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Anatomical and Histological studies of the Tongue and Histochemical features of lingual glands in kingfisher (*Halcyon smyrnensis*) and hoopoe (*Upupa epops*) with different Feeding Behaviors

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The current work investigated the anatomical and histological structure of the tongue as well as the histochemical structures of the lingual salivary glands in the carnivore kingfisher (*Halcyon smyrnensis*) and the insectivore hoopoe (*Upupa epops*). The tongue is muscular elongated pointed organ, not bifid in both species, occupy about posterior third of the bill in kingfisher and about the posterior quarter of the bill in hoopoe. It is longer and bigger in kingfisher than in hoopoe and slightly more cylinder in hoopoe. Two of backwardly directed conical papillae in each side are found at the end of the tongue corpus of kingfisher. While in hoopoe a set of a double row of large conical papillae are located at the end of the lingual corpus and directed backwardly. Histologically, stratified keratinized epithelia in the dorsum of the tongue in kingfisher and non-keratinized in hoopoe were delineated; spine-like caudally directed lingual papillae are found in the posterior part of the dorsal lingual epithelium in kingfisher only. The tongue in both species contained muco-serous glands that are distributed in the corpus and radix, while the apex is devoid of glands. These lingual glands anteriorly are mostly of compound alveolar type and compound tubule-alveolar posteriorly. A few of taste buds were noticed only above the lingual glands at the radix of the tongue in both species. Histochemically; the lingual glands revealed both neutral and acidic nature of mucin secretion in both species, the acidic carbohydrate contents implies each of sialomucins and sulphomucins. The acidity of the lingual gland was mostly increased form the corpus toward the radix in both birds.

Keywords: Kingfisher, Hoopoe, Tongue, Histology, Lingual glands, Histochemistry

INTRODUCTION

All birds are adapted to their different environments with respect to food resources; they have different feeding behavior, with corresponding differences in the size and structure of their lingual apparatus (McLelland, 1979). The histological structure and histochemical nature of the salivary glands have been described in domestic poultry species (Taib and Jarrar, 1998; Arthitvong et al. 1999; Liman et

al. 2001; Kum, 2002), and some wild birds (Jackowiak and Godynicki, 2005; Al-Mansour and Jarrar, 2007; Dehkordi et al. 2010).

Kingfishers and hoopoes compose a group of small to medium sized bright colored birds belonging to order Coraciiformes. Kingfishers is belonging to sub order Alcedines which contains three families, Alcedinidae, Halcyonidae and Cerylidae, they have a cosmopolitan distribution occupying a wide range of habitats (Knowles and

Nitchen, 1995; Woodall, 2001). The tree Kingfisher (*Halcyon smyrnensis*, Linnaeus, 1758) of the present study is a noisy bird belonging to the family Halcyonidae (Tharwat, 1997); it is widely distributed and estimated to be the world's third most common kingfisher (Fry, 1980). It is a carnivorous (piscivorous) bird, eats mostly small fish, aquatic arthropods as well as Crustacea including fresh water shrimps and crabs (Campos et al. 2000). It hovers above the water to search for its prey then dives into the water to grab and catch it (Vilches et al. 2012). Kingfishers inhabit rain forests, deciduous woodlands, savannahs, arid areas, mangrove swamps, freshwater swamps, lakes, sea shores, river valleys and estuaries. Kingfishers are diurnal, highly mobile, wide ranging and are relatively easy to observe (Knowles and Nitchen, 1995; Amat and Green, 2010).

Hoopoes (*Upupa epops*) are one of the most distinctive birds in the world; it is a migratory species, widely spread throughout most of Europe, Asia and North Africa (Kristin, 2001; Thomas et al. 2008). Most European and north Asian birds migrate to the tropics in winter, while the African populations are sedentary all year (Thomas et al. 2008). The hoopoe has traditionally been treated as a single species within the order Coraciiform, although some authors have suggested separating the hoopoe into two or more species and even its own order, Upupiformes (Hoyo et al. 2001). In the wild, hoopoes are almost completely insectivorous and use their long beak to probe into the ground for grubs and other invertebrates (Hoyo et al. 2001).

Characteristic features of the bird's tongue include the distinct median sulcus, convex lateral parts, different types of papillae, distribution of lingual glands and the crest of the backward giant conical papillae between the tongue's body and root must be taken into consideration (Jackowiak and Godynicki, 2005; Emura, 2008). Such modifications result in differing tongue's mobility and ability to slide out, extracting and manipulating food in the beak cavity (Emura et al. 2008). The present study was carried out to illustrate the anatomical and histological structure of the tongue; in addition to investigate the histochemical features of the lingual glands in each of kingfisher (*Halcyon smyrnensis*) and hoopoe (*Upupa epops*).

MATERIALS AND METHODS

The study was carried out on five specimens of white-throated kingfisher (*Halcyon smyrnensis*)

and five of hoopoe (*Upupa epops*) of both sexes. Samples of kingfishers were collected from Nile delta, at different localities in the region of River Nile branches; while samples of hoopoes were obtained from Abou Rawash (North of Giza, Egypt) during the period from September 2015 to November 2017. Animals in this study were conducted in accordance with the criteria of the investigations and Ethics Committee of the Community Laws governing the use of experimental animals.

Animals were killed by neck dislocation; dissections were done under the stereoscopic binocular microscope, and tongues were removed; examined with stereomicroscope and a digital camera was used to get photos. For histological investigations; tongue immediately immersed in 10% buffer formol, dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Transverse sections were cut at 5 μ and stained with haematoxylin and eosin. Furthermore, the mean thickness of the epithelial layers in the three different regions of the tongue (apex, body or corpus and root or radix) were measured according to Jackowiak and Godynicki (2005), with the aid of an ocular micrometer, measurements are given in micrometers. For histochemical examination; the used stains and their employments were summarized in Table (1).

Table 1: Summarized histochemical stains used in the present study.

Technique employed	Histochemical substance localized	Reference
Alcian blue (pH 1.0)	Glycoconjugates with O-sulfate (more acidic sulphonic groups)	Lev and Spicer (1964)
Alcian blue (pH 2.5)	Glycoconjugates with carboxyl (carboxylated carbohydrates)	Lev and Spicer (1964)
PAS	General neutral carbohydrates or glycoconjugates	McManus (1948)
Alcian/PAS (alcian blue pH 2.5 method followed by PAS technique)	Acidic and neutral Mucins (glycoproteins)	Spicer and Mayer (1960)
Mercuric bromophenol blue (Bp B)	Total protein.	Cremer and Tiselius (1950)

RESULTS

Anatomically; three parts are distinguished from the dorsal surface of the triangular tongue;

apex, corpus and radix in both of kingfisher (*Halcyon smyrnensis*) and hoopoe (*Upupa epops*). The measurements of the tongue areas and the lingual epithelia thickness of both species are shown in Table (2). The present work clarified that; the tongue was elongated pointed, not bifid in both species, but longer and bigger in kingfisher than in hoopoe and slightly more cylinder in hoopoe. The tongue of kingfisher occupies about the posterior third of the bill, but in hoopoe it fills about the posterior quarter of the bill.

Table (2): Measurements of the tongue parts in *H. smyrnensis* and *U. epops*. (Mean \pm SD). Where: a significant at P value <0.05 when compared with the *H. smyrnensis*. b significant at P value <0.05 when compared with the *Upupa epops*.

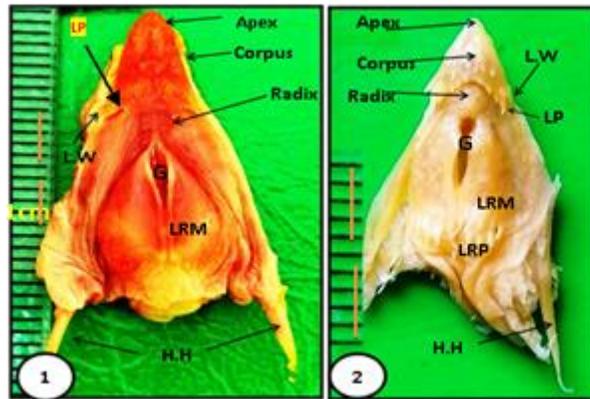
Character	<i>H. smyrnensis</i>	<i>Upupa epops</i>
Tongue length	13.57 \pm 0.065mm _a	5.68 \pm 0.13mm _b
Apex length	3.16 \pm 0.055mm _a	2.04 \pm 0.054mm _b
Corpus length	6.08 \pm 0.083mm _a	2.14 \pm 0.11mm _b
Radix length	4.33 \pm 0.016mm _a	1.5 \pm 0.071mm _b
Apex width	2.7 \pm 0.071mm _a	2.0 \pm 0.071mm _b
Corpus width	5.22 \pm 0.084mm _a	3.46 \pm 0.114mm _b
Radix width	7.06 \pm 0.114mm _a	5.1 \pm 0.071mm _b
Apex epithelia thickness	117 \pm 0.83 μ m _a	82.6 \pm 1.52 μ m _b
Corpus epithelia thickness	205 \pm 3.3 μ m _a	155.6 \pm 3.28 μ m _b
Radix epithelia thickness	121.2 \pm 0.837 μ m _a	101.6 \pm 1.14 μ m _b

There is a significant increase in the length and width of the tongue of kingfisher as compared with hoopoe tongue. The mean total length of kingfisher tongue specimens was 13.57 \pm 0.065 mm, while in *Upupa epops* tongue specimens was 5.68 \pm 0.13 mm (Table 2). On the tongue of kingfisher; two of backwardly directed filliform papillae in each side are found at the end of the tongue corpus making a V shaped letter (Fig. 1). While in hoopoe a set of a double row of large conical papillae are located at the end of the lingual corpus and directed backwardly in a wavy transverse line; two of giant papillae were found, one on each side (Fig. 2).

The histological examination revealed that the

tongue of the two species under the study demarcated the same usual histological structures; an outer epithelial layer, lamina propria, or connective tissue layer rich in blood supply, and mostly inner skeletal muscular layer. Also, the tongue is supported by a hyaline cartilage, entoglossum, which extending from the apex till the beginning of radix of the tongue; moreover, the remaining of the radix is supported by bony structure, in both birds.

The tongue of kingfisher (*Halcyon smyrnensis*) is covered by a normal stratified squamous epithelium mounting the connective tissue or lamina propria, on both dorsal and ventral surfaces. The dorsal lingual epithelium is composed of keratinized stratified squamous epithelium; that is more keratinized in the apex than corpus or radix of the tongue, while the ventral of the tongue is covered by non-keratinized stratified squamous epithelia (Plate 1: A and B). The cells of the single layered stratum basale are spherical to cuboidal with rounded nuclei. To the outside, this cell layer is followed by a compacted thick stratum spinosum displaying typical polyhedral cells with prominent nuclei. They give rise to layers of flattened cells with horizontally elongated nuclei, constituting the stratum corneum. Apart from the well keratinized covering epithelium, the lingual apex was predominated by filiform dermal papillae (Plate 1: C). The corpus of the tongue has less keratinized epithelium and contains few taste buds of bulbous form (Plate 1: D) and there are numerous orifices of compound alveolar lingual salivary glands (Plate 1: E) at the ventral border of the lingual body. The radix region of the tongue in kingfisher exhibited sharply pointed lingual papillae, lingual spine or lingual nails that are directed posteriorly (Plate 1: F). Meanwhile, keratinized epithelium on its dorsal surface exhibited many of taste buds (Plate 1: G), underneath of the epithelia large muco-serous glands, which are compound tubule-alveolar type were delineated. There are numerous orifices of the lingual salivary glands at the ventral border of the lingual body. Also, some of Herbst corpuscles were detected in the lamina propria of the radix (Plate 1: H). On the other hand, in the tongue of the *Upupa epops*, the surface of the lingual apex is slightly rough and has non-keratinized epithelium (Plate 2: A and B). The surface of the lingual corpus was smooth than that of the lingual apex; it has lingual papillae with apices pointed towards the posterior part of the tongue. (Plate 2: D).



Figures. 1&2. Photographs of the tongue and floor of the mouth of kingfisher and hoopoe showing one row of lingual papillae (LP) on the caudal margin of the paired lingual wing (LW); of Kingfisher while double rows of lingual papillae in hoopoe; Glottis fissure (G), and the laryngeal mound (LRM) with laryngeal papillae and the posterior paired of hyoid horns (HH), (1): kingfisher and (2): hoopoe.

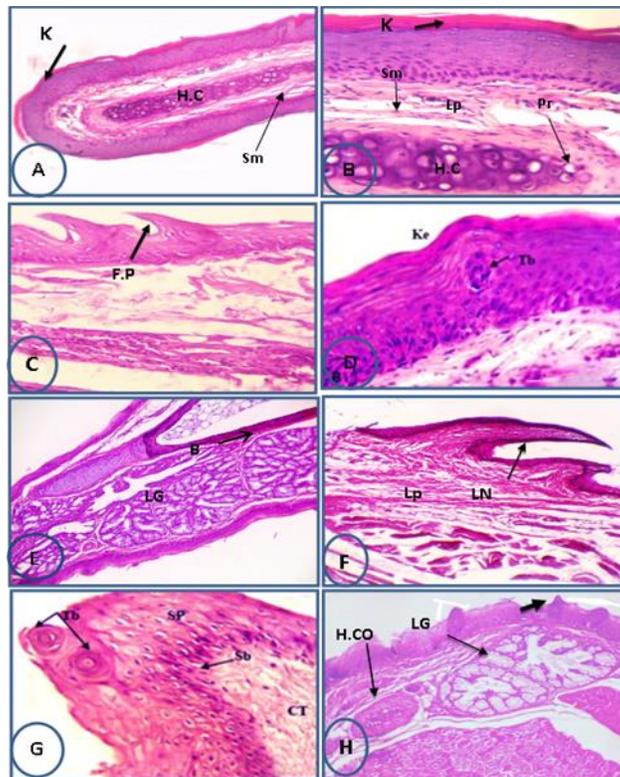


Plate 1. T.S and L.S of kingfisher tongue stained with Hx & E. A: T.S. in tongue apex showing thick keratinized dorsal epithelium (k), skeletal muscles (Sm), B: showing lamina propria (Lp), hyaline cartilage (HC), perichondrium (Pr), C: T.S. in tongue corpus showing filliform papillae (FP) on the dorsal side of the tongue, D: showing less keratinized epithelium (Ke) than the apex, and taste buds (Tb), E: showing anterior lingual glands (LG) and bony structure (B), F: L.S in tongue radix showing lingual nail (LN) in the dorsum and lamina propria (Lp), G: T.S. of tongue radix showing stratum spinosum (SP), stratum basal (Sb), intraepithelial taste buds (Tb) and sub-epithelial

connective tissue (CT), H: showing lingual papillae (thick arrow), posterior lingual glands (LG) and Herbst corpuscles (H.CO). All Figs. are 100X Mgn. except B, D: 400X, G: 40X.

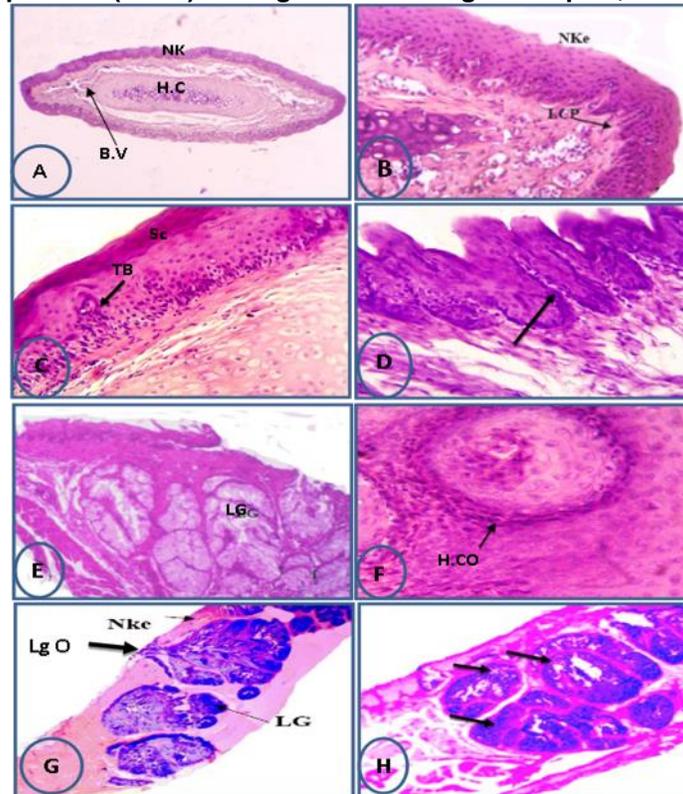


Plate 2. T.S. of hoopoe tongue regions. All Figs. are stained with Hx & E, except G & H: Alcian blue PAS stained. A: T.S. in tongue apex showing non keratinized epithelium (Nk), hyaline cartilage (HC), and blood vessels (Bv), B: showing large conical papillae (LCP), C: T.S. in tongue corpus showing Nk, stratum compactum layer (SC), taste buds (Tb) located in this border area, D: showing the anterior lingual glands (LG), E & F: T.S. of tongue radix showing NK and Herbst Corpuscles (H.CO) in the lamina propria, G: T.S. of tongue corpus stained with showing Nke, and the orifice of lingual glands (Lg. O), H: showing LG (thick arrow) stained with moderate blue color. All Figs. are 400X Mgn. except A: 100X, G: 40X.

The corpus region of hoopoe tongue showed few of taste buds (Plate 2: C) and the lamina propria contained anterior lingual gland mostly of compound alveolar type (Plate 2: E), these glands revealed a main opening in the ventral side of the tongue (Plate 2: G). The radix tongue of hoopoe showed partially keratinized epithelium beneath of which, large number of compound tubule-alveolar muco-serous glands were located. The openings of these glands are larger than those in the corpus (Plate 2: H). The lamina propria in the radix tongue of hoopoe contains some of large Herbst corpuscles (Plate 2: F).

The ventral side of the tongue is covered with non-keratinized stratified squamous epithelia along its whole length. The present study recorded that, the thickness of the lingual epithelia of *H. smyrnensis* significant increase when

compared with those of *Upupa epops* (Table 2). The histochemical staining techniques were chosen to determine the carbohydrate type of the secretions of the lingual salivary glands of kingfisher and hoopoe. The histochemical data of staining reactions employed in the present investigation are depicted in Table (2).

The data exhibited that the lingual glands in both species produce acidic and neutral mucin. The applied of Alcian blue stain, pH 1 and pH 2.5 (Plate 3: A- D), indicated that the lingual glands of both species contain acidic carbohydrates such as acidic sialomucins and sulpho mucins. Meanwhile, the acidity of the mucin secretions was mostly increased toward the base of the tongue in kingfisher as well as in hoopoe. Periodic acid-Schiff (PAS) staining was used to determine the general neutral carbohydrates including glycogen and glycoproteins, glycolipids that periodate

reactive in the secretory cells of the lingual salivary gland.

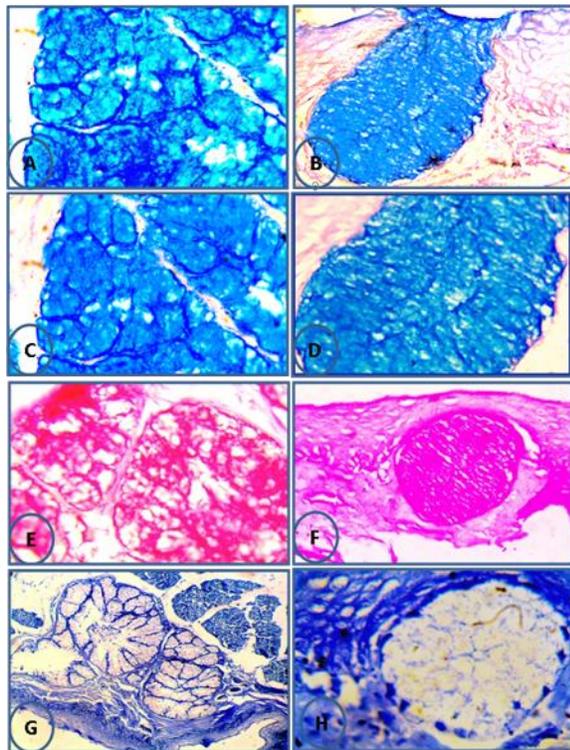


Plate 3. Histochemistry of lingual salivary glands in the tongue radix of kingfisher and hoopoe. A: T.S. in lingual salivary glands in the tongue radix of kingfisher showing Positive blue stain, B: T.S. in lingual salivary glands in the tongue radix of hoopoe showing positive Alcian blue stain, C: T.S. in tongue radix of kingfisher showing positive Alcian blue stain pH 1, D: T.S. in tongue radix of hoopoe showing positive Alcian blue stain pH 1, E: T.S. in tongue radix of kingfisher showing positive PAS stain, F: T.S. in tongue radix of hoopoe showing positive PAS stain, G: T.S. of tongue corpus of kingfisher showing the total protein contents in the lingual glands stained with Bromophenol blue, H: T.S. of tongue corpus of hoopoe showing the total protein contents in the lingual glands stained with Bromophenol blue. All Figs. are 400X Mgn. except A & G: 100X.

The lingual glands in both animals under study exhibited large amount of neutral carbohydrates including glycogen (Plate 3: E and F), with a non-significant difference between the two animals in respect to the corpus of the

tongue. While, the lingual glands in tongue radix of kingfisher revealed significant increase in the glycogen content as compared with those of hoopoe (Table 3).

Table 3. Histochemical features of the lingual glands of *H. smyrnensis* and *U. epops* (Mean of stain intensity \pm SD). a: significant at *P* value <0.05 when compared with the *H. smyrnensis*. b: significant at *P* value <0.05 when compared with the *U. epops*.

Character	<i>H. smyrnensis</i>	<i>Upupa epops</i>
Corpus		
Alcian blue (pH 1)	36.4 \pm 10.16 _a	27.6 \pm 5.3 _a
Alcian blue (pH 2.5)	145.1 \pm 20.3 _a	29.6 \pm 13.2 _b
PAS	127.8 \pm 43.3 _a	113.4 \pm 16.5 _a
Alcian/ PAS	170.4 \pm 10.2 _a	116.6 \pm 15.0 _b
Bp B	53.0 \pm 20.5 _a	27 \pm 17.4 _b
Radix		
Alcian blue (pH 1)	82.7 \pm 16.7 _a	101.46 \pm 62.5 _a
Alcian blue (pH 2.5)	168.4 \pm 39.7 _a	151.4 \pm 64.8 _a
PAS	189.51 \pm 29.9 _a	96.27 \pm 28.8 _b
Alcian/ PAS	38.1 \pm 16.4 _a	57.8 \pm 17.3 _b
Bp B	54.37 \pm 7.1 _a	71.26 \pm 32.9 _a

The total protein, indicated with mercuric bromophenol blue stain, revealed moderate contents in the corpus and radix lingual glands in both species without significant difference between of them. Plate (3: G) shows the total protein content in lingual glands in the tongue corpus of kingfisher, while (Plate 3: H) shows the protein content in lingual glands of tongue radix of hoopoe.

DISCUSSION

The tongue plays important role in feeding, which exhibits an important morphological variation adaptive to environmental conditions of each habitat. The shape and structure of the tongue differ significantly among animal species, reflecting the various functions of each respective tongue (Iwasaki, 2002; Santos et al. 2011). The present study revealed that, in the anatomy of the tongue of *Halcyon smyrnensis* and *Upupa epops*, three parts were distinguished: apex, corpus and radix; as reported with Jackowiak and Godynicki (2005), Dehkordi et al. (2010), Darwish (2012) and Nasr et al. (2012). The current study proved a significance difference between the tongues of two species studied, kingfisher tongue is longer and bigger than in hoopoe tongue. Also; morphologically the kingfisher tongue was elongated pointed and longer than in hoopoe

tongue, and this difference is belonged to the adapted to the type of food intake as recorded with Pasand et al. (2010) and El Bakaray (2011).

Campbell and Lack (1985) declared that the tongues of some fish-eating birds such as pelicans and cormorants undergo a distinct structural reduction; in accordance the present study reported that the tongue of kingfisher occupies about the posterior third of the bill, while in hoopoe it fills about the posterior quarter of the bill. The lingual papillae are commonly described in different birds, the present study showed that kingfisher has two of backwardly directed filliform papillae in each side at the end of the tongue corpus making a V shaped letter, while in hoopoe a set of a double row of large conical papillae are located at the end of the lingual corpus and directed backwardly in a wavy transverse line. The V shaped papillary crest facilitates the movement of each bolus of food towards the oesophagus (Koolos, 1986; El-Bakary, 2011) or serves to prevent regurgitation and to direct food for ingesting irrespective of food type (Erdogan and Iwasaki, 2013). Similarly, in common quail; a main row of large conical papillae is present in the posterior part of the lingual body, the apices of which are pointed towards the posterior part of the tongue (Parchami et al. 2010a). Moreover, our results showed a two of giant papillae were found, one on each side at the end of the tongue corpus of hoopoe; such giant papillae were documented in black kit (Emura, 2008); goose (Hassan et al. 2010), common quail (Parchami et al. 2010a) and golden eagle (Parchami et al. 2010b).

The present study revealed that the dorsum of the kingfisher tongue is covered with keratinized stratified epithelia, in accordance the dorsal surfaces of most of the avian tongues are covered by keratinized stratified squamous epithelium (Jackowiak, 2006; Emura et al. 2010). In contrary, the stratified epithelium of the tongue dorsum is non-keratinized, the same results were found in the tongue of laughing dove (Al-Nefey, 2015). This differences and degree of keratinization of epithelium is depending on food habitat is belonged to the adapted to the type of food intake, and these results are agreed with Jackowiak et al. (2006). In penguins, large spine-like and caudally directed lingual papillae (filiform-like papillae) are densely distributed over the entire dorsal surface of the tongue, apparently serving to catch fish (Kobayashi et al. 1998). In coincidence, similar sharp filiform papillae or spines were detected on the tongue of the kingfisher only; such that, in anatomical terminology, it is referred to as the

lingual nail or cuticula cornea lingualis (Erdogan and Iwasaki, 2013). In coincidence with the present results Kobayashi et al. (1998) advocated that, tongues of many piscivorous species are adapted to hold the slippery prey by means of numerous stiff, sharp, caudally-directed papillae. In birds, taste buds are situated deep in the epithelium and have a long taste pore, called the taste canal (Kudo et al. 2008; Erdogan and Iwasaki, 2013). The structure avian taste bud is different from that of mammals with regard to existence of the long taste canal (Crole and Soley, 2009b). Few numbers of embedded taste buds located in lingual epithelial area were observed in the posterior corpus and radix tongue of both birds under the current study. However, the taste buds of birds are distributed not only within the lingual epithelium but also within the epithelium of other parts of the oral cavity (Iwasaki, 2002). The connective tissue core or the lamina propria of the tongue contained some of Herbst corpuscle posteriorly in the radix tongue of kingfisher and hoopoe. In accordance with Crole and Soley (2009b), the present findings proved that the tongues of kingfisher and hoopoe function as a sensory organ, both for taste and touch by asset taste receptors and Herbst corpuscles, respectively.

The present study exhibited that the tongue of both species under the study is contain compound alveolar anteriorly and compound tubulo-alveolar posteriorly lingual glands distributed in the corpus and radix, while the apex has no glands. Histochemically, these lingual glands in both species produce acidic and neutral mucin. The acidic carbohydrate contents include each of sialomucins sulphomucins and glycogen. Such secretion plays an essential role in the lubricating and protection in the digestive and respiratory tract (Gargiulo et al. 2007). Glandular secretions include various classes of macromolecules, such as neutral mucins; proteoglycans containing carboxylic acid, sulfated groups; sialomucins; sulfomucins; and glycogen, which have important functions that include protection of the oral mucosa and upper digestive tract and facilitation of ingestion (Erdogan et al. 2012; Sagsoz et al. 2013) and also prevent the oral mucosa from drying by forming a barrier on the mucosal surface (Tabak et al. 1982). Moreover, the glycoproteins that cover the mucosal surface might protect the mucosa against acidic enzymatic factors (Gargiulo et al. 1991) and glycoconjugates containing sialic acid help to hydrate the mucosal surface, creating a hydrophilic environment and protecting the

mucosa against bacterial activity (Gargiulo et al. 1991; Samar et al. 2002).

CONCLUSION

From the previous the results it can be said that the anatomy as well as the histology and histochemistry of the tongue of both birds demonstrate certain specific differences of the tongues in the carnivorous kingfisher (*Halcyon smyrnensis*) and the insectivore hoopoe (*Upupa epops*) reflects a close relationship between the structures of the tongue and their feeding habits.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

FME designed and performed practical work, data analysis and also wrote the manuscript. AHA-E participate in practical work and data analysis. MIR organized experiments and reviewed the manuscript. All authors read and approved the final version.

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