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A Histochemical Study of Lungs in Iraqi Weasel *Herpestes javanicus*

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Abstract

The current study aimed to identify the histological structure of the lung in Iraqi weasels, *Herpestes javanicus*. The present study was conducted on 5 samples of weasel animals weighing between 288 and 564 g. The results of the study displayed that the trachea in *Herpestes javanicus* branches into two branches of the primary bronchi, which are similar in tissue structure to the trachea, and the results also showed that the primary bronchus branch out to be the secondary and tertiary intrapulmonary bronchus, as the cartilaginous rings turn into finger-shaped plates of hyaline cartilage and these cartilaginous plates disappear in the bronchioles, which in turn branch into the terminal bronchioles, which give a branch to the respiratory bronchioles, which in turn branch into several alveolar sacs, which lead to alveoli. The lungs are covered with the parenchymal pleura membrane, which is made up of a thin layer of connective tissue covered with a layer of mesothelial tissue. The study concluded that the lung in weasels exhibits a tissue structure similar to that of other mammals, with some special variations in this species.

Keywords: Histological, Lung, Bronchial Tree, Iraqi weasel.

1. Introduction

There are two species of weasel in Iraq, namely the weasel *Herpetes javanicus* and the Indian gray ferret. The weasel *Herpetes javanicus* is the most widespread, living in the regions covering the banks of the Tigris and Euphrates, while the Indian gray ferret is less widespread and inhabits central and northern Iraq [1].

Several studies have dealt with weasel in numerous ways [2-10], such as [11] a study in the digestive and respiratory systems of the Indian gray weasel and [12] a comparative phenotypic and anatomical study of the length and weight of the body, lung, and some digestive organs between two species of weasel .The present study aimed to study the histological structure of the lung and bronchial tree in Iraqi Weasel by using hematoxylin and eosin and some special stains (Van Giesson and periodic acid Schiff).

2. Materials and Methods

Five mature weasels of both sexes [males and females] weighing 288–564 g were used in this

study, which were obtained from the local markets in Baghdad governorate and Al-Najaf city. We dissected the lungs and immersed them in normal saline. For histological examination, the samples were fixed in 10% neutral buffered formalin for 24 hours. The tissue samples were dehydrated in graded alcohol from 70% up to 100% after washing them with distilled water. Cleared in xylene and embedded in paraffin wax. Each paraffin block was sectioned at 5 micrometers in thickness and stained with haematoxylin and eosin, Van Giesson, and Periodic Acid Schiff stain (PAS) [13]. The selective sections were photographed by using a light microscope with a camera.

3. Results

The weasel trachea branches into two branches of primary or principal bronchi that have similar structures to the trachea, one of which enters the right lung and the other the left lung. Its wall consists of three tunics, represented by the tunica mucosa, the tunica sub mucosa, cartilage layer, and the tunica adventitia **Figure 1**.

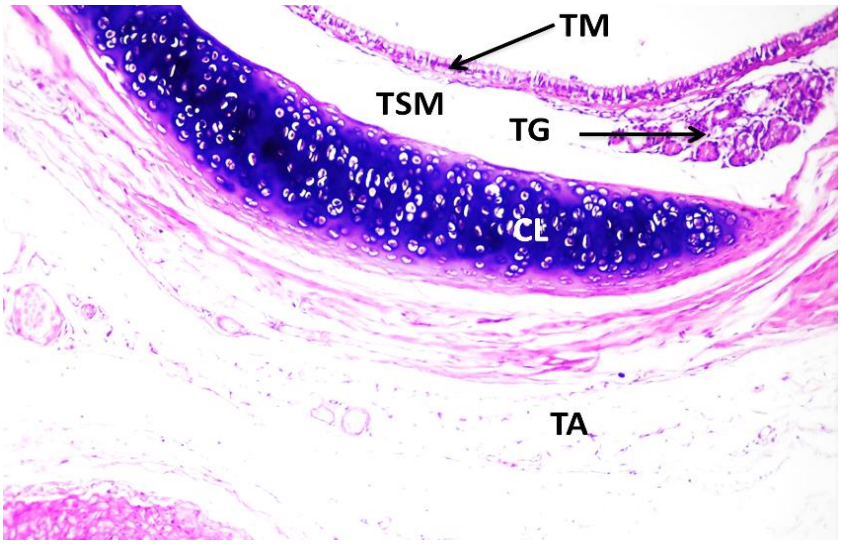

Figure 1. Source, Numbers and Percentages of *S.aureus* isolates**.**

3.1. Histological Structure of Lung

The consequences of the microscopic examination of the lung tissue showed that it has a spongy-shaped appearance that contains many sections of multiple shapes and sizes similar to the beehive, and these alveolar sacs are sometimes composed of one or multiple lobular, which represent the pulmonary alveoli. The results also showed that the pulmonary tissue is abundant in blood supply, represented by the presence of sections of blood vessels of different sizes and containing a quantity of red blood cells, as well as the appearance of transverse sections of primary, secondary, and tertiary bronchi that are in the form of semi-circular sections that show a decrease in diameter and thickness and a decrease in the number of trachea glands and the thickness of the tunics as it was gone to the examination to the caudal end of the lung, and the folds of the mucous layer of the bronchi appear in the form of oblique fingers. It was also noted that the hyaline cartilage appears in the form of an elongated finger in the primary bronchi and gradually turns into intermittent plates with a decrease in the diameter of the bronchi until it becomes circular in other areas until it disappears continuously branching, while the tunica

adventitia appears to have an abundant blood supply with vessels with sections of different diameters and sizes containing quantities of blood cells as well as the presence of adipose cells and nerves. Microscopic examination also showed the presence of a number of small terminal bronchioles surrounded by many spherical alveoli, whose cavity opens into the respiratory canals leading to the alveolar ducts, whose lining is thinner than the lining of the respiratory bronchioles, which in turn ends in a cluster of alveolar sacs in the form of a multi lobular ending in alveoli **Figure 2**.

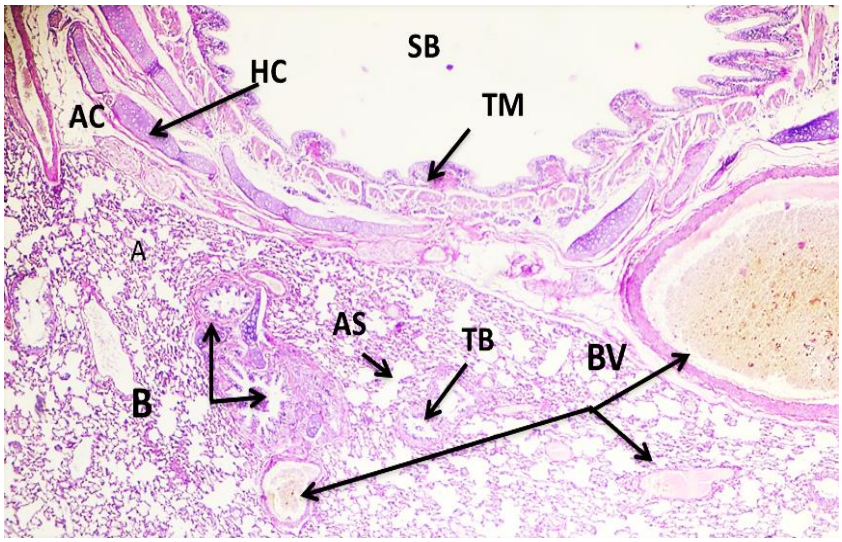

Figure 2. A cross section in the weasel lung showing the general histological structure of the lung, secondary bronchi (SB), bronchioles (B), blood vessels (BV), alveoli (A), hyaline cartilage (HC), Adipose cells (AC), alveolar sac (AS), tunica mucosa (TM), terminal bronchiole (TB), (H&E stain, 4x).

The results of the current study showed that the tunica mucosa of the primary bronchi is represented by ciliated pseudo-stratified columnar epithelial tissue, and the mucosa layer is in the form of small undulated folds prominent towards the bronchial cavity. Alternatively, the submucosa layer consists of loose connective tissue containing sero-mucous glands (mixed) and serous tracheal glands and mucous glands in small numbers. It was noted that the number of these glands decreases as the size of the bronchi decreases with the appearance of an intermittent muscularis mucosa consisting of smooth muscle fibers between the tunica mucosa and the tunica sub mucosa, either the cartilaginous layer, which in turn is intermittently represented by finger-like hyaline cartilage, or in the form of small pieces, as the results of microscopic examination showed that the adventitia layer contains loose connective tissue containing adipose cells, blood vessels, and nerves **Figure 3**.

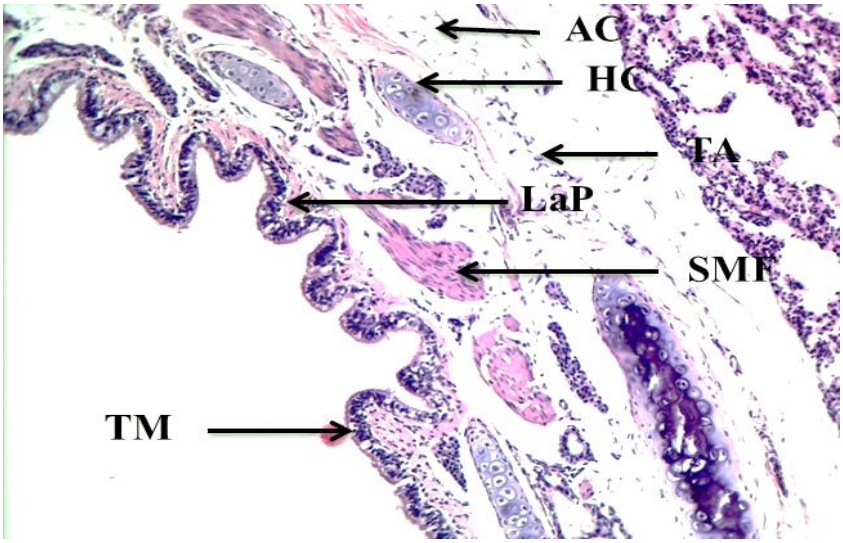

Figure 3. A cross section in the weasel lung showing the primary bronchi, tunica mucosa (TM), hyaline cartilage (HC), lamina propria (LaP), smooth muscle fibers (SMF), tunica adventitia (TA), Adipose cells (AC) (H&E stain, 10x).

The results of the present study displayed that the continuation of the decrease in diameter down to the caudal end of the lung leads to a decrease in the diameter of the bronchi and increases the depth of folds in the mucosa layer as it leads to a gradual extinction of the number of glands and the cartilaginous layer as the segmental bronchi (Tertiary bronchi) show similarity in their tissue structure to the primary bronchi, but the mucosa layer is in the form of small finger folds prominent towards the bronchial cavity, or the submucosa layer is narrow, containing scattered glandular clusters. As for the cartilaginous layer, which in turn is intermittent and contains finger-shaped hyaline cartilage or flask shape and sometimes in the form of the letter Y, the muscular layer is thicker than it is in the primary bronchi and increases in thickness as the diameter of the bronchi decreases, as the results of microscopic examination showed that the adventitia layer contains loose connective tissue containing adipose cells, blood vessels, and nerves **Figures 4.** and **5**.

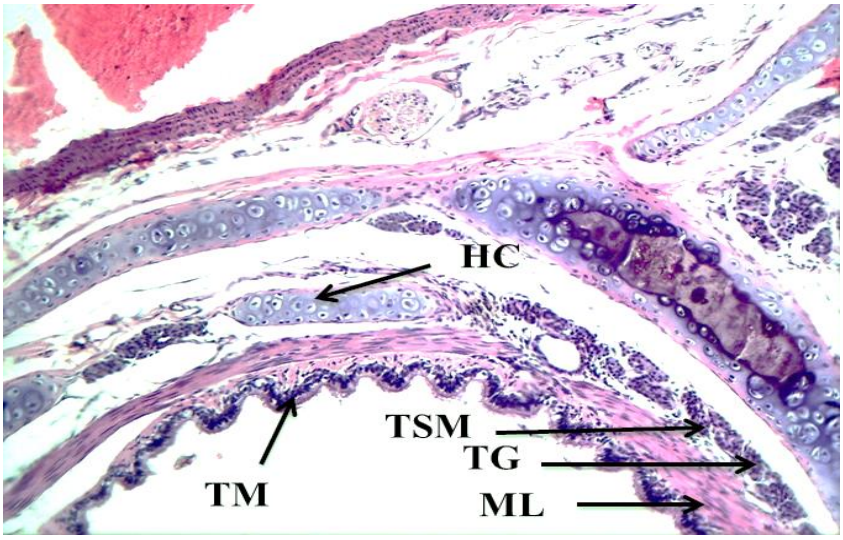

Figure 4. A cross section in the segmental bronchus, showing the tunica mucosa (TM), tunica submucosa (TSM), muscular layer (ML), hyaline cartilage (HC), trachea glands (TG),tunica adventitia (TA) (H&E stain, 10X).

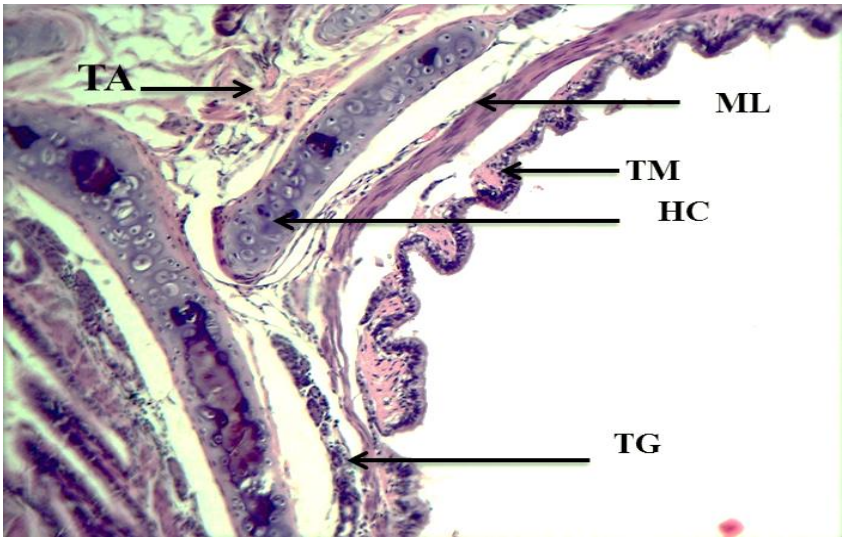

Figure 5. A section in the weasel lung showing the structure of the segmental bronchus, tunica mucosa (TM), tunica submucosa (TSM), muscular layer (ML), hyaline cartilage (HC), trachea glands (TG), tunica adventitia(TA) (H&E stain, 10 X).

The segmental continues to branch out to be bronchioles, which appear in the form of circular or oval sections, and its walls consist of a tunica mucosa represented in the form of deep folds consisting of ciliated simple cuboidal epithelial tissue, and the cartilaginous layer fades at the level of some bronchioles, while the muscular layer shows thicker than it is in the trachea and the primary, secondary, and tertiary bronchi and surroundings the epithelial lining and in the form of a circular ring, and the muscularis layer is surrounded by a thin layer of loose connective tissue, representing the tunica adventitia (**Figure 6, 7**). The results of the present study show that the terminal bronchioles have thin walls lined with cuboidal epithelial tissue free of goblet cells, based on the basement membrane, and bordered by a thin layer of loose connective tissue.

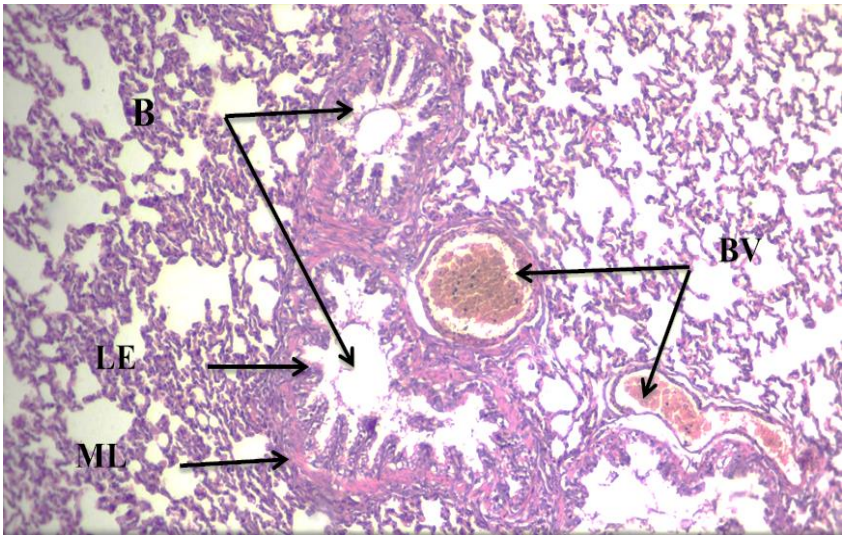

Figure 6. A section in the lung showing the structure of the bronchioles (B), lining epithelium (LE), muscular layer (ML), blood vessels (BV), (H&E stain, 10X).

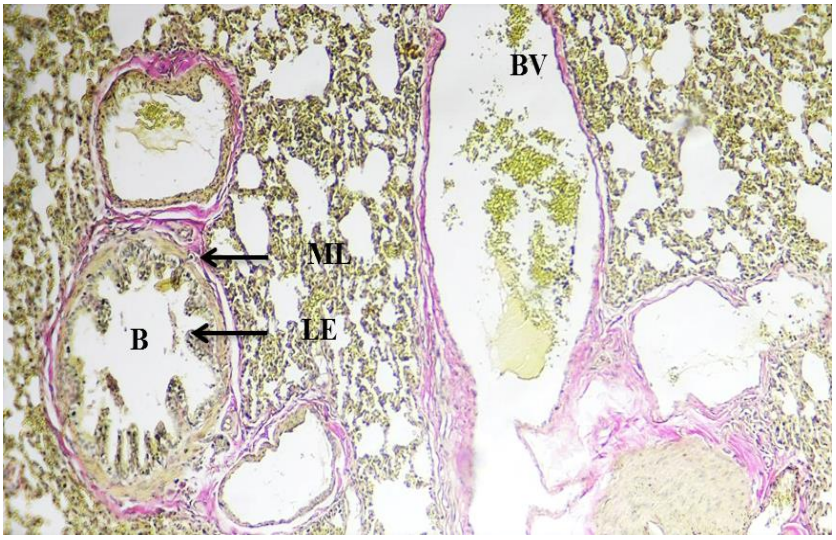

Figure 7. A cross section in the lung showing the fading of hyaline cartilage in the bronchiole (B), lining epithelium (LE), muscular layer (ML), blood vessels (BV), (Van Gieson stain, 10X).

The terminal bronchioles lead to respiratory bronchioles and they are in the form of channels with a lining consisting of simple cuboidal epithelial tissue to low cuboidal epithelial tissue, which in turn branch to form alveolar ducts, which open into alveolar sacs and contain their walls on a large number of alveoli (**Figure 8**). The results of the present study displayed that alveolar sacs appear as irregularly shaped sacs structures and form the bulk of the lung stroma, so they give a spongy texture to the lung as well as they are thin-walled as they are lined with simple squamous cells with circular or flat dark nuclei with the presence of cuboidal to circular cells near the alveolar lumen cavity (**Figure 9**).The alveolar sac, in turn, contains of a large number of alveoli pulmonary alveoli, which are separated from each other by thin walls composed of endothelial cells based on loose connective tissue containing collagenous, elastic and reticular fibers, as well as the presence of connective tissue cells and a copious network of capillary vessels spread between them. The adjacent alveolar are separated from each other by a inter alveolar septum appear circular or in the form of irregular air chambers small in size and line the pulmonary alveolar with two types of cells, namely:

1.Alveolar Cell Type I: It is represented by squamous cells with central nuclei and constitutes a large proportion of the wall of the alveoli.

2.Alveolar cells Type II: It is represented by cuboidal alveolar cells that constitute a small percentage of the cells of the wall of the surface of the alveolar and are located among the cells of the first type. They are characterized by their large size and contain lamellar bodies.

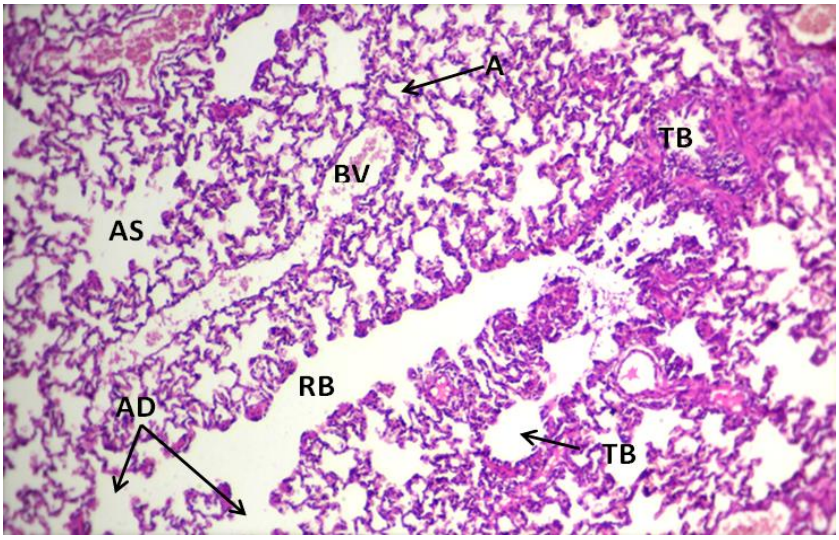

Figure 8. A cross section of the lung showing the terminal bronchioles (TB), alveolar duct (AD), alveolar sac (AS), alveolar (A), blood vessels (BV), respiratory bronchiole (RB). (H&E stain, 10x).

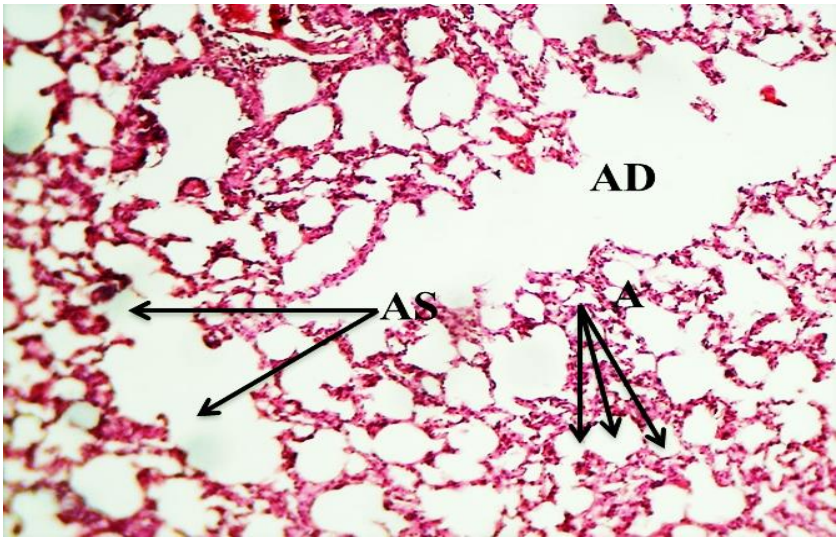

Figure 9. A cross section in the lung showing the alveolar duct (AD), alveolar sac (AS), alveolar (A), (H&E stain, 40X).

Furthermore, there is an additional type of alveolar cell called macrophage or dust cell. These cells are characterized by having a pale cytoplasm granular color containing dust particles, and the nuclei are irregularly dark in color. These cells are separated from the alveolar cells by a certain barrier or are located outside the alveolar tissue (**Figure 10**).

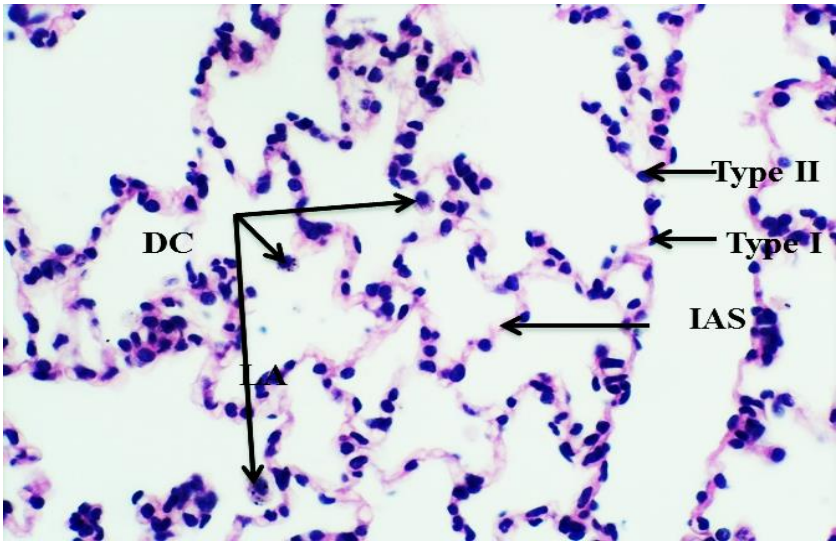

Figure 10. A cross section in the lung of the weasel showing the histological structure of the alveolus, alveolar cells Type I, alveolar cells Type II, dusty cells (DC), interalveolar septum (IAS), (H&E stain, 100X).

4. Discussion

4.1. Histological Structure of Lung

The results of histological investigation in the Iraqi weasel *Herpestes javanicus* presented that the trachea branches at the carina area into two branches of the primary bronchi that enter each lung, which appears to be a spongy shape that contains many sections of multiple shapes and sizes similar to the beehive, and these alveolar sacs are sometimes composed of one lobule or multiple lobules, which represent the pulmonary alveoli, and these results were consistent with many studies concerning the lung in mammalian [11, 14].

The bronchus wall consists of tunica mucosa, which is lined by ciliated pseudo-stratified columnar epithelial tissue contenting columnar cells, goblet cells, and basal cells, and this finding agrees with the study of the guinea pig Cavia porcellus [14] and the study of *Taphozous npudiventris* [15]. Although the results of the present study did not agree with [16] study in *Cryptotis parva*, the lining epithelium contains two types of low columnar cells, represented by columnar cells that are non-ciliated and ciliated cells, while goblet cells are not distinct, and if they exist, they are non-ciliated in shape and are buried between columnar ciliated cells that can only be distinguished by special stains. The results of the present study displayed that the tunica submucosa consists of loose connective tissue containing a small number of glands, and the cartilage layer is characterized by containing hyaline cartilage represented by finger shape or in the form of small pieces, and the tunica adventitia consists of loose connective tissue containing nerves and blood vessels. These results are generally consistent with the study of [17] in domestic rabbits, while the results of the present study appear to be partially contrary to previous studies and research related to the histological structure of the lung in mammals, as the primary bronchi are lined with ciliated epithelium, which is scattered with a small number of goblet cells. These epithelial tissue characteristics vary slightly with the epithelial tissue features of weasel in terms of the number and type of goblet cells in weasel and other mammals, and this may be due to the different tissue organization related to the functional specificity performed by the primary bronchus.

The results of the present study indicated that the primary bronchi branch into secondary and

tertiary bronchi. Tunica mucosa is characterized by an abundance of folds, while hyaline cartilage is in the form of irregularly cut plates. The tunica mucosa is separated from the hyaline cartilage with a layer of smooth muscle fibers. It is believed that the reason for the presence of folds is due to the layer of muscle fibers underneath, which is likely to be the reason for regulating the caliber of the trachea cavity. The lamina propria consists of a thin layer of connective tissue surrounded by smooth muscle fibers. The results of the recent study are partially consistent with those of [18] in domestic animals and the study of [14] in guinea pigs, *Cavia porcellus.* The results of the existing study of Weasel displayed that the wall of the bronchioles, which is characterized by containing mucous folds consisting of ciliated simple columnar epithelial tissue to ciliated simple cuboidal epithelial tissue, and the cartilaginous layer fade at the level of some bronchioles, While the muscular layer is thicker in the bronchus and spreads in different directions, this may be due to its role in controlling the flow of air to the lungs. These results are consistent with the results of the study of domestic rabbits [18]. The new study showed that the bronchioles split into smaller branches, creating what are called terminal bronchioles. These are the last parts of the weasel's air delivery system and are made up of cells that are flat and columnar or cuboidal in shape, based on the basement membrane. They are free of glands, goblet cells, and cartilage.

It is bordered by a thin layer of connective tissue, which in turn is surrounded by many air sacs, and this result is consistent with the study of [19] in guinea pigs and the study of [20] in pigs. The terminal bronchioles continue to branch into smaller branches called respiratory bronchioles, and these bronchioles are described as being thin-walled and lined with cuboidal cells or low cuboidal cells. These results of Weasel correspond to what was exposed by the results of prior studies such as [21] in domestic animals. Whereas the results of the existing study disagree with the results of the study of [22] in the camel *Camelus dromedaries*, who designated in his study that the terminal bronchioles and respiratory bronchioles are lined with simple columnar or cuboidal epithelial tissue free of goblet cells. The results of the present study are contrary to those of the mammalian [23], as it was stated that respiratory bronchioles are present in *Ruminantia* sp. and *Sus* sp., while they are poorly formed in horses *Equus* sp. and Homo sapiens, while [24] noted that respiratory bronchioles are not present in *Camelus dromedaries*. The respiratory bronchioles terminate with a thin-walled conical channel called the alveolar duct, and the final section of the alveolar duct expands to open to a number of alveolar sacs. Their walls encompass a large number of alveoli, and both the alveolar canal and the alveolar sacs are lined with simple, squamous epithelial tissue that is simpler and finer than in the respiratory bronchioles. These results are consistent with several studies, such as [18, 25, 26].

The alveolar ducts in the lungs of the mammalian lead to the alveolar sacs that represent the parenchymal tissue of the lung and give the lung a spongy texture. The alveolar sacs are different sizes and lined with simple squamous cells and represent the functional unit of gas exchange, protruding into the alveolar lumen. The alveolar sac consists of a number of air alveolars that are disjointed from each other by a thin barrier called the inter-alveolar septum. These findings are consistent with those of previous studies [26, 27].

The results of the current study showed that the lining of the pulmonary alveoli contains several types of cells, namely:

1.Alveolar cells (Type I): They are squamous cells that represent the largest proportion of the alveolar surface and on both sides of the alveolar wall.

2.Alveolar cells (Type II): They are irregular cuboid cells characterized by their large size. Furthermore, dust cells, a distinct type of alveolar cell, are separated from the alveolar cells by a certain barrier.

These results were consistent with the results of [26] in the rat Rattus norvegicus and [28] in the bat *Edilon helvum* and *Rattus norvegicus tricuspis*, as these studies revealed that endothelial cells are of three types, namely alveolar cells Type I and alveolar cells Type II, which consist of cuboidal cells comprehending granules called cytosomes that contain a substance that precludes the closure of the air alveoli, while the third type of cells is dust cells or phagocytes, which are immune cells that purify the lungs from dust and foreign materials that enter them from the external environment.

Previous studies have shown that the diameter of the bronchioles plays an important role in the flow of air to all parts of the lung, and the diameter of the bronchioles changes according to the air flow, as the diameter of the bronchioles increases in a case called broncho-dilation by stimulating sympathetic nerves, or epinephrine, which leads to airflow. The diameter of the bronchioles decreases in a condition called broncho-constriction by parasympathetic nerves, cold air, chemical radiation, and other factors that lead to decreased air flow [29, 30].

5. Conclusions

The present study concluded that the lung in Iraqi weasels exhibits a tissue structure similar to that of other mammals, with some special variations in this species.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

The samples were gained according to Local Research Ethics Committee approval in College of Education for Pure Sciences, Ibn-AL Haitham, University of Baghdad No. EC-13.

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