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Histological and Histochemical study of Lungs in Domestic Rabbits (*Oryctolagus Cuniculus*)

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Abstract

Aims: The current study was performed to explore histological features of the lung in the domestic rabbits.

Methods: The lung specimens were collected from ten healthy rabbits taken from center at College of Veterinary Medicine / University of Baghdad animal housing. Pulmonary tissue specimens were studied via routine histological techniques.

Results: Histological findings revealed division of each bronchus into intra pulmonary bronchi that were frequently divided into primary, secondary and tertiary bronchi. Subsequent subdivision of the last bronchi showed four subdivisions that were primary, secondary, tertiary (terminal) and respiratory bronchioles. The walls of these respiratory passages were showed only mucosa and submucosa tunicae. The epithelial mucosa started from bronchial ciliated pseudostratified columnar epithelium till simple squamous type at alveoli. The tunica submucosa constructed of loose connective tissue in which invested hyaline cartilage segments in the main bronchus then gradually decreased in diameter to smaller and smallest segments. The hyaline cartilage disappeared in all types of bronchioles. The respiratory bronchioles were opened into alveolar ducts which in turn opened into alveolar sacs. Numerous walls' out-pocketings of simple squamous epithelium lined alveolar duct were observed. Alveolar sac composed of a group of alveoli clusters and the alveolus was just a small space lined by simple squamous epithelium of two types; Type-I pneumocytes (flattened lining cells) and type-II pneumocytes (large lumen-bulged cells with large nuclei).

Keywords: Bronchus, Histological technique, Lung, Mason's Trichrome, Rabbit

Introduction

Rabbits are belonged to the order Lagomorpha and to the Leporidae family. Many genera were classified under such family that was *Brachylagus*, *Bunolagus*, *Nesolagus*, *Oryctolagus*, *Pentalagus*, *Poelagus*, *Romerolagus*, and *Sylvilagus*. Rabbits go by a lot of strange nonscientific names, i.e. European rabbit is the popular name for the *Oryctolagus cuniculus* species. In fact, only *O. cuniculus* spices have been

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farmed and as a result, distinct breeds have been developed from it [1].

Most of Iraq rural areas are home to large populations of wild rabbits (*O. cuniculus*) or as it is well known as the European rabbit (2). The rabbit is susceptible to varying degrees of stress because of its distinct mental and behavioral makeup. When these animals are used in laboratory research that includes creating, finding and evaluating distinct system pathways that are comparable to other animals, they are considered as very valuable models (3, 4, 5).There are several preclinical studies using rabbits to investigate potential drugs and vaccines for various human and animal illnesses (6).

Nowadays, many researches were used rabbit's respiratory system to investigate new routs of treatment against pulmonary diseases affected respiratory system of human being. So that, rabbits were serves as a useful and cheapest animal model to such aims and considerations. Pharmacologically, in this species, initial and close to the end phase airway responses enable mechanistic examination of each drug reaction and its related side effects. Researchers may use rabbits as a model since they are simple to handle and easily accessible. Additional advantages of using rabbits include their size and ability to monitor physiological changes without harming the animal (7). In the last decade, patho-pharmacological researches were documented using rabbits as an experimental model for the sack of both human and animal's health and welfare, so that, histological features of many organs such as lung and respiratory passages of the rabbits were required for depth description and monitoring (8).

Because the respiratory systems of laboratory animals are useful for identifying rare species changes, they are employed as animal models in direct scientific investigations. The lungs are the most important component of the respiratory system. They are made up of a spongy mass of tissue covered with pleura that extends from the thoracic cavity within the thorax, as well as compact soft tissue (9). The lung is divided into many lobes that vary from side to side which are connected to the trachea, which branched into the bronchi of the right and left sides inside the lung tissue. The intrapulmonary bronchus is the hills area where the bronchi enter the lungs after that. It split off into main and secondary bronchioles, which are even smaller tubes, and culminated in clusters of minuscule air sacs known as alveoli. Alveoli resembled little balloons, grapes, or branches. The lungs get a very large amount of surface area from the bubble-shaped alveoli.

Up to this point, there is a paucity of research conducted on the histological structures of the of the lungs in the local rabbits up to date. Thus, the present study intended to add knowledge on the lungs of this significant experimental animal model. The information obtained could serve as basis for other veterinary

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or medical fields such as pathology and physiology. Given the above, the current study was performed to investigate the lung histological features of the local Iraqi rabbits.

Materials and methods

Ten healthy rabbits were euthanatized in excellent health. The animals were dissected after their euthanasia and the lungs were extracted outside the body and washed well to remove all the surrounding wastes and debris, then small pieces of different sites of this organ were cut and preserved with preservation solutions (10% formalin for some specimens and Bouin's solution for others) for the subsequent purpose of histological sections preparation. Subsequently, hematoxylin and eosin stains were used to differentiate the general features of the lung tissues (pulmonary parenchyma), whereas, special stain such as Masson Trichrome was applied to specified the characteristic histochemical features of such studied tissues

Preparation and staining of pulmonary parenchyma

Tissue specimens were excised from different sites of each lung. After fixation in 10% Neutral buffer formalin solution for two days they were kept in labeled cassettes and in a tissue processor (Tissue-Tek® VIPTM 5, Sakura Finetek Inc., CA, USA). Some specimens were fixed in Bouin's solution for 16 hours. After fixation, steps were included 45 min. 80% ethanol solution, 45 min. 90% ethanol, 45 min. per a step of three absolute ethanol changes, 45 min. per a step of two xylene changes and then after 30 min. 61˚C per a step of two paraffin wax changes. Finally, a traditional paraffin block of pulmonary parenchyma was prepared. The blocks were sliced at 5 µm using a rotary microtome. The histological sections were placed on histological slides and 60° C-heated for 60 mins for assuring their adherence. The sections were exposed to two xylene changes and rehydrated using a graded descending concentration system of ethanol to remove the paraffin from the sections before staining. Finally staining with either hematoxylin and eosin (H&E) or Masson Trichrome (MTC) was performed for light microscopy (10).

Results

The lungs of the rabbit were located in the thoracic cavity and covering by the pulmonary pleura. Histologically such covering showed serous membrane constructed by tunica serosa of the lung. Results revealed that both left and right main extra-pulmonary bronchi were penetrated the lung parenchyma and continue as intra pulmonary bronchi that were divided into primary, secondary

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and tertiary bronchi in a monopodial division series. The walls of these bronchi was build up by tunica mucosa and sub mucosa which was invested with plates of hyaline cartilage gradually changed to cartilaginous smaller and smallest plates. These bronchial passages were well surrounded by pulmonary parenchyma (Fig.1). Last division of bronchi will lost the plates and forming smaller and smallest channels of airway trees called bronchioles. In fact, they were four different histologically subdivisions that were primary, secondary, tertiary and respiratory bronchioles. Actually, two asymmetrical offshoot branches emerge from each of the primary branches (dichotomous) refers to two daughter branches with the same diameters, whereas, monopodial division of bronchus refers to two daughter branches with uneven diameters.

In the present findings, the mucosa of both extrapulmonary and intrapulmonary primary bronchi was built up by folded pseudostratified columnar ciliated epithelium with goblet cells which in turn rested on lamina propria of loose connective tissue containing numerous capillaries. Muscularis mucosa was well developed and circularly arranged. The submucosa showed simple coiled tubuloacinar glands (serous & sometime mucous) and often heavily infiltrated with lymphocytes. Staining with MTC showed that bronchial wall was well supported by the presence of several hyaline cartilage plates invested in a network of dense irregular connective tissue fibers (Fig. 1). In the secondary bronchi, epithelium similar to that of the primary bronchi was observed but the tunica mucosa displayed lamina propria made of a thin layer of irregular dense connective tissue. The underneath muscularis mucosa appeared as a thicker and circularly arranged bundles of smooth muscle fibers. The supportive tunica submucosa was made of loose connective tissue with the absence of glands. The hyaline cartilage appeared as small segments (Fig. 2). While the tertiary bronchi, the epithelial mucosa was turned into ciliated simple columnar with sporadically distributed goblet cells supported by lamina propria of a thin layer of loose connective, while the submucosa displayed smallest and fewer sporadically distributed plates of hyaline cartilages (Fig. 3). Each cell in the pseudostratified epithelial layer that lines the bronchi mentioned above is crucial to ensuring that air can flow freely. In fact, defensive and preventive capabilities against foreign air particles are provided by these cells as a group. Respiratory epithelium of the large proximal airways was lined by goblet cells, basal cells and ciliated columnar cells. The tertiary bronchi were divided into four smaller respiratory channels that were in order primary, secondary, tertiary or called terminal and respiratory bronchioles. In the bronchioles, tunica mucosa was covered by simple epithelium and starting loss of goblet cells. The lamina propria was very thin layer of loose connective tissue with the presence of circularly arranged smooth

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muscle fibers. In the primary and secondary bronchioles, the epithelium was simple columnar to low simple columnar, respectively. The findings showed the presence of few goblet cells in the earlier one but not in the latter secondary bronchioles. Smooth muscle fibers of the muscularis mucosa were reduced in the secondary bronchioles into 2 to 3 layers. Both types of bronchioles were lack to the cartilage plates and glands in their walls (Fig. 4, 5). By MTC staining it showed walls that were supported by loose connective fibers with absence of glands and cartilage plates. Histological features of the tertiary (terminal) and respiratory bronchioles revealed tunica mucosa of simple cuboidal epithelium and they were supported by a thin layer of lamina propria of loose connective tissue. One layer of smooth muscle fibers was observed in the terminal type and usually absent in the last type, i.e. the respiratory bronchioles. Their walls were supported by loose connective fibers with absence of glands and cartilage plates (Fig. 6). Current results found numerous blood capillaries in the lamina propria of the mucosa of the airways in addition highly vascularized pulmonary parenchyma. Submucosal vessels are linked to a cross network of vessels outside the smooth muscles and bronchial cartilages. Fine microscopic examination revealed that each respiratory bronchiole was opened into an alveolar duct. Numerous walls' out-pocketings were found in the simple squamous epithelium that lines such alveolar ducts (Fig. 7). Alveolar ducts were running between and opened into alveolar sacs which in turn was constructed from a group of alveoli clusters and the alveolus itself was a small apace lined by simple squamous epithelium of two types, that were Type-I pneumocytes, flattened lining cells and type-II pneumocytes, that were large vacuolated cells which were processed prominently large nuclei causing a bulging into the adjacent alveolar lumen (Fig. 8). In fact, the first type pneumocytes were facing or adherent directly to the capillary wall. The basal lamina of each was adherent to the corresponding capillary endothelial cell to form blood barrier system.

The alveoli in the rabbit's pulmonary parenchyma showed well identified Type II cells. These cells are usually packed in a dense bilayer membrane they were suggested previously as very important cells.

The current findings showed the histological feature of the important airway respiratory bronchioles of the local rabbit. Between the terminal bronchial and alveolar ducts, respiratory bronchioles were partly alveolarized airways. It can be suppose that in case of their absence, insoluble particles may be transported straight from alveolar ducts to terminal bronchioles. As a result, efficacy of these may show pathological condition comes from clearance failure.

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Discussion

The lungs of current rabbits were covered by the pulmonary pleura which were showed serous membrane constructed by tunica serosa of the lungs. Usually it is imperceptible in many other animal species as in rodents whereas, slightly thick in human (11).

Results revealed that both left and right main extra-pulmonary bronchi were penetrated the lung parenchyma and continue as intra pulmonary bronchi that were divided into primary, secondary and tertiary bronchi in a monopodial division series. Such branching of airway trees was recorded previously as 25 of generations in rabbits and 32 humans (12). Actually, two asymmetrical offshoot branches emerge from each of the primary branches (dichotomous) refers to two daughter branches with the same diameters, whereas, monopodial division of bronchus refers to two daughter branches with uneven diameters. However, branching in humans is nearly fully dichotomous, while monopodial in the rabbit (13) and other animals such as rats, pigs (14) and mouse (15).

In the present findings, the mucosa of both extrapulmonary and intrapulmonary primary bronchi was built up by folded pseudostratified columnar ciliated epithelium with goblet cells which in turn rested on lamina propria of loose connective tissue containing numerous capillaries. Each cell in the pseudostratified epithelial layer that lines the bronchi mentioned above is crucial to ensuring that air can flow freely. In fact, defensive and preventive capabilities against foreign air particles are provided by these cells as a group. Respiratory epithelium of the large proximal airways was lined by goblet cells, basal cells and ciliated columnar cells. Similarly previous comparative records between rabbit and human showed that density of such epithelial cells in, human's lungs have a different higher cellular density than rabbits, which was one of the most noticeable distinctions between the two species (12).

By MTC staining, results showed bronchioles walls supported by loose connective fibers with absence of glands and cartilage plates. Similarly, previous reference recorded in African giant pouched rat the goblet cells even though there are a few in the early primary bronchioles (16).

Current findings demonstrated numerous blood capillaries in the lamina propria of the mucosa of the airways in addition highly vascularized pulmonary parenchyma. Submucosal vessels are linked to a cross network of vessels outside the smooth muscles and bronchial cartilages. Previous microscopic measurements recorded about five capillaries in each mm in rabbit lung, whereas, approximately

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seven capillaries in each mm in human's lung (17, 18, 19).

Fine microscopic examination revealed that Alveolar ducts were running between and opened into alveolar sacs which in turn was constructed from a group of alveoli clusters and the alveolus itself was a small apace lined by simple squamous epithelium of two types, that were Type-I pneumocytes, flattened lining cells and type-II pneumocytes. In fact, the first type pneumocytes were facing or adherent directly to the capillary wall. The basal lamina of each was adherent to the corresponding capillary endothelial cell to form blood barrier system. The latter system is well known for its responsibility for gasses exchange between blood (internal environment) and inhaled air (external environment) (20).

The alveoli in the rabbit's pulmonary parenchyma showed well identified Type II cells. They play a role in air-liquid interface to prevent alveolar collapse due to their production and release of surfactant phospholipids and proteins from their secretory lamellar body vesicles (20) (21).

The current findings showed the histological feature of the important airway respiratory bronchioles of the local rabbit. Between the terminal bronchial and alveolar ducts, respiratory bronchioles were partly alveolarized airways. It can be suppose that in case of their absence, insoluble particles may be transported straight from alveolar ducts to terminal bronchioles. As a result, efficacy of these may show pathological condition comes from clearance failure. In correspond, human respiratory bronchioles, unlike rabbits, seem to perform a function in the establishment of fibrosis and other conditions (22, 23, 24, 25).

Differences in both bronchial divisions style as monopodial other than dichotomous present in other species and human as well the presence distinctly of partly alveolarized airways between terminal bronchial and alveolar ducts in rabbit's lungs being in parallel with previous findings that acknowledged and indicated many differences between rabbit and other species even the laboratory animal specially those of class rodentia. The study findings were in agreement and confirmed previous records and postulations of (26) and (27, 28, 29, 30) that rabbits were different from rodents in their organs anatomically, histologically and histochemically.

Unique differences of such animal species in the present findings and previous above references in the local rabbits confirmed the reason by which rabbits were alienated and classified in new different order in the animal classification or taxonomy. Rabbits were considered rodents until 1912, but different indentation resulted in the establishment of a new order called Lagomorpha of the class mammalian (31).

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Conclusions

Other species and human as well the presence distinctly of partly alveolarized airways between terminal bronchial and alveolar ducts in rabbit's lungs being in parallel with previous findings that acknowledged and indicated many differences between rabbit and other species even the laboratory animal specially those of class rodentia. The study findings were in agreement and confirmed previous records and postulations of (23) and (27, 28, 29, 30) that rabbits were different from rodents in their organs anatomically, histologically and histochemically.

Unique differences of such animal species in the present findings and previous above references in the local rabbits confirmed the reason by which rabbits were alienated and classified in new different order in the animal classification or taxonomy. Rabbits were considered rodents until 1912, but different indentation resulted in the establishment of a new order called Lagomorpha of the class mammalian (28).

The present study provides distinct histological features of the lung tissues in the domestic rabbits, which can be used in advanced future studies to evaluate the evolutional characteristic of this animal species as a model for both human and animal health investigations and experiments.

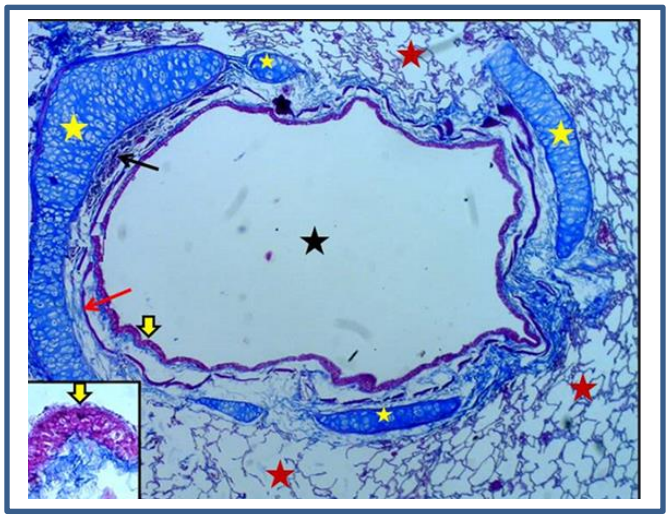

Fig. 1. Histological section showed primary bronchus characterised by pseudostratified epithelium (yellow arrows), muscularis mucosa (red arrow), submucosal glands (black arrow), wall supported by Hyaline cartiage (yellow stars), brochial lumen (black star) and pulmonary parenchyma (red stars). MTC, X200

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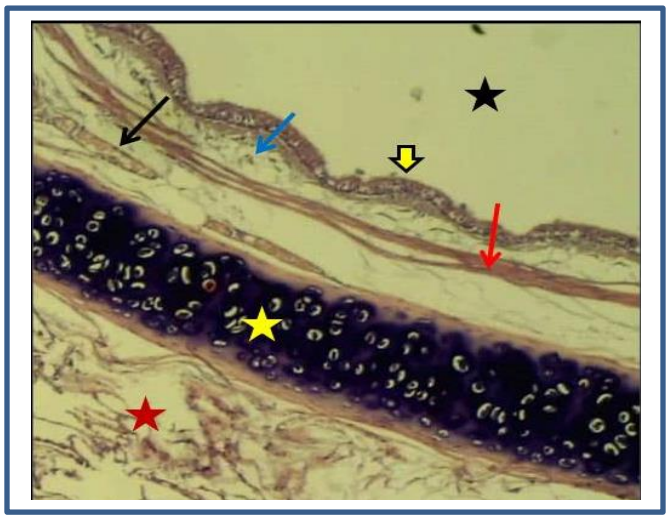

Fig. 2. Histological section showed secondary bronchus characterised by pseudostratified epithelium (yellow arrow), muscularis mucosa (red arrow), submucosal blood vessels invested in loose connective tissue (black arrow), Hyaline cartiage (yellow star), bronchial lumen (black star) and pulmonary parenchyma (red star). H&E, X400

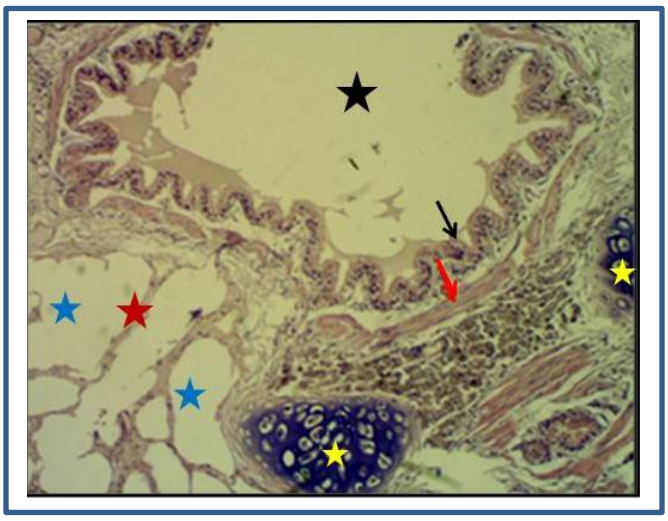

Fig. 3. Histological section showed tertiary bronchus characterised by ciliated simple columnar folded epithelium (black arrow), muscularis mucosa (red arrow), submucosal small plates of hyaline cartiage (yellow stars), bronchial lumen (black star) , many alveoli (blue stars) filling the pulmonary parenchyma (red star). H&E, X200

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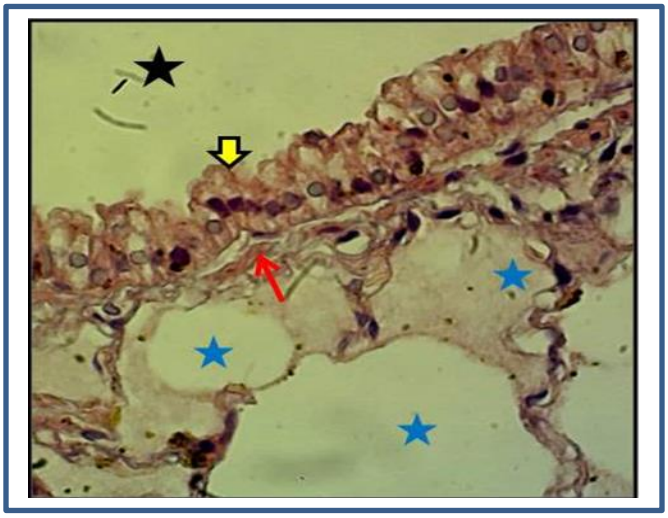

Fig. 4. Histological section showed primary bronchiole characterised by simple columnar epithelium (yellow arrow), thin layer of muscularis mucosa (red arrow), bronchial lumen (black star) and many alveoli (blue stars). H&E, X400

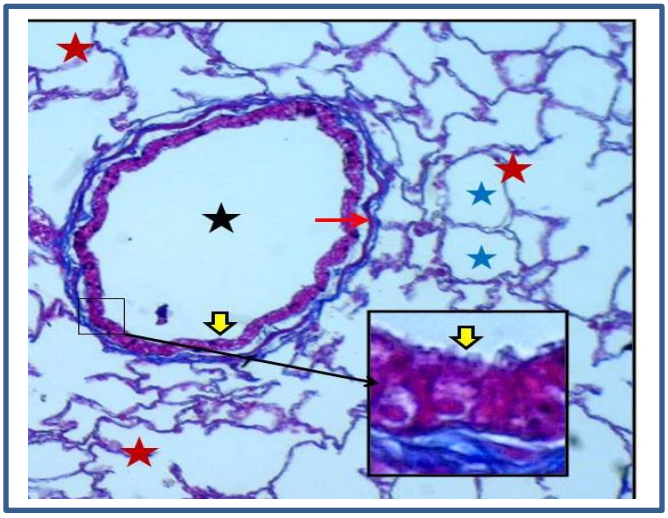

Fig. 5. Histological section showed secondary bronchiole characterised by simple low columnar epithelium (yellow arrow), very thin layer of smooth muscle fibers (red arrow), bronchial lumen (black star) and many alveoli (blue stars) forming the pulmonary parenchyema (red stars). MTC, X200

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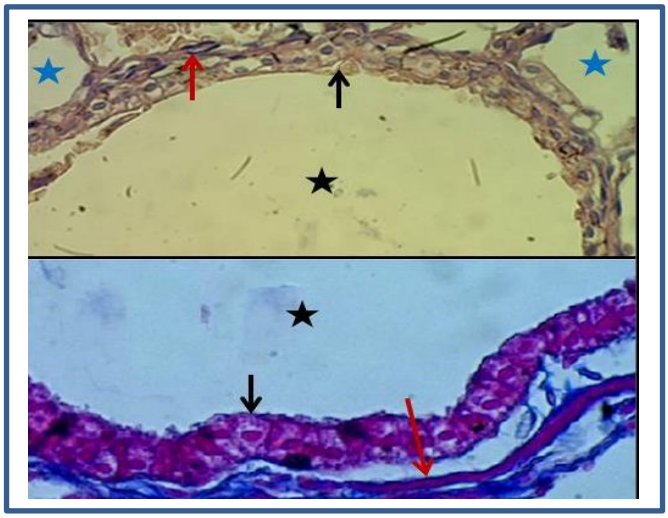

Fig. 6. Histological section showed tertiary (terminal) bronchiole characterised by simple cuboidal epithelium (black arrows), one layer of smooth muscle fibers (red arrows), bronchial lumen (black stars) and many alveoli (blue stars). H&E (upper panel), MTC (lower panel), X200

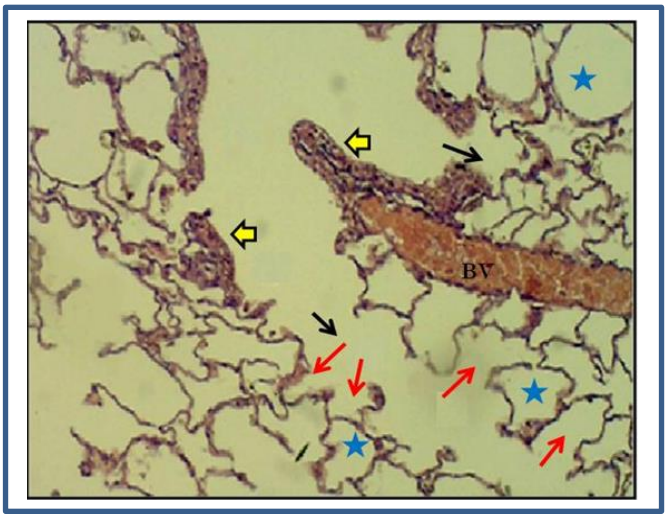

Fig. 7. Histological section showed respiratory bronchiole characterised by simple cuboidal epithelium (yellow arrows), opened into alveolar ducts (black arrows), to which opened alveolar sacs (red arrows) which in turn possessed many alveoli (blue stars) , blood vessel (BV) percised pulmonary parenchyma. H&E,X200

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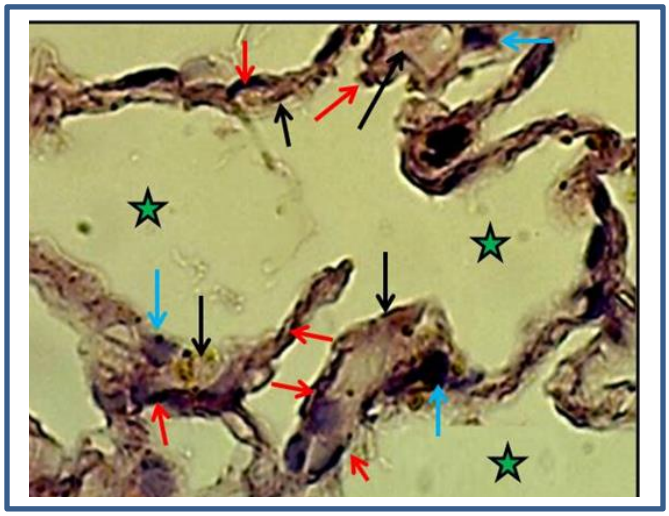

Fig. 8. Histological section showed many alveoli (green stars), their walls was formed by type I pnemocytes (red arrows) and type II pnemocytes (blue arrows). blood endothelial cells (black arrows) formed blood barior system with type I pneumocyte. H&E, X400.

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