The Pharma Innovation Journal 2022; SP-11(7): 968-973

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 968-973

www.thepharmajournal.com
Received: 02-05-2022
Accepted: 06-06-2022

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Gross and histomorphological studies of epiglottis of goat (*Capra hircus*)

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Abstract
The aim of the study is to study the macro and microstructure of the epiglottis of goat. The epiglottis of goat was the anterior of all the laryngeal cartilages which acts as primary valvular apparatus thus preventing food particles from entering the larynx during swallowing. The epiglottis was cordate leaf shaped with pointed and ventrally curved apex. The base of the epiglottis consisted of fat pad with of adipose tissue, hyoepiglotticus muscle and submucosal glands towards the lingual surface. The aryepiglottic fold connected the epiglottis with the arytenoid cartilage. The laryngeal and lingual surfaces were lined by the non- keratinized stratified squamous epithelium where propria submucosa was present between the two surfaces. The epiglottis was made up of elastic cartilage. The serous, mucous and mixed glands were present in the propria submucosa towards the lingual surface and were penetrating into the elastic cartilage.

Keywords: Epiglottis, larynx, respiratory system, elastic cartilage, goat

Introduction
Epiglottis is a single cartilage that acts as a gatekeeper of the laryngeal entrance in mammalian species (Negus, 1949) [15]. The epiglottis is essentially a mammalian structure, studying its microstructure will add important information to the field of comparative anatomy (Eshrah and Kasab, 2019) [6]. The epiglottis acts as valvular apparatus during swallowing and also plays a major role in stimulation of protective reflexes via the chemosensory cells of taste buds in epiglottis (Harding, Johnson, & McClelland, 1978) [11]. Further it acts as immune mediator, as the paraepiglottic tonsils participate in innate, cellular and humoral immunity at the local and systemic levels (Horter *et al*., 2004) [12]. The epiglottis-tongue root complex and height of the larynx enabled it as multi-tiered sphincter that helps to shape the laryngeal vocal tract in many ways (Moisik, 2008) [14]. The great Indian leader M.K. Gandhi "father of the nation" designated goats as "poor man's cow", as goats play a vital role in the economy of poor and landless labours. The respiratory diseases of goat resulted in considerable financial loss to the goat keepers (Chakraborty *et al*., 2014) [3]. Thus the knowledge of microstructure of epiglottis is essential to understand the health importance of epiglottic tonsils and pathogenesis of respiratory diseases (Casteleyn *et al*., 2008) [8]. The main objective of the present study is to understand the detail anatomical structure of epiglottis which may further helpful in impartment of preventive measures and clinical approach to the respiratory diseases.

Materials and Methods
Twelve fresh larynges of goats (four below one year, three 1 to 2 years, two 2-3 years and three 4 and above) irrespective of gender were collected from the Jiyaguda slaughter house, Hyderabad. The samples were washed and immediately transferred to the 10% formaline.
The epiglottis of six larynges were fixed for one week in 10% Neutral buffer formalin followed by decalcified using 4% EDTA and processed routinely for histology which were embedded in hard paraffin wax and the sections of 3-5 micron thick were cut using a microtome. All sections were mounted on slides and were stained with Hematoxylin and Eosin (H & E) for detailed micro-architecture, Masson's trichrome method for differentiating collagen fibers and muscle, Van Gieson's method for collagen fibers, Wilder's reticulum method for presence of any reticular fibres, Weigert's method for elastic fibers (Luna 1968), Saffranin-O staining technique for cartilage matrix identification. PAS and PAS-Ab methods for assessment of muco-polysaccharides (Singh and Sulochana, 1998).

Results and Discussion

Gross anatomy

The epiglottis of goat was unpaired cartilage which was long as stated by D.F.N. Harrison, (1995) [4], that the larynx of herbivore was protected by a relatively long epiglottis. It was cordate leaf shaped cartilage with two surfaces, two borders, apex and base. The laryngeal surface was concave transversely and convex longitudinally while the lingual surface was concave longitudinally and convex transversely and both surfaces were pitted as reported by. The rostral apex curved ventrally and was pointed (Fig 1). The base was located caudally which attached to the thyroid cartilage by the thyroepiglottic ligament and consisted of a ventral projection which was mentioned as fat pad by Eshra *et al*., (2016) [5] in donkeys and buffaloes. The lateral borders of base were connected to the arytenoid cartilage by the mucosal fold called aryepiglottic fold which extended lateral to the corniculate process (Fig 2) and cuneiform cartilage was absent while caudal end of base presented a short thick petioles (Fig.3). The hyo-epiglotticus muscle extended between the body of hyoid to the lingual surface of the epiglottis epiglottis and thyro-epiglottic ligament connected the base of the epiglottis to the rostral edge of the thyroid cartilage as found by Frey *et al*. (2006) [9] in larynx of muskox. The epiglottis was covered by a tightly adherent mucous membrane on the laryngeal surface, while the lingual surface showed the loosely attached mucosa, the subepiglottic fold which was in accordance with findings of Fulton *et al*., (2012) [10] in equine epiglottis.

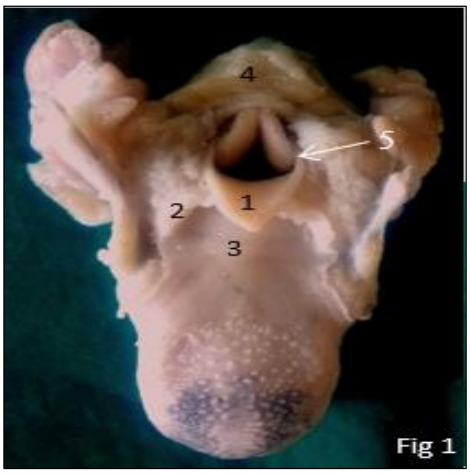

Fig 1: Larynx with intact tongue showing 1. Epiglottis 2. Posterior pillars 3. Root of the tongue 4. Pharynx, 5. Aryepiglottic folds

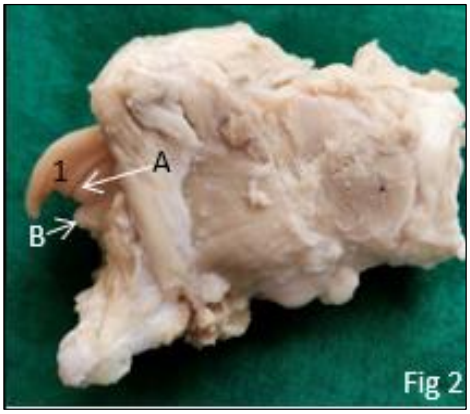

Fig 2: Lateral view of the larynx showing 1. Epiglottis, A. Lingual surface with mucosal fold B. Fat pad.

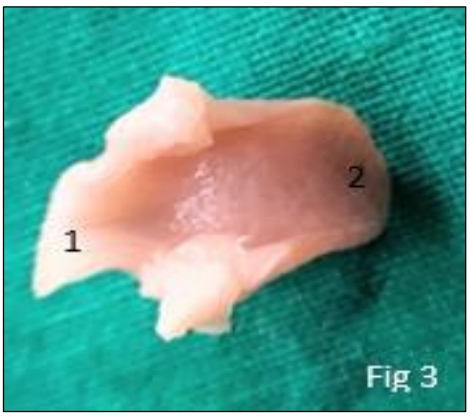

Fig 3: Dorsal view of epiglottis showing 1. Petiolous 2. Tip

The width of epiglottis at the base ranged between 16.07 mm -24.27 mm whereas thickness at tip was 2.08 mm-3.81 mm while that of base 8.19 mm-12.88 mm and length was 19.56 mm to 26.52 mm which is in agreement with the recordings of Aishwarya *et al*. (2018) [1] in Gaddi goat.

Histoarchitecture

Histologically, the epiglottis consisted of mucosa and Propria submucosa, whose thickness decreased gradually towards the tip. The mucosa consisted of epithelium and lamina propria while the lamina muscularis was absent. The lining epithelium was non-keratinized stratified squamous epithelium on both laryngeal and lingual surfaces throughout the length from tip to base (Fig-4) which was in acceptance with Eurell JA, Frappier BL (2006) [7] who stated that the laryngeal surface was lined by the non-keratinized stratified squamous epithelium till the vocal fold beyond which was lined by pseudostratified ciliated columnar epithelium. The epithelium of lingual surface was thicker than that on the laryngeal surface and the epithelial thickness varied throughout the length which was in agreement with the findings of Parkash, T. and Kumar, P., (2019) [16] in pig. The epithelium was comprised of stratum basale, stratum spinosum and stratum superficiale within outwards among which the cells of basale rested on the basement membrane, The basement membrane consisted of connective tissue fibers and that of lingual surface showed more reticular fibers that that of laryngeal surface (Fig.5). The surface of the epithelium was regular with even surface however presented undulating appearance in few areas whereas basal surface presented papillated appearance. The nuclei of the stratum basale and spinosum darkly stained and varied in shape while that of superficiale were less basophilic with flattened nuclei which oriented parallel to the length of epiglottis. The taste buds were observed throughout the length of the laryngeal surface. The lamina propria was rich in vascular supply and consisted of dense irregular connective tissue with collagen fibers, elastic fibers, reticular, fibrocytes and fibroblasts (Fig-6). Isolated lymphocytes were present throughout the length of the lamina propria while subepithelial aggregated lymphatic tissue (Fig-7) was observed in the base which was similar to that of the studies of Casteleyn *et al*., (2008) [2] in cattle where he observed the isolated primary and secondary lymphoid follicles and diffuse aggregations of lymphocytes in the mucosa at the base of the epiglottis and also described them as Laryngeal associated Lymphatic Tissue (LALT). The aggregated lymphatic tissue was covered by the lymphoepithelium.

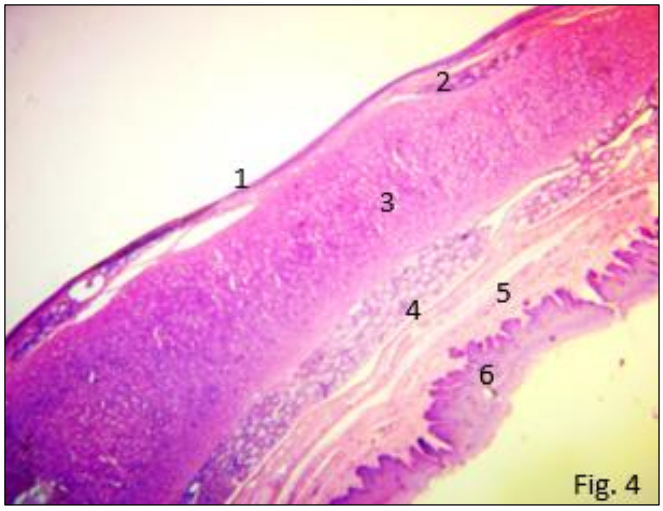

Fig 4: Epiglottis-1. Laryngeal surface 2. Propria submucosa, 3. Elastic cartilage, 4. Submucosa, 5. Lamina propria 6. Lingual surface. H & E (40x)

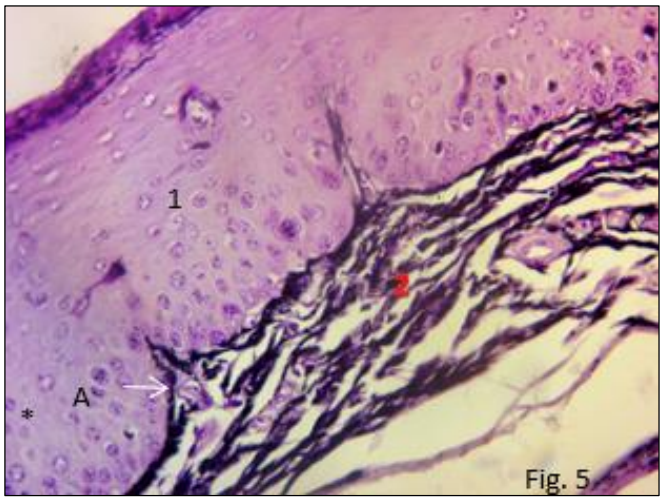

Fig 5: (400X) Lingual surface of epiglottis showing 1. Non-keratinized stratified squamous epithelium, 2. Lamina propria with reticular fibers, A-Reticular fibers of basement membrane. Wilder's stain 400X

The submucosa composed of loose connective tissue with rich vasculature, nerves, collagen fibers, adipose tissue, elastic cartilage and laryngeal glands. Excess accumulation of adipose tissue was seen towards the base of the epiglottis while microscopic structure of the submucosa of ventral fat pad (Fig. 8) revealed adipose tissue, glands, hyoepiglotticus muscle thyroepiglottic ligaments and other connective tissue which was fully in acceptance with the findings of Frey, R. and Gebler, A, (2003) [8] in the epiglottis of mangolian gazelle. The connective tissue of submucosa was in continuation with the perichondrium of the elastic cartilage.

The laryngeal glands were more towards the lingual surface while only few were present towards the laryngeal surface. The glands percolated into the elastic cartilage which resulted in discontinuous cartilage and were surrounded by the perichondrium.

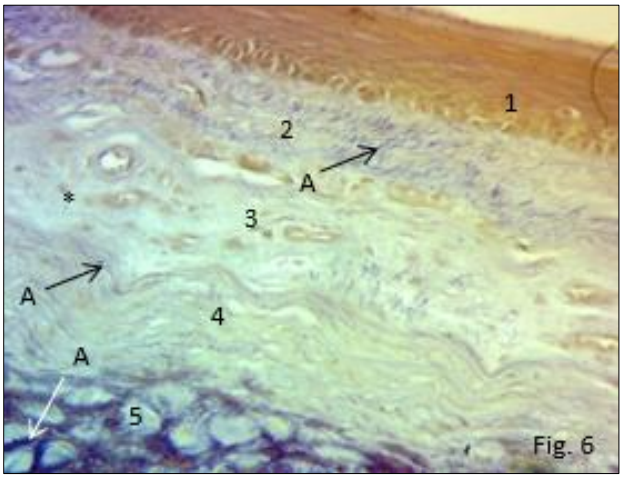

Fig 6: Laryngeal surface of Epiglottis 1. Non-keratinized stratified squamous epithelium, 2. Lamina propria showing elastic fibers 3. Submucosa with sparse elastic fibers 4. perichondrium, A-elastic fibers-Wiegert"s stain (400X)

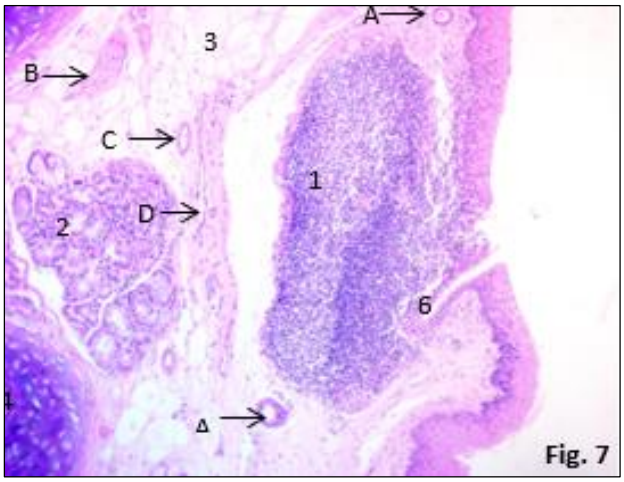

Fig. 7: Base of the epiglottis showing laryngeal surface with 1. Lymphnode 2. Submucosal mixed glands, 3. Adipose tissue 4. Elastic cartilage 6. Duct opening onto the laryngeal surface A Duct of the laryngeal glands B Nerve, Arrow-Vein, C-artery. D –Vein. H& E (100X)

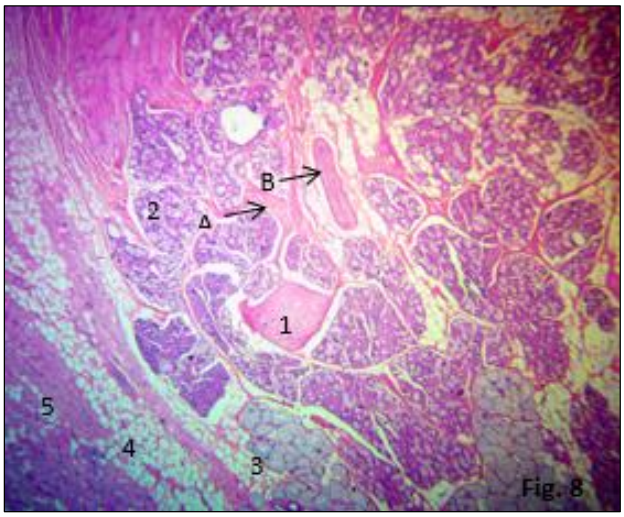

Fig 8: Base of the epiglottis showing the Fat pad 1. Cartilgenous plate 2. Mixed glands 3. Mucous glands 4. Addipose tissue 5. Hyoepiglotticus muscle, A-Inter lobular septa, B-Artery, H& E (40X)

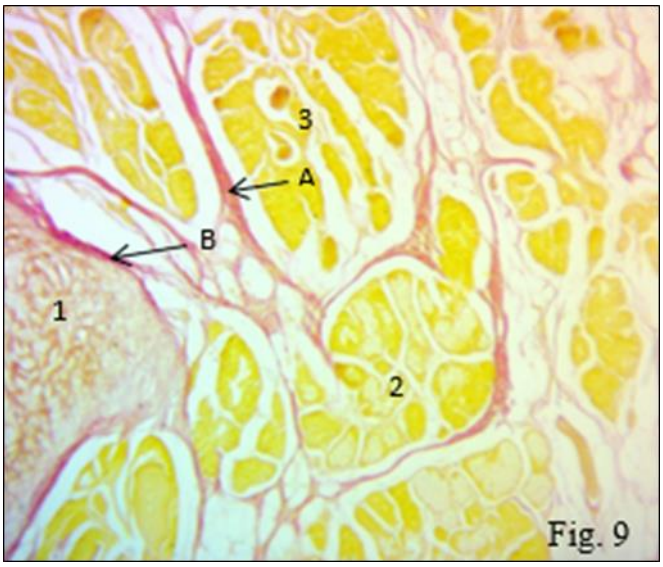

Fig 9: Epiglottis showing 1. Elastic cartilage 2. Mixed glands 3. Serous glands, A - interlobular septa with collagen fibers, B - collagen fibers of the perichondrium, Vangieson's 100X

The glands were mucous, serous and mixed were in the form of lobules separated by the connective tissue which composed of connective tissue fibers and adipose tissue (Fig-9). The mucous glands were branched tubulo-alveolar type while serous and mixed glands were acinar type. The serous acini, mucous acini with serous demilunes and mucous alveoli were evident whereas the later was confined to the submucosa of the lingual surface. The acini were lined by the pyramidal cells with centrally located nucleus in serous glands while the mucous glands were lined by the columnar cells whose nucleus was located peripherally (Fig-10). The glands towards laryngeal surface were mixed and serous while the lingual surface showed serous, mucous glands and mixed glands, which is partially in agreement with Singla. S and Kumar. P, 2021 [18] who observed more serous glands in the base. The intralobular ducts were lined by low cuboidal epithelium whereas interlobular ducts were lined by simple cuboidal while the major ducts were lined by high cuboidal to two layered cuboidal epithelium which gradually changed to stratified Squamous epithelium epithelium at the terminal opening (Fig.11).

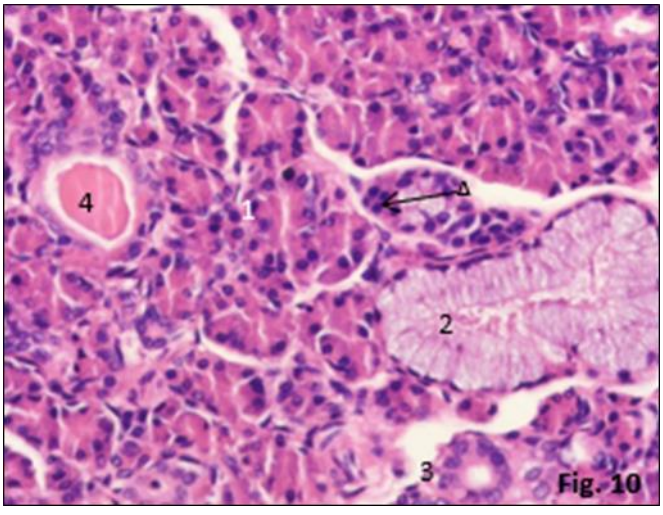

Fig 10: Showing the submucosal glands 1. Serous glands 2. Mucous glands, 3. Intercalated duct 4. Intra lobular duct and A-mixed gland with serous Demilune H& E 400x

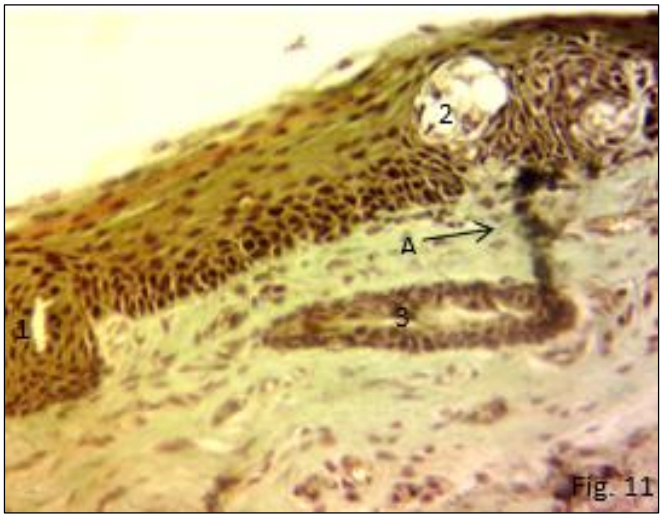

Fig 11: Laryngeal surface of epiglottis showing 1. External opening of the duct lined by stratified squamous epithelium on to the surface 2. Taste bud 3. Major duct with stratified cuboidal epithelium in the lamina propria, A- Duct opening onto the laryngeal surface. Saffron O(400X)

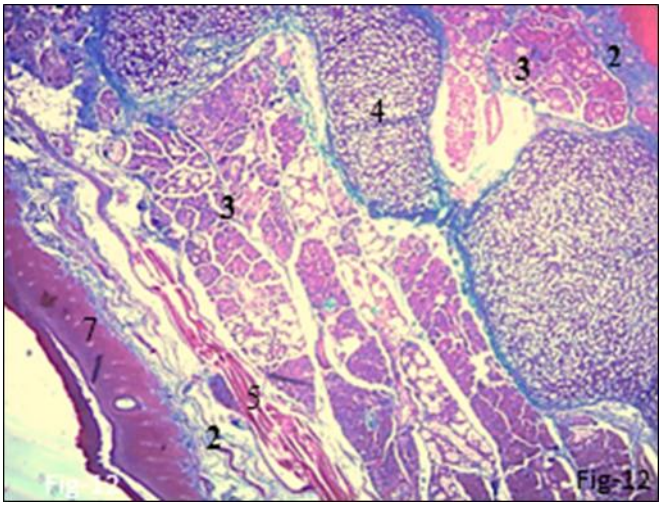

Fig 12: Epiglottis showing 1. Laryngeal surface lined by Non-keratinized stratified squamous epithelium, 2. Lamina propria, 3. Submucosal glands, 4. Elastic Cartilage, 5. Hyoepiglotticus muscle, 7. Non-keratinized stratified squamous epithelium. Masson" Trichrome(40x)

The hyoepiglotticus muscle (Fig. 12) was striated muscle that extended in the submucosa of lingual surface which was seen throughout the length of the epiglottis but was absent in the tip and thickness of the muscle gradually reduced towards the apex.

The elastic cartilage was not continuous and was in the form of discontinuous plates with glands, adipose tissue and connective tissue extended into it (Fig-13). The chondrocytes were located in the lacunae which were single however few cell nests were observed while the chondroblasts were located towards the periphery. The matrix composed of connective tissue fibers and sparse ground substance. The elastic fibers were predominant and were lightly

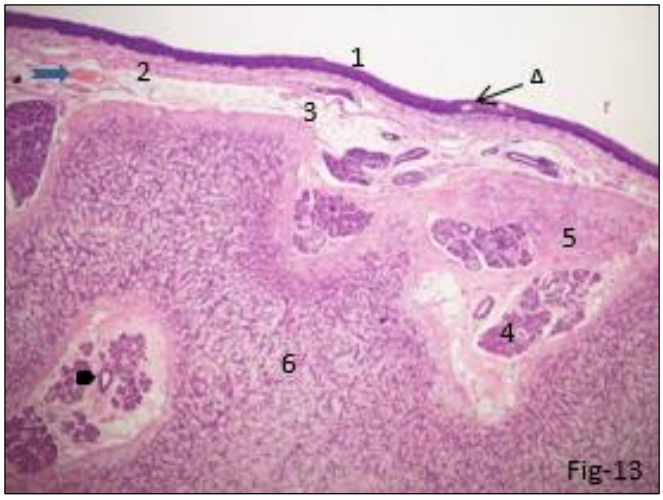

Fig 13: Epiglottis showing the 1. Epithelium 2. Lamina propria 3. Submucosa with loose connective tissue 4. Mixed glands percolated into the elastic cartilage 5. Perichondrium surrounding the glands 6. Elastic cartilage (Taste buds-arrow, artery-bold arrow, arrow head interlobular duct) H& E (40X)

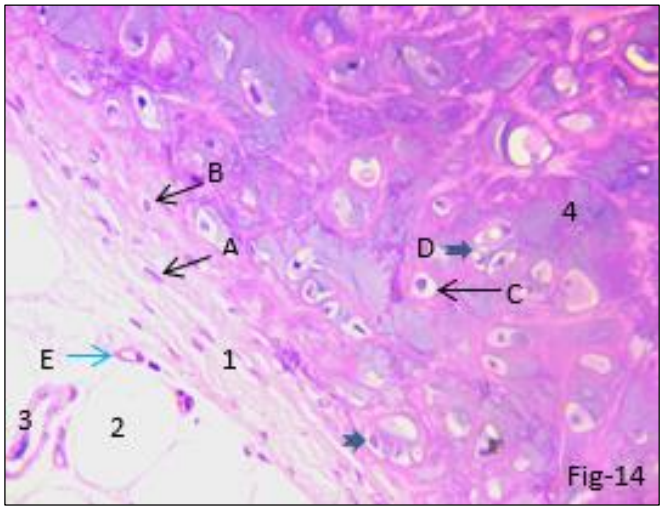

Fig 14: Elastic cartilage showing 1. Eosinophilic ground substance 2. Adipose cell 3. Small Artery 4. Ground substance A-Fibrocyte, B-Chondroblast, C-head-Lacunae with Chondrocytes surrounded by capsule D-Cell nest E-Capillary in the perichondrium

eosinophilic, thick, branched and anastomosed which resulted in dense network in the deeper zone while the periphery zone consisted of thin fibers that formed wide meshed network. The collagen fibers were few which were masked by the ground substance and were intermingled with the elastic fibers (Fig-14). The perichondrium consisted of collagen fibers, elastic fibers and reticular fibers (Fig-15). The blood vessels and lymphatic vessels were seen in the perichondrium but not extended into the cartilage.

Histochemistry

The chondrocytes of epiglottis showed strong positive reaction to PAS which indicated the presence of mucopolysaccharides (Fig-16). The ground substance of elastic cartilage was positive to PAS AB revealed that it consisted of hyaluronic acid, sulphated polysaccharides,

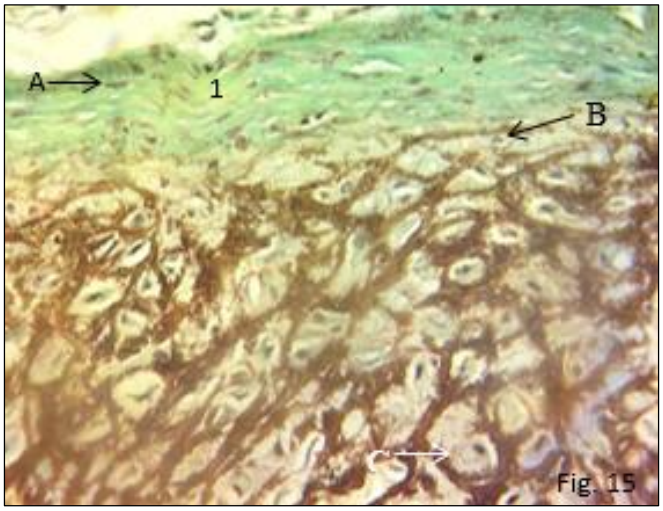

Fig 15: Elastic cartilage showing 1. Perichondium, A-Fibrocytes, B-chondroblasts, C-chondrocyte showing capsule within the lacunae-Saffron O-400X

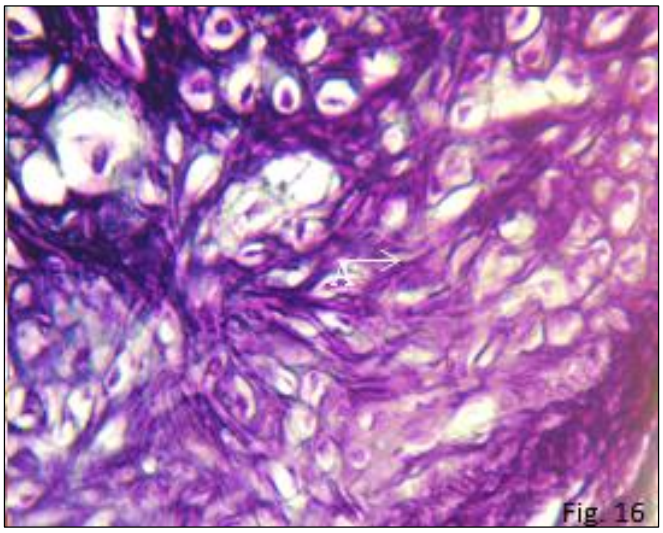

Fig 16: Elastic cartage showing A-reticular fibers-Wilder's stain400X

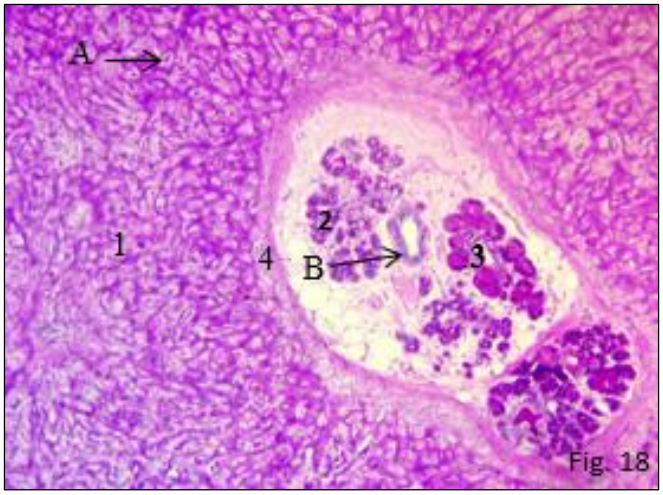

Fig 17: Epiglottis showing positive reaction to PAS 1. Cartilage with penetrated glands 2. Mixed glands 3.serous glands, 4. Perichondrium A-Chondrocytes B- Interlobular duct, PAS (40X)

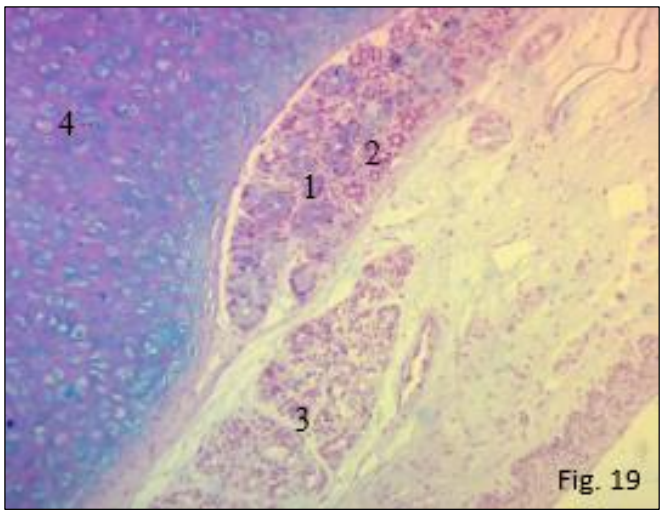

Fig 18: Epiglottis showing positive reaction to PAS Alcian blue 1. Mucous glands with acid mucopolysacchrides 2. Serous glands with neutral polysaccharides, 3. Glands showing negative reation 4. Cartilage with acid mucopolysaccharides and sulfated proteoglycans and hyaluronic acid, PAS Alcian Blue (40X)

acidic and neutral mucopolysaccharides. The mucous and serous glands consisted of neutral mucopolysaccharides whereas the mucous part of the mixed glands showed positive to acid mucopolysaccharides and few glands towards the laryngeal surface were negative to PAS AB (Fig -17). Present results of PAS and PAS AB of epiglottis were dissimilar to the observations of Singla. S and Kumar. P., 2021 [18] in goat where the chondrocytes of epiglottis were strongly PAS positive for glycogen and mucous acini were positive to the acidic mucopolysaccharides on the ventral surface.

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