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Comparative anatomical, histometrical, and histochemical study of esophagus between ring-necked parakeet (*Psittacula krameri*) and black-shouldered kite (*Elanus caeruleus*)

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Abstract

The present study aimed to investigate the esophagus's anatomical, histometrical, histochemical aspects in the Ring-necked parakeet and Black-shouldered Kite. The esophagus in the parakeet was shorter and narrower than the kite esophagus. The crop was a well-developed sac shape in the parakeet, while the crop was hardly recognizable in the kite. Histological study shows that the esophagus in both species was composed of four layers arranged from inner to outer (mucosa, submucosa, musculosa, finally serosa). These layers showed variances in the height of the fold, the glands number, and the wall layers' thickness. The mucosa in the esophagus of the kite consisted of non-keratinized stratified squamous epithelium, while in the parakeet, mucosa consisted of keratinized stratified squamous epithelium. The esophageal glands were compound tubuloalveolar in the parakeet, while in the kite, there were simple alveolar glands. The folds in the kite were longest and contained more esophageal glands, while the parakeet glands were more prominent and had fewer numbers within the submucosa. The submucosa layer in the parakeet was thicker than that of the kite. The histochemical results showed a positive reaction of esophageal glands with Alcian blue (PH 2.5), while reacting negatively with Periodic Acid Schiff (PAS) stains due to the acidic mucopolysaccharides secretions nature.

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Introduction

According to the type of diet consumed, avian species are classified into granivorous and carnivorous birds (1). The Ring-necked parakeet (*Psittacula krameri*) is a medium-sized bird in the family Psittacidae. It is a granivorous bird, feed on fruits, vegetables, nuts, seeds (2). The Black-shouldered kite (*Elanus caeruleus*) is a medium-sized bird of prey within the family Accipitridae, which includes many other diurnal birds such as hawks and eagles. It is a carnivorous bird, feed on mice, grasshoppers, rats, small reptiles, rabbits (3). The avian digestive tract shows different characteristics among various species according to feeding type and dietary habits (4). The esophagus long tubular quite wide and highly distensible organ (5). It lies dorsal to the trachea in the frontal area of the neck and then runs to the right side (6). It is connected cranially to the pharynx while caudally connected with the glandular stomach. The esophagus is used to transport feeding material between them (7). To accommodate a large amount of food, the esophagus is long and highly distensible due to the presence of longitudinal folds (8). The word esophagus is derived from the Greek words: oisein, to carry, and phagein, to eat (9). In Psittacine birds, the esophagus is generally divided into a cervical esophagus, crop, and thoracic esophagus (10). The crop of birds is an esophageal diverticulum and varies in size depending on the species and the bird's age (11). In

Grey-backed Shrike, the esophagus is divided into the cervical and thoracic parts, and the crop is absent (4).

The current work is aimed to report the anatomical, histometrical, histochemical comparative structures of the esophagus between the Ring-necked parakeet (granivorous bird) and Black-shouldered kite (carnivores' bird) and correlate this with the food type.

Materials and methods

Twenty adult birds were used (10 Ring-necked parakeets and 10 Black-shouldered Kites). The birds were brought from Suq Al-ghazl in Baghdad and checked for their health status. The birds were anesthetized by using chloroform. The tissue samples of the esophagus were removed and immersed in neutral buffered formalin for 24h. After fixation, the specimens were dehydrated with alcohol, cleared with xylene, embedded in paraffin wax, sections of 5 mm thickness, stained routinely with Hematoxylin and Eosin for general histological study and special stains of periodic acid Schiff (PAS) - Alcian blue at pH 2.5 for histochemical study (12). Later, the sections were examined by an Olympus microscope. The pictures took by the digital microscopic camera for historical analysis. The automated computer-adopted image analysis system Image J® was used.

Ethical approve

Informed consent according to the Declaration of Helsinki was obtained from the ethics committee of the college of Science, Mustansiriyah University Ref. No. BCSMU/0821/0002.

Results

Anatomical description

Ring-necked parakeet *(Psittacula krameri)*

The esophagus was divided into three parts, cervical part, crop, and thoracic part. The cervical part begins with the end of the pharynx and runs on the right side of the neck. In the caudal area of the neck, it opens into a sack-like structure called the crop. It was well developed in a Ring-necked parakeet and used as a storage area. It has bulges to the right across the midline of the neck. The last part of the esophagus was the thoracic part, which connects with the proventriculus. The cervical esophagus was longer than the thoracic esophagus. The total length of the esophagus in the Ring-necked parakeet was 4 cm.

Black-winged Kite *(Elanus caeruleus*)

The esophagus is divided into two regions, cervical esophagus, and thoracic esophagus. It is located at the right side of the neck dorsally to the trachea, and it extends from the pharynx to the glandular stomach (proventriculus). The esophagus was long and highly distensible due to many longitudinal folds to accommodate large amounts of the food. These folds were extensive. The crop was hardly recognizable. The length of the esophagus in the Black-winged kite was 5 cm.

Histological structure

Ring-necked parakeet (*Psittacula krameri*)

The esophagus consists of four histological layers arranged from inner to outer: mucosa, submucosa, musculosa, serosa (Figure 1). The mucosa of the esophageal wall was formed of thick keratinized stratified squamous epithelium (Figure 2). The mean thickness values of mucosa were 123.5±9.3 µm (Table 1). Its mucosal folds were externally covered by epithelium and internally filled with loose connective tissue constituting the lamina propria (Figure 2). The mean of length values of these folds was 323.9±31.2 µm and the width 241.1±13.8 µm. The muscularis mucosa was present as a layer of smooth muscle fibers. The muscle fibers were only oriented longitudinally, the exact orientation of the folds of the mucosa. The tunica submucosa was a loose connective tissue beneath the muscular mucosa containing compound tubuloalveolar mucous glands (Figure 2). The mean thickness values of submucosa were 245.1±22.5 µm. The mean of length values of the glands was 207.1±26.2 µm and the width 231.1±11.3 µm. The musculosa consisted of smooth muscle fibers with inner circular and outer longitudinal muscles (Figure 1). The mean thickness values of the musculosa were 281.3±10.2 µm. The tunica serosa surrounded it, formed by connective tissue containing blood vessels, nerve fibers, fatty tissue (Figure 1). The mean thickness values of serosa were 62.4±3.9 µm. The esophageal gland's secretions were acidic mucopolysaccharides due to the positive reaction with PAS-Alcian blue stains.

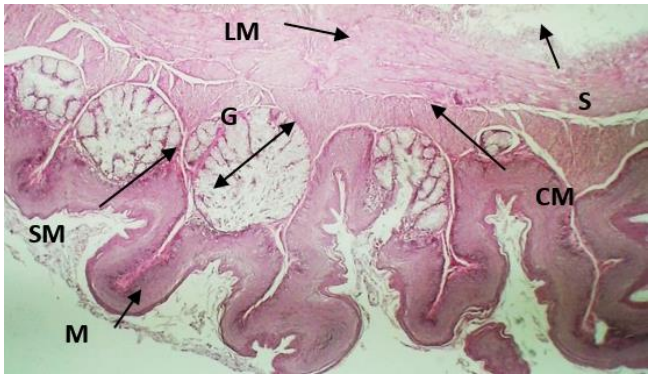

Figure 1: Cross-section in the esophagus wall of the Ring-necked parakeet shows: mucosa (M), submucosa (SM), circular muscles (CM), longitudinal muscles (LM), serosa (S), and esophageal glands (G) within (H&E) 40X.

Table 1: Measurement of the esophagus layers in the ring-necked parakeet and the black-shouldered kite

Length and width of layers	Ring-necked parakeet (mean µm ± SE)		Black-shouldered kite (mean µm ± SE)	
	Esophagus	Crop	Cervical esophagus	Thoracic esophagus
Longitudinal folds length	323.9±31.2	310.8±45.86	728.9±37.19	1308.2±83.36
Longitudinal folds width	241.1±13.8	213.2±10.18	418.1±23.18	494.5±15.57
Glands length	207.1±26.2	absent	106.2±8.66	196.04±16.65
Glands width	231.1±11.3	Absent	135.7±8.19	177.5±9.28
Mucosa thickness	123.5±9.3	84.7±3.02	108.2±8.97	85.47±3.49
Submucosa thickness	245.1±22.5	Very thin	141.6±9.58	178.3±13.51
Musculosa thickness	281.3±10.2	256.8±16.83	394.4±21.05	435.98±18.52
Serosa thickness	62.4±3.9	74.9±4.12	99.96±5.76	170.6±8.75

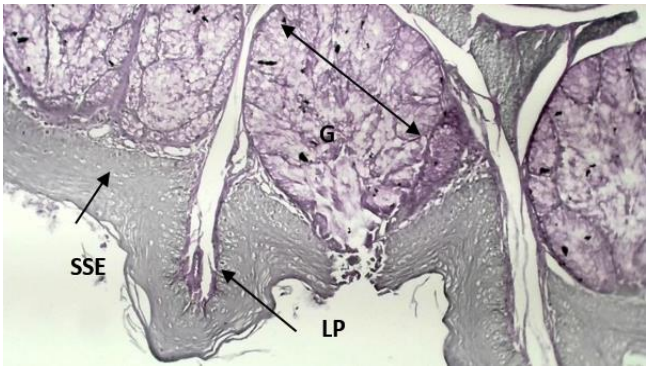

Figure 2: Cross-section in the esophageal glands of the Ring-necked parakeet shows: keratinized stratified squamous epithelium (SSE), lamina propria (LP), tunica submucosa consists of loose connective tissue with compound tubuloalveolar mucous glands (G) in blue color (PAS-Alcian blue) 100X.

The crop was structurally similar to the esophagus, except for the absence of mucous glands in the crop. The crop folds appeared with a few vast waves in shape (Figures 3 and 4). The mean length values of these folds were 310.8±45.86 µm and the width 213.2±10.18 µm. The mean mucosa thickness value is 84.7±3.02 µm. The submucosa was a weak layer. The muscularis mucosa was challenging to differentiate from the musculosa layer due to the almost total absence of the smooth muscle fibers under the lamina propria. The musculosa had two muscular layers, inner circular muscular fibers and outer longitudinal muscular fibers (Figure 3), the mean thickness values of the musculosa were 256.8±16.83 µm. Serosa, formed by loose connective tissue, blood vessels, and nerves, covered the organ externally (Figure 3). The mean of serosa thickness values 74.9±4.12 µm.

Black-shouldered Kite *(Elanus caeruleus*)

The esophagus consists of four distinct functional layers arranged from inner to outer: mucosa, submucosa, musculosa, and the outer serosa (Figure 5). In the cervical part (Figure 6), the mucosa consists of numerous longitudinal folds of different shapes and depths. The mean of length values of these folds 728.9±37.19 µm and the width 418.1±23.18 µm. It was lined by non-keratinized stratified squamous epithelium. The lamina propria of mucosae consisted of loose connective tissue with lymphatic tissue (Figure 6). The mean of mucosa thickness values 108.2±8.97 µm. The esophagus glands were simple alveolar glands opening to the inner surface of the wall through ducts (Figure 6). The mean of glands length values 106.2±8.66 µm and the width 135.7±8.19 µm. The high column cells with the flat basal nucleus lined the glandular lumens of esophageal glands. The glands were surrounded by a thin layer of smooth muscle fibers (Figure 6). The lamina propria separates from the submucosa by muscularis mucosa. The submucosa consists of loose connective tissue with blood vessels and nerves (Figure 6). In the cervical part, the mean of submucosa thickness values 141.6±9.58 µm. The musculosa was consists of two layers of smooth muscle fibers: inner circular muscles and outer longitudinal muscles. The mean musculosa thickness value is 394.4±21.05 µm. It surrounded the tunica serosa formed by connective tissue containing blood vessels, nerve fibers, and fatty tissue (Figure 5). The serosa mean thickness values were 99.96±5.76 µm. The thoracic part of the esophagus (Figure 7) mucosa was lined with non-keratinized squamous stratified epithelium. The thoracic esophagus possessed the highest folds than the cervical part. The means length values of these folds 1308.2±83.36 µm and the width 494.5±15.57 µm. The glands number in the thoracic part was more than the cervical part. The means length values of these glands were 196.04±16.65 µm and the width 177.5±9.28 µm. The mean thickness value of mucosa is 85.47±3.49 µm. The submucosa, musculosa, and serosa histological structure differ from the cervical part. The submucosa means thickness values were 178.3±13.51 µm. The musculosa and serosa mean thickness values were 435.98±18.52 µm, and 170.6±8.75 µm, respectively. The esophageal gland's Secretions were acidic mucopolysaccharides due to the positive reaction with PAS- Alcian blue stains (Figure 6).

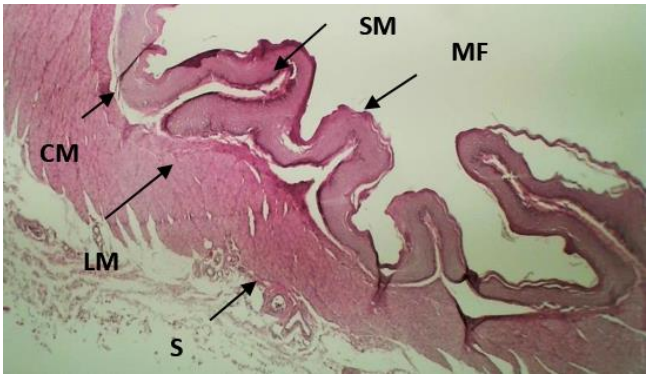

Figure 3: Cross-section in the crop wall of the Ring-necked parakeet shows: mucosa folds (MF) appeared with a shallow and wide wave in shape, submucosa (SM) was fragile, circular muscles (CM), longitudinal muscles (LM) with two layers, inner circular and outer longitudinal muscular fibers, serosa (S) with loose connective tissue, vessels, nerves (H&E) 40X.

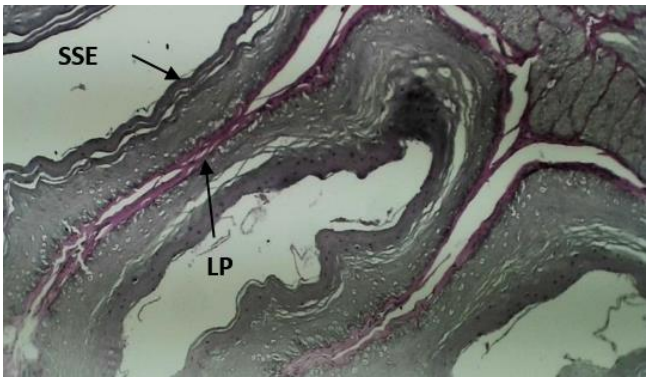

Figure 4: Cross-section in the crop folds of the Ring-necked parakeet shows: mucosa with keratinized stratified squamous epithelium (SSE), and no mucous glands were present in the lamina propria (LP). (PAS-Alcian blue) 100X.

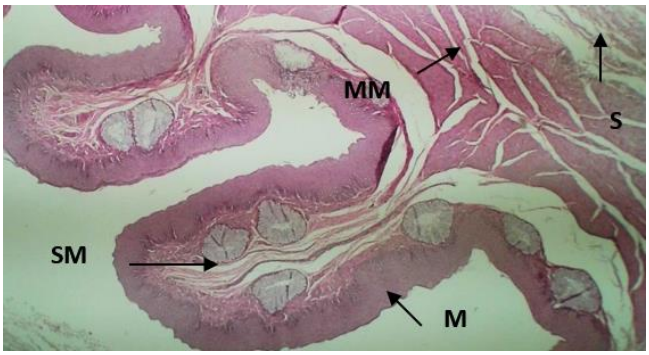

Figure 5: Cross-section in the esophagus wall of the Black-shouldered kite shows mucosa (M), submucosa (SM), musculosa (MM), and serosa (S) (H&E) 40X.

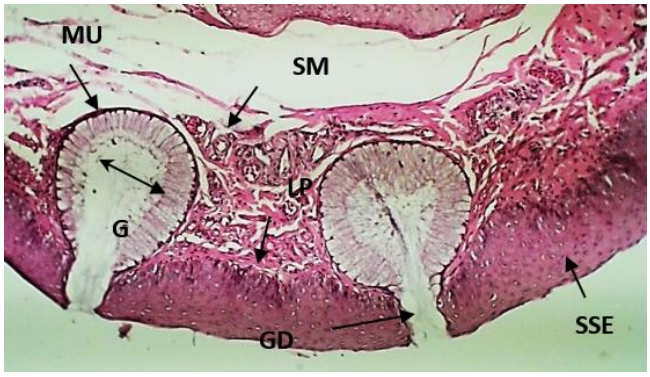

Figure 6: Cross-section in the esophageal glands of the Black-shouldered Kite shows non-keratinized stratified squamous epithelium (SSE), lamina propria mucosae (LP), submucosa (SM), and simple alveolar glands (G), lined by high columnar cells with a flat basal nucleus and surrounded by smooth muscles (MU), esophagus gland duct (GD) (H&E) 100X.

Figure 7: Cross-section in the thoracic esophagus folds of the Black-shouldered Kite shows the highest mucosa folds (MF) and more glands (G) than the cervical part (PAS-Alcian blue) 40X.

Discussion

In both species esophagus was a long muscular tube situated between the pharynx and glandular stomach. It was located to the right side of the neck dorsally to the trachea and opens into the proventriculus. This result is in agreement with Al-Kinany (13) and Rossi (14). The esophagus in the Ring-necked parakeet was shorter and narrower than that of the Black-shouldered Kite. These results agreed with Taha and Al-Duleemy (15) in Zebra finch and Starling. The length of the esophagus is related to the size of the food intake because the long esophagus can expand more than the short esophagus. In the Ring-necked parakeet, the esophagus is divided into three parts cervical part, crop, and thoracic part. These findings agreed with Hena (16), who study both the

Quail and the pigeon esophagus. In the Black-shouldered Kite, the esophagus is divided into cervical and thoracic part only (8). In the both species, the cervical part is longer than thoracic part. These results were compatible with the results of Hasoon and Haba (5).

The crop in the Ring-necked parakeet was a well-developed sac shape similar to Japanese quail and Dove crop which appear as a flexible out pocket (17,18), while the crop was hardly recognizable in the Black-shouldered kite similar to Ostriches (19). The variances among the esophagus and crop resulted from the difference in the function of each region which differed in the type of food consumed by the two birds (20). Carnivorous birds needed a long esophagus to swallow and stored food along the esophagus, while the granivorous birds needed to comprehensive store represented by the crop (21). The present study agrees with the results of Al-Juboory (20), who studied the esophagus in the *Columba palumbus*, and *Tyto alba* and with Elshaer (22), who studied the esophagus in the Kingfisher and Hoopoes.

The esophagus in both species of birds is composed of four layers, mucosa, submucosa, muscular, and serosa. These results were compatible with the results of Shehan (23), who studied the esophagus in Geese, and Parisa (24), who studied the alimentary canal of Pheasant. While, these findings disagreed with Zhu (4) in Grey-backed shrike who mentioned that the esophagus is composed of three layers, mucosa, musculosa, and serosa, while submucosa was absent.

These layers showed variances in the folds' height, number, and size of the glands and the thickness of the esophageal wall layers. The mucosa in the cervical esophagus of the Black-shouldered Kite consisted of non-keratinized stratified squamous epithelial. These results were consistent with Shehan (23), who studied the esophagus in geese, and Abd-Elnaeem (25), who studied the esophagus in the King fisher. While in the Ring-necked parakeet, mucosa consisted of keratinized stratifies squamous epithelium. This present study agrees with the results of Ali (26) in *Lorasmelono cephalus* and with Rajabi (27) in the parakeet and Rock dove.

The esophagus wall in two species contained longitudinal folds. This result was compatible with the results of Tomar (28). In the Black-shouldered Kite, the folds were longer and contained more simple alveolar esophageal glands. The same condition had been found in little crake by Omar (29). In the Ring-necked parakeet, inner folds were shorter, and esophageal glands were more significant and less compound tubuloalveolar within the submucosa. This finding agrees with the previous result of Madhu (30) in emu, and these results were confirmed by Zaher (6) in quail. The lamina propria separates from the submucosa by muscularis mucosa in both species. The muscularis mucosa was present as a layer of smooth muscle fibers only. These results were compatible with the results of Parchami and Dehkordi (31), who study the esophagus wall in common quail. The muscle fibers were oriented longitudinally, the exact orientation of the folds of the mucosa. These results were compatible with the results of Sokolowska (32).

Submucosa in the Ring-necked parakeet was thicker than that of the Black-shouldered kite, while the two other layers were thinner in the Ring-necked parakeet. In both species, the submucosa consists of loose connective tissue with blood vessels, lymphatics, and nerve fibers. The same results were confirmed by Parchami and Dehkordi (31), who studied esophagus wall in common Quail. The musculosa consisted of two layers of smooth muscle fibers with inner circular and outer longitudinal muscles in both species. These findings agreed with Klasing (33), who studied the esophagus in budgerigar and agreed with Qureshi (34), who studied the digestive tract in duck. In contrast Rodrigues (35), who studied the esophagus in blue and yellow macaws and Srisai (36), who studied the esophagus in germain's swiftlet, disagreed with these findings.

The serosa in the two species was formed by connective tissue containing blood vessels and nerve fibers. These results were compatible with the results of Kadhim and Mohamed (37), who studied the esophagus in the homing pigeon. This finding disagrees with the result of Shalaby (38). The crop in the Ring-necked parakeet was well-developed, but no mucous glands were present. The same findings were observed by Reavill (10) and with Kausar (39) in domestic pigeon, and disagreed with the study of Zaher (6) in common quail. Histochemically, applying PAS-AB (pH 2.5) stain, the esophageal glands were given blue color with Alcian blue stains and no reaction with PAS stain. This reaction indicated the presence of high content of acidic mucin secretions. These findings were in agreement with Taki-El-Deen (40) and disagreement with Yovchev and Penchev (41). The mucin was playing an important role in the mucosal protection as a pre-epithelial barrier (42).

Conclusion

The esophagus in the two birds was the beginning of the digestive tract. It was essential because the food is a passage from the pharynx to the proventriculus through it. The esophagus is relatively long, with mucous glands and several folds that increase the esophagus's inner surface and assist food movement. It is characterized by a crop that serves as a reservoir for food storage in a ring-necked parakeet. No crop was observed in the esophagus of the black-shouldered kite. The absence of crops may be due to the food may be stored throughout the length of the esophagus. The esophagus diameter and crop shape differed depending on the type of nutrition.

Acknowledgment

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Conflict of interest

The results of the current study are part of the requirements of Ph.D. in Department of Biology, College of Sciences, Mustansiriyah University in Zoology, for the first author. Also, we are the authors of this manuscript, declare and confirm that no significant or other relationship with any official institution.

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دراسة تشريحية وقياسية وكيميائية نسيجية مقارنة للمريء بين الدرة مطوقة العنق والحدأة سوداء الكتف

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الخلاصة

هدفت الدراسة الحالية إلى التحقق من الجوانب التشريحية والنسيجية القياسية والكيمياء النسيجية للمريء في الدرة مطوقة العنق والحدأة سوداء الكتف. المريء في الدرة أضيق وأقصر من مرئ الحدأة. الحوصلة في الدرة كانت عبارة عن كيس متطور بشكل جيد بينما في الحدأة كانت الحوصلة صعبة التمييز. أشارت الدراسة النسيجية إلى أن المريء في كلا النوعين يتألف من أربع طبقات نسيجية تترتب من الداخل إلى الخارج (المخاطية، تحت المخاطية، العضلية وأخيرا المصلية). أظهرت هذه الطبقات تباينا في عدد وارتفاع هذه الطيات ووجود الغدد المريئية وعدد هذه الغدد وسمك طبقات جدار المريء. تتكون المخاطية في مريء الحدأة من ظهارة حرشفية مطبقة غير متقرنة بينما في الدرة تتكون المخاطية من ظهارة حرشفية مطبقة متقرنة. الغدد المريئية في الدرة من النوع الحوصلي الأنبوبي المركب بينما في الحدأة تكون الغدد حوصلية بسيطة. الطيات الداخلية في الحدأة أطول وتحتوي على غدد مريئية كثيرة بينما في الدرة الغدد أكبر وبأعداد اقل وتكون موجودة في الطبقة تحت المخاطية. طبقة تحت المخاطية في الدرة تكون اسمك مما هي عليه في الحدأة. أشارت نتائج الكيمياء النسجية إلى التفاعل الإيجابي للغدد المريئية مع صبغة اليشين بلو بينما تفاعلت سلباً مع صبغة حامض بيرودك - شيف وذلك تبعا للطبيعة الحامضية للإفرازات المخاطية متعددة السكريات.

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