Academia Open
Vol 10 No 2 (2025): December (in progress)
DOI: 10.21070/acopen.10.2025.11564 . Article type: (Science)

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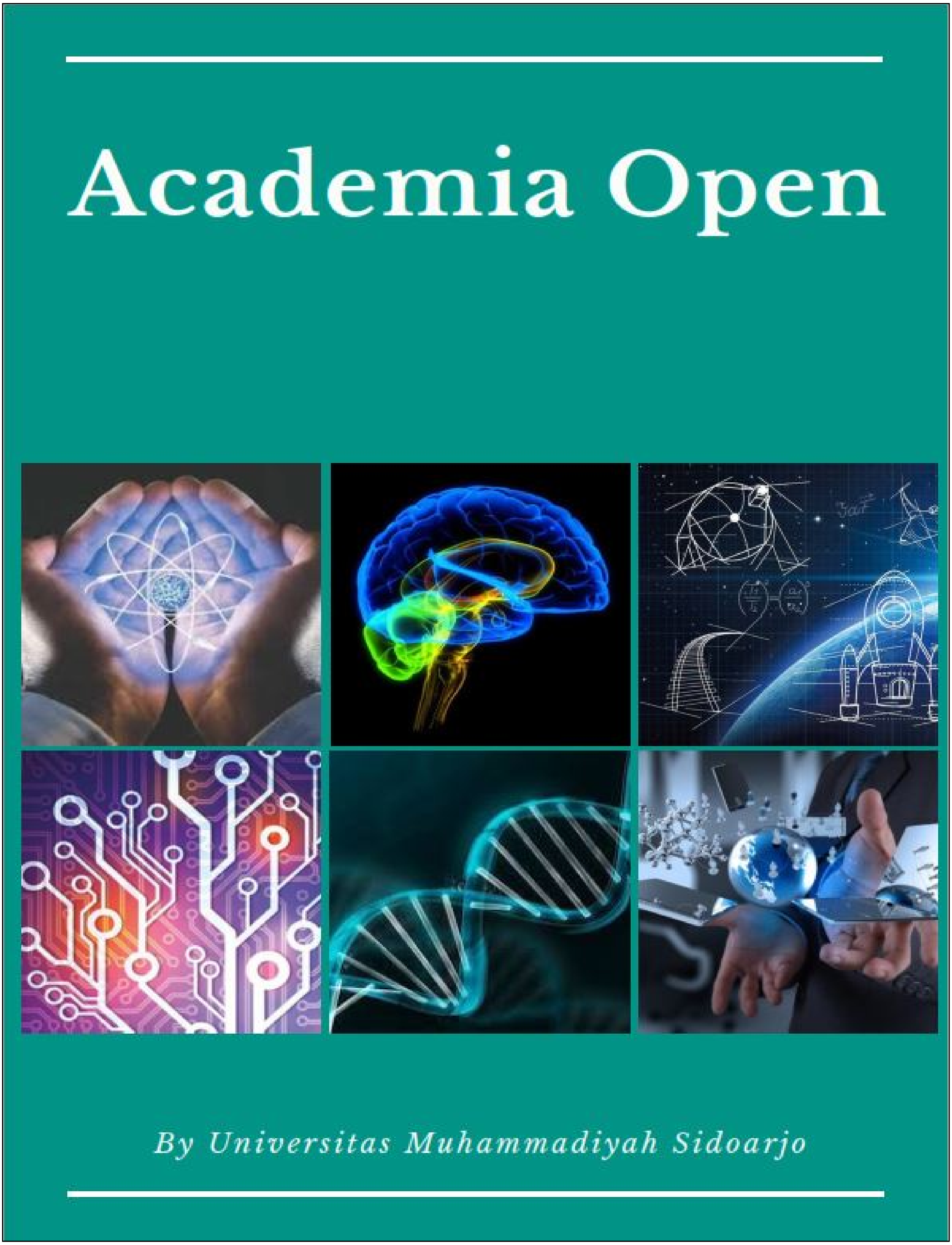

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Keratinization and Muscular Variation in the Abdominal Esophagus of Rabbit and Guinea Pig

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Abstract

Background: The esophagus exhibits species-specific structural adaptations related to diet and function, particularly in its abdominal segment. **Method**: Samples from adult rabbit and guinea pig esophagi were processed using routine histological techniques and stained with hematoxylin and eosin. Measurements of epithelial height and muscularis thickness were statistically analyzed ($P \leq 0.05$). **Results**: Significant histomorphometric differences were observed between the two species in epithelial and muscular thickness, with the exception of the submucosal layer. **Aims**: To compare the histological architecture of the abdominal esophagus in adult rabbits and guinea pigs, focusing on epithelial keratinization and muscular organization. **Findings**: Both species showed keratinized stratified squamous epithelium, but the degree of keratinization and muscle layer arrangement varied. The rabbit showed partial keratinization and a thicker muscularis externa, while the guinea pig had full keratinization and complex muscular layering. **Novelty**: This study provides the first direct comparative analysis of the abdominal esophageal segment between these two rodent species, highlighting structural distinctions potentially linked to dietary habits. **Impact**: The findings offer valuable insights for veterinary anatomy, comparative histology, and may inform experimental models involving the gastrointestinal tract in small mammals.

Highlights:

- Species Comparison: Highlights structural differences in the esophagus between rabbits and guinea pigs.
- Keratinization Pattern: Shows variation in epithelial keratinization linked to species-specific function.
- Veterinary Insight: Provides data useful for experimental and anatomical studies in small mammals.

Keywords: Esophagus, Rabbit , Histology, Histochemical.

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Published date: 2025-07-09 00:00:00

Introduction

The Lagomorpha group's rabbit (Oryctolagus cuniculus) is the animal most closely related to humans, after primates. It is a more acceptable laboratory mammal than primates in terms of husbandry, ease of breeding, cost-effectiveness, and ethical and legal conveniences. According to[1], rabbits are better laboratory animals than mice or rats because of their phylogenetic resemblance to humans, size, blood volume, responsiveness, and other congruences that enable them to more closely resemble human physiological traits in biomedical research. Rabbits are considered as important and nutritious source of animal protein on a global scale[2].

The guinea pig, which was domesticated between 3000 and 6000 years ago in the South American highlands primarily for meat, is now a significant source of protein in some rural populations. It is also widely used in many scientific domains, such as toxicology, allergies, respiratory conditions, nutrition, hearing, product testing, and medical quality control. An important laboratory animal used in biological model research[3], [4]. For example, guinea pigs are important for toxicity and vaccination testing since their immunological genes are more similar to those of humans than mice, making them a good model in immune system research[5].

The esophagus extends straight from the pharynx to the cardia of the stomach. The esophagus was dorsal to the trachea until it entered the stomach. According to its location in the body, the esophagus is separated into three parts: the cervical, thoracic, and abdominal[6], [7].

Method

A. Collection of Specimens

The present investigation was conducted in the Department of Anatomy, Histology, and Embryology, College of Veterinary Medicine, University of Al-Muthanna. The study period ran from September 15, 2024, until June 1, 2025. Ten juvenile rabbits were present. The rabbits, which weighed between 150 and 250 grams, were collected from local markets and each one was examined by a veterinarian to ensure their health.

B. Surgical Procedures , Histological and Histochemical Study

Before the animal was put to sleep, two milliliters of chloroform ($CHCl_3$) were put on cotton and placed near its nose[8]. Using the appropriate tools, such as scissors, forceps, and dissection scalpels, the rabbits' abdomens were then gently sliced along their length to remove the esophagus[9], [10].

Each animal's esophagus was removed after anesthesia, and flowing water was used to clean the cavities. The cervical regions and the esophagus were the sites of the sample collection. After that, histological preparation was done according to the usual protocol[11].

C. Sample Fixation

The esophageal specimens, intended for histological examination, were kept in a 10% formalin solution after extraction. The fixing process took 48 hours to complete. After fixing, the specimens were washed with tap water for two to three hours to remove the formalin solution.

D. Dehydration

The specimens were subjected to escalating ethyl alcohol concentrations for two hours at each concentration, starting with 70%, 80%, 90%, 100%, and 100%. This was done in an attempt to gradually remove the water with in the tissue.

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E. Clearing

The specimens were exposed to xylene twice for an hour each in order to improve their transparency and remove the alcohol solution.

F. Infiltration

After being cleaned, the samples were put in glass containers with a 1:1 mixture of melted paraffin wax and xylene in an electric oven set to 59 to 60 degrees Celsius. The specimens were then transferred to various containers that held only melted paraffin wax. In order to ensure that the specimens were fully impregnated, the wax was changed twice, separated by one and a half hours.

G. Embedding

The specimens were placed in special metal molds using paraffin wax. A heated needle was set over a flame to remove any air bubbles that might have surrounded the specimens. The specimens were removed from the mold and stored until they were cut into sections after the molds had had time to solidify at room temperature.

H. Trimming and Sectioning

The paraffin blocks containing the specimens were cut with a keen scalpel. After mounting the paraffin block for sectioning in a Rotary Microtome, the specimens were cut to a thickness of 5 micrometers. To encourage the best possible tissue spreading, the cut ribbons were placed in a water bath that was kept at 45°C. After that, the tissue fragments were put on sanitized glass slides, allowed to dry for an hour at 37°C on a hot plate, and then stored at room temperature until the next day.

I. Staining

The histochemical properties of the cells in each segment of the stomach and esophagus are described by [12] using a range of stains, such as Masson's trichrome, Alcian blue, and Periodic acid Schiff (PAS).

1. Eosin and Mayer Haematoxylin Stain(H&E): It was a common dye used to show the general structure of the tissue in histological examinations.
2. Masson Trichrome Stain: The purpose of this stain is to show how smooth muscle and connective tissue fibers appear
3. Periodic Acid-Schiff (PAS) Stain: [12]: This stain was utilized to demonstrate of neutral muco-polysaccharide secretion
4. Alcian Blue stain :Acid muco-polysaccharide secretion was demonstrated

J. Mounting

The slices are put on a glass slide with Mayer's albumin (a blend of glycerin and egg albumin) at a 1:1 ratio. To prevent deterioration (the growth of bacteria and fungi), a few drops of thymul were applied, and the slides were then dried on a hot plate at 40ºC for 24 hours.

K. Histological Measurements

Every component of the esophagus was measured histologically using an ocular micrometer and a light microscope. These measures comprised the thickness of each section's tunica adventitia, tunica muscularis (longitudinal and circular layers), tunica submucosa, and tunica mucosa (epithelium, lamina propria, and muscularis mucosa).

L. Statistical Analysis

The mean values with the standard error (SE) have been used to report the numerical,results.

Results and Discussion

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A. Adult Rabbit

The esophagus mucosa of keratinized squauamous epithelium makes up the rabbit wall. The esophageal tunica submucosa was composed of loose to moderately dense connective tissue that contained collagen and elastic fibers. The three layers of the muscularis, the inner longitudinal muscle layer and the outer longitudinal muscle layer, were thicker and had more loose connective tissue. All of the previously indicated strata were fully developed using hematoxylin and eosin stain (Figure1,2).Submucosal glands were absent as also reported earlier by [13] in guinea pig and rabbits, [14]in African gaint rat and[15] in guinea pig.

Throughout the esophageal course, keratinized stratified squamous epithelium constantly produced keratin protein, but pre-keratin in the underlying strata gradually decreased from the cervical to the abdominal course (Figure 3,4).The degree of keratinization varied depending on the species, according to [16][17] It was observed that the esophagus of the African giant rat lacked lamina muscularis and was made of non-keratinized squamous epithelium [15]reported that in guinea pig. There were no submucosal glands seen along the esophageal length(Figure1,2). Nonetheless, submucosal glands were found in the esophagus of dogs and pigs[18]**.** In guinea pigs, the absence of submucosal glands in the esophagus was also observed [19]**.** With an epithelial height of 2700 µm and a markedly enlarged muscularis externa of 10,803 µm (Table 6), (Figures1,2).

Histological research revealed that the esophagus epithelium of rabbits was somewhat keratinized, which is in line with observations made in a number of rodent species, including guinea both chinchillas and pigs[20]**.** However, [21]showed that rabbits had a higher degree of keratinization, suggesting that nutrition significantly affects epithelial characteristics. In contrast, the stratified squamous epithelium of humans and cats is not keratinized [22]**.** In line with other rat models, the muscularis mucosae was seen as a thin longitudinal layer of smooth muscle. Statistical analysis indicated significant histomorphometric differences between the adult guinea pigs and rabbits at the level of ($P \leq 0.05$) except for the submucosal layer(Table 6),

B. Adult Guinea Pig

According to histological findings, the esophagus is composed of four tunics: adventitia/serosa, muscularis, submucosa, and mucosa in its three divisions (cervical, thoracic, and abdominal). The mucosa created a lumen in the shape of a star (Figure5,6).This is similar to the finding made by [22] ,that the crosssection of the rabbit esophagus lumen resembles a star.

It has five layers: stratum corneum, lucidum, granulosum, spinosum, and basale, and is bordered by keratinized stratified squamous epithelium (Figure 7,8).as also observed by[14] and[16] inguinea pig, However, it was discovered that the African gaint rat's esophageal epithelium was not keratinized [15].

The tunica muscularis of the abdominal esophagus was somewhat different from the muscularis of the esophagus because three layers of the muscularis were apparent in some areas of the esophagus as an inner and outer longitudinal middle circular layer. the last two esophageal segments. In some areas of the oesophageal abdominal segments, four layers were discernible: circular, longitudinal, inner to outer, and inner to outer. (Figure 5–6).These findings are in agreement with [16] who also documented variability in muscular layering in the abdominal segment of the guinea pig esophagus.

Tunica	Adult Rabbit	Adult Guinea Pig	t	Sig.
total Mucosa	2279.667 ± 10a	2196.667 ± 16a	1.354	0.247
Keratine	95.667 ± 12a	400 ± 15b	-10.450	0.000

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epithelium	2000 ± 10a	1000 ± 20b	12.247	0.000
Lamina propria	88.333 ± 22a	300 ± 11b	-7.146	0.002
Muscularis mucosae	95.667 ± 11a	496.667 ± 50b	-12.520	0.000
total Submucosa	1000 ± 23a	600 ± 14b	4.899	0.008
total Muscularis	9000 ± 33a	4600 ± 22b	7.537	0.002
internal	3700 ± 12a	2000 ± 30b	10.410	0.000
middle	4300 ± 32a	2000 ± 22b	14.085	0.000
external	1450 ± 10a	600 ± 14b	20.821	0.000
Serosa	1000 ± 22a	2550 ± 33b	-24.012	0.000

Table 1. Show histomorphometric parameter of Esophagus in in adult Rabbit and adult guinea pig. Values represent : µm (Mean ± SE). Different letter means signifcant differences (p<0.05) between different parameter.

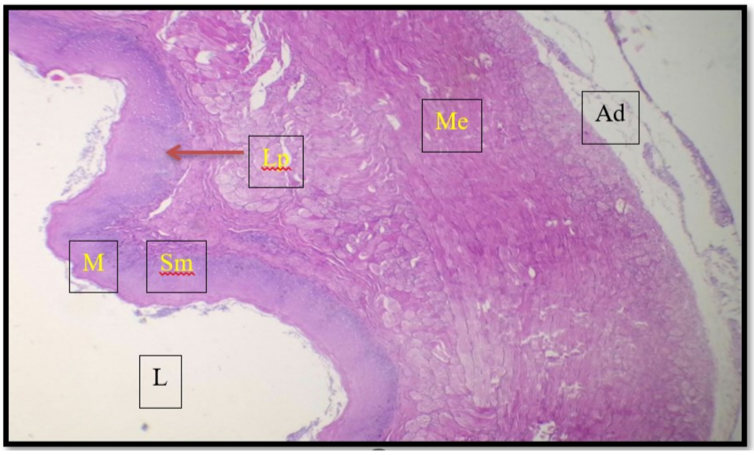

Figure 1. Histological cross section of the esophagus in adult rabbit shows abdominal part: : (L) Lumen, (M) mucosa , (Lp) lamina propria, (Sm) submucosa , (Me) muscualaris, (Ad) serosa , H&E stain X4.

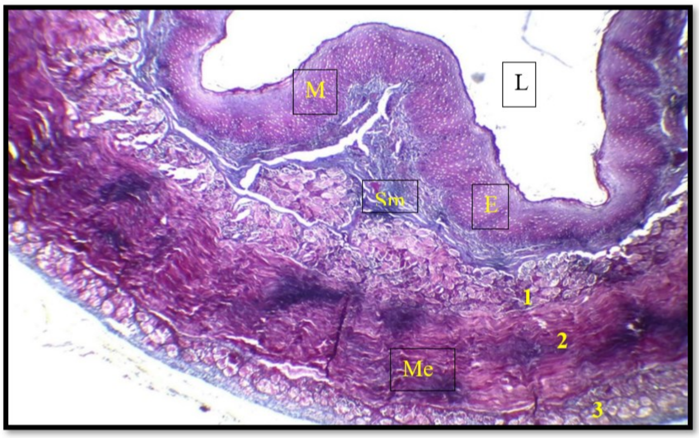

Figure 2. Histological cross section of the esophagus in adult rabbit shows abdominal part: (L) Lumen, (M) mucosa , (Sm) submucosa , (Me) muscualaris externa layers (1, 2, 3),, (Lp) lamina propria, (K) keratin , Masson's Trichrome stain X 4.

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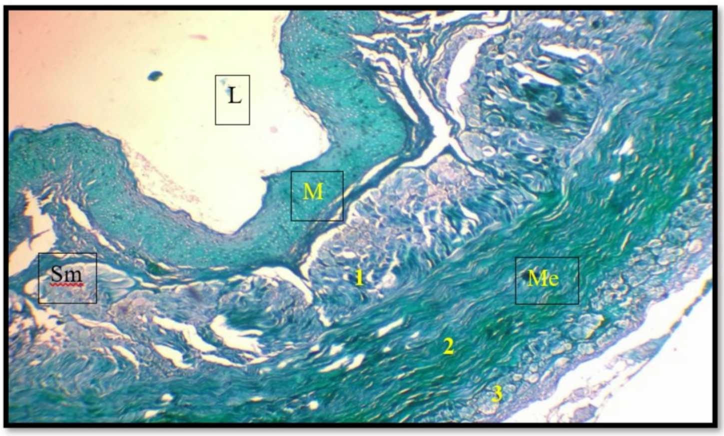

Figure 3. Histological cross section of the esophagus in adult rabbit shows abdominal part: (L) Lumen, (M) mucosa , (S) submucosa , (Me) muscularis externa layers (1, 2, 3), positive strong reaction response No esophageal glands, Alcian Blue stain X 4.

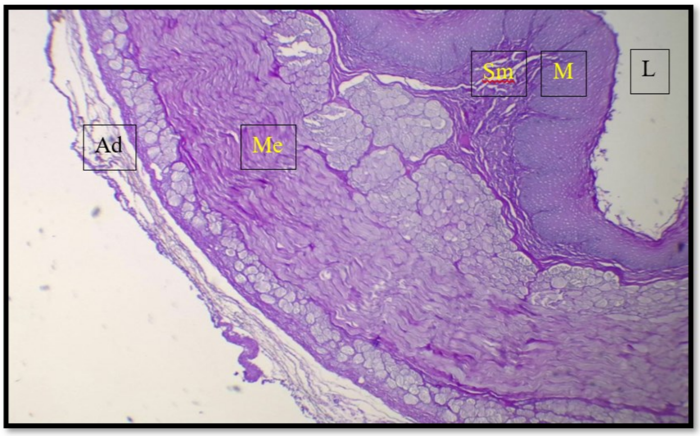

Figure 4. Histological cross section of the esophagus in adult rabbit shows abdominal part: (L) Lumen, (M) mucosa , (Sm) submucosa , (Me) muscualaris, (Ad) serosa, positive strong reaction response No esophageal glands PAS stain X4.

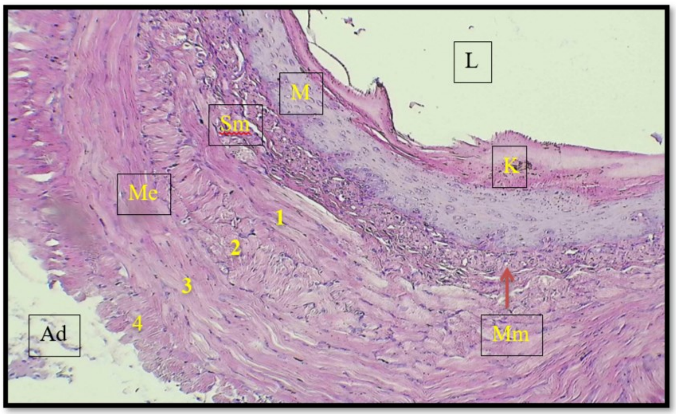

Figure 5. Histological cross section of the esophagus in adult guinea pigs shows abdominal part: (L) Lumen, (M) mucosa, (Mm) muscualaris mucosa , (Sm) submucosa, (Ad) serosa (K) keratin, (Me) muscualaris, esophagus of guinea pig showing the 4 layers of tunica muscularis inner circular and longitudinal(1,2) and outer circular and longitinal(3,4) muscular layers, H&E stain X10.

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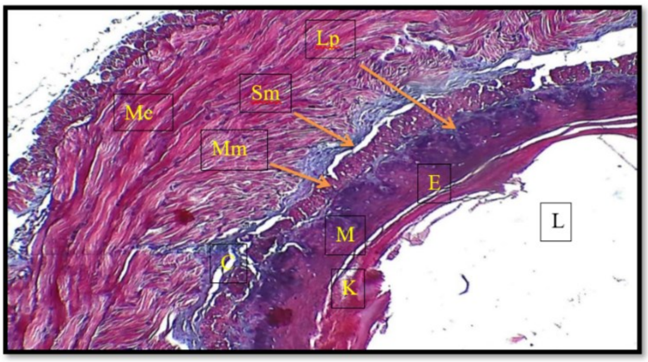

Figure 6. Histological cross section of the esophagus in adult guinea pigs shows abdominal part: (L) Lumen, (M) mucosa , (Sm) submucosa , (Me) muscualaris, (E)Stratified squamous epithelium, (Lp) lamina propria, (K) keratin , (Mm) muscualaris mucosa (C) collagen fibres, Masson's Trichrome stain X 4.

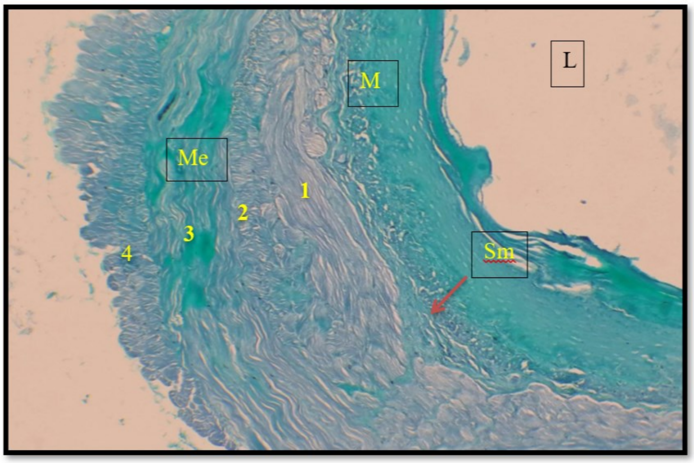

Figure 7. Histological cross section of the esophagus in adult guinea pigs shows abdominal part: (L) Lumen, (M) mucosa , (S) submucosa , (Me) muscularis externa layers (1, 2, 3,4), positive weak reaction response No esophageal glands, Alcian Blue stain X 4.

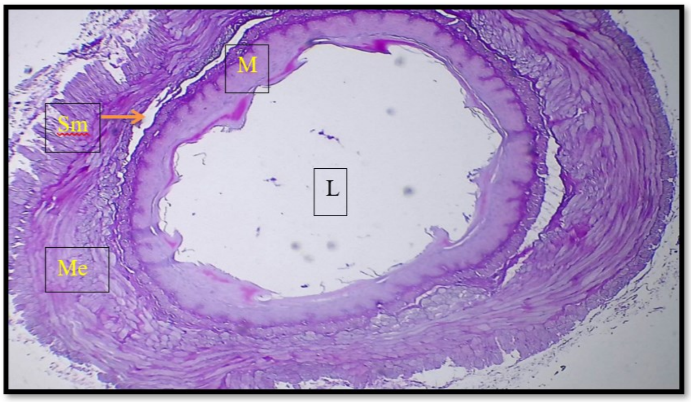

Figure 8. Histological cross section of the esophagus in adult guinea pigs shows abdominal part: (L) Lumen, (M) mucosa , (Sm) submucosa , (Me) muscualaris positive strong reaction response No esophageal glands .PAS stain X 4.

Conclusion

The esophagus of adult rabbits and guinea pigs shares common structural features, such as keratinized stratified squamous epithelium and absence of submucosal glands. However, notable differences were

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observed in the degree of keratinization, mucosal folding, and muscular layer arrangement, particularly in the abdominal segment. The rabbit esophagus exhibited a thicker muscularis externa, while the guinea pig showed more complex muscular layering and a fully keratinized epithelium. These anatomical differences likely reflect species-specific dietary and functional adaptations, and provide important comparative insights into rodent gastrointestinal morphology.

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