



Histomorphological and carbohydrate histochemical study of the pancreas in native ducks (*Anas Platyrhynchos*)

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Abstract

In the current study, the histomorphological and histochemical characteristics of the pancreas in native ducks are examined with a particular emphasis on the structure of pancreatic acini and Langerhans islets (LI). Five adult male native ducks (*Anas Platyrhynchos*) are examined from the local market of Mosul. All samples are processed routinely for histological and histochemical analyses. Hematoxylin & Eosin, PAS-AB (pH 2.5), Toluidine blue, and Masson's Trichrome stains are used. The morphological examination reveals that male ducks' pancreas is made up of three major lobes (dorsal, ventral, and third lobes). The pancreas is a small, semi-wide structure with a pale to white pinkish tone located between the duodenum branches. The findings show that the pancreas is enveloped in a thin layer of loose connective tissue capsule and mesothelial; septa extend from the capsule into parenchyma dividing it into many lobules. The study concludes that the pancreatic lobules are composed of a large area of exocrine acini and a small amount of LI, with an exocrine component consisting of round or oval acini with zymogen granules. The endocrine component is represented by three distinct LI types and lacks defined boundaries. Alpha islets are formed of alpha cells (ACs) and a few delta cells (DCs); beta islets are composed of peripherally located beta cells (BCs) and a few (DCs); and mixed islets are composed of alpha, beta, and delta cells. The present study sheds light on the morphological, histological, and histochemical characteristics of the indigenous duck pancreas. However, future research should focus on the pancreas of other bird species as well.

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Introduction

In the digestive system, the pancreas is a key glandular organ. The initial investigations establish that most bird species have three distinct pancreatic cell types (A, B, and D cells) (1). In addition, some previous studies report that the avian pancreas is separated from the right side of the abdominal cavity in all birds. It is positioned between the descending and ascending duodenal limbs (2-3). Additionally, some authors report that it is unique among animals to have two or more lobes (4-5). For example, the dorsal, ventral, third, and splenic lobes of the early hatched

goose (*Anser anser*) are four (6). There are dorsal and ventral lobes in red jungle fowl (7) as well as a small thin lobe called the splenic lobe and a delicate lobe as a subdivision of the ventral lobe called the third lobe, or two dorsal and ventral lobes in Houbara bustard (8). Chickens have four pancreatic lobes: ventral, dorsal, third, and splenic (9). In falcon (10) demonstrates that the avian pancreas can be divided into exocrine portions that comprise the majority of the pancreatic mass and is composed of the simplest to most complex cellular types; acinar cellular portions; ductal cellular portions; and endocrine portions further divided into light and dark islet portions. Besides, in pancreas of the long-

legged buzzard (*Buteo rufinus*) (11) indicated that the endocrine system controls blood sugar levels, while the exocrine system releases a lot of important enzymes and electrolytes.

According to the above survey, there is a need for more histochemical and histomorphological investigations of the pancreas in some birds, especially native ducks. Therefore, the current study aims to identify the histomorphological and histochemical characteristics of pancreas, as well as to examine the histological anatomy of pancreatic acini and Langerhans islets in native ducks (*Anas Platyrhynchos*).

Materials and methods

Animals and tissue preparation

Five healthy adult male ducks weighing 2–2.5 kg are used in this study obtained from the market in the city of Mosul. The birds were slaughtered after being deeply anesthetized by chloroform inhalation and the abdominal cavity was exposed to view the abdominal viscera (12). The total length of the pancreatic lobes was fixed. After conducting topographic examinations, the entire pancreatic lobes were removed and washed with normal saline. Finally, morphological studies were carried out. The pancreas was washed with distilled water, and it was divided into three anatomical parts: the dorsal, ventral, and third lobes. The length of each lobe was measured with the aid of a Vernier caliper. The pancreas was then fixed in 10% neutral buffer formalin for 48 hrs. The samples were routinely processed, and the standard paraffin embedding was done and serially sectioned with 5–7 μ m thickness (12–13). The tissue sections were deparaffinized and stained with hematoxylin and eosin to study the general histological structure of the pancreas, Masson's trichrome stain to show the connective tissue, periodic acid Schiff reagents PAS-AB (pH 2.5) to detect mucopolysaccharides and Toluidine blue to detect glycosaminoglycan in the pancreas tissue of the local male duck (14–16). Some measurements related to the research were taken from several slides such as 1- capsule thickness, 2- diameter of secretory units, 3- diameter of Langerhans islands and their cells, 4- number of cells that line the secretory units, and 5- diameter of blood vessels (artery and vein). The results were compared with the three lobes of the gland.

Photographs of the sections

All tissue sections were photographed, and the micro morphometric parameters were analyzed by using an OMAX 8.0MP digital USB microscope camera with advanced image analysis software (OMAX Toup View 3.7). The camera was fitted to Microscope-Olympus-CX31 and the calibration of the objective lenses to the software was done with the aid of a stage micrometer.

Statistical analysis

All the findings were analyzed using SPSS software. The morphological parameters were compared by one-way ANOVA, and the Tukey's test was used as a post-doc test. The difference was considered significant when P values were less than 0.001.

Results

The pancreas in the local duck is a pale pinkish, lobulated organ. Each lobe is shaped like a ribbon. The pancreas is positioned between the ascending and descending limbs of the duodenum and is completely encompassed by the pancreatic duodenal ligament (Figure 1). The histological examination of the duck pancreas reveals that a capsule lined with a single layer of mesothelial cells and supported by a thin layer of loose connective tissue encircles the entire gland and extends from capsule to parenchyma, dividing it into three main lobes; the dorsal lobe measured 6.83 ± 0.76 cm long, the ventral lobe measured 4.72 ± 0.34 cm long, and the third lobe measured 3.97 ± 0.63 cm long. The present investigation discovers a difference in the thickness of the capsule that covers the pancreas lobes (Figures 2 and 3). The histological appearance of the pancreas reveals that the exocrine section (exocrine secretory acini) occupies a greater area than the endocrine portion (islets of Langerhans), which is encircled by a network of fibers (Figure 4).

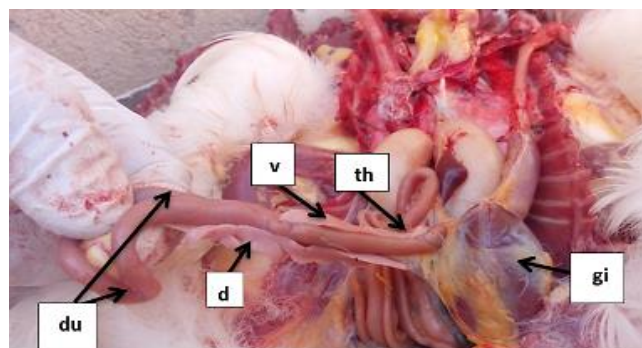


Figure 1: Anatomical position of the bird under consideration (v- Ventral lobe, d-Dorsal lobe, th- Third lobe, du- Duodenum, gi- Gizzard).

The current study demonstrates that the secretory acini of the exocrine portion in the pancreas in the local ducks are composed of serous tubuloacinar glands that differ in size and shape of cells from the tall columnar to polygonal with large basophilic zymogen granules. The secretory unit also differs in diameter (Table 1) in all lobules, and it has a ductal system that begins with the center. The mucosa is the deepest layer, bordered by columnar epithelium with goblet cells and lamina propria connective tissue. In the mucosa layer, temporary creases can be seen. The muscular layer is the

second layer. It is composed of longitudinal smooth-muscle fibers on the inside and thick circular smooth muscle fibers on the outside. The muscular and mucosal layers are encased in the adventitia, a thin connective tissue layer.

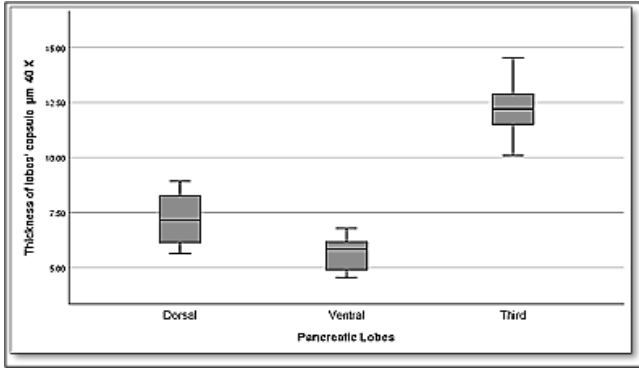


Figure 2: Diagram showing the thickness of capsule in three pancreatic lobes of native ducks.

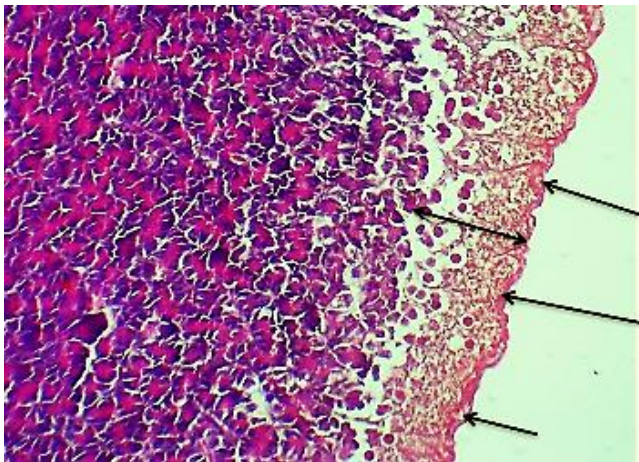


Figure 3: Microphotograph illustrates the parenchyma of the third lobe of the pancreas covered by a thin layer of connective tissue capsule (double head arrow) with mesothelial cells (black arrows). H&E, X40.

Table 1: Average of diameters of secretory units and number of cells that line the secretory units of the pancreas in native ducks

Lobes	Diameter of secretory units (μm)	Number of cells that line the secretory units
Dorsal	** 21.70±2.40	9-11
Ventral	** 24.05±1.19	10-12
Third	** 27.92±2.39	8-12

**Indicates a significant difference between the three lobes at P<0.01.

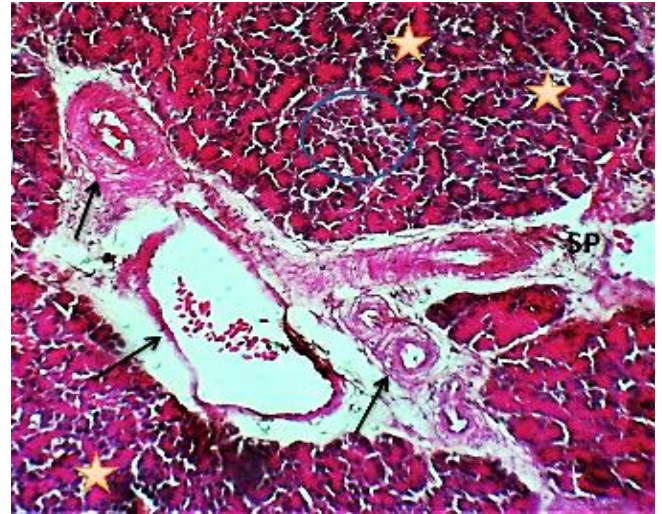


Figure 4: Histological section shows the parenchyma of the third lobe of the pancreas with blood vessels (black arrows) within the connective tissue around it (septa) (sp) among pancreatic acini (orange stars). Note the endocrine portion (blue circle). H&E, X40.

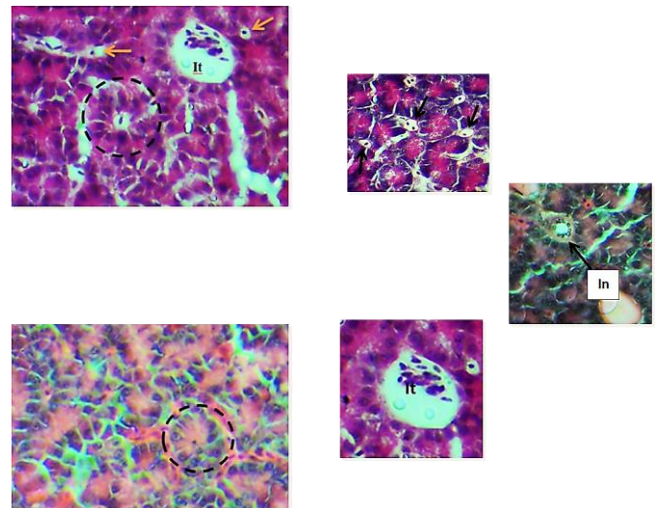


Figure 5: Histological images show the parenchyma of the dorsal lobe of the pancreas with the pancreatic acini (dotted circles) and solitary endocrine cells (orange arrows), the Centro acinar cells (black arrows) in the center of acini, intercalated duct (In), interlobular duct (It). H&E, X100.

The endocrine portion of the duck pancreas had two types of Langerhans islets of various sizes and shapes (Table 2), scattered in small groups of cells with almost no clear boundaries between the islets of Langerhans and the exocrine portion (Figure 7). Some of these islets were only a few cells gathered and embedded between the acini; Alpha

islets predominate in the dorsal lobe; they are made up of Alpha and Delta cells, and Beta islets predominate in the ventral lobe; they are made up of Beta and Delta cells. The presence of numerous endocrine islets of both types, Alpha islets, and Beta islets, distinguishes the third lobe (Figure 8).

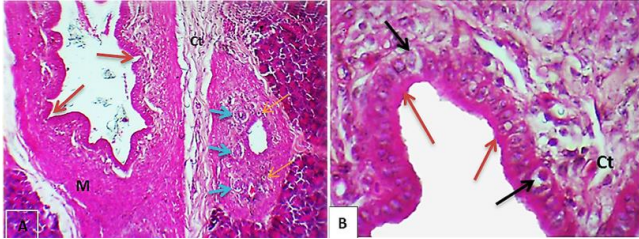


Figure 6: Microphotography illustrates the main pancreatic duct (A). Note the longitudinal folds in its mucosa lined by simple columnar epithelium (red arrows) and solitary endocrine cells (orange arrows) with goblet cells (black arrows) (B), the thick muscular wall of the duct (M), the surrounding connective tissue (Ct), and the beginning of the interlobular duct (It) (blue arrows). H&E, (A) X40, (B) X100.

Table 2: Average Diameter of Langerhans islets with Alpha and Beta cells diameters of the pancreas in native ducks

Lobes	Langerhans islets (µm)	Alpha cells (µm)	Beta cells (µm)
Dorsal	** 55.55±11.12	* 6.61±0.59	* 4.19±0.32
Ventral	**74.75±5.03	* 5.00±0.33	* 3.09±0.11
Third	** 86.47±6.02	* 4.78±0.64	* 3.27±0.23

*Indicates a significant difference between the three lobes at P<0.05. **Indicates a significant difference between the three lobes at P<0.01.

The study shows that the Langerhans islets include large alpha cells that have a pale nucleus, usually at the peripheral position, and small beta cells that with a dark and large central nucleus arranged to form cords or irregular rows of polygonal cells and have round nuclei. Beta cells are smaller than alpha cells with basophilic cytoplasm. Delta cells are numerous irregular cells with large spherical nuclei scattered between the peripheral alpha and central beta cells (Figures 8 and 9).

Male duck pancreas ganglia are visible in all lobes of the pancreas. The ganglionic cells (solitary endocrine cells) are large, with rounded nuclei and small nucleoli (Figures 5 and 6). Their cytoplasm shows prominent Nissl granules. The ganglion cells are surrounded by many small, darkly stained glial cells. Some are discovered in the interlobular connective tissue septa, which are surrounded by numerous blood vessels, the latter of which vary in size with different

lobules (Figure 4) (Table 3). Staining with Masson's trichrome, the surrounding stromal connective tissue is stained green, specifically the regions around blood vessels and running ducts (Figure 10).

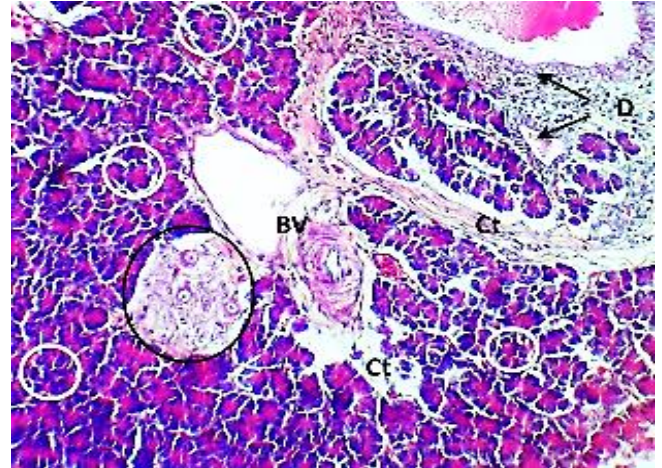


Figure 7: Histological picture shows the parenchyma of the dorsal lobe of the pancreas with the endocrine (black circle) and exocrine portions of the pancreas (white circles), blood vessels (BV), connective tissue (Ct) between lobules and ducts (D). H&E, X40.

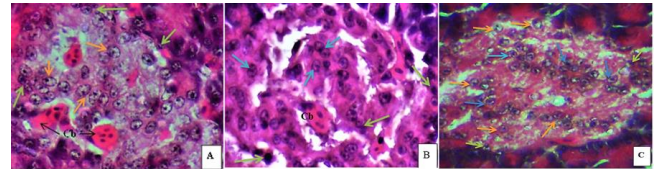


Figure 8: Microphotography illustrates the island of Langerhans (A-dorsal lobe) (B-ventral lobe) (C-third lobe) containing (orange arrows) Alpha cells, (Blue arrows) Beta cells and (green arrows) Delta cells, capillary blood vessels (Cb). H&E, X400.

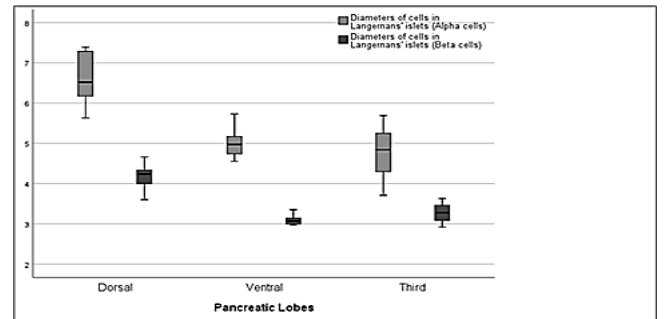


Figure 9: The diagram shows the mean diameter of Alpha and Beta-cell in Langerhans islets in three lobes of the pancreas in native ducks.

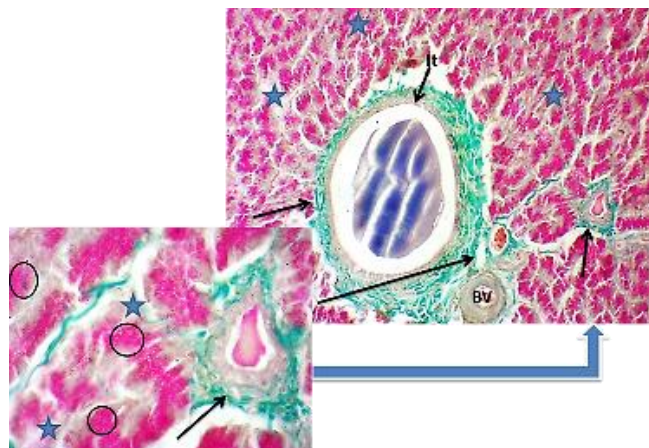


Figure 10: Tissue section of ventral lobe in the pancreas of adult ducks. It shows dense connective tissue stained green (black arrows) around the blood vessels (BV), the connective tissue around the interlobular duct (It), connective tissue fibers intervening between acini (blue stars), as seen in the enlarged side image zymogen granules in pancreatic acini (black circles). Masson's Trichrome stain, X100, X400.

Table 3: Measurements of the mean diameter of the artery and vein of the pancreas in native ducks

Lobes	Diameters of arteries micrometer unit (μm)	Diameter of veins micrometer unit (μm)
Dorsal	*** 50.002 \pm 4.272	** 130.108 \pm 7.793
Ventral	*** 81.518 \pm 15.474	** 176.388 \pm 68.016
Third	** 100.906 \pm 4.332	** 157.800 \pm 16.020

** Indicates a significant difference between the three lobes at $P < 0.01$. *** Indicates a significant difference between the three lobes at $P < 0.001$.

Histochemical results reveal weak reactions of periodic acid–Schiff (PAS) staining with the secretory unit indicating the presence of a little number of neutral mucopolysaccharides. The secretory unit, on the other hand, has a strong reaction with AB (pH 2.5) indicating the presence of a large number of acidic mucopolysaccharides (mucin) in the secretory unit. Histochemical results of the duct show the negative reaction of periodic acid–Schiff (PAS) indicates the absence of neutral mucopolysaccharides while the ducks show a strong reaction with AB (pH 2.5) indicating the presence of a large number of acidic mucopolysaccharides in all ducks.

Histochemical results of the island of Langerhans show the negative reaction of Alfa cells with PAS stain and moderate reaction with AB (pH 2.5) (nucleus only) indicating the absence of neutral mucopolysaccharides and a moderate amount of acidic mucopolysaccharides in Alfa cell only while the Histochemical reaction of Beta-cell were

negative with PAS stain and strong reaction with AB (pH 2.5) stain indicated the presence of a large number of acidic mucopolysaccharides in Beta cells and absence of neutral mucopolysaccharides in Beta-cell as shown in (Figure 11). As for the use of Toluidine blue stain, we note that it gave a negative result with the islets of Langerhans in its different cells, except for the Beta cells which had a weak reaction with toluidine blue (light pink) indicating that the presence of the little amount of glycosaminoglycan's while the secretory units gave a negative result with this stain as shown in (Figure 12).

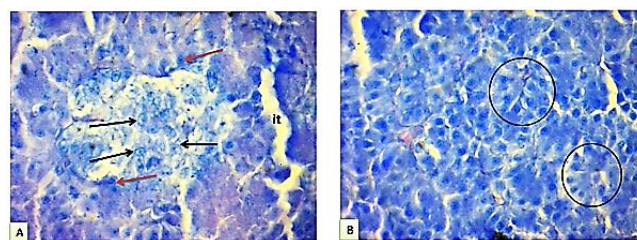


Figure 11: Microphotography illustrates the ventral lobe, (A) island of Langerhans, (black arrows) Beta cells and (brown arrows) Delta cells and the intralobular duct (It), (B) shows secretory units (black circles). PAS-AB (pH 2.5), A X400, B X100.

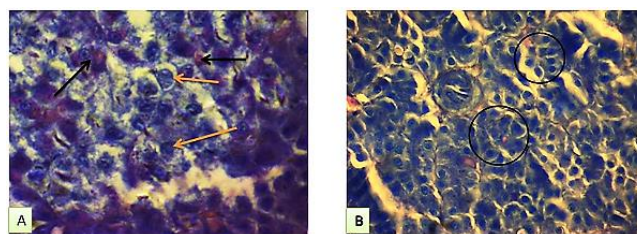


Figure 12: Microphotography illustrates the dorsal lobe, (A) island of Langerhans, (orange arrows) Alfa cells and (black arrows) Delta cells, (B) shows secretory units (black circles). Toluidine blue, A X400, B X100.

Discussion

The pancreas of the local duck is found to have a lobulated organ with ribbon-like lobes. The pancreas is placed in the area between the duodenal descending and ascending limbs and is completely encompassed by the pancreatic duodenal ligament. It is lined by mesothelium and is covered by a thin layer of loose connective tissue capsule. The test results are consistent with the previous findings on the pancreas of common quail and red jungle fowl (4,8) but are inconsistent with the previous findings on the glandular derivatives of captive bustards' (17) and golden eagle's (*Aquila Chrysaetos*) pancreas (18).

The connective tissue Septa runs from the capsule to the parenchyma, splitting it into three major lobes. The dorsal, ventral, and third lobes are found to be comparable to those described in the adult Kestrel pancreas study (19). This goes in contrast to the results of (6). According to them, describing the pancreases in early hatched geese (*Anser anser*) as having four lobes means that each of these pancreatic lobes has its intercalated duct, intralobular duct, interlobular duct, and main duct that extends from the pancreas to a point in the duodenum where it empties its contents.

The present work establishes the ductal system of the indigenous duck pancreas, beginning with centroacinar cells at the center of pancreatic secretory acini and progressing through the intercalated duct to the intralobular duct. Both ducts are located within the pancreatic lobes and were lined with simple cuboidal epithelium, whereas the interlobular ducts were located between the pancreatic lobes and were lined with simple columnar epithelium. The interlobular ducts are joined to form the main pancreatic duct, which is lined with columnar epithelium containing goblet cells. These findings are consistent with those of (20), who demonstrates that the gross anatomy of the pancreatic lobes and ducts in six breeds of domestic ducks and six species of wild ducks in China are distinct from those of (21) in pigeon pancreas, where the intercalated ducts are lined with a simple flattened epithelium. Moreover, the intralobular ducts are lined with a tall columnar epithelium. The intralobular and interlobular ducts are made up of two distinctive tissues: a columnar epithelium with goblet cells that line the ducts and is bordered by connective tissue; and simple columnar epithelium tissue that lines the main duct lines. (22) found that all portions of the pancreas's duct system lack goblet cells in their lining epithelium at all analyzed ages.

The histological examination revealed that the pancreas of the local duck is divided into two segments: exocrine and endocrine segments that are positioned within the meshwork of reticular fibers. These findings confirm with (23) findings in the ostrich pancreatic (*Struthio camellias*) and (24) findings in the duck pancreas (*Anas boscas*).

The exocrine glands of the local duck are organized in the form of tubuloacinar glands with cells ranging in size and shape from tall columnar to polygonal and with diameters varying across all lobules. This acinus covers a significant area of the pancreas and has centroacinar cells identified as one or two nuclei in the pancreatic acini's center. The exocrine portion's ducts vary in size and are lined with simple epithelium. These ducts, particularly the larger ones, are surrounded by a circular muscle layer. These findings validate with (23) in ostrich pancreatic and (21) findings in pigeon pancreas.

The endocrine portion of the local duck pancreas contains two Langerhans islets of varying sizes and shapes, scattered as small clusters of cells surrounded by reticular fibers and a

network of sinusoid-like blood capillaries lined by endothelial cells; Alpha islets predominate in the dorsal lobe. Beta islets, on the other hand, predominate the ventral lobe. They are composed of Beta and Delta cells. The third lobe is characterized by the presence of a large number of both Alpha and Beta endocrine islets. These findings corroborate with those of (6) for the pancreatic islets of the Houbara bustard (*Chlamydotis undulate*) and (25) for the pancreas of the porcupine (*Hystri cristata*), in which they demonstrate the presence of Alpha, Beta, and Delta cells in the pancreatic islets' center.

Delta cells are also detected in the pancreas of Mynah (*Acridotheres tristis*) in both Alpha and Beta islets (26). These findings in local ducks are consistent with those obtained from prior investigations. Researcher (27) reports that the Langerhans islets in the Abu-Gura pancreas are of the Alpha, Beta, and mixed types. On the contrary, while (28) describes the pancreas of geese as having three Langerhans islets and the secretory acini in the exocrine parts as being composed of a variable number of pyramidal cells with basophilic zymogene granules, while the intralobular ducts are lined.

Blood vessels vary in size between lobules, and ganglionic nerve cells are present in the perivascular connective tissue surrounding major blood vessels. They are identified by their oval pale nuclei with a prominent nucleolus. These findings are consistent with those of (19) in adult Kestrel pancreatic and (29) in rat pancreas. According to those scientists, ganglia are identified as a clump of cells located between the parenchymal tissue acini and the interlobular connective tissue or as a massive ganglion next to the pancreatic lobule.

The histochemical staining with periodic acid–Schiff (PAS) staining demonstrates mild responses with the secretory unit in the native duck (*Anas Platyrhynchos*), indicating the presence of a limited amount of neutral mucopolysaccharides and negative reactions in ducts. While the secretory units and ducts exhibit robust reactivity to AB (pH 2.5), these reactions imply the presence of a high concentration of acidic mucopolysaccharides (mucin) within the secretory unit. The histochemical analysis of the Langerhans Island reveals that Alfa and Beta cells have a negative reaction to PAS stain and a moderate to high sensitivity to AB (pH 2.5), respectively. According to several researchers, histochemical data demonstrate that the periodic acid–Schiff (P.A.S.) stain reacts moderately with the apical surfaces of the intercalated and interlobular duct cells, but strongly with the cytoplasm of major pancreatic duct cells and their secretions (30).

Accordingly, the alpha islets are primarily composed of moderately stained alpha cells and several weakly stained beta cells. The beta islets mildly stained with TB dye is densely packed with beta cells and sparsely packed with alpha and delta cells (1). The use of the toluidine blue stain

in this study demonstrates that Langerhans islets are negative in all cells to save the Beta cells, which exhibit a weak reactivity with toluidine blue (light pink), while the secretory units are negative.

Conclusion

This work aims to provide a histomorphological and histochemical characterization of the pancreas in native ducks, with an emphasis on the histological anatomy of pancreatic acini and Langerhans islets. Five mature male native ducks from the species are used in this study (*Anas Platyrhynchos*). According to the current study results, the pancreas is a small, semi-wide structure with a pale to white pinkish color located between the duodenal descending and ascending limbs. Additionally, blood vessels vary in size among lobules. Ganglionic nerve cells can also be detected in the connective tissue around major blood arteries. These findings corroborate the previous observations in the pancreas of rats. Finally, this study sheds light on the morphological, histological, and histochemical characteristics of the indigenous duck pancreas. However, future research should focus on the pancreas of other bird species as well.

Conflict of interest

The authors declare no competing interests

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دراسة نسيجية قياسية ونسجية كيميائية لكرهيدرات البنكرياس في البط المحلي

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الخلاصة

يتم في الدراسة الحالية فحص الخصائص النسيجية الكيميائية والشكلية القياسية لمتن البنكرياس في البط المحلي مع التركيز على العينات البنكرياسية وجزر لانكرهانز. تم الحصول على خمس عينات بنكرياس لذكر البط من السوق المحلية وخضعت جميع العينات للطرق الروتينية في التحضيرات النسيجية لغرض إجراء التحليل النسيجي والنسجي الكيميائي. وتم استخدام صبغة الهيماتوكسلين - ايسين وملون الماسون ثلاثي الصبغ والتوليد الأزرق وملون حامض البريوديك - شيف مع الاليشين الأزرق ٢,٥. يظهر الفحص الشكلي أن البنكرياس في البط يتكون من ثلاثة فصوص كبيرة وهي الظهري والبطني والثالث. وعلى ما يبدو فإن البنكرياس يظهر أيضا بهيئة تركيب صغير شبه واسع وبلون أبيض-وردي الى وردي-شاحب يقع بين فرعي العفج. تبين النتائج أن البنكرياس يكون مغلف بطبقة رقيقة من النسيج الضام الرخو المحاط بالظهارة المتوسطة وتمتد من المحفظة حويصلات نحو متن العضو لتقسمه الى عدة فصيصات. وتكشف الدراسة أن فصيصات البنكرياس مؤلفة من مساحة كبيرة من عينات الإفراز الخارجي مع كمية قليلة من جزر لانكرهانز وان وحدات الإفراز الخارجي تتمثل بعنات تراوحت بين دائرية الى بيضوية تحوي خلاياها على حبيبات الزيموجين، في حين تتمثل وحدات الإفراز الداخلي بثلاثة أنواع من جزيرات لانكرهانز غير واضحة الحدود، إذ تكونت جزر الفا من خلايا الفا مع القليل من خلايا ديلتا. وتتألف جزر بيتا من خلايا بيتا المحيطة بالتموضع والقليل من خلايا ديلتا، في حين تتألف الجزر المختلطة من خلايا صمية من النوعين الفا وبيتا وكذلك ديلتا. أخيراً، تلقي هذه الدراسة الضوء على الخصائص المورفولوجية والنسجية والكيميائية النسيجية لبنكرياس البط المحلي. ومع ذلك يجب أن تركز الأبحاث المستقبلية على بنكرياس أنواع أخرى من الطيور أيضاً.