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Anatomical and Histological Study of Trachea in Iraqi Naked –Rumped Tomb Bat (*Taphozous nudiventris*)

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Abstract

The present study was conducted on five healthy naked- Rumped Tomb Bats their weights were ranged between (45.8-53 gm). The Result of the anatomical study revealed that the trachea of the bat was non-folding cartilage tube and flexible. It composed of series of incomplete cartilaginous rings arranged on the ventral surface of the neck, their numbers about (15-17 cartilage rings). The trachea divided into three parts and these parts differs in anatomical characteristics in terms of diameters and links. The first part was the upper part which have less diameter than the middle and lower part, as its diameter reached into (1190.82 \pm 69.51 mm), while the middle part was larger diameter (1635.56 \pm 101.24 mm) and the lower part was wider diameter (1681.53 \pm 54.40). Trachea was lined by respiratory epithelium (ciliated pseudostratified columnar epithelium) with a few goblet cells. Lamina propria –sub mucosa of the trachea was supported by hyaline cartilage and it is composed of loose connective tissue, with bundles of collagen fibers and adipose cells.

INTRODUCTION

The respiratory system is responsible for gas exchange in the body. It provides the body cells with O_2 necessary for its activities and saves them from CO_2 (1). The respiratory system in the mammals divided into two parts, the first is conducting portion, which is the part responsible for delivering air from the surrounding air to the respiratory pharynx,throat, tract including (the nose, and trachea, bronchi)(2,3). The second is respiratory portion which performs gas exchange function and includes (respiratory bronchioles, alveolar ducts, alveolar sacs and alveoli)(4-6). The Studies have shown that trachea in mammals composed from series of cartilaginous rings in the form of a letter (c-shaped) and divided into two parts(7,8).It is numbered according to the areas of positioning; cervical trachea extends from the full ring into branch within the chest, contains 38-43 ring of hyaline cartilage, and forms incomplete back rings. The second area is the abdominal trachea located at the center of the dorsalventral part of the right and left of the trachea that enters the lung (9-11) and it connects the ligaments of the rings between adjacent cartilaginous rings(12). The cervical trachea connected with the ventral and both sides by the thyroid muscle and it bounded by longitudinal muscle (13).(14) study explained that the posterior tracheal open consists of smooth muscle fibers pressed on the food mass in the esophagus that is located behind the trachea, the trachea is also located in the cervical region of the thyroid gland and blood vessels. Tracheal wall in general consists of respiratory epithelium (ciliated pseudo stratified columnar epithelium) with goblet cells. Under epithelium, the lamina propria and sub mucosa composed of loose connective tissue containing tracheal glands, which are serous and mucous type, while hyaline cartilage covered externally by tunica adventitia or serosa(15). A studies of wiled goat's trachea showed that the trachea lined by a layer of pseudo stratified ciliated epithelium and it was noted that the epithelial cells was more individual cells producing mucus, while goblet cells a relatively small and acidic secretion (16,17). Moussa, E. and Hassan, S.A.(2015) mentioned that in red fox the ciliary cells in the central part of the trachea contains more goblet cells which result from cell division of chondrocytes(18). Cartilage matrix is usually provided by a perichondrium, whose inner layer is chondrogenic, containing cells with the ability to become chondroblasts and its outer portion is dense irregular connective tissue(19).

MATERIAL AND METHODS

In the current study,5 animals were used to identify the morphological description and histological structure for the trachea of Naked-Rumped tomb bat *Taphozous nudiventris*, which it was collected from Al-Najaf city.

The animals were anesthetized using chloroform. The animals were cut and the trachea was extracted from the samples, then it was fixed in aqueous Bouin's solution for (22-24) hour after that it was washed with 70% ethyl alcohol and finally it was kept until the completion of the steps of preparing sections. The dehydration process was carried out by sampling a series of ascending concentrations of ethyl alcohol starting from the concentration 70%,80%,90%,100% for 30 minutes per concentration and it was repeated the process twice in 100% for the purpose of remove water. The samples were cleared using xylene, then they were embedded with paraffin wax and they were sectioned with rotary microtome thickness of (6) micrometer. The serial sections were then placed on clean glass slides containing drops of distilled water and the sections were transferred into the hot plate at 37° c to dry (20). After that the sections were prepared to stain with the following stains:-

- 1- HematoxylinHaris&Eosin stain
- 2-periodic acid-shiff (PAS) stain
- 3-Massons trichrome stain
- 4-Alizarine red s stain

Statistical analysis

The data were analyzed using the SPSS program v.16 (social package of social science) used the one way ANOVA test to find least significant difference at $p \le 0.05$ as well as of the independent t- test. The results were presented as mean \pm standard error. The thickness of each main layers lining the tracheal wall.

RESULT

Trachea of Naked-Rumped tomb bat Taphozous nudiventris was appeared as non-folding cartilage tube and flexible, composed of series of incomplete cartilaginous rings arranged on the ventral surface of the neck, their number about (15-17 cartilage ring) when using a methylene blue stain (Fig.1). The trachea accompanied with the heart at the level of its base and it branched into two branches, the primary bronchi enter the right and left lobes of lung (Fig.2). The first part was the upper part which have less diameter than the middle and lower part and its diameter reached into $(1190.82 \pm 69.51 \text{ mm})$, the middle part was larger diameter (1635.56 ±101.24 mm) and the lower part was wider diameter (1681.53 ±54.40)(Fig.3).The cartilaginous rings of the trachea open in the posterior surface corresponding to the esophagus. The ends of the cartilaginous rings contain loose connective tissue and bundles of smooth fibers called trachealis muscles, which lies between the mucous membrane lining the trachea and the loose connective tissue that are attached to the inner surface of the cartilage rings(Fig.4). The trachea of the bat is a hollow organ composed of Tunica mucosa, Tunica. submucosa and Tunica adventitia that contain the cartilage rings(Fig.5). The trachea lined by respiratory epithelium (ciliated pseudo stratified columnar epithelium) with a few goblet cells(Fig.6). Lamina propria and sub mucosa of the trachea was supported by hyaline cartilage and it was composed of loose connective tissue, with bundles of collagen fibers and adipose cells (Fig.7). The matrix (ground substance) of the tracheal cartilages it contained the scattered lacunae that bounded chondrocytes and it was entirely surrounded by perichondrium which were continued with the adventitial coat of the trachea (Fig.8). The mean thickness of the tunica mucosa $(90.62\pm$ 11.75mm), while the tunica sub mucosa was thicker $(388.81 \pm 77.70 \text{ mm})$ than the tunica mucosa, but the tunica adventitia thickness was (386.56±28.85mm)(Fig.9).



Figure. (1): ventral view of trachea showing the number of cartilaginous rings (Methylene blue stain).

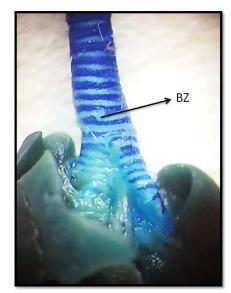


Figure (2): ventral view of trachea showing (BZ:Branch zone) of cartilaginous rings of Trachea.

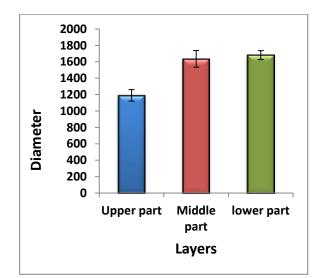


Figure (3): The mean and standard error(Mean±SE) for the diameters of the three parts of trachea (upper part, middle part, lower part).

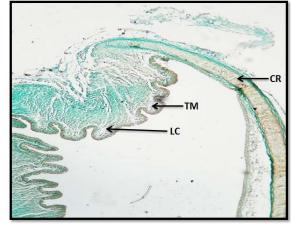
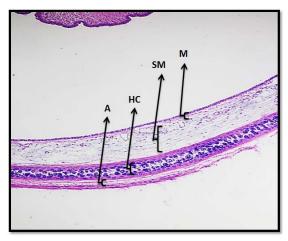
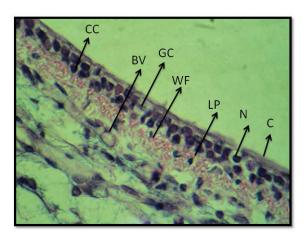


Figure (4): Cross section of the trachea in the Bat *Taphozous nudiventris* showing :area of contact cartilage rings (CR) with trachealis muscle(TM) and loose connective tissue(LC)(Alizarin stain 20X).



Figure(5): Cross section of the trachea in the Bat *Taphozous nudiventris* showing :tunica mucosa(M),tunica sub mucosa(SM),hyaline cartilage(HC),tunica adventitia(A)(H&E stain 10X).



Figure(6): Cross section of the trachea in the Bat *Taphozous nudiventris* showing: cilia(C),goblet cell(GC),nuclei of cells(N),columnar cells(CC),lamina propria(LP),white fibers(WF),blood vessels(BV)(H&E stain 100X).

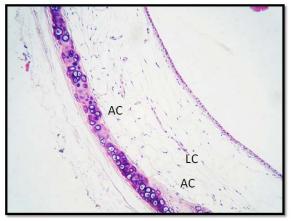
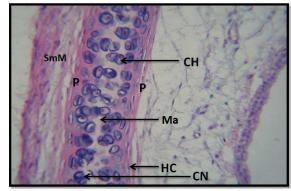


Figure (7): Cross section of the trachea in the Bat *Taphozous nudiventris* showing: loose connective tissue (LC), adipose cells (AC)(H&E stain 10X).



Figure(8):Cross section of the trachea in the Bat *Taphozous nudiventris* showing :Hyaline cartilage(HC),Chondrocytes(CH),Perichondrium(P), Cell nest(CN),matrix(Ma),Smooth muscle fibers(SmM)(H&E stain 40x).

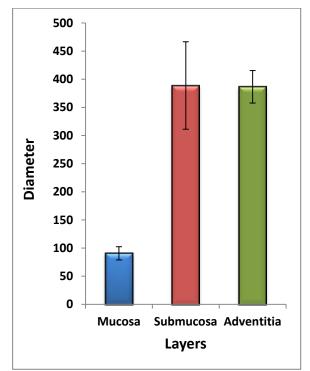


Figure (9): Shows the mean and standard error (Mean±SE) for the thickness of three tunica of trachea (Mucosa, sub mucosa, tunica adventitia).

DISCUSSION

The results of the current study showed that the trachea in the naked rumped tomb bat *Taphozous nudiventris* appears as a cartilaginous and flexible (unable to bend) tube. This tube extending from the end of the cricoids cartilage ring of the larynx at the level of the second cervical vertebrae, when the link area thyroid gland in the ventral surface of the first three cartilage rings. This result is confirmed by the sources and research reported in the studies related with different mammalian as the studies (21-23).

Reference of studies on birds including such as (24) study on male pigeon (*Columba domestica*) which showed that the trachea is branched into two branches in the syrinx tube. The syrinx tube is located at the base of the trachea to the primary bronchi, both of which are entered into the lung by a chamber located on the barrier surface of the right and left lungs.

The results of the current study is disagreement with the study of the male adult pigeon, explaining that the trachea in the naked rumped tomb bat, appears to be associated with the heart at the base level and branched into two branches of the primary bronchus, one enters the right lung and the other to the left lung. This description also resembles that of (25, 23) in their studied on trachea of the cat *Felis catus*.

The trachea is divided into three parts, the first part is the upper part, which has a diameter rate (1190.82 \pm 69.51 mm), as it was lower than the middle and lower parts, with the diameter rate of the middle part (1635.56 \pm 101.24 mm). The lower part recorded the highest rate in comparison of the upper and middle parts diameters, reaching (1681.53 \pm 54.40 mm).

The results of the present study inconsistent with the results of the different types studies of mammalians for trachea diameters. It was observed a significant variation on trachea diameters from on animals with other. This difference is related to the structural construction and different sizes of animals based on requirements of functional need. The results of (26) study on transverse diameter of the Ovis aries were showed that the upper third diameter is greater than the middle third diameter and the lower third with a diameter of the upper third (26.77 ±1.2 mm), the middlethird diameter (24.38 \pm 1.5 mm) and the diameter of the lower third (22.5 \pm 2.7 mm). While trachea diameter in the camel (Camelus dromedaries) showed a clear difference, with a diameter of $(77.1 \pm 0.35 \text{ mm})$ (27). Whereas the trachea diameter in humans from the age of 22-88 years is $(6.8\pm35.6 \text{ mm})$ in males and $(24.7 \pm 6.1 \text{ mm})$ in females (28).

The trachea rings are opened in the posterior surface that corresponds of the esophagus and the ends of the cartilage rings connect the loose connective tissue and the smooth muscle fibers that represented the tracheal muscle, which is in agreement with several studies that mentioned on the histological structure of the trachea (29). The results of microscopial examination demonstrated that the trachea consists histologically of three tunicae represented by mucous, submucosa and adventitia that containing the cartilage rings. This result is consistent with the results of many studies in various vertebrates (30,31).

Several studies, which examined the histological structure of the trachea in different mammals, confirmed that the mucosa is composed of pseudo stratified ciliated columnar epithelium that contain of three cells types (columnar, basal and goblet cells) which are large in size and diffuse in the tunica mucosa (32,33,34,35). This fact did not fully agree with the results of the present study about the trachea in Nached –Rumped Tomb Bat of goblet cells. The goblet cells were scattered slightly in the middle and lower part of the ciliated columnar cells, this results similar to (36) study in bull. The matrix (ground substance) of the tracheal cartilages contained the scattered lacunae which bounded the chondrocytes and it was entirely surrounded by perichondrium that were continued with the adventitial coat of the trachea, this result agree with the results of (10,19).

The results of the present study showed that there was a significant difference between the thickness of the tunica mucosa when compared with the submucosa and the adventitia, as they were thicker than the tunica mucous. Whereas the mucous thickness was (90.62 ± 11.75 mm), tunica submucosa thickness was (388.81 ± 77.70 mm) which was thicker than the tunica mucosa, while the tunica adventitia has reached its thickness into (386.56 ± 28.85 mm) and it has not been observed between them and the submucosa, a significant difference.

The results of the present study are in agreement with the results of (26) and contrary to the results of (37) to study the turkey *Melagaris gallopava* whereas The hyaline cartilage layer was remarkably thick as it reached the thickness ($464 \pm 11 \text{ mm}$) and this may be due to the ossification of this layer as well as containment of the wall of the turkey trachea on a thick layer of the muscular tunica, which consists of a thick circular muscle layer. In the naked rumped tomb bat, the muscular tunica was poorly formed as it only appears thickness in the area that connects the cartilage rings, which is the tracheal muscle which consists of smooth muscle fibers that may be associated with the pressure on mass of food passing through the esophagus (10).

The study (38) showed partial compatibility with the results of the current study in the thickness of the hyaline cartilage as the thickness of the hyaline cartilage in Guinea chicken with the thickness of the hyaline cartilage in naked rumped tomb bat. While the thickness of the hyaline cartilage in the chicken water was less thick than the cartilage in the naked rumped tomb bat and this may be due to the presence of the ossification.

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