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Research Article

Anatomical and Histomorphological Study of the Trachea and Lung in Indigenous Guinea Pigs (*Caviaporcellus*)

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ABSTRACT

The present study was carried out to investigate the anatomical and histological features of the trachea and lung in an indigenous adult guinea pigs. The current study was conducted on thirty local guinea pigs in both sexes that divided into three equal groups, the first group used for anatomical observations, the second used for cast preparation, while the third one used for histological study. Anatomically the trachea was cartilaginous organ made up of C-shaped cartilaginous rings which attached by an annular ligament. The trachea expands from the second cervical vertebrae level to the 4rth thoracic vertebral level wherever it bifurcates into the right and left principal bronchi. The total number of tracheal rings, total weight and total size of lung, the total length and diameter of the trachea in male were higher than that in female. The resin cast revealed the bronchial tree showed (4) primary bronchi in the right lung and (3) primary bronchi in the left lung. The right lung consists of (4), while the left lung consists of (3) lobes. Histologically the tracheal wall was consisting of four tunicae. The mucosa was lined by pseudostratified ciliated columnar epithelium with goblet cells which positively react with PAS stain. Tunica mucosa was separate from hyaline cartilage by submucosa which contains few tubule acinar mucous glands. The trachealis muscle was attached at the inner side of the rings. The primary bronchi were similar to the trachea but with smaller diameter, and were divided into primary, secondary and tertiary intra- pulmonary bronchi where the cartilaginous rings changes into some plates of hyaline cartilage that disappear in the bronchioles which ends at a terminal bronchioles that directly open in alveolar ductless, alveolar sacs and alveoli while the respiratory bronchioles were absent. The alveoli were open into the alveolar ducts and alveolar sacs. The alveoli lined by type I and II pneumocyte.

Key words: Trachea, Lung, An indigenous, Adult guinea pigs

INTRODUCTION

Guinea pigs an experimental animal used as a model for human diseases than other rodents (Williams *et al.*, 2009). The biological characteristics of this animal make this animalasa valuable model for research includingmedicine, biological and immunological fields (Abidu-Figueiredo *et al.*, 2008).

The respiratory tract anatomy variesdepend on species (Legaspi, 2010). The trachea is a cartilaginous structure made of hyalinecartilage, attached by a fibro-muscular membrane. It composed from many of hyalinecartilages (C-shaped) in different animals species, these cartilages have free ends on the dorsal side. Muscles of the tracheal closed each of the cartilage (Dabanoglu and Kara, 2001). The bronchial tree consists of intrapulmonarybronchi, bronchioles, terminal and respiratory bronchioles. Furthermore, Alveolar ducts and air sacs (Legaspi, 2010). The main action of the lung is the separate diffusion ofO2 and remove the CO2 and respiratory gaseous exchange (Glenny and Robertson, 2011). This study object to investigate the anatomical and histological features of the lungs and trachea in guinea pigs and to describe the bronchial tree by uses Resin Technique.

MATERIALS AND METHODS

Experimental design

Twenty healthyadult guinea pigs (*Caviaporcellus*) of both sexes were divided into three equal groups. Each group consist of 10 (5 male and 5 female). The first group used for atopographic relationship, gross anatomy of trachea, lung, length and diameter of trachea using the electronic digital Vernier caliber, weight, volume of lung and counting of tracheal rings, the second used for bronchial tree making corrosive resin cast and the third group used for the histological study.

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Cast technique

The injection of resin was done by hands pressure using syringe of 20ml, the specimens were injectedwith 1ml, 1. 25ml and 1. 50ml, 1. 75ml and 2 ml of resin. After complete solidification, the specimens were macerated using a solution of 40% KOH for 4-5 days then washed and dried by hot air to become ready for examination (Nassar, 2012).

Histological study

The specimen was taken from all lobes of both lungs. And from the primary bronchus (left and right). Then fixed in 10% formalin, dehydrated by using ascending concentration of alcohol (70,80,90,100%). two-run of each, cleared was achieved through xylene in two steps for 30 minutes for each step, infiltrated then embedded in paraffin wax, sectioned in 6-7 µm then the sections were stained with Harris Hematoxylin and Eosin stain and Periodic Acid Schiff (Luna, 1968). The statistical analysis was applied using two ways ANOVA and means asignificant difference at P≤0.05. In using statistical package for social sciences (T-Test).

RESULTS AND DISCUSSION

Anatomical study

Topography and gross anatomy of the trachea

Trachea: The currentstudyinvestigates trachea in both sexes of guinea pigs showed as a pliable cylindrical tube hollow made up of a many of hyaline cartilage rings which confirm by an annular ligament and their ring edges andmuscle filled itas (Fig. 1).

The trachea is organlie at the ventral aspect of the neck at midline, and it extended forward to the back from the cricoid cartilage of larynx to the carina at the 4th thoracic vertebra, this finding agrees with (Al-Anbaki, 2013) in the rabbit. It was bifurcated at the level of 4th thoracic vertebra into right and left principal bronchi (Fig. 1) as observed by Nasser (2012) in cats.

The mean total tracheal length in male was higher than that in the female. In the male, the mean number of tracheal rings was less than in female (Table 1). The tracheal rings were to a noval shape semi-circular in its cross-section. This finding didn't like as trachea in the dog (Jackson and Krevan, 1984). Topographically the trachea can be separate into cervical and thoracic portions (Fig. 1).

The length, rings number in the cervical region of the trachea, length, rings number in the thoracic region in female was higher than in male (Table 1). The tracheal lengths and the plates number of the trachea vary between species and within species, in dog 42 to 46 plate, cat, 38 to 43 plate (Getty, 1975).

The mean tracheal and right principal bronchusdia meter in male was higher than in female, the left, principal bronchus diameter in female was higher than in male (Tabe1). No significant difference between them at P≤0. 05 in all parameters.

Lung: The lungs of both sexes revealed as paired, pink, spongy organs, surrounding the heart, and its shape is identical to the thoracic cavity shape, as in domestic animals (Pasquini *et al*., 1997).

Bothlungs have a dorsal, apex, base and it has caudal and ventral borders, the ventral and caudal borders were pointed and flatted, while, the dorsal border was roundedthick. The right lung apex was rounded, largewhile the left lung was pointed and small, as observed in rabbit by (Al-Anbaki, 2013). The lungs have three surfaces, the costal, medial and the diaphragmatic which were convex, narrow surfaces as in ruminants by (Thrall, 2002).

The lung was divided into districted lobes by interlobar fissures, the right lung has apical, middle, caudal, and accessory lobes (Fig. 2), as found in goat by Habib and Mohammed (2010), but similar to the observation of Nasser (2012) in the cat. The situation of the heart the make right lung was larger than the left lung. This result same with results of Legaspi (2010) in the dog. Also, the left lung consists of (3) lobes including caudal, middle and apical as (Fig. 2). These resultscontrast with asobserved in cats. Where the left lung wasconsist of left apical lobe which consists of(parts of caudal and cranial), And the caudal lobe (Oliveira *et al*. , 2001),that disagree with(Kennedy *et al*. , 1978) in hamsterwhich possesses only one lobe.

The mean body weight, total weight of lungs, weight of the right and left lung in male (Table 2), were higher than in the female. The mean weights of middle, caudal and accessory lobes of right lung in male were higher than that in female (Table 3). The mean weights of apical and caudal lobes of the left lung in male were higher than that in female (Table3). The mean total volume of the right lung, apical and accessory lobes in male were higher than that in the female, and the mean total volume of the left lung. In the left lung, the mean volume of the middle and caudal lobes in male was higher than female (Table 4). Significant differences are studies at P≤0.05 in all parameters between two sexes.

Table 1: Morphometric parameters of trachea in both sexes of guinea pigs (mm).

Parameters	Male (Mean±SE)	Female (Mean±SE)
Total length of trachea	41.164±0.32A	40.214±0.37 A
Numbers of tracheal rings	35.8±0.343 A	36 ±0.672 A
Length of the cervical region	28.742± 0.320 A	29.246±0.721 A
Number of cervical rings region	23±0.36 A	25±0.29 A
Length of the thoracic region	10.242±0.418 A	10.826±0.589 A
Number of thoracic rings region	12.8±0.349 A	11±0.192 A
Diameter of trachea	6.064±0.084 A	5.932±0.036 A
Diameter of right principal bronchus	4.073±0.0750 A	3.984±0.067 A
Diameter of left, principal bronchus	3.8560±0.0460 A	4.011±0.033 A

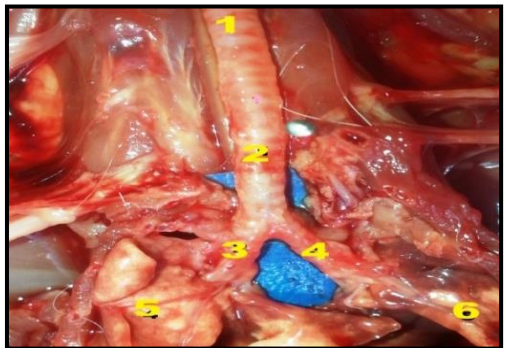

Fig. 1: Topographic and the gross anatomy of trachea in male guinea pigs. 1-Trachea (cervical region) .2-thoracic region 3-right primary bronchi 4- Left primary bronchi. 5- Right lung. 6-Left lung.

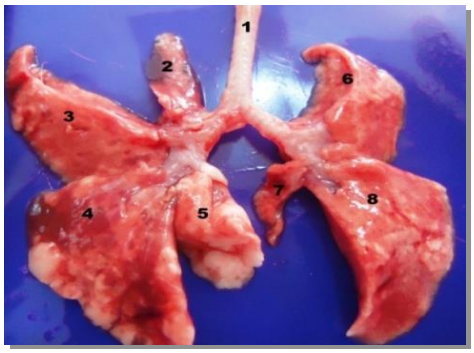

Fig. 2: Topographic and the gross anatomy of trachea in male guinea pigs. 1-Trachea (cervical region) .2-thoracic region 3-right primary bronchi 4- Left primary bronchi. 5- Right lung. 6-Left lung.

Fig. 3: A, B. C. :The resin cast for the left bronchial tree which illustrated of the direction of the ramification of the bronchial tree Shows :1-Trachea 2-right principal bronchus 3-left principal bronchus 4- R. apical primary bronchi 5-R.Middle primary bronchi 6-R.Accessory primary bronchi 7-R.Caudal primary bronchi 8-L.Apical primary bronchi 9-L.Middle primary bronchi 10-L.Caudal primary bronchi.

Table 2: Morphometric parameters of the lung in both sexes of guinea pigs:(g)

Parameters	Males Mean±SE	Females Mean±SE
body weight	415±22.19 A	389.8±33.739 A
Total weight of lungs	3.45±0.120 A	3.29 ±0.221 A
Total Weight of right lung	1.860 ±0.033 A	1.846±0.085 A
Total Weight of left lung	1.584 ±0.046 A	1.578±0.060 A

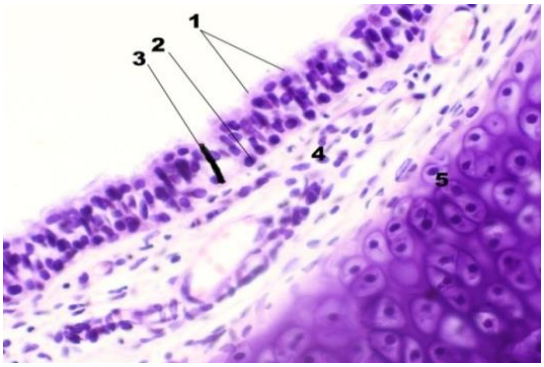

Fig. 4: Histological section of trachea in female guinea pigs Shows :1-Cilia 2-Basal cell 3-mucosa 4- lamina proper 5-Hyaline cartilage H α E stain(X 100).

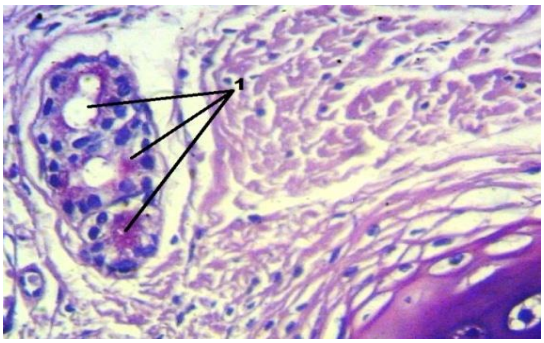

Fig. 5: Histological section in trachea of male guinea pigs shows: 1- the mucous tracheal glands (arrow) in the submucosa (PAS) stain (X400).

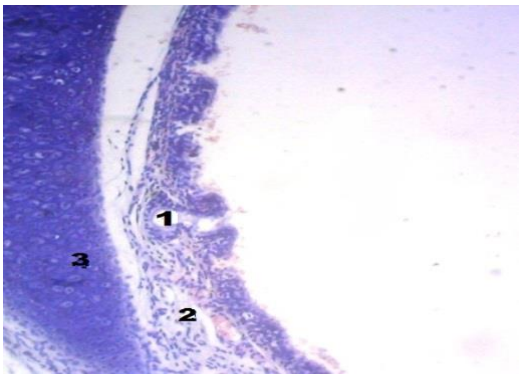

Fig. 6: Histological section of trachea in female guinea pigs shows: 1-Tracheal glands 2 -Tunica submucosa.3 -hyaline cartilage H α E (X 40).

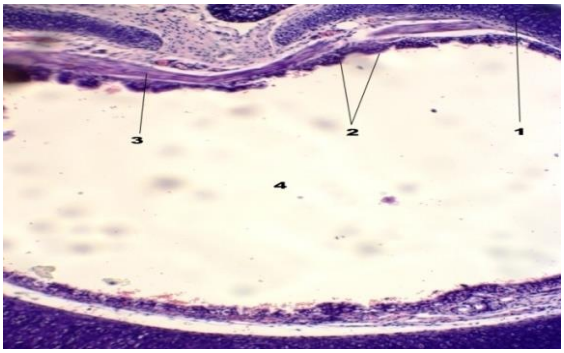

Fig. 7: Histological section of cross section in trachea of male guinea pigs shows: 1-hyaline cartilage 2-Tunica mucosa 3-Trachealis muscle 4- Lumen of trachea H and E (X40).

The present study investigates the forming the trachea and bronchial tree. The trachea consists of a left and right bronchi. The right bronchus was formed from four primary bronchi, the apical, middle, accessory and the caudal lobe. The right principal bronchus gives the right primary bronchus to the apical lobe (Fig. 3A, B, C), this finding was identical to the that reported in cats by (Nasser, 2012). The middle and accessory bronchus raised behind the apical bronchus, in the right lung the middle bronchus enter the middle lobe, and the accessory bronchus was passed toward the accessory lobe (Fig. 3B). The right caudal principal bronchus directed to the caudal lobe (Fig. 8 B), these results same with results of (Al-Anbaki, 2013) in the rabbit. The right principal bronchus drives the right primary bronchus that branches into secondary and tertiary bronchi which created the secondary and tertiary bronchioles.

The left, principal bronchus split into the left primary apical bronchus. Primary, middle bronchus and primary caudal bronchus for the left apical, middle lobe and caudal lobes respectively (Fig. 3 A, B, C), this finding was in contrast with that recorded in pigs lung by (Schwarzkopf *et al*., 2010). Secondary formed from left primary bronchi, while tertiary bronchi formed from Secondary, then they give the bronchiole at the end.

Histological Results

Trachea

In this study, the trachea in both sexes of guinea pigs was identical and observed as a hollow tubular firm organ composed of mucosa, submucosa, muscularis (cartilaginous layer) and adventitia. Pseudostratified ciliated columnar epithelia werelined the mucosa. It composed of ciliated columnar cells, goblet cells and basal cells that rest on a basement membrane (Fig. 4), as found in caiman (Santos *et al*., 2011).

The most abundant cell types were the ciliated columnar cells which showed as tall columnar cells, with apical cilia on its surfaces which extend into the tracheal lumen, slightly stained cytoplasm with large oval nuclei which apically located (Fig. 4), the same finding by Yang *et al.* (2010) in Yak. The goblet cells were abundant, possess goblet shape, and basal nuclei. The goblet cells were PAS positive which giving rise to magenta colour due to it's a mucopolysaccharide. Mucous secretion act as an epithelial protective barrier (Buchner and Maxwell, 1993).

The basal cells showed triangular and small cells rested on the base membrane but did not reach the lumen, as found in goat by (Habib and Mahammed, 2010**),** the tracheal epithelium was same as observed in other laboratory animals (Reznik, 1990).

Not well-developed lamina propria which appeared asa thin loose connective tissue layer made up of elastic and collagenous fibres. A very thin layer of muscularis mucosa with a few smooth muscle fibres, such result found in rabbit by (Al-Anbaki, 2013).

The tunica submucosa observed a layer of loose connective tissue. Blood vessels and very few, small, tubular – acinar submucosal mucous glands that showed appositively react with PAS (Fig. 5). The glands opened by a slit-shaped duct into the lumen of the trachea (Fig. 6). The guinea pig revealed a unique characterin the glands density, this finding disagreedwith that reported in rabbit, mice and hamster were the submucosal glands were absent (Widdicombe *et al*., 2001).

The current study demonstrated low or very rare submucosal glands while in camelwere found a large number of submucosal glands (Raji and Naserpour, 2007). Many of tracheal glands secrete mucous lined by simple columnar tocuboidal cells or with large round basally situated nuclei and wide lumen (Fig. 5). This result agreed with Widdicombe and Pecson (2002**)**in thehorse, where it was founded mucous was secreated, where the mucus has an important role in preventing the dust accumulation (Thornton and Shechan, 2004).

The goblet cells producing mucus is also it secreted by the submucosal gland,and it produces moisture and lubrication and providing normal mucociliary clearance (Buchner-Maxwell, 1993).

The muscular cartilaginous layer merges with hyaline cartilage per ichondriumand fibroblastic tissue which found among the cartilaginous rings. The hyaline cartilage contains chondrocytes in an amorphous matrix, similar observation in ruminant by (Habib and Mahammed, 2010).

The cartilaginous rings were C-shaped, opened dorsally, filled by connective tissue and a smooth trachealmuscle thatconnect the inner side of the rings (Fig. 7), as observed in other species (Ibe *et al.*, 2011**).** Tunica adventitia covered the cartilage andshowed a loose connective tissue as observed by (Al-Anbaki, 2013) in rabbits.

The tracheal bifurcation at the carina formed the right and left, principal bronchus was formed histologically from:

The mucosawaslinedwithpseudostratified ciliated columnar epithelia. The submucosa contains loose connective tissue with tubular-acinar mucus glands. The fibrocartilaginous layer contains hyaline cartilage, while the adventitia composed of loose connective tissue, similar results were reported by Robinson *et al.* (1986) in ferret and deer bronchi (Saari, 1997).

The primary bronchi give secondary bronchi then give tertiary bronchi, histological of the secondary bronchi was made up of Folded Mucosa that lined by a pseudostratified columnar ciliated epithelium with goblet cells that gives a positive reaction with PAS (Fig. 8). Lamina propria thin was a distinguished smooth muscle fiberslayer which separated the lamina propria from the submucosa, difficult to distinguish submucosal glands were very few.

The tracheal and primary bronchi cartilaginous rings were replaced by a separated hyaline cartilage plates (Fig. 8). The adventitia surrounded directly by the lung parenchyma and contains blood vessels, as observed in domesticated animals (Caceci**,** 2008).

The secondary bronchi give the tertiary bronchi that distinguish by folded mucosa withlininggoblet cells in the simple columnar ciliated epithelium tissue that reacted positively with PAS. The smaller bronchi the epithelium become low simple columnar ciliated epithelium, a similar result was observed in the hamsterby (Kennedy *et al.*, 1978). Lamina propria composed of elastic fibers, smooth muscle and connective tissue.

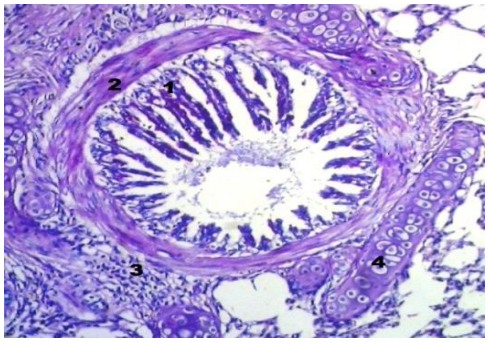

Fig. 8: Histological section of secondary bronchi in female guinea pigs shows:1-Tunica mucosa 2-Smooth muscle 3-Tunica submucosa 4-Hyaline cartilage. (PAS) stain (X100).

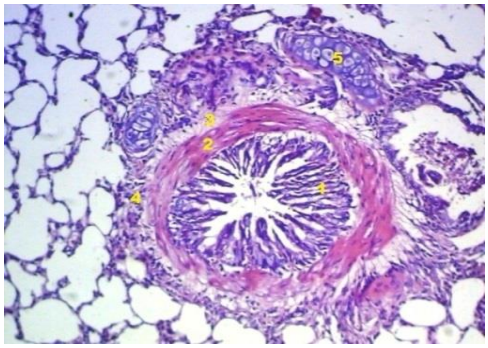

Fig. 9: Histological section of tertiary bronchi in male guinea pigs shows: 1-Low simple columnar epithelium.2-Smooth muscle 3-Tunica submucosa 4-Tunica adventitia 5-Small irregular hyaline cartilage. H α E. (X100).

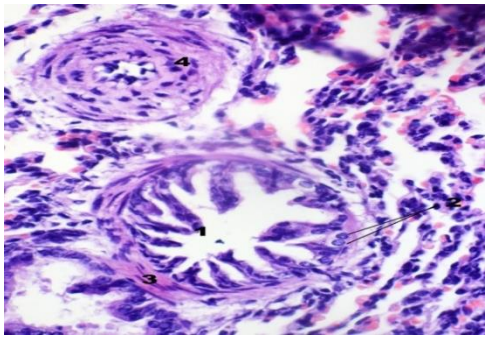

Fig. 10: Histological section in lung of male guinea pigs shows the bronchiole.1-simple columnar ciliated epithelium 2 Clara cells 3-Smooth muscle4-branch of pulmonary artery. H α E. (X100).

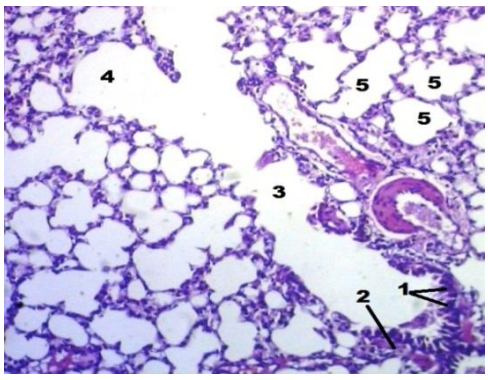

Fig. 11: Histological section in lung of male guinea pigs shows Terminal bronchiole.1-Simple cuboidal epithelium2-Smooth muscle 3-Alveolar duct 4-Alveolar sac 5- Alveoli .H α E.(X 100).

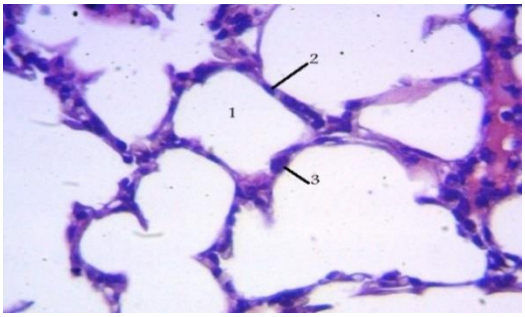

Fig. 12: Histological section in lung of male guinea pigs shows: 1-Alveoli 2-type I pneumocyte 3-type II-pneumocyte H α E. (X 400).

Irregular hyaline cartilage plates smaller than that in the secondary bronchi, the connective tissue of the submucosa merges with the adventitia (Fig. 9) as observed by Mariassy and Plopper (1983) in sheep. The lining epithelium of the bronchioles waschanged to become low simple column arciliated epithelium with goblet cells. It displaysa Clara cells which appeared dome shape, light cytoplasm and centralnucleus, also it was lack of the glands and the hyaline cartilage plates in its walls (Fig. 10), as reported by(Samuelson, 2007).

The thin loose connective tissueof the lamina propria which surrounded by a layer of smooth muscle fibers and lackingsubmucosal glands, cartilaginous plates was absence and the adventitia was immersed with lung parenchyma (Fig. 11). Thesefeatures were similar to those noticed in goats by (Habib and Muhammad, 2010).

The terminal bronchioles mucosa was lined with a simple ciliated columnar to the simple cuboidal epithelium and goblet cells were absent (Fig. 11). Lacking smooth muscle layer of the lamina propria, similar observation it was found in pig by (Kalita, 2014). The terminal bronchioles attached directly into alveolar ducts that leads to alveolar sacs and into alveoli (Fig. 11), the same result found in rabbit and gerbil by (Ibe *et al.*, 2011).

The alveolar ducts were along upright tubular frame, lined with simple squamous epithelium and showed many out-pocketing of alveoli. Alveolar ducts leadto alveolar sacs, which composed of alveolar cluster around collectiveairspace (Fig. 11) as found in goat by (Baba and Choudhary, 2008).

Currently, lung parenchyma was composed of alveoli, blood capillaries and interalveolar septum. The alveoli display as small spherical air spaces surrounded partly by highly thin epithelium which formed the mainlining epithelium, type I pneumocyte which showed as squamous cells with a centrally located nucleus (Fig. 12) and the type II pneumocyte, which observed as rounded or cuboidal cells scattered between the type I pneumocyte (Fig. 12). As shown in rats by (Dahlin *et al.*, 2004) andin goat (Carvalho and Goncalres, 2011). Type I pneumocyte of alveoli and stated to be mainly accountable for an interface between the air and blood to permit gas exchange (Banks, 1993).

Pneumocytetype II had secrete mucus, mucus work as lubrication of the epithelial layer, result reduces in the surface tension and prevents alveolar collapse through the exhalation (Plopper and Adams, 1993). (Kahwa *et al.*, 1997) Showed that pneumocytes type II and I types were found in same species. The interalveolar septa were made up of one layer of epithelium, very thin connective tissue

layer that composed of fine collagen, blood capillaries and reticular, elastic fiber sunderlying the epithelium, this result was agreed with (Baba and Choudhary, 2008) in goats. The visceral pleura was made up of thin connective tissue and a mesothelium, like the finding of (Habib and Mohammed, 2010) in goat.

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