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A comparative, histomorphological and histochemical study of lower respiratory system at different ages postnatally in male albino rats

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***Abstract*---**Histomorphological and histochemical study, by light microscopy, was undertaken on the lower respiratory system of the albino rat at two different ages (1st and 4th months). The trachea and lungs were weighted, measured and stained with Hematoxylin and eosin, Masson's Trichrome stain and PAS stain. The results revealed there's significant differences in weight of body and lung of rat pups represented by gradually increase of weight and lung respectively, at 1st and 4th month. On the other hand there were no statistical differences in trachea weights at 1st and 4th month. The diameter of trachea, bronchioles and alveolar sac significantly changes. In contrast the tracheal lumen showed no significant changes in diameter in 1st and 4th months. Thickness of mucosa, sub-mucosa and tracheal cartilage significantly changes. The most thicker of mucosa showed at 1st month while they reduced gradually at 4th. By the other hand the results of thickness measuring of interstitial tissue showed no significant changes.

***Keywords*---**Rat, histomorphological, histochemical, lung and trachea.

Introduction

The respiratory system is a complex biological system comprised of several organs that facilitate the inhalation and exhalation of oxygen and carbon dioxide in living organisms. For all air-breathing vertebrates, respiration is handled by the lungs, but these are far from the only components of the respiratory system (Akers and Denbow, 2013).The lungs are conical in shape, has an apex and a base with two

surfaces. The outer surface of is covered by the ribs (Moore *et al.,* 2014). On medial side, the bronchi, blood vessels, and lymphatic vessels enter at the hilum of the lungs (Hayman et al., 2012). Apex projects into root of the neck. It is covered by cervical pleura. It is grooved anteriorly by subclavian artery. Base lies on its diaphragmatic surface. Anterior border is sharp thin and overlaps the heart, presents a cardiac notch and lingula. Posterior border is rounded thick and lies beside the vertebral column. Inferior border is thick and costal surface is convex. Costal surface is covered by costal pleura, which separates lung from ribs, costal cartilages & intercostal muscles. Medial surface is divided into two parts anterior (mediastinal) part that contains a hilum in the middle. Posterior (vertebral) part is related to bodies of thoracic vertebrae, intervertebral discs, posterior intercostal vessels & sympathetic trunk (Warren, 2021). The respiratory system subdivides into a conducting portion and a respiratory portion. The majority of the respiratory tree, from the nasal cavity to the bronchi, is lined by pseudostratified columnar ciliated epithelium. The bronchioles are lined by simple columnar to the cuboidal epithelium, and the alveoli possess a lining of thin squamous epithelium that allows for gas exchange (Kia'i and Bajaj, 2021).The lower respiratory system develop when originate as an evagination from the endoderm along the floor of the pharynx. The evagination is designated the laryngotracheal groove. From lateral walls of the laryngotracheal groove, ridges grow medially, and fuse along the midline, establishing a tracheoesophageal septum. The septum separates a laryngotracheal tube (future trachea & lung buds) from the esophagus (Hyttel *et al.,* 2009; Sadler, 2015).

Materials and methods:

Animals: Sixty healthy male albino rats regardless to their aged twenty male (1 months age) twenty male (2 months age) and twenty male (4 months) obtained from animal house of college of veterinary medicine Al-Qasim green university. Then housed in individual cages , fed as well and giving them water before their euthanasia and dissection. Rats were divided into two parts, the first part including ten rats of each age focused on the morphological part. The second one also including ten rats of each age focused on the histometrical and histochemical parts section.

Preparation of Specimens : Rats were weighted by digital balance, then they were anesthetized using Ketamine 15 mg/ K.g of body weight intramuscular injection (Schindala,1999).The trachea and lung was observed and collected by abdominal laparotomy and cranial displacement of sternum, then it was carefully dissected along with its total morphology, after that it was washed by normal saline solution to remove blood or any other adhering debris. The trachea and lung was identified and photographed *in situ* using digital camera.

Morphological Study: For gross observation twenty rats were used, which divided into two equal parts , ten male (1 months), ten males(2 months) and ten males (4 months), which were represented the study of position , shape, color The parameters of this part. Body weight, Weight of lung, Weight of trachea shape of lung and shape of trachea. All measurements mentioned above were listed in tables. The total length of trachea and lung were conducted in centimeters by using the electronic Vernier scale, the weights were measured in

grams by using sensitive electron balance.
Histological Study:-Thirty Rats were used for histological observation (ten rats of each age).The specimens performed according to (Suvarna *et al.*,2012).

Staining : The following stains were used in present study

1-Harris Hematoxylin and Eosin Stain: These are naturally occurring substances that have been use in the history of histopathology **(Titford, 2009)**.

2-Masson's Trichrome stain For the staining the connective tissue fibers. Historical assessment on the use of various stains in histology indicates that most pathologists were attracted by stains that gave multicolored results on the tissue specimens. As such, trichrome stains were developed from this need **(Shostak, 2013).**

3-Periodic Acid Schiff (PAS): performed according to **(Ngokere et al., 2016).**
Statistical Analysis : -The data were statistically analyzed using SPSS (version 16.0) (Field, 2013). All numerical results have expressed as the mean values ± standard error (SE). For comparisons, the statistical significance has assessed by ANOVA by statistician Ronald Fisher . The significance level was set at ($p<0.05$).

Results and Discussions:

Correlation of body, lung and tracheal weights with age of male rats: Table 1 illustrated the relation between weights of lung, trachea and body of rat pups during 1st and 4th month of life. The statistical analysis revealed there's significant differences in weight of body and lung of rat pups during postnatal stages (1 and 4months) represented by gradually increase of weight and lung respectively, at 1st and 4th month. On the other hand the present study revealed no statistical differences in trachea weights at 1st and 4th month.

Table (1): The weight of body, trachea and lung of the male albino rat in different age (1 and 4) months.

Age / organ	Body Weight	Trachea Weight	Lung Weight
One month	121.200 ± 3.891 *	0.160 ± 0.00103	0.806 ± 0.00286 *
Four month	418.400 ± 8.773*	0.328 ± 0.00915	2.452 ± 0.000707*

The numbers represent the mean ± standard error. *There is a statistically significant difference ($P \leq 0.05$).

Diameter of trachea and tracheal lumen in rat : Table (2) shows the diameter of trachea during deferent periods in postnatal stage; The results revealed there's significant changes in diameters of trachea, these increasing in diameter due to the growing up of pups and increase the needing to O2 consumption. While the statistical analysis of tracheal lumen showed that there were no significant changes in diameter in 1st and 4th months.

Table (2): The diameter of trachea and tracheal lumen of the male albino rat in different age (1 and 4) months.

Age / Diameter	Trachea	Tracheal lumen
One month	0.042±0.003 *	0.862±0.874
Four month	0.039±0.001 *	0.844±0.986

The numbers represent the mean ± standard error. *There is a statistically significant difference (P ≤0.05).

Diameter of bronchioles and alveolar sac in rat : Table(3) shows the diameter of bronchiole and alveolar sac during deferent periods of rat pups age; The results revealed there's significant age-related changes in diameter of bronchioles from 1st month to forth month. For alveolar sac there were significant increase in diameter in animals at 4th month comparing with 1st month.

Table (3):Show the diameter of bronchioles and alveolar sac of the male albino rat pups in different age (1 and 4) months.

Age / Diameter	Bronchioles	Alveolar sac
One month	0.239±0.014 *	0.032±0.004 *
Four month	0.170±0.004 *	0.058±0.002 *

The numbers represent the mean ± standard error. *There is a statistically significant difference (P ≤0.05).

Thickness of mucosa and submucosa : The results in table (4) revealed there were significant differences in thickness of mucosa and sub-mucosa of lower respiratory system in male rat pups during two different periods of study. The most thicker of mucosa showed at 1st month while they reduced gradually at 4th month. By the other hand the results of thickness measuring of sub-mucosa showed that the most thicker of sub-mucosa showed at 4th compared with 1st month.

Table (4): Show the thickness of mucosa and sub-mucosa of the male albino rat pups in different age (1 and 4) months.

Age / Thick.	Mucosa	Sub-mucosa
One month	0.047±0.001 *	0.065±0.066 *
Four month	0.032±0.001 *	0.074±0.076 *

The numbers represent the mean ± standard error. *There is a statistically significant difference (P ≤0.05).

Thickness of tracheal cartilage and interstitial tissue: The results in table (5) revealed there is significant differences in thickness of tracheal cartilage during

postnatal life in different periods of study. Furthermore the statistical analysis revealed the most thicker cartilage appeared at 1st when compared with those in 4th month, while the most thicker interstitial tissue recorded at 4th month of rats life. For interstitial tissue the results showed that there were no significant differences in thickness during two ages.

Table (5): Show the thickness of tracheal cartilage and interstitial tissue of the male albino rat pups in different age (1 and 4) months.

Age / Thick.	Tracheal cartilage	Interstitial tissue
One month	0.154±0.0008 *	0.013±0.001
Four month	0.134±0.001 *	0.014±0.0001

The numbers represent the mean ± standard error. *There is a statistically significant difference (P ≤ 0.05).

Discussion

The present study revealed that the gradually increasing in body weight of body, trachea and lung pups with sequential morphogenesis and differentiation stages and this finding were in agreement with Noakes **(**2009**)** and Van Engelen *et al.* **(**1995**)** who reported that the increase in weight and size of the pups is due to increased period of lactation and the completion of growth of organs and limbs. According to Spangenberg *et al.* **(**2014**)** reported that around day 14th the pups started to eat solid feed, which meant that feed intake for lactation week 3 was influenced by the intake of the pups and causes increase body weight and size. One of the major functions of trachea and bronchial epithelium is mucociliary clearance of inhaled harmful particles. This important lung-protective function depends both on the availability and subsequent efficiency of mucosal glands, and ciliary actions (Ibe *et al.*, 2011). Choi *et al.*(2000) recorded a linear relationship between tracheal diameter and number of mucous glands in mammals and concluded that increased size of conductive airway (marked by increased tracheal diameter). Our finding agrees with Ishaq **(**1980**)** in dog , Caccamo *et al.* **(**2007**)** in cat , Frandson *et al.* **(**2009**)** in ruminant , Al-Anbaki **(**2013**)** in rabbit and Al-Jebory (2018) in mice . Ibe *et al.* (2011) recorded a tracheal diameter of 5.44 mm (± 0.03) with an average of 5.32 mm in the rostral trachea in the African Giant pouched rat. In the present study, tracheal mucous glands were predominant in the rostral trachea. Widdicombe *et al.*(2001) did not record any tracheal gland in mice and attributed this to their small tracheal diameter of approximately 1 mm. They observed tracheal glands in other rodents such as hamster, rat and guinea pig with wider tracheal diameter of approximately 1.5 mm, 2.5 mm and 2.5 mm respectively. The change of diameter occur duo to increase in lung size by increase gas flow with alveolarization continuously. This results agree with Hyde *et al.* (2004) in deferent mammalian and Joshi and Kotecha (2007) who reported that in humans, the small bronchioles expanded significantly in size with increasing age and height up to age of 22 years but the increase in size thereafter was slow significant (Joshi and Kotecha, 2007).

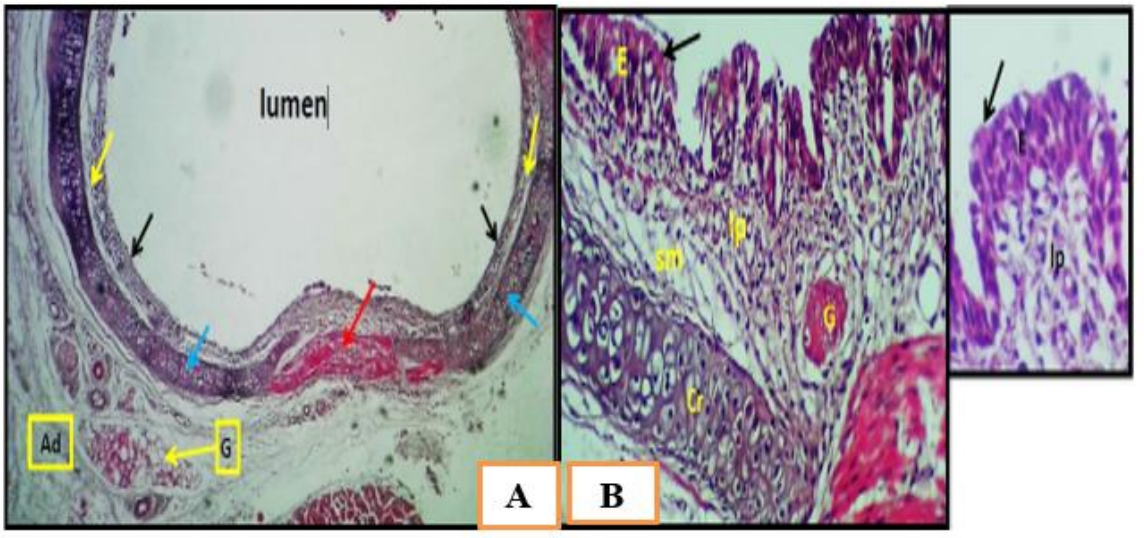

Figure 1: Cross section of trachea in male rat at (1 month) shows: **(A):** Mucosa (black arrow), sub-mucosa (yellow arrow), hyaline cartilage (blue arrow), smooth muscle (red arrow), seromusous gland (G), tunica adventitia (Ad). **(B):** Psedo stratified columnar epithelium (E), cilia (black arrow), lamina properia (lp), sub-mucosa (sm), hyaline cartilage (cr). H&E stain. 10x. B: Magnified section. 40x., 10x.

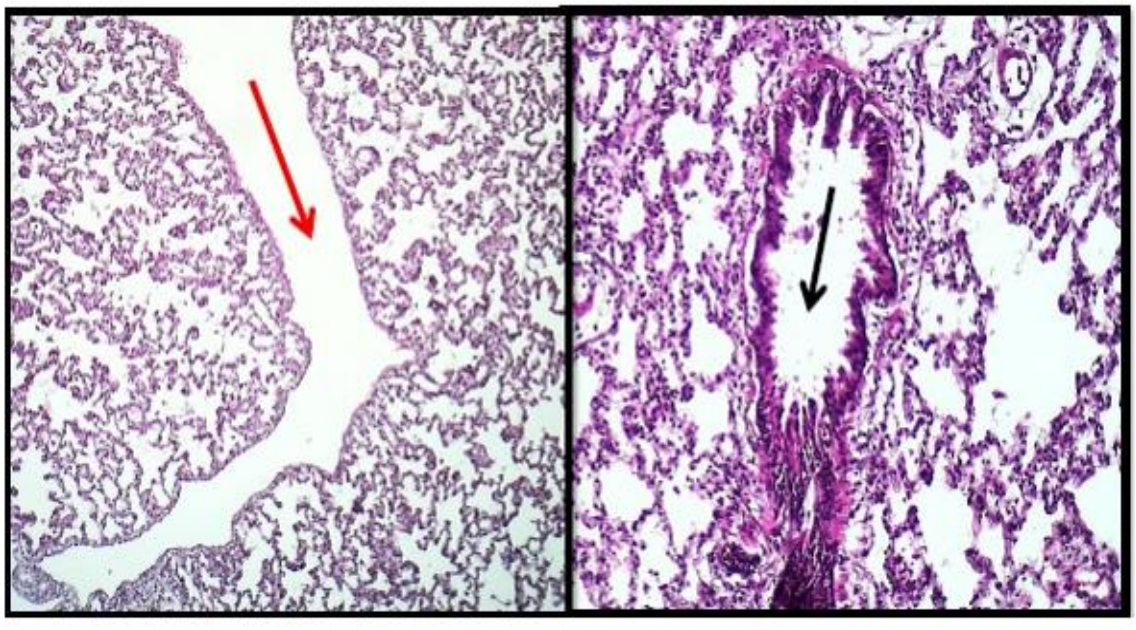

Figure 2: Cross section of lung in male albino rat at (1 month) shows: respiratory bronchiole (red arrow), terminal bronchiole (black arrow). H&E stain. 4x.

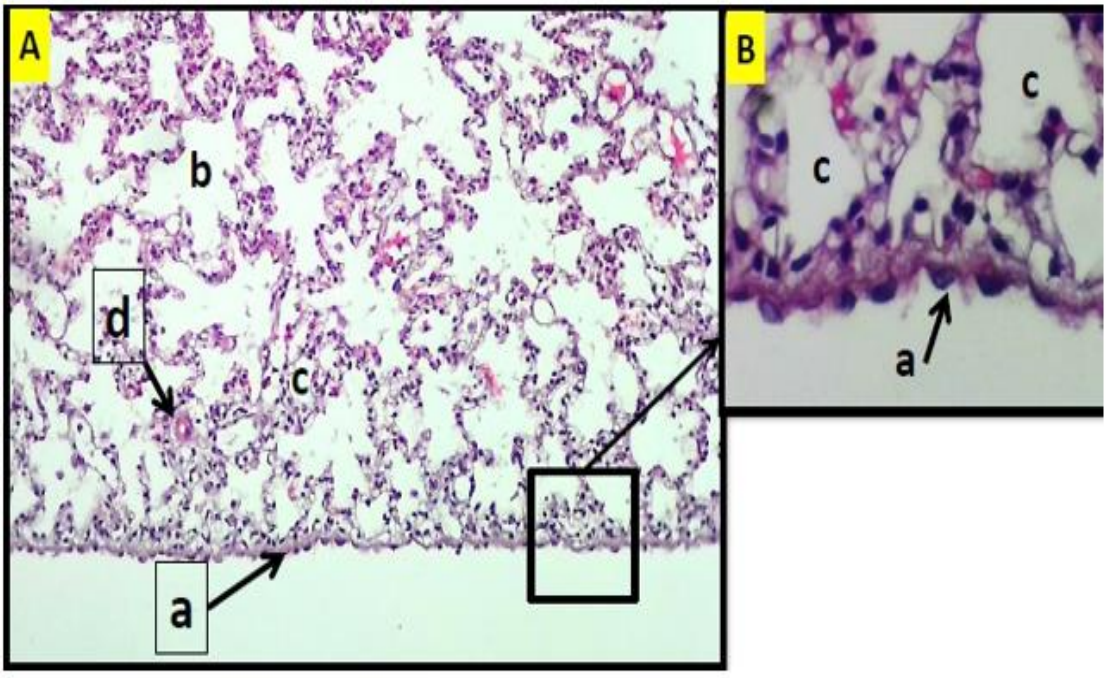

Figure 3:A: Cross section of lung in male albino rat at (1 month) shows: mesothelium cell (a), blood vessels (d), alveoli (c) , alveolar duct (b).H&E stain. 10x. B: Magnified section. H&E stain. 40x.

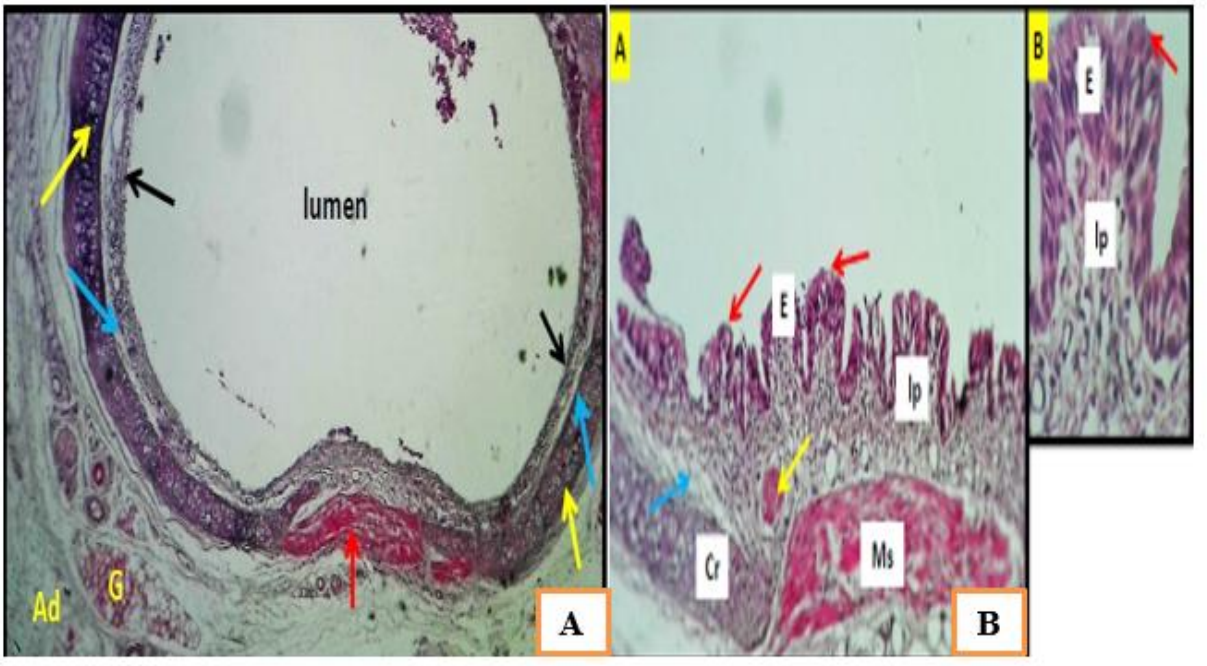

Figure 4: Cross section of trachea of male albino rat at (4 month) shows **(A):** Mucosa (black arrow), sub-mucosa (blue arrow), hyaline cartilage (yellow arrow), smooth muscle (red arrow), gland (G), tunica adventitia (Ad). H&E stain. 4x. **(B):** A: Mucosa (E), lamina properia (lp), sub-mucosa (blue arrow), dark gland (yellow arrow), hyaline cartilage (Cr), smooth muscle (Ms), H&E stain. 10x. B: Magnified section. 40x.

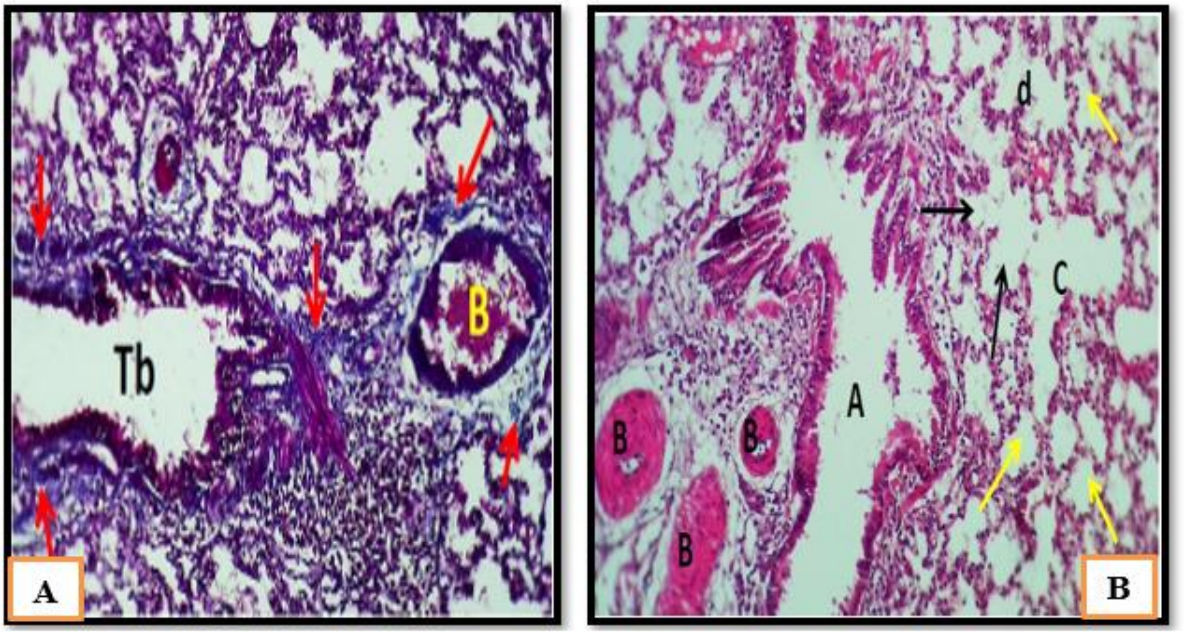

Figure 5: Cross section of lung in male albino rat at (4 month) shows (A): terminal bronchi (A), blood vessels (B), alveolar duct (d), alveolus (black arrow), alveoli (yellow arrow). H&E stain. 10x. (B): the collagen bundles around the terminal bronchi and the blood vessels (red arrow), terminal bronchi (Tb), blood vessels (B). Masson trichrom stain. 10x.

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