



## Anatomical and histological study of tongue in ground squirrel

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### Abstract

The aim of this study was to investigate the anatomical and histological structure of the tongue in ground squirrels. This animal is widely distributed all over the world, especially in the Middle East, but no study has been reported on its tongue structure. In this investigation, we studied five squirrels that were died due to the road accidents. The freshly samples were collected and fixed in 10% formalin solution and after being transferred to the laboratory, the tongue tissue was separated and was studied anatomically. Then, the samples entered the tissue passage stages, and 5-7  $\mu\text{m}$  thick sections were prepared from the paraffin blocks and stained by the hematoxylin-eosin method. The results of this study showed that the surface of the tongue is covered by filiform papillae. Between this papillae, Fungiform papillae without taste buds were visible from the apex to root of the tongue. Three numbers of circumvallate papillae were also visible at the root of the tongue, which were the only chemical papillae of the tongue in this rodent. Most of the tongue structure was made up of skeletal muscles. Lingual glands were serous and mucous secretory units and could be seen only in root of tongue. According to the results of the present study, despite the unique differences in the tongue structure in the ground squirrel, it seems that in general, the tongue structure in this ground squirrel is similar to other squirrel families, especially the Iranian squirrel.

**Keywords:** Lingual papillae, Lingual glands, Ground squirrel, Histology, Morphometry

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## Introduction

Ground squirrel (*Marmotini*) is the name of a squirrel's family. Unlike other squirrels, these rodents live on the ground or underground. These animals are medium in size, have a short tail and big eyes and can weigh up to 8 kg. Most of the ground squirrel species live socially and in large groups in the plains. These animals can stand up on their hind legs when they feel threatened. In the standing position, they are more proficient in finding dangers and observing the surroundings. If these animals feel serious danger, they announce the existence of danger to others by producing special sounds (Rabin *et al.*, 2006).

The tongue as an important organ in the digestive system, plays an important role in the way of consuming food, directing it and the secretions of the digestive system. This muscular organ is covered by stratified squamous epithelium, and in addition, it is also responsible for the sense of taste by having various papillae (Colville and Bassert, 2008; Dyce *et al.*, 2009). Studies show that the main difference in the structure of the tongue among different animals is related to the type and distribution of its papillae (Sonntag, 1922; Fawcett and Bloom, 1986).

In the dorsal surface of the tongue, there may be different types of papillae, such as filiform, fungiform, foliate and circumvallate papillae. These lingual papillae are divided into two mechanical types (filiform, conical and lenticular papillae) and chemical papillae (fungiform and circumvallate papillae).

The distribution of these papillae mostly depends on the eating habits of different species (Fonseca *et al.*, 2011).

No study has been conducted on the anatomical and histological structure of the tongue structure in the ground squirrel, So, this was investigated in the present study.

## Methods and methods

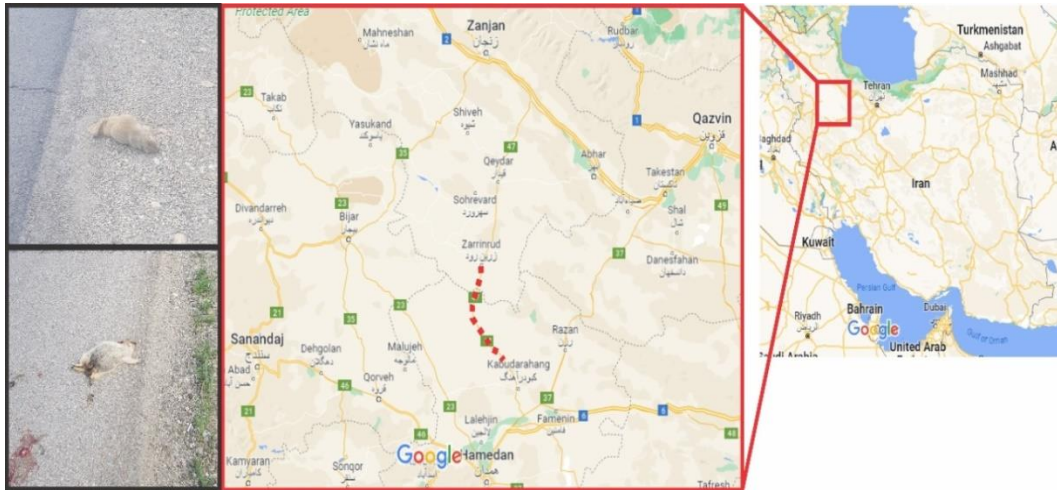
In the present study, we investigated five squirrels in the early March of 2022 to the late June of 2023. The samples were collected from Zarin-Roud city (Khodabande- Zanzan province) to Kabudarahang city (Hamadan province) road accidents that had died before (Fig. 1). Freshly Samples were collected and fixed in 10% buffered formalin solution and after being transferred to the laboratory, the tongue tissue was separated and was studied anatomically. Then, the samples entered the stages of tissue passage (including the dehydration, clarification and impregnation with melted paraffin). Next, paraffin blocks were prepared from the samples and using a microtome, slides with a thickness of 5 to 7  $\mu$ m were serially prepared and stained by the hematoxylin-eosin (H&E) method. The prepared slides were examined and photographed by optical microscope, Dino Lite camera and Dino capture software V. 2 (Kalantari-Hesari *et al.*, 2022).

## Results

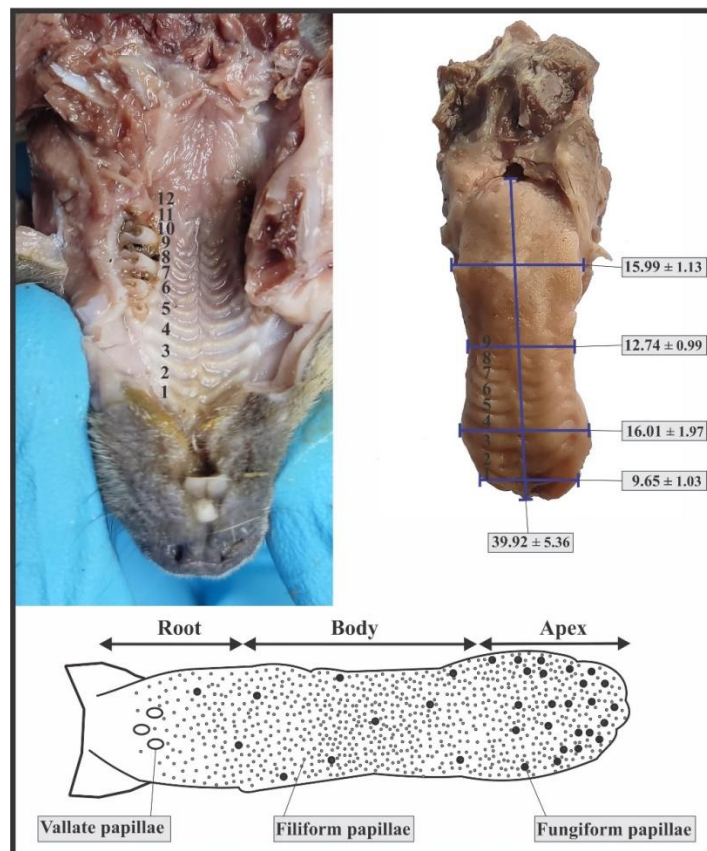
The results of the anatomical study of the tongue in the ground squirrel show its foamy shape, which can be divided into

three parts: apex, body and root. The morphometric parameters of the tongue are presented in Figure 2. The depressions on the tongue were complementary to the hard palatine

ridge. The number of tongue ridge was 9 and the number of palatine ridge was 12. The midline and lingual cartilage were not observed in this animal (Fig. 2).



**Figure 1:** Five died ground squirrels collected in the early March of 2022 to the late June of 2023 (from Zarin-Roud city (Khodabandeh- Zanzan province) to Kabudarahang city (Hamadan Province)).

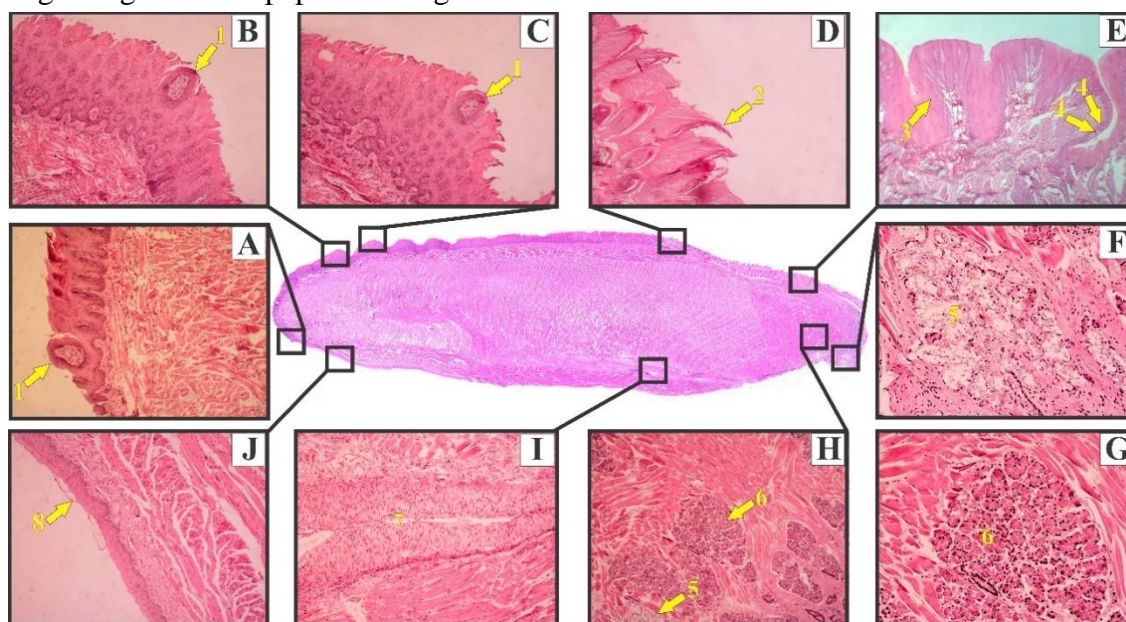


**Figure 2:** Anatomical structure of the hard palate and tongue in ground squirrel. The number of tongue ridge was 9 and the number of palatine ridge was 12.

Many filiform and fungiform papillae and three circumvallate papillae were visible on the dorsal surface of the tongue. Also, the number of fungiform papillae was more in the apex than the body part, and the lowest number of fungiform papillae was visible in the root part.

The results of the histological study showed that both the dorsal and ventral surface of the tongue are covered with stratified squamous epithelium, and the dorsal surface has a more degree of keratinization than the ventral surface. Also, the study of the distribution of tongue papillae showed that the beginning of the papillae in ground

squirrels is from the cranial-ventral part of the tongue. Most of the surface of the tongue is covered by filiform papillae. The tip of these papillae is bent towards the posterior part of the tongue and a thin layer of keratinized layer covers it (Fig. 3, part D). Between the filiform papillae, fungiform papillae without taste buds were visible from the apex of the tongue to the root. It seems that these fungiform papillae were visible in two kinds, some of these papillae were prominent from the surface of the tongue, but some of these papillae to be deep in the tissue of the tongue (Fig. 3, Parts A to C and Fig. 4).



**Figure 3:** Cross section of the ground squirrel tongue. H&E staining. The beginning of lingual papillae starts from the cranial-ventral part of the tongue.

**A:** fungiform papillae (arrow No. 1) protruding from the surface of the tongue. **B and C:** fungiform papillae (arrow No. 1) indented on the tongue surface. **D:** filiform papillae protruding from the surface of the tongue (arrow No. 2). **E:** circumvallate papillae (arrow No. 3) with taste buds (arrow No. 4). **F, G and H:** mucous (arrow No. 5) and serous (arrow No. 6) glands of the tongue accumulated at the root. **I:** Sublingual vein in ground squirrel tongue (arrow No. 7). **J:** stratified squamous epithelium with a little amount of stratum corneum covering the ventral part of the tongue (arrow No. 8).

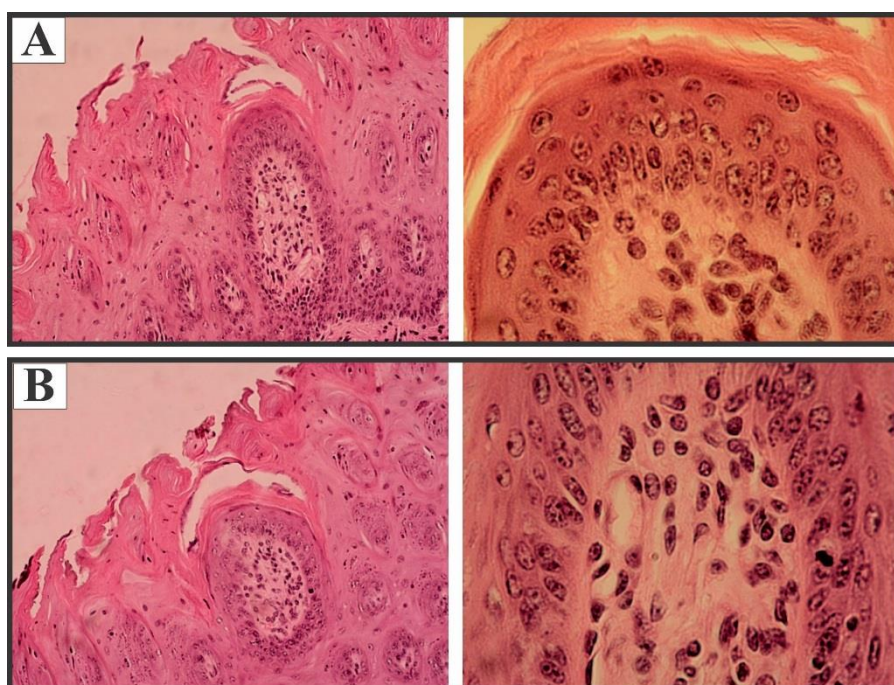
Three numbers of circumvallate papillae were also visible at the root of the

tongue, which were the only chemical papillae of the tongue in this rodent (Fig.

3, part E). In the investigation of the histological structure of the tongue of this animal, no lenticular papillae were observed. In the structure of this animal tongue, due to the absence of mucosal muscle, mucosa and submucosa are unified and mainly consist of dense connective tissue and skeletal muscles with different orientations. The mucosa-submucosa of the tongue was devoid of any glands in the apex and body, and the

accumulation of glands could be seen only in the root of the tongue. These glands were separate serous and mucous units (Fig. 3, parts F, G and H). The sublingual vein was clearly visible in the ventral part of the tongue structure of this rodent (Fig. 3, part I).

The fungiform papillae in the tongue of this rodent did not have taste buds (Fig. 4).



**Figure 4:** fungiform papillae embedded in the tongue tissue of ground squirrel. H&E staining. Magnification  $\times 100$  and  $\times 400$ . No taste bud was observed in the structure of these papillae.

## Discussion

The oral cavity is surrounded by the tongue. Usually, in all animals, the surface of the tongue is covered with a stratified squamous epithelium (keratinized or non-keratinized). The dorsal surface of the tongue in most animals is covered with prominent structures called lingual papillae (Sonntag, 1924).

The structure of the tongue in the ground squirrel showed that its shape is foaming and consists of three parts: apex, body and root, and two dorsal and ventral surfaces. The entire dorsal and ventral surface of the tongue was covered with keratinized stratified squamous epithelium. Most of the dorsal surface of the tongue is covered by filiform papillae. Among the filiform papillae, fungiform papillae were visible from the

apex to the root of the tongue. The number of three circumvallate papillae was also observed in the root of the tongue. The major part of the mucosa-submucosa structure of the tongue was composed of skeletal muscles with different orientations. While tongue glands of serous and mucous type were visible only at the root of the tongue. These glands were located in the form of separate serous and mucous secretory units and located between of the skeletal muscles.

The width of the tongue body in rodents is usually the same or slightly smaller than the other parts of the tongue. In the investigation of the tongue of *Sciurus vulgaris*, this organ is reported to be relatively long and narrow (Ünsaldi, 2010). The tongue of the African giant rat is reported to resemble a spatula, similar to the tongue of a horse (Sisson and Grossman, 1953).

Anatomical investigation of the tongue of Jentink's flying squirrel (*Hylopetes platyurus*) (Wihadmadayami *et al.*, 2020), rats (Harłajczuk *et al.*, 2018), Japanese giant flying squirrels (Emura *et al.*, 1999), Iranian flying squirrels (Sadeghinezhad *et al.*, 2016), Patagonian cavy (Emura *et al.*, 2011) and long-eared hedgehogs (Parchami *et al.*, 2018) have shown that the tongue in these rodents is wide and flat and consists of three separate parts: the apex, the body (corpus) as the main part of the tongue and the root (or radix linguae) which is the caudal part of the tongue and connected to the larynx.

The tongue of the long-eared hedgehog (*Hemiechinus auritus*) has a

rectangular shape with a clearly elongated body, and the root of the tongue is also widened (Parchami *et al.*, 2018). Also, the shape of the apex in rat's tongue is reported to be round (Iwasaki *et al.*, 1997). In the study of the anatomical structure of the tongue of the European Hedgehog (*Erinaceus europaeus*), its shape is reported to be a flat cylinder with a wider apex. The division of the tongue into three parts of the apex, body and root was also present in other reports (Akbari *et al.*, 2018). The anatomically results of the present study were consistent with previous studies.

The median sulcus on the tip of the tongue of many rodents, including mice, spotted rats, rats, bank voles, and American beavers, has been reported as a special feature with different lengths and widths (Jackowiak and Godynicki, 2005; Shindo *et al.*, 2006) while rodents such as: Guinea pig (Kobayashi, 1990), Patagonian cavy (Emura *et al.*, 2011), capybara (Watanabe *et al.*, 1997), rock cavy (Santos *et al.*, 2015), Brandt's Hedgehog (Goodarzi and Azarhoosh, 2016), European hedgehog (*Erinaceus europaeus*) (Akbari *et al.*, 2018) do not have a median groove or sulcus medianus. In the present study, the median groove was not observed on the ground squirrel's tongue.

The results of the present study on the ground squirrel's tongue showed that the shape and arrangement of tongue papillae is similar to other squirrel species (Sadeghinezhad *et al.*, 2016); but the fungiform papillae were visible in two kind, prominent and embedded in

the tongue tissue. On the other hand, in the present study, fungiform papillae lacked taste buds.

The review of previous studies indicates that the epithelium of the tongue in the dorsal and ventral parts is of the stratified squamous epithelium type, and depending on the animal species and food habits, the ventral surface may be keratinized or non-keratinized (Herrel *et al.*, 2005; Hussein and Al-Asadi, 2010). But the dorsal surface in all studied species is of keratinized stratified squamous epithelium (Wassif, 2001; Crole and Soley, 2009; Igboke and Okolie, 2009; Jamniczky *et al.*, 2009). In the present study, the entire mucous membrane of the dorsal and ventral surface of the tongue was covered with keratinized stratified squamous epithelium.

Investigation the structure of filiform papillae in other studies shows that these papillae are bent towards the root of the tongue, which is mostly due to the function of this type of papillae to guide food to the pharynx (Trzcielińska-Lorych *et al.*, 2009; Karan *et al.*, 2011). In the present study, the filiform papillae were bent towards the root of the tongue, which is consistent with other reports. Also, in all the available reports in this field, filiform papillae constitute the most lingual papillae in terms of number and extent of distribution (Iino and Kobayashi, 1988; Iwasaki, 2002; Jackowiak and Godynicki, 2005), which was consistent with the results of the present study.

The distribution of fungiform papillae in the present study started from

the cranial-ventral part of the tongue and continued to its caudal part. This distribution of fungiform papillae was consistent with other reports on squirrel and hedgehog species (Parchami *et al.*, 2018; Sadeghinezhad *et al.*, 2018). Although the presence of taste buds in fungiform papillae has been reported in most studies (Herrel *et al.*, 2005; Parchami *et al.*, 2018; Sadeghinezhad *et al.*, 2018), but in the present study, these papillae lack taste buds, were not consistent with previous studies.

In the present study, the number and structure of circumvallate papillae, no significant difference was observed with other squirrel species. Also, in the study of the tongue glands of the Iranian squirrel, it has been reported that these glands are present in the root and body and with two serous and mucous natures (Sadeghinezhad *et al.*, 2018), while in the present study, the tongue glands can only be found in the root of the tongue. They were seen, but in terms of secretory units, they consisted of two types of serous and mucous units.

In many reports, the presence of leaf-shaped papillae on the tongue surface has also been reported (Jabbar, 2014). In the present study, these papillae were not observed in the structure of the tongue, so it was not consistent with the previous studies.

Finally, it can be concluded that the structure of the tongue in the ground squirrel is more similar to the Iranian squirrel, with the difference that the fungiform papillae observed in the present study lack taste buds and some of these papillae are completely

embedded in the tongue tissue. Also, the median groove and leaf-shaped papillae are absent in the ground squirrel's tongue, and the tongue glands are present only at the root of the tongue.

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