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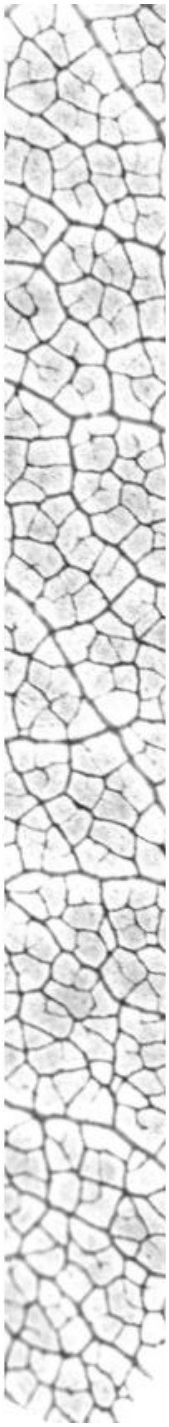

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Comparative histological and morphological feature between rabbit (*Oryctolagus cuniculus*) and dog (*Local breed*) in dorsal surface of tongue parts

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Abstract
This study aimed to provide a histological future different for tongue (apex and root) between of rabbit and dog. Histological features were assessed by morphological measurement, histological characteristics and histological description. The morphological measurement included tongue length, Thick of apex and Root. However, the dog was appeared significant increase ($p \leq 0.05$) in tongue length, root thickness compare with rabbit. While the histological characteristics included determination each of keratinized layer, epithelial layer, Lamina properia, Sub-mucosa and muscles external, which showed that apex and root measurement of the rabbit tongue were recorded increase significantly ($p \leq 0.05$) in each of keratinized layer and epithelial layer, while recorded decrease significantly ($p \leq 0.05$) in each of Sub-mucosa and muscles external compare with dog. While the lamina properia layer not record significantly different ($p \geq 0.05$) between the rabbit and dog. The histological description showed that the epithelial tissue in dog was thinner than in rabbit, while the prickle layers appeared to contain a less number of elongated columnar cells than in the rabbit, also the granular layer were as less wide region as the rabbit. However the apex papillae of the dog tongue were larger than a rabbit. The lamina propria in the rabbit as projection were elongated in the epithelium tissue and numerous, while the dog has an irregular corrugate projection and less deep in epithelial than the rabbit. The muscular layer in rabbit consisted of internal muscle fibers arranged longitudinally with oblique fibers, while the external muscle fibers arranged transversely and intervened with oblique muscle fibers. On the contrary, in dog the internal layer consisted of a band of transverse and oblique muscles, while external muscle consists of longitudinal muscles. The root of the tongue was appeared that the basal later in dog appeared elongated columnar cells, while the rabbit was cuboidal cell, also the cornifed layer in rabbits was consisted of many parakeratinized layers, while in the dog was thinner layer. The type of lingual gland in dog was serous and mucous gland and extended between submucosa and muscular layer. While, the lingual gland in rabbit intervened in muscular layer and consisted of serous glands only. The muscular layer in the dog and rabbit was similarly consisting of internal and external muscle layer.

Keywords: rabbit, dog, tongue, dorsal surface

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INTRODUCTION

The alimentary canal started with oral cavity which is considered the entrance to the digestive tube. Oral cavity proper is surrounded by palate on the roof while laterally bordered by teeth and gum in two sides and tongue which is covered the floor of the cavity (Dyce, 2018). The structure of the tongue and other organ in oral cavity varies depend to the habit of feeding (Iwasaki, 2002).

The tongue is a mobile and muscular organ connected with the end of the oral cavity by root (third part of the tongue). Whereas, the anterior part of tongue is the apex which is free and have various shapes depend on the type of animal also the dorsal median groove of the apex divided the surface into two equal parts (Mahabady, 2010).

However, the tongue in all animal species is covered with specific functional units called papillae. These papillae in turn varied in according to their location, function and animal species. The New Zealand white rabbit contains three parts in elongated tongue and four surfaces (dorsal, ventral and two lateral sides). The apex

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represents frequently more than 2/5 of the tongue length. As well as the dorsal surface of the apex includes a median groove. In wild rabbit the dorsal surface of the tongue has a median groove and transverse ridge. Whereas, in local rabbit the tongue lack of median groove and transverse ridges. The wide lingual roots which is short, narrow and smooth part. The structure of the tongue develops with the type of nourishment. While, the lingual papillae differentiate in shape, location and function (Bate, 2004). Generally, papillae can be classified into six types due to the shape which are foliate, conical, vallate, filiform, fungiform and lenticular papillae. The papillae in these three parts have two functions, mechanical (filiform papillae) and other types of papillae are gustatory which include foliate, circumvallate and fungiform. Furthermore, these papillae have different shapes and the location is varied along the tongue (Abumandour, 2013, Kadhim, 2016). In addition; the filiform papillae cleared of taste buds, but all other type of lingual papillae in rabbits have taste buds (Ibrahim, 2017).

Histologically, the tongue has a thick layer of keratinized stratified squamous epithelium. The next layer is lamina propria which consists of connective tissue. The tongue of wild rabbit covered by thick layer of stratified squamous keratinized epithelium., Also have a thick mucosa and sub- mucosa (AL-Mahmodi, 2016). Whereas, the tongue of local rabbit lack of median groove. The mucosa and sub mucosa layer is thin. This layer is followed by muscular stratum which is skeletal muscles, this thick layer appears in three positions; longitudinal, transfer or oblique that count on animal type. In wild rabbit the circular layer of muscle internally and longitudinal muscular externally. Whereas, the local rabbit muscular layer contains the circular muscle externally and the longitudinal internally. Also the tongue embraces two types of serous and mucous glands which are Von Ebner gland and Weber gland (Singh, 2018).

The tongue in dog is a movable and muscular organ. The shape of the tongue is thick and wide from back and thin in front. Furthermore, it is red, bright color. The dorsal surface of tongue has sulcus median linguae. The papillae distributed in all parts of the tongue and each type of papillae have varies shape (Dyce, 2018).

It is evident from previous studies that attempts have been implemented to explain the morphology and anatomy of the tongue of rabbit. However, the details of the histological composition of oral cavity structures were not given much attention. Moreover, the comparative and histological investigation of these components still required more elucidation that in order to obtain a comprehensive perception of the effects of variation of feeding habits and food types on the dissimilarities and adaptations of animals to cope with their natural requirements. So the current study aimed to show the histological comparison between rabbit and dog.

MATERIALS AND METHODS

Collecting specimens

Two species of domestic animals were used for the current study. All animals were clinically normal and healthy. Three adult males of each species were prepared from each dog (*Local breed*) and rabbit (Oryctolagus cuniculus). The ages of animals are different. In the dog the age was between 9 and 11 months while, in rabbit ranged 6and 9 months respectively.

The rabbits were collected from local Basra market have 3kg weight for each one. The dogs were bought from local market and have 15 kg weight for each dog. Before euthanasia the rabbits were injected in intramuscular by ketamine 0.45 mg/kg and xylazine 0.75 mg/kg for each one. The rabbit was lose their consciousness after the time of injection and then opened the thoracic cavity and bleeding by puncher in the right atrium. Also the dogs were intramuscular injected by 2.25mg/kg ketamine and 3.75mg/kg xylazine to lose their consciousness before killing them.

The specimens were collected from the oral cavity of experiment animals which are the tongue. The tongues were dissected by separating the extrinsic muscles (hypoglossal, genioglossal and styloglossal) from the tongue and then used to study morphometric features that were the length and thick by using digital vernier.

The specimen taken from two specimens of tongue from the apex and root of tongue with dimensions was 5x5x5 mm in size.

Histological preparation

The specimens were collected from two parts of tongue washed by normal saline solution (0.9%) to cleaned them, after that fixed immediately in buffer formalin 10% at room temperature for 24-48 hrs. for light microscope study. Then, histological processes started included; dehydration which contains serial of different concentrations of ethanol one hours for each step. Clearing two steps of xylene, one hour for each step. After that, the specimens were embedded in melted paraffin (56̊C). Serial section of 5um thickness were stain by Hematoxilin and Eosin and Mallory stain followed (Luna, 1968 and Kiernan, 2015).

RESULTS AND DISCUSSION

Morphology and Histology measurement

The tongue filled the ground of the mouth of the lower jaw extended in rostral shape in both rabbit and dog. **Table 1** presented the variation in tongue morphological measurements in both animals. It is clear that the length of the tongue differed significantly ($p \leq 0.0001$) between animals, the mean total length in rabbit was (50.090 ± 1.348) mm which was shorter than the mean of tongue length in dog (128.186 ± 5.405) mm. The comparison between rabbit and dog in the thickness of the apex of

Table 1. Morphological measurements of tongue length, and thickness of apex and root in rabbit and dog (mean ± SD) (N=3)

Morphological Measurements	Animal		Sig
	Rabbit (mm)	Dog (mm)	
Tongue Length	50.090± 1.348	128.186± 5.405	0.0001
Thick of apex	3.826± 0.506	3.036± 0.197	0.066
Thick of Root	6.926± 0.613	26.920± 0.900	0.0001

Table 2. The histological layer thickness at the apex of tongue in rabbit and dog (mean ± SD) (N=15)

Tissue type	Animal		Sig
	Rabbit (µm)	Dog (µm)	
Keratinized layer	2.693± 2.053	1.413± 0.738	0.031
Epithelial layer	9.493± 4.813	6.773± 1.892	0.001
Lamina properia	6.746± 1.409	6.773± 1.950	0.966
Sub-mucosa	11.013± 2.622	14.106± 3.286	0.008
Muscles externa	108.533±28.938	202.266± 59.251	0.0001

Table 3. Thickness of histological layers at the root of tongue in rabbit and dog (mean ± SD) (N=15)

Tissue type	Animal		Sig
	Rabbit (µm)	Dog (µm)	
Keratinized layer	2.960± 1.911	1.120± 0.662	0.001
Epithelial layer	10.720± 3.434	3.306± 0.874	0.0001
Lamina properia	2.986± 0.722	6.800± 2.775	0.0001
Sub-mucosa	8.426± 3.879	25.963± 1.471	0.0001
Muscles external	275.200± 101.457	453.466± 10.595	0.0001

tongue showed no a considerable difference. Turning to the thick of tongue in root part, in dog was (26.920 ±0.900) mm, which revealed a noticeable different thickness (p≤0.0001) than root in rabbit (6.926 ± 0.613) mm.

Table 2 described changes in thickness of histological layers in the apex of tongue between rabbit and dog. It is clear that the thickness of keratinized layer in rabbit was (2.693 ±2.053) mm wider significantly (p≤ 0.031) than the mean of keratinized layer in the apex of tongue of dog (1.413 ± 0.738) mm. Whereas, the mean epithelial layer in apex of tongue of rabbit was (9.493 ± 4.813) mm that more than a half of the mean epithelial layer in apex of tongue in dog (4.266 ± 1.892) mm. While, the thickness of lamina propria in both animals did not differ significantly. In sight of the thickness of sub-mucosa layer in apex of tongue of rabbit was (11.013 ± 2.622) mm which was significantly (p≤ 0.008) less thickness than the sub-mucosa layer in the apex of tongue of dog (14.106 ± 3.286) mm. The thickness of muscular layer in the apex of the tongue of dog was approximately as double as the thickness of the muscular layer in the apex of the tongue of rabbit which were (108.533 ± 28.938) and (202.266 ± 59.251) mm respectively.

Turning to the thickness of histological layers in root of tongue between rabbit and dog was shown in **Table 3**. The mean thickness of keratinized layer in root of tongue in dog was lesser significantly (p≤0.001) than that in rabbit. Also the mean width of epithelial layer in rabbit recorded huge difference of the same layer in dog which were (10.720± 3.434) and (3.306 ± 0.874) mm respectively. While, a significant difference was detected

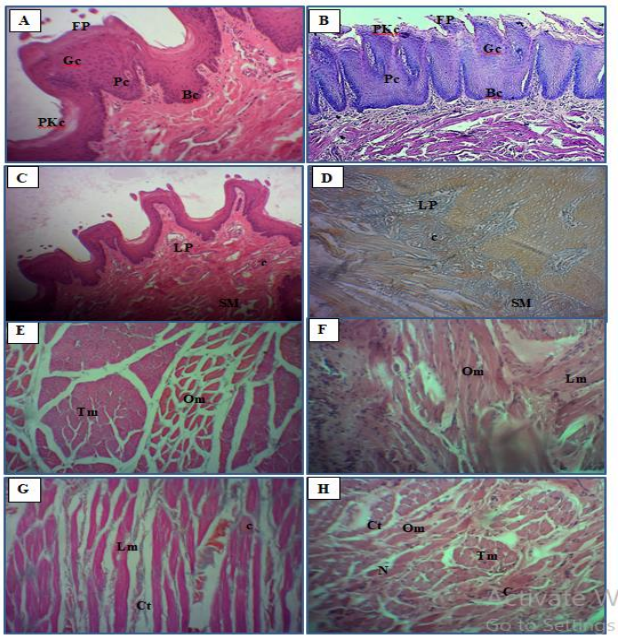

Fig. 1. Transverse section in the dorsal surface of the apex of tongue showed (A) the epithelial tissue of dog, (B) the epithelial tissue of rabbit (Mallory stain), (C) the lamina propria and sub-mucosa in dog, (D) lamina propria and sub-mucosa in rabbit (Mallory stain), (E) internal muscular layer in dog, (F)internal muscle in rabbit, (G) external muscular layer in dog, (H) external muscular layer in rabbit. (FP) filiform papillae, (PKc) parakeratinized cells, (Gc) granular layers, (Pc) prickle layers and (Bc) basal layer. (LP) lamina propria layer, (SM) sub-mucosalayer and (c) capillary. (Tm)transverse muscle , (Om) oblique muscle. (Lm) longitudinal muscle, (Ct) connective tissue and (N) nerve (H&E, X 100).

in the mean thickness of the lamina propria layer in dog (6.800 ± 2.775) mm which was larger than the thickness in lamina propria layer in rabbit (2.986 ± 0.722) mm. In both of the sub-mucosa and muscular layers thickness there were a significant difference (p≤ 0.0001) in both rabbit and dog.

Histology description

The apex of tongue

Fig.1-A demonstrates the dorsal surface of the apex of tongue in rabbit. It is clear that the epithelial tissue was stratified squamous. The basal layer of this tissue consisted of a single line of columnar shape cells which contained oval shape nuclei. Then the next above layer was prickle which includes larger cells than the cells in basal layer. The above layers comprised of flattened cells which were wider than the prickle layer and were denser in number called granular layers. The subsequent layers were cornified. The cells in these layers were flattened, large and showed eosinophilic characteristics. The nuclei appeared in the upper layer as results of parakeratinization process. The filiform emerged as projections from the cornified layer which were numerous and had a conical shape (**Fig.1-B**). While, in the dorsal surface of the apex of tongue in dog,

the epithelial tissue was thinner than the epithelial tissue in rabbit. Likewise, the basal layer of this tissue consisted of a single line of columnar cells and oval nuclei like basal cells in epithelial layer of apex of tongue in rabbit. Also above these layers, the prickle layers appeared to contain less numbers of elongated columnar cells than in the prickle layer of rabbit. However, the size of cells in this layer was larger in dog than in rabbit. The granular layers were as less wide region as in rabbit. But, the cells were flattened and wide. Above of the granular layers, the cornified layers were para keratinized and thinner region in comparison with rabbit. The filiform papillae were observed in cornified region which were numerous and contain two apices (**Fig. 1-A**). In general, these papillae in the apex of the tongue of dog were larger than in rabbit. It was recognizable that the lamina propria layer in dog extended to constitute the core of filifom papillae while this feature did not recognized in rabbit.

The layer under the epithelial tissue was lamina propria. The layer intervened with epithelial tissue by the projection called papillae. These projections in the rabbit were elongated in the epithelial tissue and numerous. While in dog were irregular corrugate projection and less deep in epithelial than in the rabbit. In general, the lamina propria is divided into two parts, the first portion is named superficial papillary portion can be discriminated between the basal membrane and connective tissue which takes silver stain. The papillary portion contained reticular fibers that differ in length and width and less dense than that in the portion under. The second portion consisted of dense reticular fibers and fibroblast cells. No variation between rabbit and dog in lamina propria layer was detected (**Fig. 1-C**), (**Fig. 1-D**). The sub-mucosa layer was beneath the lamina propria and contained dense network of connective tissue and had a capillaries. The sub-mucosa layer in rabbit was less thickness than the sub-mucosa in dog (**Fig. 1-D**). Furthermore, the fibers were more dense and extra capillary in dog in comparison with rabbit (**Fig. 1-C**).

The muscular layer consisted of skeletal muscle. The internal muscle fibers arranged longitudinally with oblique fibers (**Fig. 1-F**). While, the external muscle fibers arranged transversely and intervened with oblique muscle fibers. As well as nerves and capillaries occurred in loose connective tissue between muscles bands (**Fig. 1-H**). On the contrary, in dog the internal layer consisted of band of transverse muscles followed by oblique band (**Fig. 1-E**), while, the longitudinal muscles comprised the external part of muscle layer. The muscular layer contained connective tissue between bands that contained nerve and capillary (**Fig.1-G**).

Root of tongue

Fig. 2 demonstrates the root of the tongue in rabbit and dog. It is clear that the epithelial tissue in dog contains the basal layer which was elongated columnar cells with oval nucleus. The basal layer was single line of cells. However, the prickle layer contain an elongated cells and numerous. While, the granular layers follow the prickle layers compose of flattened, wide and numerous cells. The cornified layers contain flattened cells (more than granular layers) and content the nuclei. Also, the cornified layer is parakeratinized cells consist thin layer and contain conical papillae (**Fig. 2-A**).

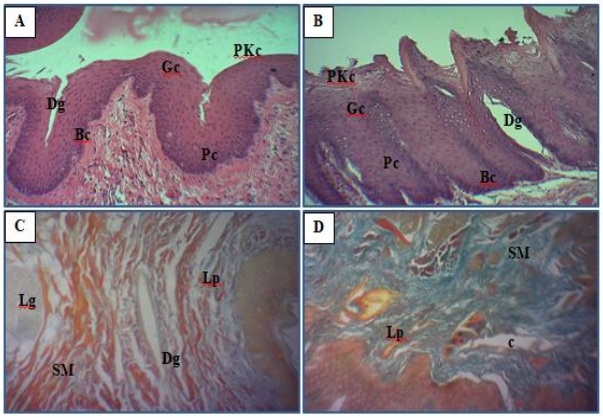

Fig. 2. Transverse section in the dorsal surface of the root of tongue showed : (A) epithelial tissue in dog, (B) epithelial tissue in rabbit, (C) lamina propria and submucosa in dog (Mallory stain), (D) lamina propria and submucosa in rabbit (Mallory stain).(PKc) parakeratinized cells, (Gc)granular layers, (Pc) prickle layers, (Bc)basal layer, (Dg)duct gland, (Lg) lingual gland, (LP)lamina propria, (SM)submucosa and (c) capillary, (H&E,X100)

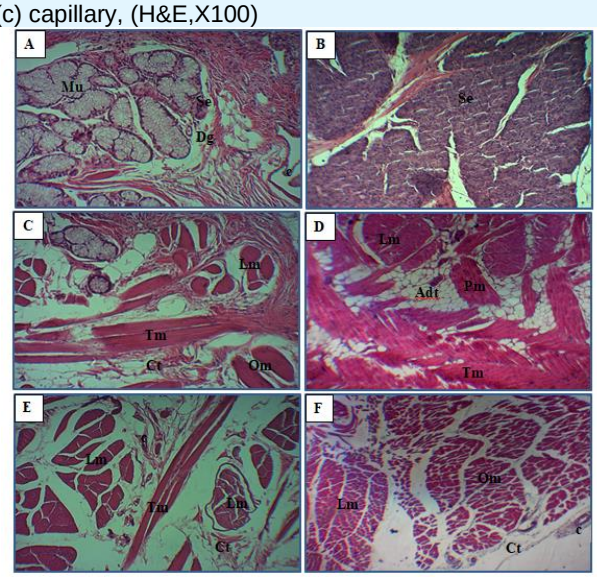

Fig. 3. Transverse section in the dorsal surface of the root of tongue showed: (A)lingual gland in dog , (B)lingual gland in rabbit, (C)internal muscle in dog, (D) internal muscle in rabbit, (E) external muscle in dog , (F) external muscle in rabbit. (Mu) mucous acini, (Se)serous acini, (Dg) duct gland, (Lm) longitudinal muscle, (Tm) transverse muscle, (Pm)perpendicular muscle, (Om) oblique muscle, (Ct) connective tissue, (Adt) adipose tissue and (c) capillary (H&E,X100)

While, the epithelial layer in rabbit consisted of single line of basal layer. The basal layer cells shape was cuboidal and oval nuclei. This layer was Followed by the prickle layers that contain elongated shape cells and thicker in prickle layer than in dog. The granular layers were as the same as in granular layers in dog. While, the cornified layers consisted of many para keratinized layer (**Fig. 2-B**). Similarity, The lamina propria and sub-mucosa layer in both rabbit and dog contained connective tissue rich of fibers, capillaries and duct gland in dog (**Fig. 2-C** and **D**). However, serous and mucous glands extended from sub-mucosa layer to muscular layer in dog (**Fig. 3-A**). While, in rabbit the serous glands appeared in muscular layer (**Fig. 3-B**). However, the thickness of these layers differed between rabbit and dog. he muscular layers in rabbit contained longitudinal muscles followed by perpendicular and transverse muscles respectively (**Fig. 3-D**). Whereas, in dog the muscular layer consisted of adipose tissue intervene longitudinal, transverse and oblique muscles respectively in the internal layer (**Fig. 3-C**). While, the external muscular layer consisted of bundles of longitudinal and transverse muscles in dog (**Fig. 3-E**), whereas, the external muscular layer in rabbit contained bundles of longitudinal and oblique muscles (**Fig. 3-F**). Both in rabbit and dog, the capillaries and nerves were in connective tissue between muscles.

CONCLUSION

The tongues varied in morphological characteristic features between the studied animals. The thickness of epithelial layers in tongue varied between studied animals and the dog recorded the lowest thickness between them. The lingual gland in rabbit differed in others studied animals, it was serous lingual gland.

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