

Morphohistological study of the tongue in local mice species by using special stain

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Abstract: The study of tongue in adult healthy males local mice *Mus musculus* included the morphological description and histoarchitecture at the light microscopic level by using Periodic Acid Schiff Reagent (PAS) stain method. The results revealed that the tongue appeared as elongated slender organ with rounded bisected anterior end and had three parts: rostral rounded bisected part (apex), wide long and bisected middle part (body) and caudal part (root). The dorsal surface of the tongue was rough with the presence of deep median groove. An elevated rounded torus linguae found on the posterior third of the dorsal surface of the tongue. The microscopic examination revealed that the central core of the tongue was occupied by two main groups of the middle vertical muscle fibers, oblique muscle fibers interwoven with the superior and inferior longitudinal muscle fibers and three parts of transverse blocks of muscle fibers, encircled by the oral mucosa. The collagenous fibers of the lamina propria appeared as a distinct rings surrounds the muscle fibers fasciculi. Five types of lingual papillae [filiform, fungiform, circumvallate, conical and lenticular papillae] with different density and shape covered the whole dorsal surface of the tongue. The filiform papillae were the narrowest and the most numerous of all lingual papillae, they appeared at three shapes, the long fork shaped; bristle like shaped and short cone shaped filiform papillae. The fungiform papillae were fewer and wider than the filiform papillae and they were scattered randomly among them. One circumvallate papillae was found on the front of the root part of the tongue. The conical papillae distributed on both sides of the torus linguae, they had conical shaped and the epithelial covering was heavy keratinized stratified squamous epithelium containing no taste buds. The lenticular papillae were found in the middle regions of the torus linguae, they were smaller than the conical papillae, and the epithelial covering was heavy keratinized stratified squamous epithelium containing no taste buds.

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1. Introduction

The tongue is an organ capable of various muscular movement located in the floor of the oral cavity, connected posteriorly by the frenulum linguae and is free at its cranial side (1,2). It is important in taste, mastication, swallowing and speech (3,4). The shape and topography of the tongue differ among mammals species reflecting their greatly influenced by feeding habits and enviromental conditions (5,6,7,8). The papillary system found on the dorsal surface of the mammalin tongue classified into gastatory papillae (fungiform, circumvallate and foliate) and mechanical papillae (filiform, conical and lenticular), the gastatory papillae had taste buds and pores, whereas the mechanical papillae play a role in tongue surface protection, withstand constant abrasion and desccation, food grinding and mastication, etc (9,10). The microscopic description of the lingual papillae have been made by (11,12,13,14,15). The aim of this study is to describe the gross and microscopic structure of the tongue in local mice *Mus musculus*, the most diversified species of rodents which adaptable to several enviroment and diet conditions and which had

been used widely in the scientific field as stated by (16).

2. Materials and Methods

Adult healthy males local mice *Mus musculus* were used in this study obtained from the spining market during winter season. The experimental animals were anesthetized under cholroform inhalation then transferred to the dissection board. The mouth had been dissected, followed by harvested the tongue from the bases of the buccal cavity by using a sharp scissors and for each experimental animals. The tongue carefully extended to their full length after stripped from the surrounded connective tissue. Topography of the tongue was noted, and photographed. For histological preparation, tongue specimens were fixed in 10% formalin for 24 hours, washed, dehydrated through ascending grades of ethanol alcohol, cleared and embeded in paraffin wax. Six micron thick sections were cut by using rotary microtome. The paraffin wax was removed by immersing the slides in xylene. The slides were passed through descending grades of alcohol, distilled water and stained with Periodic Acid Schiff Reagent (PAS)

stain methods. Tongue tissue sections were cleared and mounted as per usual methods (17). The images of tongue tissue sections were taken by using light microscope with digital camera canon, Japan.

3. Results \ Gross description

Morphological observations revealed that the tongue in local mice was white grayish in color, elongated slender shape with rounded bisected anterior end. The tongue had three parts, they are: rostral rounded bisected part (apex), wide, long bisected middle part (body) and caudal part (root). The dorsal surface of tongue was rough and characterized by the presence of deep median groove which divided the apex and the body into equal portions, Fig.(1). it was found an elevated rounded torus linguae on the posterior third of the dorsal surface of the tongue, Fig.(1). The ventral surface of the tongue was smooth with distinct rings separating the transverse blocks, as seen in Fig.(2).

3. Results \ Light microscopic examination

The histological examination by light microscope and by using special stain (PAS) revealed that the central core of the tongue in the experimental mice was occupied by wide fasciculi and blocks of striated muscle fibers encircled by the oral mucosa. The muscle fibers fasciculi arranged in three directions criss cross with each other and intermingled with the loose connective tissue and collagenous fibers of the lamina propria of the oral mucosa. The collagenous fibers appeared as a distinct rings surrounds the muscle fibers blocks and fasciculi, Fig.(3). The rings can be recognized by naked eye as seen in Fig.(2). Fig.(4) shows the superior and inferior longitudinal muscle fibers; middle vertical muscle fibers and transverse blocks muscle fibers, the later classified into three parts: the first part extends from the middle to the apical part of the tongue, the second part was connected to the superior longitudinal muscle fibers of the tongue and the third part which fans out from the middle to the root of the tongue. Fig.(5) shows the complex muscular architecture, the two main groups of the middle vertical muscle fibers and the oblique muscle fibers which interwoven with the superior and inferior longitudinal muscle fibers and which extended to the torus linguae that found on the posterior third of the dorsal surface of the tongue. The mucosa of the dorsal surface of the tongue is thrown into numerous elevations which called lingual papillae, they gave the dorsal surface rough appearance.

Five types of lingual papillae [filiform, fungiform, circumvallate, conical and lenticular papillae] with different density and shape covered the whole dorsal surface of the tongue, each papillae was formed of an epithelial cap and connective tissue core

derived from the underlying lamina propria. Dense irregular connective tissue, capillaries, nerves as well as mucous glands and immune cells (mast cells, lymphocytes and macrophages) constitute the lamina propria which interdigitations with the epithelium.

The filiform papillae were the narrowest and the most numerous of all lingual papillae, they were densely distributed at the apex and body parts being less numerous at the level of circumvallate papillae and rarely in the caudal region of the root part of the tongue. They were wide and large in their base, oriented posteriorly and covered with heavy keratinized stratified squamous epithelium with no taste buds. Fig.(6) shows three shapes of the filiform papillae, the long fork shaped; bristle like shaped filiform papillae which noticed numerous and the less numerous small, short cone shaped filiform papillae.

The fungiform papillae were fewer and wider than the filiform papillae and they were scattered randomly among them. They had mushroom shaped with stalk and rounded head, the epithelial covering was thin non keratinized stratified squamous epithelium containing few taste buds and they had richly vascular connective tissue core, Fig.(7).

One circumvallate papillae was found on the front of the root part of the tongue, it was larger than any of other lingual papillae and surrounded by a circular trench, the epithelial covering was thin non keratinized stratified squamous epithelium containing taste buds, Fig.(8).

Microscopically taste buds appeared as onion like, ovoid shaped, pale staining multicellular structures. The cells that forming the taste buds were three types: type 1 the basal cells were population of small spherical cells located at the base of the taste buds; type 2 the supporting or sustentacular cells were elongated columnar cells with ovoid shaped and lightly stained nucleus, they formed the outer wall of the taste buds; type 3 gustatory or sensory cells were located in the center of the taste buds, each cell had elongated darkly stained nucleus and it was bear a taste hairs that project into a small opening within the luminal surface of the epithelium called the taste pore, Fig.(8).

The conical papillae distributed on both sides of the torus linguae, they had wide conical shaped and the epithelial covering was heavy keratinized stratified squamous epithelium containing no taste buds, Fig.(9).

The lenticular papillae were found in the middle regions of the torus linguae, they were smaller than the conical papillae, and the epithelial covering was heavy keratinized stratified squamous epithelium containing no taste buds, Fig.(9).

The ventral surface was smooth and the epithelium was consisted of thin non keratinized stratified squamous epithelium free from lingual papillae. The underlying lamina propria consisted of areolar connective tissue with collagen fibers, elastic fibers, immune cells, capillaries and blood vessels. Adipose tissue found in the spaces between muscle fibers, as seen in Fig.(10).

4. Discussion

The morphological results of the present study revealed that the tongue in local mice *Mus musculus* had elongated slender shaped with rounded bisected anterior end. In comparison with those of other mammals, it was seen as elongated flat, broad and round anteriorly in *Oryctolagus cuniculus* (5,6); triangular shaped tapered anteriorly, wide caudally in *Mellivora capensis* (7); spatula shaped, broad cranially and narrow caudally in *Cricetomys gambianus* (8). The present study had shown that the tongue had three parts, rostral rounded bisected part (apex); wide, long bisected middle part (body) and caudal part (root). The deep median groove divided the apex part and the body part of the tongue into equal portions. Similar observation was found by (5,6) in *Oryctolagus cuniculus* (14) in Wistar rats; (7) in *Mellivora capensis* and (18) Iraqi goat *Capra hircus*. Some investigators have different results about the median groove, they demonstrated that it was absent along the entire surface of the dorsal surface of the tongue like (19) in cape hyrax *Procavia capensis*; (11) in *Rousettus aegyptiacus*; (15,21) in hedgehog *Hemiechinus auritus*; (20) in *Oligoryzomys nigripes*. It was noticed that an elevated ovoid torus linguae located on the posterior third of the dorsal surface of the tongue. Similar observation seen on bank vole *Clethrionomys glareolus* (12); rat *Arvicanthis niloticus* (13); geoyid rodent *Geomys bursarius* (14). The torus linguae was absent in lesser mouse deer *Tragulus javanicus* (9).

The histological examination by light microscope and by using special stain (PAS) revealed that the central core of the tongue in the experimental mice was occupied by wide fasciculi and blocks of striated muscle fibers encircled by the oral mucosa. The muscle fibers fasciculi arranged in three directions criss cross with each other and intermingled with the loose connective tissue and collagenous fibers of the lamina propria of the oral mucosa. (2) stated that the penetration of the lamina propria in the spaces of the the muscle fibers fasciculi and blocks made the mucosa strongly adherent to the muscle fibers whereas the function of the interdigitations of the lamina propria with the epithelium was to increase the mechanical connection (5). In the present study the collagenous fibers appeared as a distinct rings surrounds the muscle fibers blocks and fasciculi. The

collagenous ring that associated with the complex muscular architecture was believed to confer on the tongue tensile, strength that needed for protraction and retraction as stated by (10) in rat *Rattus norvegicus*; Bat *Eidolon helvum*; Pangolin *Manis tricuspis* and (23) in *Oryctolagus cuniculus*.

The present study showed five types of lingual papillae, the gustatory papillae (fungiform, circumvallate) and mechanical papillae (filiform, conical and lenticular) with different density and shape covered the whole dorsal surface of the tongue. Similar observation were made by; (12) in *Clethrionomys glareolus*; (7) in *Mellivora capensis* the bank vole and (18) in Iraqi goat *Capra hircus*. The number of the lingual papillae types varies within the mammalian species, like Lesser mouse deer *Tragulus javanicus* (9); rat *Arvicanthis niloticus* (13); *Rousettus aegyptiacus* (11); *Oryctolagus cuniculus*, Egyptian fruit bat *Rousettus aegyptiacus* (24); golden headed lion tamarins *Leontopithecus chrysomelas*, they possessed only four types of lingual papillae (filiform, fungiform, circumvallate and foliate), while *Zalophus* (25); Wistar rats (14) and the two species of rat *Rattus norvegicus* & *Rattus wistar* (26), they possessed only three types of lingual papillae (filiform, fungiform and circumvallate). (7) stated that the variations in some mammalian lingual papillae may be concerned with the feeding habits and the type of food eating. The entire epithelial surface of the Pangolin *Manis tricuspis* was non papillated, this may suggest that its tongue was not specialized for manipulation of food within the oral cavity and gustation through the tongue primarily serves to trap insects with its sticky surface as stated by (10).

The present study showed that the filiform papillae appeared the narrowest and the most numerous of all lingual papillae. Similar observation was made by (28) in rabbit *Oryctolagus cuniculus*; (27) in red sokoto goats *Capra hircus* (4) in albino rats; (13) in rat *Arvicanthis niloticus*; (11) in *Rousettus aegyptiacus*; (14) in Wistar rats; (21) New Zealand white rabbit *Oryctolagus cuniculus*, Egyptian fruit bat *Rousettus aegyptiacus* and (26) in two species of rat *Rattus norvegicus* & *Rattus wistar*. (9) stated that the distribution and arrangement of filiform papillae gave the tongue a rough surface adapted for grinding, movement of foods and mastication. Three shaped of filiform Papillae type were found in this study. Similar finding was made by (12) in the bank vole *Clethrionomys glareolus*; (10) in rat *Rattus norvegicus*, bat *Eidolon helvum*; (7) in *Mellivora capensis*; (24) in *Rousettus aegyptiacus*; (26) in two species of rat *Rattus norvegicus* & *Rattus wistar* and (28) in hedgehog *Hemiechinus auritus*. In the present study the three shapes of the filiform type were, the long fork shaped papillae; the curve shaped filiform

papillae with blunt end which noticed numerous and the less numerous small, short cone shaped filiform papillae. Some investigators mentioned different forms of the filiform papillae type, like cylindrical shaped, large conical shaped and small conical shaped in turkey mice(16); the bristle like shaped, conical like shaped and crown like shaped in rat *Rattus norvegicus*, bat *Eidolon helvum* (10); single, bifurcated and serrated shaped in *Mellivora capensis* (7); fork shaped; saw shaped and curve shaped in hedgehog *Hemiechinus auritus* (21); small hair shaped, giant with smooth surface and bifid shaped in *Rousettus aegyptiacus* (24). (10) stated that the morphological and structural differences in the filiform papillae related to there various functional differences seen in the tongue of mammalian species, the bristle like shaped filiform papillae function in rats *Rattus norvegicus* was to increase the friction between the tongue and food substances, facilitating the movement of particles by the tongue within the oral cavity, whereas the cone shaped and crown shaped filiform papillae function in the bats *Eidolon helvum* was to retain food in the mouth during flight and probably aid in oral absorption due to its increased surface area.

This study revealed the presence of one circumvallate papillae type located on the front of the root part of the tongue. Similar finding was found in Wistar rats (14); in hedgehog *Hemiechinus auritus* (15); *Mellivora capensis* (7); Iraqi goat *Capra hircus* (18) in two species of rat *Rattus norvegicus* & *Rattus wistar* (26). The number of the circumvallate papillae varies within the tongue of the mammalian species, two circumvallate papillae found in wild boar and pig (30); rat *Arvicantis niloticus* (13); New Zealand white rabbits tongue *Oryctolagus curiculus* (6); brown throated sloth *Bradypus variegates* (29). Three circumvallate papillae found in *Zalophus* (25); the bank vole *Clethrionomys glareolus* (12); golden headed lion tamarins *Leontopithecus chrysomelas* (31); hedgehog *Hemiechinus auritus* (21). The circumvallate papillae was absent in Cape hyrax *Procavia capensis* (19).

The conical papillae distributed on both sides of the torus linguae while the lenticular papillae were found in the middle regions of the torus linguae. These papillae were absent in Lesser mouse deer, *Tragulus javanicus* due probably to the absence of the torus linguae as stated by (9).

A heavy keratinized stratified squamous epithelium covered the conical and lenticular papillae of the tongue in the experimental local mice were noticed in this study. It may suggest that the epithelium can withstand constant abrasion, protection and desiccation that it is exposed to by reason of the food type of this species which includes hard pebbles

as stated by (10), hence the extensive variation in the keratinization of the tongue in some mammals like rat *Rattus norvegicus* and bat *Eidolon helvum* not only suggests functional adaptation but also phylogenetic adaptation as related to their diets.

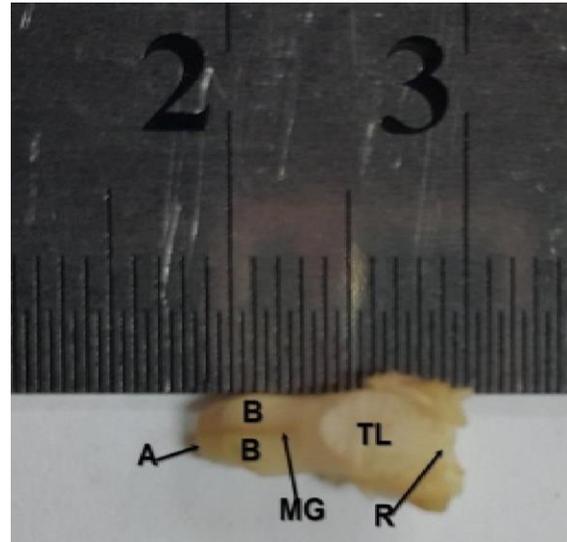


Fig. (1) Dorsal view of the entire tongue in local mice illustrates: A. Apex part; B. Body part; MG Median Groove; R. Root; TL Torus Linguae.



Fig. (2) Ventral view of the entire tongue in local mice illustrates: DR. Distinct Ring; TB. Transverse Block.

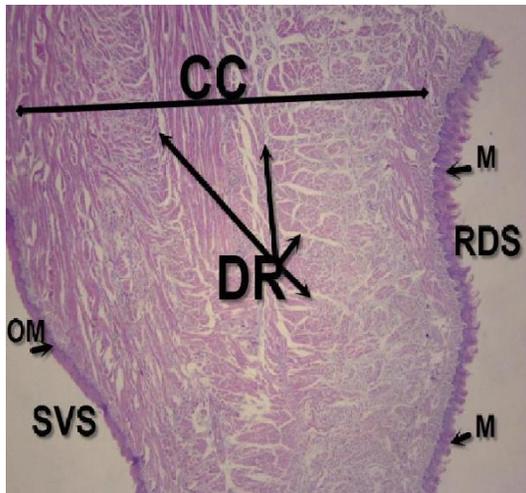


Fig. (3) Sagittal section of the tongue in local mice illustrates: CC. Central Core of muscle fibers; DR. Distinct Rings; M. Mucosa; OM. Oral mucosa; RDS Rough Dorsal Surface; SVS. Smooth Ventral Surface. PAS stain (x 10).

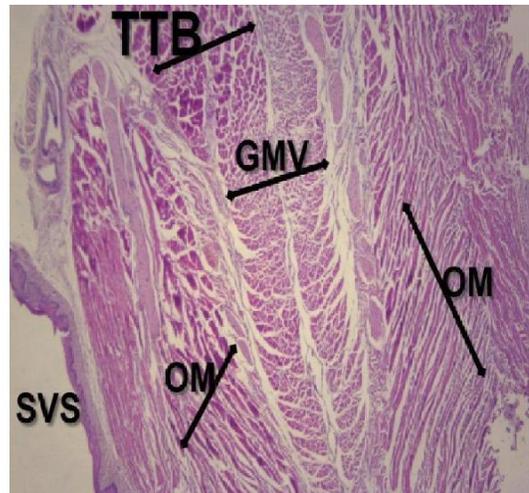


Fig.(5) Sagittal section through the torus linguae of the tongue in local mice illustrates: GMV. Groups of Middle Vertical muscle; OM. Oblique Muscle; SVS. Smooth Ventral Surface; TTB. Third Transverse Bloc. PAS stain (x20)

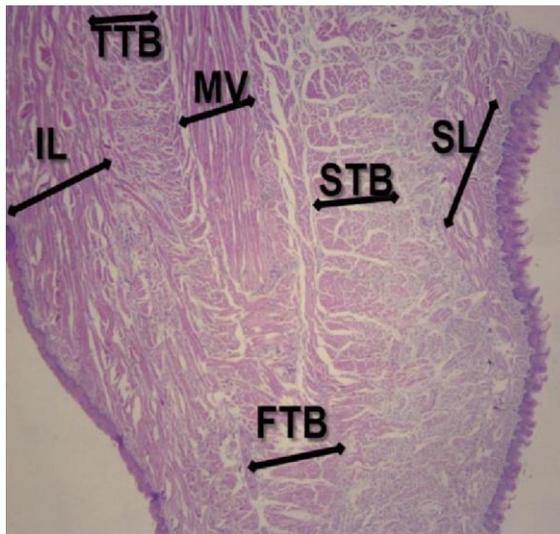


Fig. (4) Sagittal section of the tongue in local mice illustrates: FTB. First Transverse Block; IL. Inferior Longitudinal Muscle; MV. Middle Vertical Muscle; SL. Superior Longitudinal Muscle STB. Second Transverse muscle Block; TTB. Third Transverse Block. PAS stain (x10)

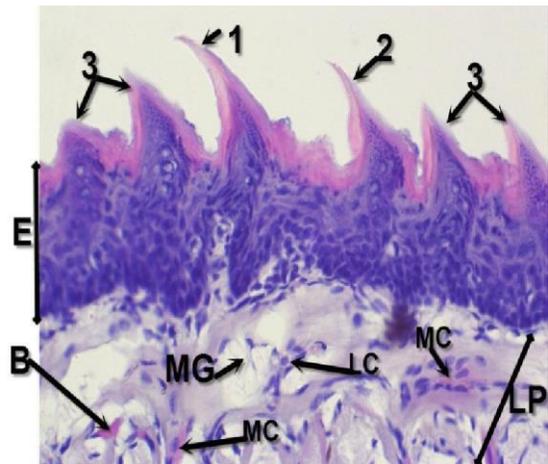


Fig. (6) Longitudinal section of the apex of tongue in local mice illustrates: 1.long fork shaped filiform papillae; 2. Bristle like shaped filiform papillae; 3short cone shaped filiform papillae; B. Blood Vessel;E. Epithelium; MC. Mast Cell; MG. Mucous Gland; LC. Lymphocyte; LP. Lamina Propria. PAS stain (x 40).

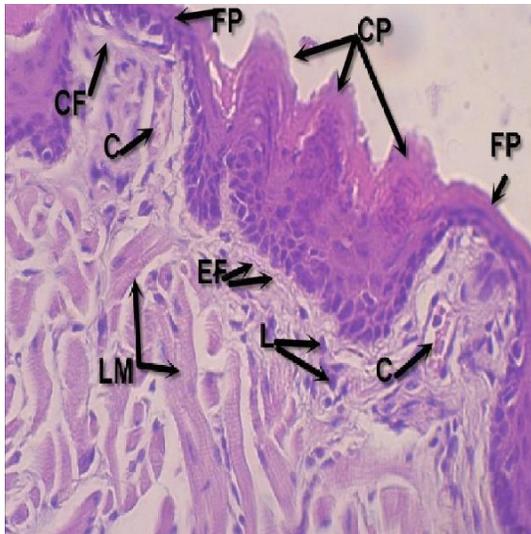


Fig. (7) Sagittal section through the body of tongue in local mice illustrates: C. Capillary; CF. Collagen Fibers; CP. Conical Papillae; EF. Elastic Fibers; FP. Fungiform Papillae; L. Lymphocyte; LM. Longitudinal Muscle PAS stain (x 40).

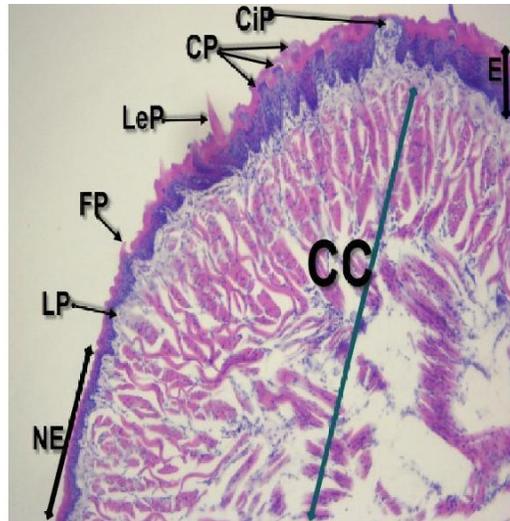


Fig. (9) Transverse section through the posterior region of the root of the tongue in local mice illustrates: CC. Central Core of muscle fibers; CP. Conical Papillae; CiP. Circumvallate Papillae; E. Epithelium; FP. Filiform Papillae; LeP. Lenticular Papillae; LP. Lamina Propria; NE. Non keratinized Epithelium. PAS stain (x10).

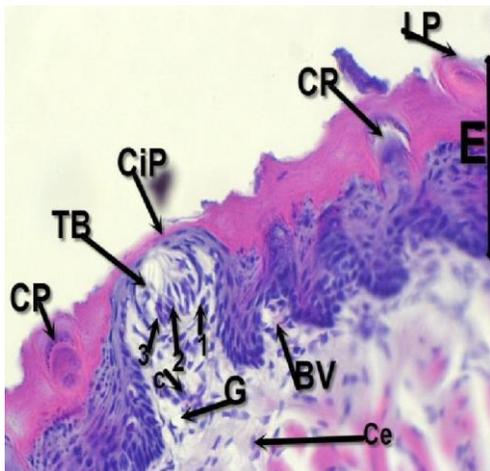


Fig. (8) Sagittal section through the root of tongue in local mice illustrates: BV. Blood Vessel; C. Capillary; Ce. Lymphocyte; CiP. Circumvallate Papillae; CP. Conical Papillae; E. Epithelium; G. Mucous Gland; LP. Lenticular Papillae; TB. Taste Buds 1-Basal cell, 2- Supporting Cell and 3- Gustatory Cell. PAS stain (x40).

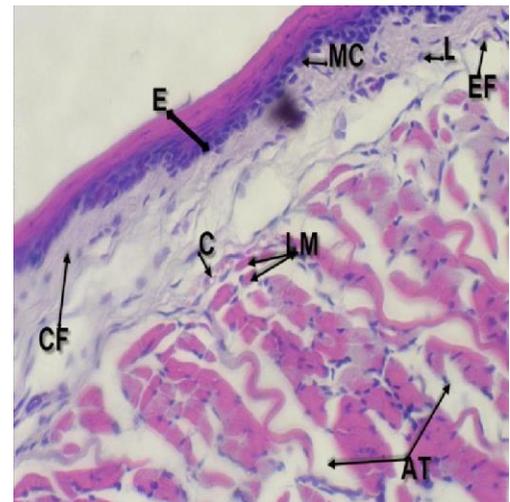


Fig. (10) Transverse section through the ventral surface of the tongue in local mice illustrates: AT. Adipose Tissue; C. Capillary; CF. Collagen Fibers; E. Epithelium; EF. Elastic Fibers; L. Lymphocyte; L M. Longitudinal Muscle; MC. Mast Cell. PAS stain (x40).

Conclusion

The tongue in local mice was white grayish in color, elongated slender shaped with rounded bisected anterior end and had three parts. Deep median groove and elevated ovoid torus linguae found on the dorsal surface. Microscopically the central core of the tongue

was occupied by two main groups of the middle vertical muscle fibers, oblique muscle fibers interwoven with the superior and inferior longitudinal muscle fibers and three parts of transverse blocks of muscle fibers, encircled by the oral mucosa. Lingual papillae found on the dorsal surface of the tongue classified as two types of gustatory lingual papillae (fungiform, circumvallate) covered by thin non keratinized stratified squamous epithelium containing taste buds and three types of mechanical lingual papillae (three shaped filiform papillae, conical papillae and lenticular papillae) covered by heavy keratinized stratified squamous epithelium containing no taste buds. Three shapes of the filiform papillae found in this study, the long fork shaped; bristle like shaped filiform papillae and small, short cone shaped filiform papillae.

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