tongue. Fine regular lines were clearly detected on the interpapillary epithelial surface (Fig.12)

##### b) *Fungiform papillae*:

Fungiform papillae were projected above dorsal lingual surface and compressed between filiform papillae. The dome shaped fungiform papillae exhibited elevated margins of its epithelial cells with rounded broad irregular keratinized epithelial surface. A centrally located well-defined taste bud was seen with a regular gustatory pore surrounded by a shallow indentation (Fig.13).



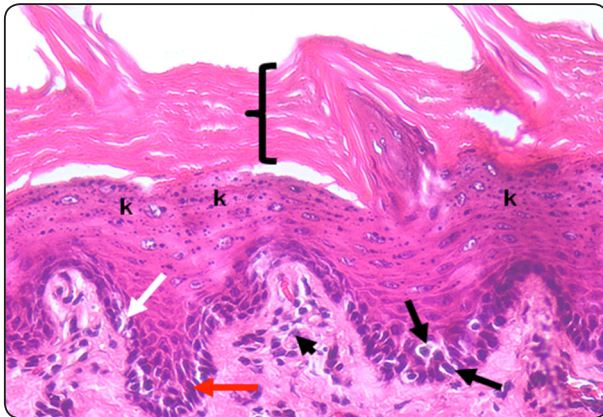


Fig. (8) Photomicrograph of the dorsal lingual surface of group IV showing well developed filiform papillae with a uniform epithelial covering, basilar hyperplasia (red arrow), few clear cells (black arrows), few vacuolated cells (white arrow), apparent increase of keratohyaline granules (k), moderate hyperkeratosis (bracket) and well- developed lamina propria with few inflammatory cells (arrow head). (H&E, Orig. Mag.X 200)

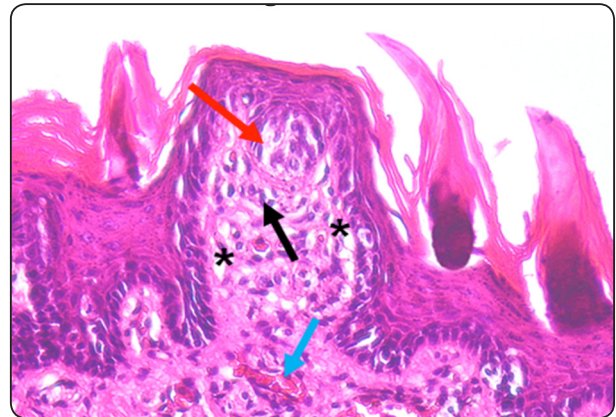


Fig. (9) Photomicrograph of the dorsal lingual surface of group IV showing regularly shaped fungiform papilla, a taste bud with few vacuolated cells (red arrow), lamina propria with degenerative areas (asterisks), congested blood vessels (blue arrow), and some inflammatory cells (black arrow) (H&E, Orig. Mag.X 200)

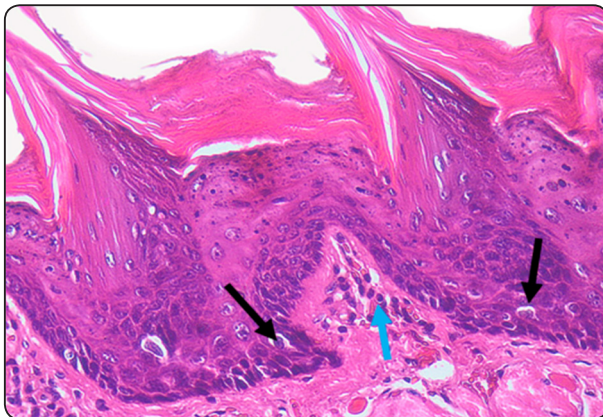


Fig. (10) Photomicrograph of the dorsal lingual surface of group V showing well structured filiform papillae with few scanty clear cells (black arrows), properly formed lamina propria and few inflammatory cells (blue arrow). (H&E, Orig. Mag.X 200)

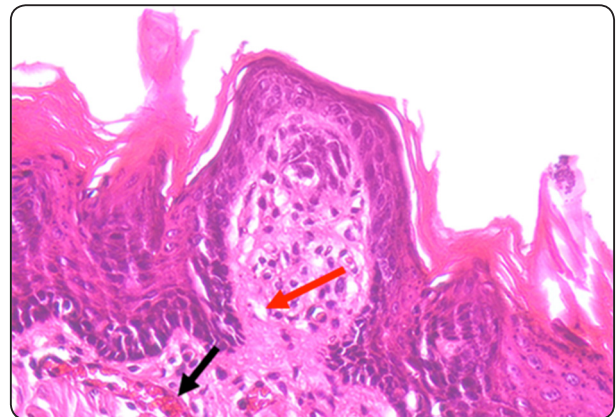


Fig. (11) Photomicrograph of the dorsal lingual surface of group V showing mushroom-like fungiform papilla and taste bud, few dilated lengthened thin walled blood vessels (black arrow) and few areas of degeneration (red arrow). (H&E, Orig. Mag.X 200)



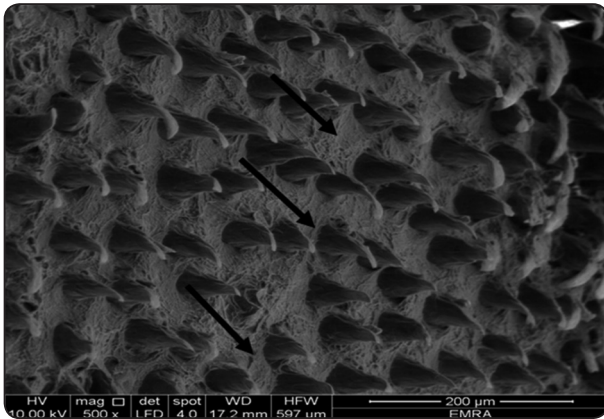


Fig. (12) Scanning electron micrograph of the dorsal lingual surface of group I showing regularly distributed parallel and thread-like filiform papillae with keratinized tips. The interpapillary surface exhibited fine regular lines (arrows) (Orig. Mag. x 500)

**Group II (Diabetic control):**

**a) Filiform papillae:**

The dorsal tongue surface of diabetic rats presented filiform papillae with markedly disturbed orientation. Wide areas of papillary degeneration were clearly evident. Most of the remaining papillae revealed hyperkeratosis, while few others were covered by constricted keratin. The tips of some filiform papillae were blunt and eroded. The

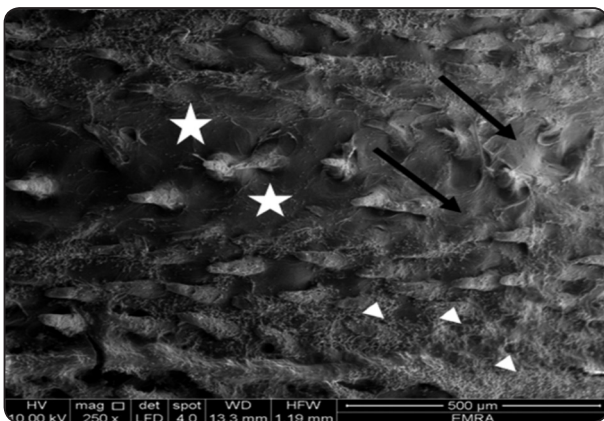


Fig. (14) Scanning electron micrograph of the dorsal lingual surface of group II showing wide areas of papillary degeneration (stars) and disturbed orientation of filiform papillae. Irregular interpapillary ridges (arrows) and surface deposits of microplacae (arrow heads) (Orig. Mag. x 250)

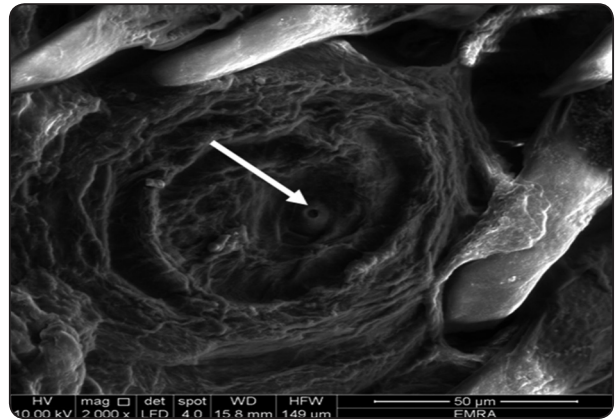


Fig. (13) Scanning electron micrograph of the dorsal lingual surface of group I showing fungiform papilla with a well defined taste bud and gustatory pore (arrow) (Orig. Mag. x 2000).

interpapillary ridges appeared irregular, sharp with heavy keratinization in some areas. The surface of the tongue was covered with translucent granular deposits resembling microplacae (Figs. 14 & 15).

**b) Fungiform papillae:**

When compared to the control group, the fungiform papillae appeared depressed with marked loss of the normal microridge appearance of their epithelial cells. Rough irregular areas of

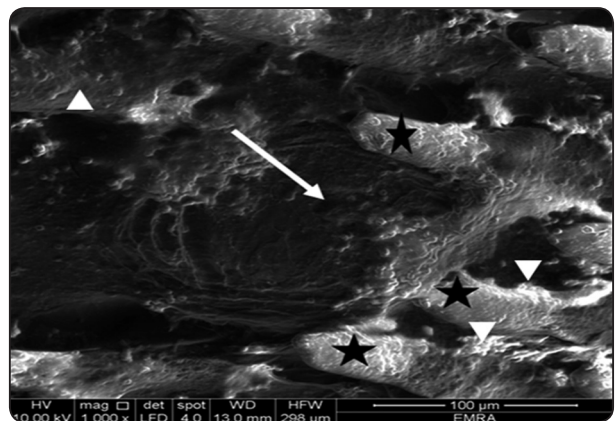


Fig. (15) Scanning electron micrograph of the dorsal lingual surface of group II showing keratinized blunt ends of filiform papillae together with areas of hyperkeratosis (stars), disfigured depressed fungiform papilla, ill-defined taste bud & gustatory pore (arrow) and microplacae surface deposits (arrow heads) (Orig. Mag. x 1000).

microplacae were clearly demonstrated on top of the papillary epithelial covering. The gustatory pore was ill-defined and shrunken resembling a crater like pattern; together with atrophy of the taste bud. Marked areas of epithelial degeneration could be also detected nearby the papillary outline (Fig. 15).

### Group III (*Metformin*):

#### a) *Filiform papillae*:

Compared to group II, the metformin treated rats revealed interlacing filiform papillae projecting in different directions with blunt tips of some papillae. Numerous papillae presented hyperkeratosis of the epithelial covering. Degenerated areas but to a lesser extent were still recognized on the dorsal lingual surface. The interpapillary ridges were apparently irregular (Fig. 16).

#### b) *Fungiform papillae*:

The fungiform papillae were moderately projecting beyond the dorsal tongue surface in relation to the diabetic group. The papillae appeared with a well-defined outline and a slightly wrinkled keratinized epithelial covering. Some of

the regenerating taste buds displayed a distorted gustatory pore on the summit of the papillae (Fig. 17)

### Group IV (*Forxiga*):

#### a) *Filiform papillae*:

The filiform papillae of this group were almost directed to the posterior normal direction. Some areas of hyperkeratosis were observed as well as the blunt tips in few papillae. The areas of papillary degeneration were apparently decreased on the dorsal lingual surfaces. Moreover, irregularities of the interpapillary ridges were prominent in some areas (Fig. 18).

#### b) *Fungiform papillae*:

The fungiform papillae of group IV projected above the dorsal tongue surface with a uniform outline. The keratinized epithelial covering appeared with slight irregularities and elevated epithelial microridges. The taste bud at the summit of the papillae exhibited a well-defined gustatory pore (Fig. 19).

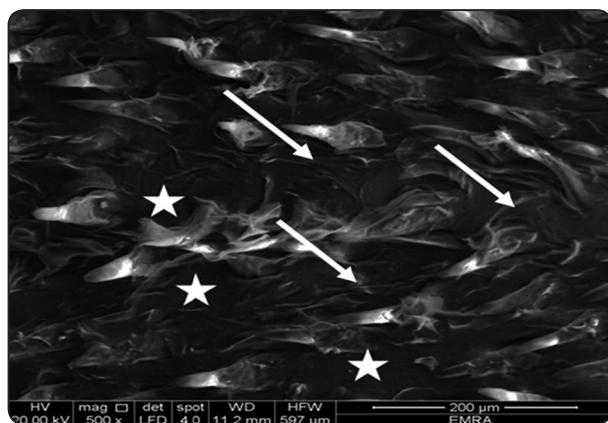


Fig. (16) Scanning electron micrograph of the dorsal lingual surface of group III showing interlacing filiform papillae with blunt tips of some papillae, areas of papillary degeneration (stars) and irregular interpapillary ridges (arrows) (Orig. Mag.x 500)

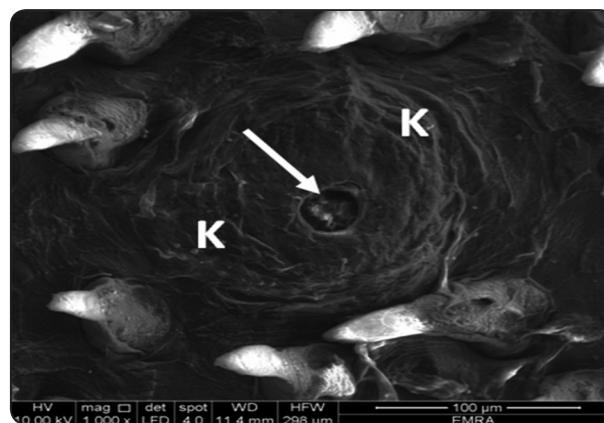


Fig. (17) Scanning electron micrograph of the dorsal lingual surface of group III showing moderately projecting fungiform papilla, wrinkled keratinized epithelial covering (K), well- defined taste bud and distorted gustatory pore (arrow) (Orig. Mag. x 1000)

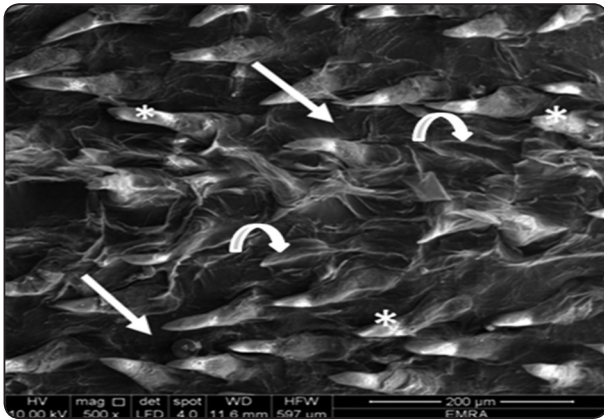


Fig. (18) Scanning electron micrograph of the dorsal lingual surface of group IV showing filiform papillae directed posteriorly with blunt tips of few papillae (asterisks), minor areas of papillary degeneration (arrows), irregularity of some interpapillary ridges (curved arrows) (Orig. Mag. x 500)

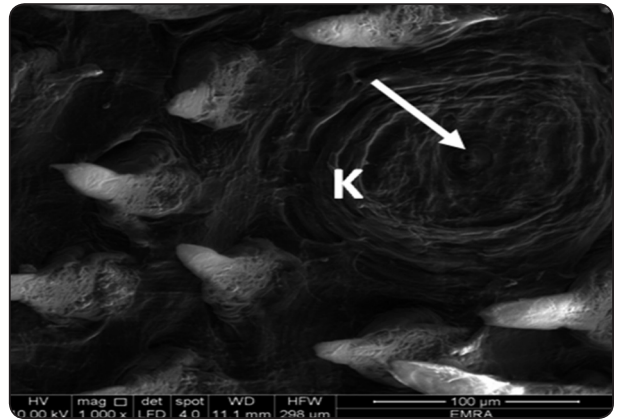


Fig. (19) Scanning electron micrograph of the dorsal lingual surface of group IV showing fungiform papilla with slight irregularities of the keratinized epithelial covering (k), a well- defined taste bud and gustatory pore (arrow) (Orig. Mag. x 1000)

**Group V (Tamarind):**

**a) Filiform papillae:**

The dorsal lingual surface of group V revealed thread like filiform papillae with a posterior normal direction. Minor areas of degeneration and few others with hyperkeratosis were manifested. The interpapillary ridges displayed only few areas of irregularities (Fig. 20).

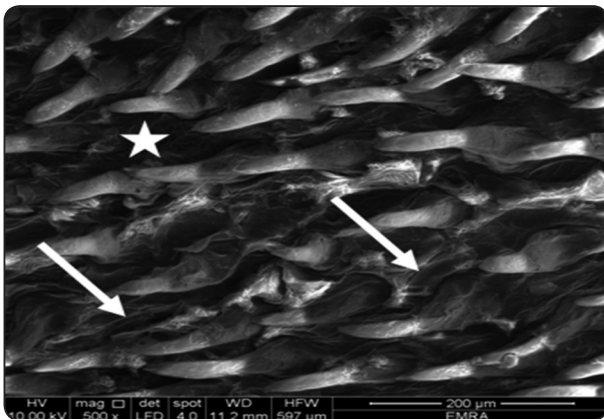


Fig. (20) Scanning electron micrograph of the dorsal lingual surface of group V showing filiform papillae with normal posterior direction, few papillary degenerative areas (star), interpapillary ridges with few irregularities (arrows) (Orig. Mag. x 500)

**b) Fungiform papillae:**

The fungiform papillae of this group exhibited almost normal architecture similar to the control group with approximately regular indentations of the keratinized epithelial covering. Well-defined slightly depressed taste bud and gustatory pore were also detected (Fig. 21).

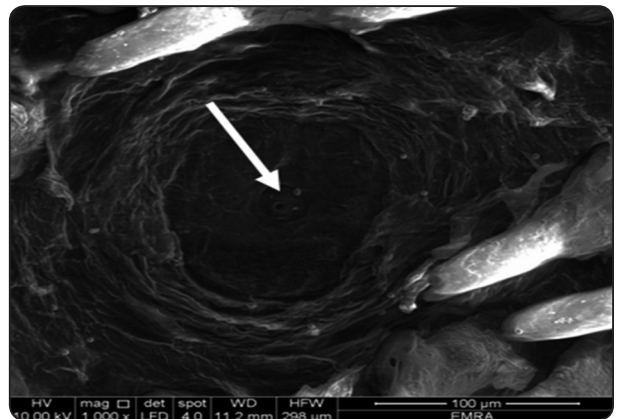


Fig. (21) Scanning electron micrograph of the dorsal lingual surface of group V showing fungiform papilla with well- defined taste bud and gustatory pore (arrow) (Orig. Mag. x 1000)



## DISCUSSION

Recent increase in diabetic population is a major problem because of its complications that may contribute to high mortality rate<sup>(28)</sup>. The dorsal tongue surface was thought to be a mirror of the general health particularly filiform papillae and their epithelial cells that showed a high metabolic activity. Thus, any enzymatic disturbances and/or vascular changes associated with many diseases such as diabetes gave rise to atrophy or loss of the papillae<sup>(26)</sup>. In this study, we evaluated the effect of HF/STZ induced T2DM and different anti-diabetic agents on the histology of rats' dorsal tongue surface using light microscope (LM) and environmental scanning electron microscope (ESEM). ESEM was used in this study because it was easier to view delicate samples with minimal preparation, mechanical disruption and preparation associated artifacts<sup>(29)</sup>.

In the present study, histological and ultrastructural results of diabetic group showed loss of normal architecture of filiform papillae, areas of destructed papillae with desquamated epithelial covering or complete papillary loss. These observed papillary changes were attributed to chronic inflammation, decreased salivary production, increased salivary glucose levels and alteration in nerve supply occurred secondary to insulin deficiency<sup>(30,31)</sup>. In addition, the diabetes associated micro-angiopathy and narrowing of blood vessels caused tissue hypoxia and consequent atrophic changes of papillary epithelium<sup>(32)</sup>.

Regarding the filiform papillae, our H&E findings revealed apparent increase in the epithelial thickness in diabetic rats' samples with marked cellular polymorphism and disorganization. Basilar hyperplasia, vacuolar degeneration as well as nuclear binucleation, karyorrhexis and pyknosis were also detected. In accordance with our results, it was suggested that irritation of epithelial cells by diabetes led to acceleration of the death and

replacement rate thus the mitotic figure of lingual epithelial cells was also increased<sup>(33)</sup>. In addition, the increase of binucleated cells that was attributed to disturbed cell division in hyperplastic epithelium in some pathologic conditions occurred in the early stages of apoptosis<sup>(34-36)</sup>. On the other hand, the cell vacuolation mediated by overproduction of reactive oxygen species resulted from intracellular water and electrolytes infusion disturbing cell membrane permeability and insulting the cell membranes<sup>(37)</sup>. Thus, these degenerative epithelial changes could be correlated to the close association between diabetes induction, oxidative stress, mitochondrial dysfunction, and apoptotic cell death via intracellular calcium overload in epithelial cells<sup>(38)</sup>. Persistent hyperglycemia caused glycation of phospholipids, lipid peroxidation and protein oxidation of cell membrane and/ or organelles<sup>(39)</sup>. Therefore, oxidative damage of DNA, proteins and fatty acids together with marked reduction of enzymatic and non enzymatic cellular antioxidants defenses played a great role in the pathogenesis of diabetes chronic complications including vascular and cellular damage<sup>(40)</sup>.

On the other hand, our data revealed hyperkeratosis with surface corrugation in both light and scanning electron microscopic specimens of diabetic group. This was clarified by the less demonstrable excursion of lipid-like material and hydrolytic enzymes from keratohyaline granules of hyperplastic epithelium in some pathologic conditions that in turn reduced the desquamation process<sup>(41)</sup>. Moreover, *Rodgers et al.*<sup>(42)</sup> and *Akai et al.*<sup>(43)</sup> marked an increase in the expression of keratin complexes gene and its proteins in diabetic mice because of the Ca<sup>2+</sup> induced differentiation of cells with the increased glucose level. *Popelet al.*<sup>(32)</sup> found that keratin accumulations on the dorsal lingual epithelium in diabetic rats played a role in fixation of microflora on the tongue mucosal surface and possibly led to the development of inflammatory process.

Also, there was an apparent increase of clear cells in basal and parabasal cell layer which could be attributed to the persistent subepithelial inflammatory infiltrates and edema at this experimental period evoking the migration of some inflammatory cells from lamina propria to epithelium<sup>(44)</sup>.

The herein study demonstrated wide degenerative areas in lamina propria of diabetic tongue specimens with few fibroblasts and intense inflammatory cells infiltrates. In accordance with our observations, *Caldeira et al.*<sup>(45)</sup> detected unusual increase of neutrophil elastase (serine proteinase) exocytosis in type 2 obese diabetic mice that potentiated tissue damage including degradation of proteoglycans, plasma proteins, elastin and fibronectin. Furthermore, it also modulated the function of inflammatory cells such as lymphocytes activation and platelet aggregation<sup>(46)</sup>. The elevated blood glucose level was also documented to attenuate fibroblastic proliferation resulting in atrophic changes<sup>(43)</sup>.

Moreover, apparent thick walled engorged blood vessels were also spotted in H&E sections of diabetic group. *Kemeny et al.*<sup>(47)</sup> proved that hyperglycaemia resulted in endothelial dysfunction, reduction of nitric oxide production, and synthesis of reactive oxygen species, causing arterial stiffening. Also, *Nagmoti et al.*<sup>(25)</sup> stated that the manifested hyperlipidemia in type 1 & 2 diabetic rats was illustrated by the significant increase in plasma cholesterol, triglycerides and lipoprotein and gave rise to secondary complications such as blood vessels atherosclerosis.

Variation in the architecture of fungiform papillae with marked loss of their normal mushroom shape was evident in diabetic group of this study. Elongated papillae with narrow tips were seen in H&E sections; while SEM examination displayed depressed papillae in addition to loss of the normal epithelial microridges. Concomitant with these results, *Nagato et al.*<sup>(48)</sup> observed that denervated

rat fungiform papillae underwent atrophy so that it may appear similar to filiform papillae.

In this work, the rough irregular microplicae detected on fungiform surface and in between filiform papillae by SEM in diabetic rats were consistent with the observations of *Asikainen et al.*<sup>(49)</sup>. The authors reported that the structural complexity of microplicae (MPLs) of oral mucosal cells was a common observation in tongue between filiform papillae. MPL structure and function present underlying basis for membrane associated mucin, thus protection of mucosal surface is secured. It was found that irregularities in the microplicae indicate atrophied tongue which promotes streptococcal aggregation and hence increases susceptibility to infection.

Histological and ultrastructural examination of diabetic group presented signs of degenerative fungiform taste buds with ill-defined and shrunken gustatory pore. In parallel to these results, *Bocci*<sup>(50)</sup> and *Pai et al.*<sup>(51)</sup> related the similar taste buds changes in fungiform and vallate papillae to the significant reduced innervations and neuropathy defects in diabetic rats that may worsen to the atrophy of gustatory pore epithelial structures or even pore disappearance in case of fungiform denervation.

Regarding metformin treated diabetic group, the filiform and fungiform papillae began to regain their normal architecture. Slight improvement in the papillary epithelial covering was detected so that mild basilar hyperplasia, hyperchromatic nuclei as well as few vacuolation and clear cells were noted. Areas of degeneration were still recognized in the scanning electron microscopic examination. It is conceivable to suggest that this mild regenerative ability of metformin is related to its ability to inhibit the formation of advanced glycation end products. It may also enhance the free-radical defense mechanism, thus preventing the diabetes associated oxidative damage and cell injury. Furthermore, *Sung et al.*<sup>(52)</sup> and *Salman et al.*<sup>(53)</sup> added that

metformin activates adenosine monophosphate-activated protein kinase (AMPK) which is essential in protection of cellular functions under energy-restricted environment. Metformin administration increased the glutathione contents (a free radical scavenger) in body cells of STZ-diabetic rats <sup>(54)</sup>. In addition, metformin had an anti-apoptotic effect illustrated by its role in the reduction of caspase enzymes activities <sup>(55)</sup>.

The lamina propria in metformin group showed less degenerative areas, moderate inflammatory cells as well as dilated relatively thin walled blood vessels. The improved changes of this group could be correlated to the anti-inflammatory and antioxidant activities of metformin<sup>(56)</sup>. Concurrently, *Alhaidair et al.*<sup>(57)</sup> confirmed that metformin prevented several pro-inflammatory cytokines synthesis as tumor necrosis factor alpha, interleukin-6 and 8 that in turn inhibited vascular dysfunction. It was reported that matrix metalloproteinase enzyme that accelerated matrix degradation in pathological conditions such as diabetes, was weakly labeled in metformin treated rats. Some risk factors that directly associated with declined insulin resistance were still stated such as vascular angiopathy and increased blood lipid levels as well as the risk for hypoglycemic events <sup>(58)</sup>.

In this study, the forxiga treated group displayed marked development of the papillae. The criteria for active epithelial maturation were clearly manifested with few recognized clear cells. SEM results revealed apparent decrease in the areas of papillary degeneration. Moreover, the taste buds exhibited a well-defined gustatory pore. These remarkable histological and ultrastructural improvements can be related to the antioxidative effect of forxiga in SGLT2 inhibition that sequentially diminished oxidative stress and oxygen consumption induced by diabetes <sup>(59-61)</sup>. In addition, the independent actions of forxiga on glucose and lipid metabolism were also proved <sup>(8, 62)</sup>. Furthermore, it was declared that dapagliflozin strongly attenuated apoptosis that was induced by DM <sup>(62, 63)</sup> which might account for the improvement observed in the papillary epithelium in this study.

Well-developed lamina propria, thin walled blood vessels as well as few infiltrates of inflammatory cells were observed in forxiga-treated group of our study. These observations were correlated to the anti-inflammatory effect of SGLT2 inhibitors in type 2 diabetic mice <sup>(61, 62, 64)</sup>. *Ott et al.* <sup>(65)</sup> speculated that dapagliflozin attenuated the expression of numerous pro-inflammatory genes, such as osteopontin and Icam-1, confirming its anti-inflammatory effect. Forxiga revealed significant improvement in blood pressure, decrease in capillaries hyperperfusion and triglycerides <sup>(58)</sup>. Hence, it prevented the thickening and stiffening of arteriolar wall improving vascular tone and tissue hypoxia <sup>(65)</sup>, thus resulting in restoration of the papillary epithelium.

Concerning the previously mentioned complications associated with the hypoglycemic drugs used in this study in treatment of T2DM, together with their reported enhancement of cancer which might limit their utilization; alternative herbal treatments became mandatory <sup>(66)</sup>.

In this research, histological and SEM findings of filiform and fungiform papillae of Tamarind seeds extract (TSE) treated rats showed marked improvement in their distribution, direction, size and shape as well as the fungiform taste bud and its pore simulating the control group. H&E sections revealed restoration of the integrity and thickness of papillary epithelium with scanty clear cells. In accordance with our findings, *Attah et al.* <sup>(67)</sup> found that tamarind restored the mitotic activity and phenotypes of epithelial cells as well as epithelium thickness in wound healing of rat skin. It was reported that tamarind indica possesses high contents of phenolic acids, flavonoids and polyphenols. These bioactive compounds have potent antioxidant protective activity in scavenging free radicals <sup>(19)</sup> inhibiting lipid peroxidation and attenuating intracellular reactive oxygen species (ROS) protecting the cells from injury. Moreover, TSE up regulated the expression of antioxidant enzymes under mild oxidative stress <sup>(40)</sup>. On the other hand, tamarind exhibited an anti-apoptotic effect as it caused



membrane stabilization, restored mitochondrial function, decreased glutathione consumption and prevented intracellular  $\text{Ca}^{2+}$  overload, caspase-3 activation and DNA fragmentation. It was also documented that polysaccharides of tamarind possessed immunomodulatory effect via increasing phagocytosis and inhibiting leukocyte migration and proliferation<sup>(17,20,38)</sup>.

The herein study showed well-structured lamina propria with fewer matrix degenerative areas and inflammatory cells. This significant ameliorative effect of tamarind extract could be attributed to its regulatory anti-inflammatory and pharmacological activities. *Fook et al.*<sup>(46)</sup> confirmed the regulatory effect of tamarind on neutrophils and in reducing neutrophil serine proteinase via the selective inhibition of platelet-activating factor receptor and stimuli in addition to the high potency of exogenous serine proteinase inhibitor extracted from tamarind seeds. Therefore, tamarind was demonstrated as potential therapy for some inflammatory diseases. Additionally, it was reported that alkaloids of tamarind seeds had pharmacological effects at low concentration resulting in stimulation of fibroblastic proliferation and collagen formation. Moreover, the released copper content from tamarind seeds regulated the synthesis of lysyl oxidase involved in collagen synthesis<sup>(68)</sup>.

In the ongoing work, few dilated congested blood vessels together with lengthened thin walled vessels were detected in the tamarind group compared to the other groups. These observations were interpreted by *Meyer et al.*<sup>(69)</sup> who considered the positive relation between the length of basal cell layer of epithelium and capillaries as an adequate adaptation to meet the nutritional needs of the increased metabolic activity of the epithelium. The antioxidant activity and insulin level restoration by tamarind extract flavonoids and polyphenols in type 1 and 2 diabetic rats significantly decreased the serum cholesterol, lipoproteins and triglycerides. This hypolipidemic effect was achieved through inhibition of lipolysis and/or enzymatic activity of

cholesterol and triglycerides biosynthesis. Hence, TSE was effective in preventing atherosclerosis and cardiovascular diseases<sup>(17-19)</sup>.

In conclusion, the use of TSE in treatment of T2DM has proven its efficacy in restoring the normal architecture of tongue papillae versus the commonly used antidiabetic drugs, thus minimizing the oral complications associated with diabetes.

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