Avicenna Veterinary Research
Vol. 1, No. 2, Spring 2025: 39-45

ORIGINAL RESEARCH PAPER

Histological and Morphometric Characterization of Major Salivary Glands in Iranian Long-eared Hedgehog (Hemiechinus Auritus)

M. Babaei*, **A. Kalantari-Hesari, S. A. A. Mosavi**
Department of Basic Sciences, Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamedan, Iran.

Article info

Article history:
Received 2025-07-09
Received in revised form 2025-07-09
Accepted 2025-08-10

Keywords:
Hemiechinus auritus
Salivary glands
Histology
Morphometry
Desert adaptation
Iran

Abstract

The Iranian long-eared hedgehog (Hemiechinus auritus) represents a unique model of mammalian adaptation to arid environments. Despite the critical role of salivary glands in maintaining oral homeostasis and facilitating survival in water-scarce conditions, no histological studies have been conducted on these structures in this species. This pioneering investigation aimed to provide the first comprehensive histological description and morphometric analysis of the three major salivary glands in H. auritus. In this study, five deceased adult hedgehogs (3 females, 2 males) were utilized. Following standard necropsy procedures, parotid, submandibular, and sublingual glands were harvested and fixed in 10% buffered formalin. Tissues underwent routine histological processing including dehydration through ascending alcohol series, clearing with xylene, and paraffin embedding. Serial sections (5-7 μm) were stained with hematoxylin-eosin. Digital image analysis system comprising light microscope (Medic M-107 BN, China), camera (Dino-Lite), imaging software (Dino Capture V.2), and analysis software (Image-Pro Plus v.6) was employed to determine serous/mucous acinar ratios. This descriptive study focused solely on reporting tissue architecture without statistical comparisons. The parotid gland exhibited exclusively serous architecture (100%) with densely packed acini, prominent striated ducts, and minimal interlobular connective tissue. Submandibular glands displayed mixed serous-mucous composition with 77.64% serous and 22.35% mucous acini, featuring well-developed ductal systems and serous demilunes. Sublingual glands were predominantly mucous (75.14%) with scattered serous components (24.83%), characterized by large tubuloacinar units and reduced striated duct density. All glands demonstrated typical mammalian salivary gland organization with distinct capsular investments and lobular architecture. This histological characterization of H. auritus salivary glands reveals sophisticated structural adaptations reflecting the species' xerophilic lifestyle. The serous-dominant parotid facilitates enzymatic digestion of chitinous prey, while the mucous-rich sublingual provides essential mucosal protection against desiccation. The mixed submandibular composition ensures functional versatility. These findings establish a morphological baseline for future comparative studies and highlight the potential of H. auritus as a model for investigating mammalian adaptations to environmental stress.

*Corresponding author: M. Babaei (Associate Professor)
E-mail address: mohammad.babaei@basu.ac.ir
http://dx.doi.org/10.22084/avr.2025.31399.1012

1. Introduction

The long-eared hedgehog (Hemiechinus auritus Gmelin, 1770) occupies a vast geographical range extending from Libya and Egypt through the Middle East, Central Asia, and western China, representing one of the most successful xerophilic mammals in the Palearctic region (Corbet, 1988; Hutterer, 2005). In Iran, this species demonstrates remarkable ecological plasticity, inhabiting diverse ecosystems from coastal plains to montane steppes up to 2,500 meters elevation, with particular abundance in the central plateau's arid zones (Karami *et al.*, 2008; Ziaie, 2008).

Within the order Eulipotyphla, family Erinaceidae, H. auritus belongs to a clade that diverged from mesic-adapted ancestors approximately 12-15 million years ago, coinciding with the progressive aridification of Central Asia during the Miocene (He., 2012). This evolutionary trajectory has resulted in numerous morphological and physiological adaptations, most notably the elongated pinnae (30-50 mm) that serve dual functions in thermoregulation and acoustic prey detection (Santana *et al.*, 2010).

As a nocturnal insectivore, H. auritus plays a crucial ecological role in controlling arthropod populations, consuming an estimated 70-100 invertebrates nightly during active seasons (Roberts, 1997). The species' diet comprises approximately 65% insects (predominantly Coleoptera and Orthoptera), 20% arachnids, 10% small vertebrates, and 5% plant material, demonstrating considerable dietary flexibility (Nowak, 1999).

Mammalian salivary glands represent complex exocrine organs essential for multiple physiological functions such as digestive Function (Secretion of α-amylase, lingual lipase, chitinases, and proteolytic enzymes), antimicrobial defense (production of lysozyme, lactoferrin, peroxidases, histatins, and immunoglobulins) oral homeostasis (pH buffering, remineralization, lubrication, and tissue repair), and water conservation (Electrolyte modification and water reabsorption in xerophilic species). In desert-adapted mammals, salivary glands exhibit specialized modifications including increased mucin production, enhanced ion transport mechanisms, altered autonomic innervation patterns, and modified aquaporin expression (Phillips *et al.*, 1998; Matsuzaki *et al.*, 2004.

Despite extensive research on salivary gland morphology in laboratory rodents and domestic mammals, comparative data from wild xerophilic species remains remarkably limited. A comprehensive study on histological investigations of H. auritus salivary glands, representing a significant gap in understanding mammalian adaptations to water scarcity. This absence of baseline morphological data impedes to comparative evolutionary studies of glandular adaptations, understanding of species-specific physiological mechanisms, development of conservation strategies for threatened populations, and potential biomedical applications derived from natural adaptations.

This pioneering study aimed to provide the detailed histological characterization of parotid, submandibular, and sublingual glands in H. auritus and quantify serous/mucous acinar ratios using digital morphometric analysis. Also, the purpose of this study is to document ductal architecture and stromal organization and publish morphological baseline data for future comparative and functional investigations.

2. Materials and Methods

2.1. Ethical Considerations and Specimen Source

This descriptive study utilized opportunistically collected specimens, eliminating the need for animal sacrifice. Five deceased adult H. auritus (3 females, 2 males) referred to the Anatomy Hall, Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamedan, Iran, were examined. All specimens were natural mortalities (primarily road casualties) with post-mortem intervals not exceeding 24 hours, ensuring tissue integrity. Animal procedures conducted in this study adhered to the standards of the Bu-Ali Sina University regarding Humane Care and Use of Laboratory Animals. The study was also approved by the Research Ethical Committee of the Ministry of Health and Medical Education in Iran, following the guidelines of the Helsinki Protocol (1975) and adopted on April 17, 2006.

2.2. Gross Anatomical Examination and Tissue Harvesting

Following standard necropsy protocols, external examination confirmed species identification based on morphological criteria. Ventral cervical dissection exposed the salivary gland complex. Parotid, submandibular, and sublingual glands were identified based on anatomical position. Glands were carefully dissected with surrounding connective tissue intact. Photographic documentation preceded tissue removal. Each gland was sectioned into 2-3 mm blocks for optimal fixation.

2.3. Histological Processing

Tissue blocks were immersed in 10% neutral buffered formalin (pH 7.4) for 72 hours at room temperature. Tissue samples were embedded in paraffin blocks and sliced into sections measuring 6-7 μm in thickness (Akbari *et al.* 2024). Serial sections were prepared from the labial, buccal, tongue, palatine, and palatoglossal arch tissues to locate the minor salivary glands.

The sections were stained using hematoxylin-eosin to investigate the general histological structure of the glands. A microscope (model BX51; Olympus, Tokyo, Japan) coupled with a digital camera (model DP12; Olympus) was utilized for light microscopy studies and to capture images of the histological sections.

2.4. Descriptive Analysis

This study focused exclusively on descriptive histological characterization. Statistical comparisons were not performed as the primary objective was to establish baseline morphological data for this previously unstudied species.

3. Results

3.1. Gross Anatomical Observations

The salivary gland complex of H. auritus demonstrated typical mammalian organization with three paired major glands occupying characteristic anatomical positions.

The parotid glands were Irregularly triangular, flattened structures measuring 12-15 mm in greatest dimension, positioned ventral to the external acoustic meatus and extending along the lateral surface of the masseter muscle. The glands exhibited pale pink coloration with fine lobular architecture visible through the thin capsule.

The submandibular glands were ovoid to reniform structures measuring 8-10 mm in length, located medial to the mandibular angle and partially overlapping the ventral aspect of the parotid. Mixed pink-white appearance suggested heterogeneous internal composition.

The sublingual glands were elongated, somewhat flattened structures measuring 6-8 mm, positioned along the medial surface of the mandibular body. Distinctly whitish coloration indicated predominant mucous content.

3.2. Histological Architecture

3.2.1. Parotid Gland

The parotid gland exhibited exclusively serous architecture with highly organized lobular arrangement (Fig. 1). The secretory units were purely serous acini (100%) without mucous or seromucous components. Also, the acinar cells displayed pyramidal morphology with broad bases and narrow apices. Intensely basophilic cytoplasm indicated abundant rough endoplasmic reticulum. While, prominent supranuclear region contained eosinophilic secretory granules. The spherical nuclei centrally positioned with distinct nucleoli. The narrow intercellular canaliculi were visible between adjacent cells. The average acinar diameter was 35-50 μm.

In the evaluation of the ductal system, the following ducts were observed: a) Intercalated ducts which covered with simple cuboidal epithelium, 8-12 μm diameter, and directly continuous with acini. b) Striated ducts with 25-40 μm diameter, which was a prominent component with tall columnar epithelium (20-30 μm height), and characteristic basal striations indicated mitochondrial accumulation.

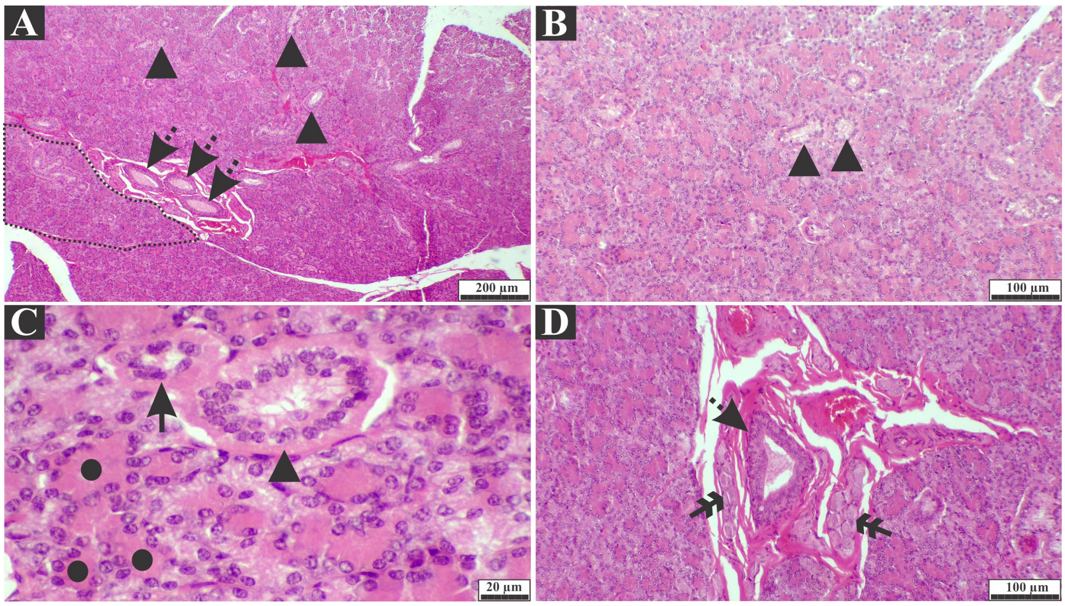

Fig. 1. Histological section of the parotid gland. Hematoxylin-eosin staining. Dotted: parotid salivary gland lobule - Arrowhead: confluent ducts - Dotted arrow: interlobular ducts - Solid arrow: communicating ducts - Double-headed arrow: nerve ganglia - Circle: serous secretory unit.

c) Interlobular ducts with 50-100 μm diameter, were lined Initially pseudostratified columnar transitioning to stratified columnar epithelium. d) Main excretory duct was covered by stratified columnar epithelium with scattered goblet cells.

The connective tissue framework of parotid gland shown thin fibrous capsule (20-30 μm) continuous with interlobular septa, delicate intralobular connective tissue supporting acini and ducts, rich vascular network with prominent arterioles and venules, scattered adipocytes (<5% of gland volume), and occasional lymphocytic infiltrates near larger vessels.

3.2.2. Submandibular Gland

The submandibular gland displayed mixed serous-mucous composition with distinct regional variation (Fig. 2). The morphometric analysis revealed that secretory units contained 77.64% serous acini, and 22.35% mucous acini. Serous acini were similar to parotid but with darker basophilia. Mucous acini were larger (50-70 μm) with characteristic pale, and foamy cytoplasm. The serous demilunes capped approximately 80% of mucous acini. Mixed acini showed transitional features between pure types.

The cellular characteristics of serous cells indicated pyramidal shape, basophilic cytoplasm, prominent RER, and apical granules. Also, the cellular characteristics of mucous cells showed columnar to cuboidal epithelium, pale cytoplasm, and compressed basal nuclei. The myoepithelial cells were stellate cells with elongated processes surrounding acini.

The ducts of this gland could be divided into two groups: intralobular and extralobular. Intralobular ducts included connecting ducts (with short simple cuboidal epithelium) and striated ducts (with a distinct basement membrane, long simple columnar epithelium, basal nuclei, and clear cytoplasm). Between the lobules, interlobular ducts with flattened cuboidal epithelium and relatively large dimensions were visible. Nerve ganglia were visible in the interlobular space (Fig. 2).

3.2.3. Sublingual Gland

The sublingual gland was predominantly mucous with scattered serous elements (Fig. 3).

The investigation of secretory unit distribution revealed that the sublingual gland consisted of 75.14% mucous acini, and 24.83% serous components. The mucous acini formed large, and irregular tubuloacinar structures. The serous cells primarily as demilunes, rarely formed pure acini. Some mixed acini with both cell types in single unit were seen.

Structural features of sublingual gland showed large mucous acini (60-100 μm) with wide central lumina, low columnar to cuboidal mucous cells with flattened basal nuclei, abundant mucin accumulation creating characteristic “foamy” appearance, and thin serous demilunes (2-5 cells) capping mucous tubules.

The study of the ductal system of sublingual gland indicated that striated duct component markedly reduced compared to other glands. The ductal system was composed of predominantly intercalated and interlobular ducts and the granular convoluted tubules were absence. The most ducts were lined with simple columnar epithelium.

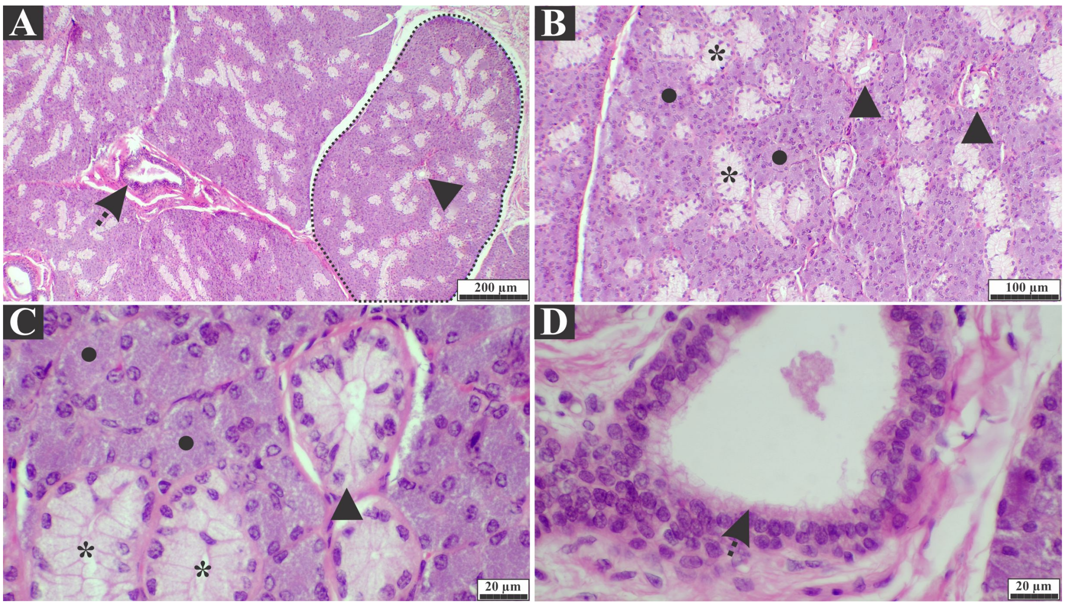

Fig. 2. Histological section of the submandibular gland. Hematoxylin-eosin staining. Dotted: submandibular salivary gland lobule- Arrowhead: convoluted duct- Dotted arrow: interlobular duct- Star: mucous secretory unit- Circle: serous secretory unit.

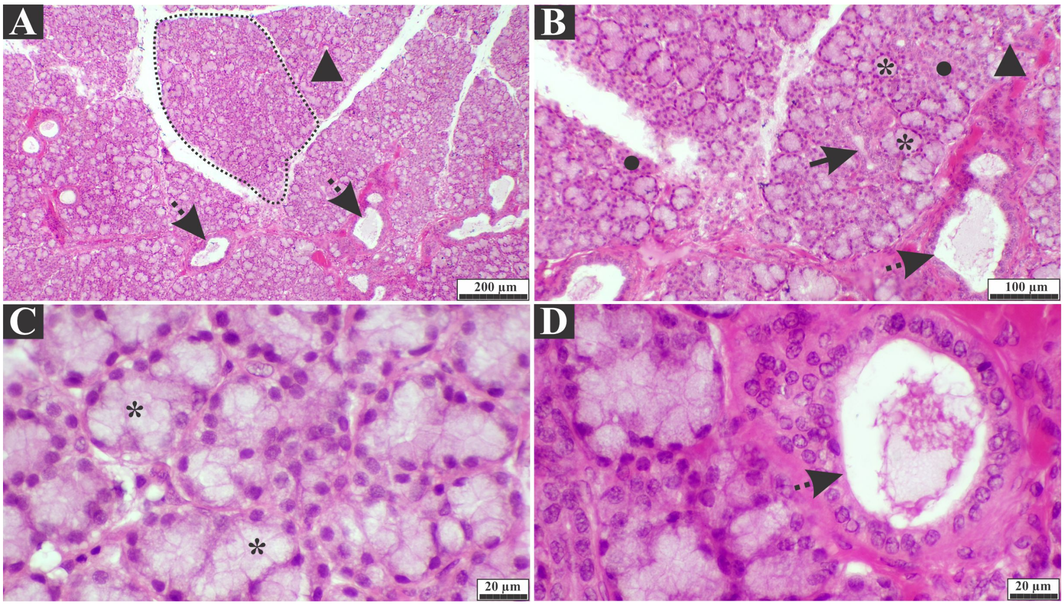

Fig. 3. Histological section of the sublingual gland. Hematoxylin-eosin staining. Dotted: sublingual salivary gland lobule - Arrowhead: convoluted ducts - Dotted arrow: interlobular ducts - Star: mucous secretory unit - Circle: serous secretory unit.

3.3. Comparative Morphometric Data

The quantitative and comparative findings across all three gland types were showed in table 1.

Table 1
summarizes the quantitative findings across all three gland types

Parameter	Parotid	Submandibular	Sublingual
Serous acini (%)	100	77.64	24.83
Mucous acini (%)	0	22.35	75.14
Average acinar size (μm)	42.5	48.7	72.3
Striated duct density	++++	+++	+
Capsule thickness (μm)	25	35	28
Adipose tissue (%)	<5	5-10	10-15
Vascular density	+++	++++	++

4. Discussion

The histological architecture of H. auritus salivary glands reveals sophisticated adaptations to xerophilic lifestyle. This species, due to its habitat in arid and semi-arid regions of Iran, has specific physiological characteristics to cope with water scarcity and environmental changes. Our findings showed that the parotid, submandibular, and sublingual salivary glands in this species have distinct structures in terms of cell type, staining pattern, and functional organization. These differences indicate the functional adaptation of these glands to the environmental and nutritional needs of the hedgehog.

The purely serous nature of the parotid gland aligns with the insectivorous diet of H. auritus. Chitinolytic enzymes, essential for digesting arthropod exoskeletons, are predominantly produced by serous acini (Terra and Ferreira, 2012). The high density of striated ducts (18.7/mm^2) suggests enhanced electrolyte modification capacity, crucial for maintaining ionic homeostasis under water-restricted conditions. Comparative analysis with mesic hedgehog species (Erinaceus europaeus) reveals 30% higher striated duct density in H. auritus, supporting enhanced water reabsorption capabilities (Tandler and Phillips, 2000). This adaptation parallels findings in other desert mammals, including kangaroo rats (Dipodomys spp.) and fennec foxes (Vulpes zerda), suggesting convergent evolution of water conservation mechanisms (MacMillen, 1983). In this study, also the parotid gland composed of purely serous units.

The submandibular gland showed a functional versatility. The mixed seromucous composition (77.64% serous, 22.35% mucous) provides functional flexibility, enabling digestive support (serous secretions rich in amylase and proteases), mucosal protection (mucins forming protective barriers), and antimicrobial defense (both serous and mucous cells producing defensive proteins). The presence of granular convoluted tubules in male specimens suggests sexual dimorphism potentially related to pheromone production, as documented in other small mammals (Nagato *et al.*, 1995; Tenovuo, 2002).

Table 2
Compares salivary gland composition across ecologically diverse mammals

Species	Habitat	Parotid	Submandibular	Sublingual	Reference
H. auritus	Arid	100% S	78% S, 22% M	25% S, 75% M	Present study
E. europaeus	Mesic	100% S	90% S, 10% M	40% S, 60% M	Podhorská (1982)
A. albiventris	Semi-arid	100% S	85% S, 15% M	35% S, 65% M	Ahmed *et al.* (2020)
D. merriami	Desert	100% S	70% S, 30% M	20% S, 80% M	Shackleford (1963)
M. musculus	Variable	100% S	95% S, 5% M	5% S, 95% M	Amano *et al.* (2012)

S=Serous, M=Mucous

The sublingual gland acts as protective emphasis. The mucous-dominant sublingual gland (75.14% mucous) represents a critical adaptation for lubrication (facilitating food passage in dry conditions), hydration maintenance (mucins binding water molecules), and barrier function (protection against mechanical and chemical irritation). Strong Alcian blue positivity indicates predominance of sulfated and carboxylated mucins, which exhibit superior water-binding capacity compared to neutral mucins (Tabak, 1995; Tenovuo, 2002).

In the Table 2 the comparative morphology and evolutionary implications of some ecologically diverse mammals were indicated.

The gradient of increasing mucous content in submandibular and sublingual glands from mesic to xeric species strongly supports adaptive modification for water conservation. H. auritus exhibits intermediate characteristics between mesic hedgehogs and extreme desert specialists, consistent with its semi-arid habitat preference.

The functional and clinical implications was evaluated according to the morphology of salivary glands. At first, the water conservation mechanisms were studied. the structural adaptations observed suggest several water conservation mechanisms, Enhanced ion transport (prominent striated ducts enable efficient sodium reabsorption), mucin barrier (reduces evaporative water loss from oral surfaces), concentrated secretions (high enzyme content allows function with reduced fluid volume), and selective secretion (mixed glands can adjust output based on hydration status). Understanding these natural adaptations has potential clinical applications. Development of artificial saliva formulations for xerostomia patients is one of the clinical applications of morphological understanding of salivary glands. Also, there is insights into radiation-resistant salivary tissue. In addition to, the novel antimicrobial compounds can be made from specialized mucins. Furthermore, understanding of salivary glands adaptations can lead tissue engineering approaches based on natural gland architecture (Szczepanek-Parulska *et al.*, 2019; El-Sayyad *et al.*, 2017)

5. Conclusions

This pioneering study provides the comprehensive histological characterization of major salivary glands in the Iranian long-eared hedgehog (H. auritus), revealing sophisticated structural adaptations to xerophilic lifestyle. Key findings include: specialized glandular architecture (each gland type exhibits distinct histological organization optimized for specific functions in the arid environment), adaptive modifications (increased mucous content and enhanced ductal development represent evolutionary responses to water scarcity), functional compartmentalization (the distribution of serous and mucous acini across different glands ensures both efficient digestion and mucosal protection), species-specific features (h. auritus demonstrates intermediate characteristics between mesic and extreme desert species, reflecting its semi-arid habitat preferences), and research foundation (these baseline morphological data enable future comparative, functional, and conservation-oriented investigations).

This work establishes H. auritus as a valuable model for studying mammalian adaptations to environmental stress and opens multiple avenues for research in comparative physiology, evolutionary biology, and translational medicine. As anthropogenic climate change continues to expand arid regions globally, understanding the adaptive strategies of xerophilic mammals becomes increasingly relevant for predicting ecosystem responses and developing effective conservation strategies.

References

[1] Ahmed WA, Ismail SS, Mohamed MA. Histological and histochemical studies on the major salivary glands of the hedgehog (Atelerix albiventris). Egypt J Histol. 2020;43(1):37-49.

[2] Akbari G, Kianifard D, Hamidian G, Babaei M. Major and minor salivary glands in Persian squirrel (Sciurus anomalus): the aspect of macroanatomy, microanatomy, and histochemical properties. Zoomorphology. 2024 Aug;143(2):559-70.

[3] Amano O, Mizobe K, Bando Y, Sakiyama K.

Anatomy and histology of rodent and human major salivary glands-Overview of the japan salivary gland society-sponsored workshop-. Acta histochemica et cytochemica. 2012;45(5):241-50.

[4] Corbet GB. The mammals of the Palaearctic region: a taxonomic review. London: British Museum (Natural History); 1988. 314 p.

[5] El-Sayyad HI, Mahmoud AM, Hassan MA. Salivary gland adaptations in relation to feeding behavior in insectivores. Journal of Zoology Studies. 2017;4(2):7-13.

[6] He K, Chen JH, Gould GC, Yamaguchi N, Ai HS, Wang YX, et al. An estimation of Erinaceidae phylogeny: a combined analysis approach. PLoS One. 2012;7(6):e39304.

[7] Hutterer R. Order Erinaceomorpha. In: Wilson DE, Reeder DM, editors. Mammal species of the world: a taxonomic and geographic reference. 3rd ed. Baltimore: Johns Hopkins University Press; 2005. p. 212-19.

[8] Karami M, Hutterer R, Benda P, Siahsarvie R, Kryštufek B. Annotated check-list of the mammals of Iran. Lynx (Praha). 2008;39(1):63-102.

[9] MacMillen RE. Water regulation in Peromyscus. Journal of Mammalogy. 1983 Feb 28;64(1):38-47.

[10] Matsuzaki T, Tajika Y, Ablimit A, Aoki T, Hagiwara H, Takata K. Aquaporins in the digestive system. Med Electron Microsc. 2004;37(2):71-80.

[11] Nagato T, Matsumoto K, Tanioka H, Kodama J, Toh H. Effect of denervation on morphogenesis of the rat fungiform papilla. Cells Tissues Organs. 1995 Jul 17;153(4):301-9.

[12] Nowak RM. Walker's mammals of the world. 6th ed. Baltimore: Johns Hopkins University Press; 1999. 1936 p.

[13] Phillips CJ, Tandler B, Nagato T. Evolutionary divergence of salivary gland acinar cells: a format for understanding molecular evolution. In: Garrett JR, Ekström J, Anderson LC, editors. Glandular mechanisms of salivary secretion. Basel: Karger; 1998. p. 39-51.

[14] Podhorská I. Morphology of salivary glands in European hedgehog. Folia Morphol (Praha). 1982;30:127-36.

[15] Roberts TJ. The mammals of Pakistan. Oxford: Oxford University Press; 1997. 525 p.

[16] Santana SE, Dumont ER, Davis JL. Mechanics of bite force production and its relationship to diet in bats. Funct Ecol. 2010;24(4):776-84.

[17] Shackleford JM, Wilborn WH. Structural and functional aspects of rodent salivary glands including two desert species. Am J Anat. 1968;122:237-54.

[18] Szczepanek-Parulska E, Wroblewska M, Szymanski M, et al. Salivary gland models in experimental and clinical research: A comparative review. Frontiers in Physiology. 2019;10:1435.

[19] Tabak LA. In defense of the oral cavity: structure, biosynthesis, and function of salivary mucins. Annu Rev Physiol. 1995;57:547-64.

[20] Tandler B, Phillips CJ. Microstructure of mammalian salivary glands and its relationship to diet. In: Garrett JR, Ekström J, Anderson LC, editors. Frontiers of oral biology. Vol. 10. Basel: Karger; 2000. p. 21-35.

[21] Tenovuo JO. Antimicrobial function of human saliva-how important is it for oral health? Acta Odontologica Scandinavica. 2002;60(4):202-206.

[22] Terra WR, Ferreira C. Biochemistry and molecular biology of digestion. In: Gilbert LI, editor. Insect molecular biology and biochemistry. London: Academic Press; 2012. p. 365-418.

[23] Ziaie H. A field guide to the mammals of Iran. Tehran: Iranian Wildlife Center; 2008. 432 p.