



Histological and Immunohistochemical Characterization of the Pancreas in Indian Roller (*Coracias benghalensis*)

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ABSTRACT

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The Indian Roller (*Coracias benghalensis*) is a widely distributed avian species in the Southeast Asia region, known for its ecological importance and striking plumage. Despite its prevalence, limited information exists regarding the anatomical and functional aspects of its internal organs, particularly the pancreas. It aims to understand the structural organization and cellular composition of the pancreatic tissue, contributing to the field of comparative anatomy and avian physiology. The study involved 15 adult male live birds aged over one year. The birds were anesthetized with chloroform, excised, and had their pancreatic gland excised. The study found that alpha islets were present in the dorsal, ventral, and splenic lobes, while beta islets were present in the ventral and splenic lobes. Mixed islets were present in all lobes, and the percentage of alpha, beta, and mixed islets across all pancreatic lobes was also high. Histological slides were prepared, stained, and immunohistochemistry was conducted using primary and secondary antibodies to glucagon and insulin hormones. The pancreas is a mixed gland with an exocrine and endocrine part, surrounded by a thin capsule. It has trabeculae that extend from the capsular tissue to the interior. The exocrine pancreas consists of acini and the ductal system, while the endocrine part consists of islets of Langerhans. Immunohistochemistry results indicate three types of pancreases: dark islands containing alpha and delta cells, light islands containing beta cells, and mixed islands containing alpha, beta, and delta cells.

1. INTRODUCTION

The study of the digestive system in birds and other animal groups is of great importance due to the various adaptations and the morphological and histological differences observed in different parts of the system. The feeding mechanism also plays a crucial role in determining the success of birds' adaptation to their environment. The type of food and the way it is obtained directly affect the formation of these differences, which provide the body with the nutrition necessary to carry out daily activities [1-3].

Many researchers have studied the gut in various vertebrates, including birds, from different morphological and histological aspects, as well as studies that focused on functional aspects [4-6].

The pancreas in vertebrates is one of the major glands of the digestive system. It is a flat and elongated organ. Among digestive organs, the pancreas plays a dual role, including the regulation of blood glucose levels. The pancreas is a mixed gland composed of two parts: the exocrine pancreas and the endocrine portion [7, 8]. The exocrine pancreas, the largest portion of the gland, secretes digestive enzymes that flow through the pancreatic ducts into the duodenum, and its activity is regulated by neural and hormonal signals [9, 10]. The endocrine part is represented by the islets of Langerhans,

which are responsible for the secretion of hormones, including glucagon and insulin, that help regulate blood glucose levels [11, 12].

Some studies related to immunohistochemical studies have addressed the determination of the distribution of endocrine cells that produce the hormones glucagon, insulin, and somatostatin in the bird pancreas. This technique revealed the presence of three types of islets in the endocrine region: dark, light, and mixed islets, each characterized by immunoreactive cells for glucagon, insulin, and somatostatin [13-15].

The Indian Roller (*Coracias benghalensis*) belongs to the class Aves, order Coraciiformes, family Coraciidae, and genus *Coracias*. The Indian Roller is characterized by a large head and a full body, with its head appearing inconspicuous at rest, but its bright blue colors stand out clearly in flight [16].

Its main habitat is agricultural environments, light forests, and pastures. This bird catches insects and frogs and dives into the water to search for fish, as insects make up the majority of its diet, which it consumes throughout the year, thus acting as one of the active biological control agents against agricultural insect pests [17, 18].

Coracias benghalensis is a widely distributed avian and is known for its ecological importance and striking plumage. Despite its prevalence, limited information exists regarding the anatomical and functional aspects of its internal organs,

particularly the pancreas, a vital organ involved in both endocrine and exocrine functions. Therefore, the aim of this study was to determine the morphological, histological, and histochemical characteristics of the pancreas, as well as to describe its morphological dimensions in a migratory bird species inhabiting the Iraqi environment, represented by the Indian Roller (*Coracias benghalensis*).

2. MATERIALS AND METHODS

This study was carried out on 15 male birds of the Indian Roller aged than one year, weighing 170±2.32 gm. The samples were collected in the area of Al-Yusufiyah, Najaf Al-Ashraf and Al-Qazel market in Baghdad during the period from September 2024 to the end of December 2024, this bird was classified in the Natural History Museum and the scientific name was proven, the studied animals were anesthetized with chloroform, then the pancreases were dissected and removed from them, after that the samples were fixed with fixative solutions (10% neutral formalin and Bouin's solution for 24-48 hours) and then the sequential steps were performed in the preparation of tissue sections. The tissue sections were then stained with routine and special stains (hematoxylin-eosin, periodic acid-Schiff (PAS), Masson trichrome stain (MT), and Mallory Heidenhain-Azan stain according to Luna [19] and Suvarna et al. [20]. The histological sections were examined and photographed using a compound light microscope equipped with a digital camera and various magnification levels that met the requirements of the current study.

The immunohistochemistry technique of the hormones glucagon and insulin was performed on formalin-fixed tissues embedded in paraffin wax. After removing paraffin wax with xylene (three times for 4 minutes each time) and rehydrating the tissues with ethanol (twice in 100% ethanol, twice in 95% ethanol, once in 70% ethanol, for 5 minutes each time). The sections were placed in a phosphate buffer solution (PBS) pH 7 inside a glass jar, after which it was placed in a water bath with a temperature of 97°C for 30 minutes. Then the sections were washed with PBS solution. H₂O₂ 3% was added after washing with PBS solution for one minute, and the first antibody was added and incubated for 20 minutes at room temperature. Rabbit insulin (product number E-AB-67409) and rabbit glucagon (product number E-AB-13230) (both supplemented by Elabscience company, USA) were used separately. Diluted in a ratio of 1:50 in PBS and applied overnight at 4°C, after washing with PBS solution, the paired enzyme horseradish peroxidase was added and incubated for 20 minutes at room temperature.

The sections were washed 4 times with the daring solution,

30 µL of DAB (3,3'-Diaminobenzidine) chromogen was added to 1 ml of substrate DAB and mixed, and added to the sections, incubated for 10 minutes, washed with PBS solution 4 times, and analyzed using a light microscope. Image J software has been used to quantify of coloration brown in images by using 5 replicates for each sample.

IBM SPSS version 25.0 was used to do the statistical analysis, and the data were tabulated in a datasheet. Continuous variables' means and standard errors were given, and the analysis of variance (ANOVA) and least significant difference (LSD) tests were used to determine whether there were any significant differences. The definition of statistical significance was a probability value (p≤ 0.05) [21].

3. RESULTS

3.1 Features of pancreatic islands

At a probability level of p≤0.05, the current study's findings on the percentage measurements of alpha, beta, and mixed islets in the Indian Roller bird revealed significant differences. The percentage of alpha islets in the dorsal, ventral, and splenic lobes was 5.92±44.01%, 3.86±14.79%, and 4.78±34.11%, respectively. In contrast, the percentage of beta islets was 2.34±13.31% in the dorsal lobe, 0.79±5.71% in the ventral lobe, and 1.97±8.24% in the splenic lobe. Meanwhile, the percentage of mixed islets in the dorsal, ventral, and splenic lobes was 12.47±41.96%, 3.00±23.17%, and 6.31±27.28%, respectively. The percentage of alpha, beta, and mixed islets across all pancreatic lobes ranged from 5.88±51.54%, 2.69±13.09%, and 5.51±27.93%, respectively (Figure 1 and Table 1).

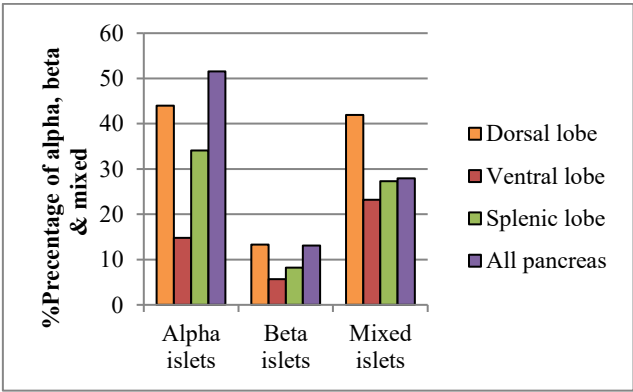


Figure 1. The rate of diameters and the percentage of islands of alpha, beta, and mixed for each of the three types of the Indian Roller bird, *Coracias benghalensis*

Table 1. The rate of diameters and the percentage of islands of alpha, beta, and mixed for each of the three types of the Indian Roller bird, *Coracias benghalensis*

Studied Features	Dorsal Lobe	Ventral Lobe	Splenic Lobe	All Pancreas	Diameter of Pancreatic Islets	P value
Type of Langerhans	S.E±M %	S.E±M %	S.E±M %	S.E±M %	S.E±M µm	
Alpha islets	5.92±44.01 ^a	3.86±14.79 ^a	4.78±34.11 ^a	5.88±51.54 ^a	167.85±425.86 ^a	0.016 [*]
Beta islets	2.34±13.31 ^b	0.79±5.71 ^b	1.97±8.24 ^b	2.69±13.09 ^b	0.89±10.74 ^b	0.05 [*]
Mixed islets	12.47±41.96 ^a	3.00±23.17 ^c	6.31±27.28 ^a	5.51±27.93 ^c	69.89±156.90 ^c	0.05 [*]
P value [#]	0.038 [*]	0.01 [*]	0.018 [*]	0.01 [*]	0.046 [*]	0.00 [*]

The values represent the mean and standard error (S.E±M), the data represent (15 replicates), different letters indicate the presence of significant differences at a probability level of p≤0.05, and similar letters indicate the absence of significant differences.

3.2 Histological and histochemical study

The results of the current study showed that the pancreas of the Indian Roller bird is a compound tubuloacinar gland. The pancreas is surrounded externally by a capsule consisting of thin connective tissue composed of collagen fibers and a small number of elastic fibers, and reticular fibers, and extends from the trabeculae of the capsular tissue to the interior of the pancreatic tissue, which is composed of connective tissue.

The capsule was stained pink when treated with the H&E stain, blue when treated with the Masson trichrome (MT) stain, and red when treated with the Mallory-Heidenhain-Azan stain due to the presence of connective tissue fibers (Figures 2(A, B, C)). The pancreatic was a mixed gland consisting of two portions: the exocrine pancreas and the endocrine part, the exocrine pancreas is represented by the secretory units and ductal system, the secretory units constitute the major part of the glandular tissue surface and are represented by the acini and each acinus represents a serous secretory unit composed of a series of pyramidal cells into a long columnar shape based on a thin basement membrane, as the tip of the narrow cells head towards a small cavity, which is the cavity of the acinus.

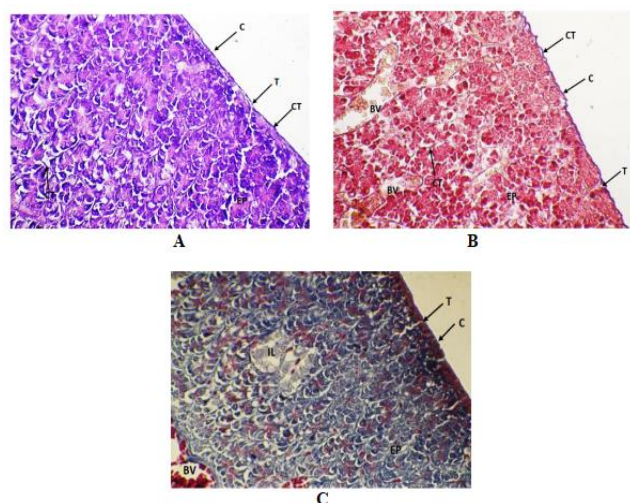


Figure 2. Cross section in the dorsal lobe of the Indian Roller pancreas showing: Capsular (C), Trabeculae (T), Exocrine pancreas (EP), Connective tissue (CT), Blood vessels (BV), Islets of Langerhans (IL), A-H&E stain, B-MT stain, C- Mallory Heidenhain-Azan stain (A, B, C, 40x)

Each cell has a single round basal nucleus with a clear dark nuclear color, and the cytoplasm surrounding the nuclei is characterized by a denser and darker color, while the apical cytoplasm appears less dense and is characterized by containing zymogen granules, and the number of cells in each serous acinus is between 6-8 cells. The acini are stained dark purple, and the zymogen granules appear pink when the tissue sections are stained with routine H&E stains, purple when stained with PAS, and red when stained with Mallory Heidenhain's azan (Figures 3(A, B, C)). The acini varied in size and shape, including large and small, round and oval, and elongated acini that occurred in the ventral and dorsal lobes, while the majority of acini were found irregularly in the splenic lobe.

The results of the current study of the Indian Roller pancreas show that the ductal system through which the products of the secretory units enter the duodenum begins with a group of cells called centroacinar cells that fill the acinar cavity. These

cells have an oval shape with central round nuclei and are characterized by clear nuclei (Figure 4).

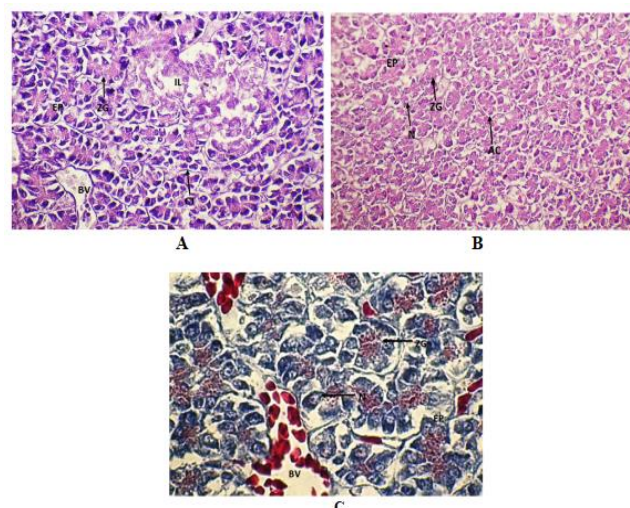


Figure 3. Cross section in the dorsal lobe of the Indian Roller pancreas showing: Exocrine pancreas (EP), Connective tissue (CT), Zymogen granules (ZG), Blood vessels (BV), Endocrine part of Langerhans islets (IL), Acinus (AC), Cell nuclei (N), A-H&E stain, B-PAS stain, C- Mallory Heidenhain-Azan stain, (A, B, 40x and C, 100x)

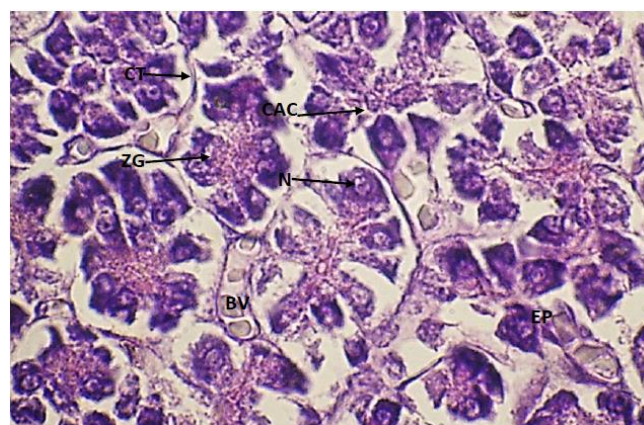


Figure 4. Cross section in the dorsal lobe of the Indian Roller pancreas showing: Exocrine pancreas (EP), Connective tissue (CT), Zymogen granules (ZG), Blood vessels (BV), Cell nuclei (N), Centroacinar cells (CAC) (H&E stain, 100x)

The centroacinar cells are connected to the intercalated duct, which is lined with a single row of simple squamous epithelium (Figure 5). The intercalated duct is connected to another duct that is larger in diameter than itself and is called the intralobular duct. It is lined with simple cuboidal epithelial tissue whose cells have round nuclei that are located near the base of the cell and lie between the units of the external excretory portion (Figure 6).

The intralobular duct is connected to the interlobular duct, which has a larger diameter than the intralobular duct and is lined with simple cuboidal epithelial tissue a low columnar epithelial tissue that has cells with round nuclei near the cell base. This duct is located within the connective tissue trabeculae that extend from the capsule surrounding the pancreas and contains sections or branches of blood vessels represented by arteries and small and medium-sized veins (Figures 7 and 8).

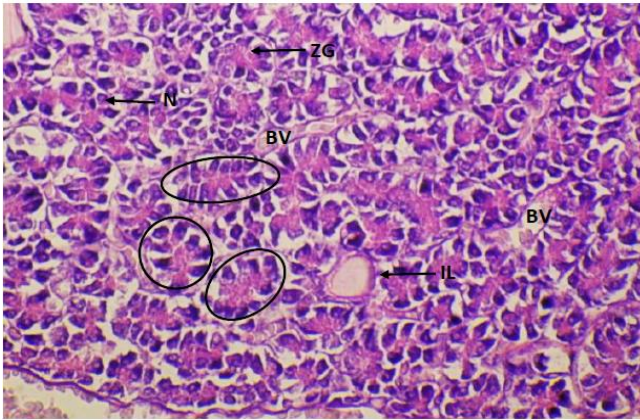


Figure 5. Cross section in the dorsal lobe of the Indian Roller pancreas showing: Exocrine pancreas (EP), the shape of the round, oval, elongated acinus, Zymogen granules (ZG), Blood vessels (BV), Cell nuclei (N), intercalated duct (IL) (H&E stain, 40x)

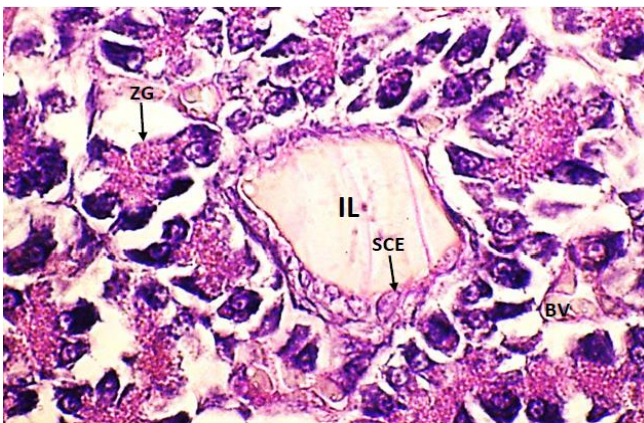


Figure 6. Cross section in the ventral lobe of the Indian Roller pancreas showing the exocrine pancreas, Zymogen granules (ZG), Blood vessels (BV), Simple cuboidal epithelial tissue (SCE), Intralobular duct (IL) (H&E stain, 100x)



Figure 7. Cross section in the ventral lobe of the Indian Roller pancreas showing the exocrine pancreas, Blood vessels (BV), Simple cuboidal epithelial tissue (SCE), Interlobular duct (INL), Connective tissue (CT) (H&E stain, 40x)

The interlobular ducts empty their secretory contents into the main duct, which in turn empties its contents into the

duodenum. It is characterized by the size of its cavity and is lined with pseudostratified columnar epithelium, followed by a thick layer consisting of several layers of connective tissue interspersed with smooth muscle fibers, referred to as the muscular layer, followed by a thin layer of connective tissue referred to as the tunica adventitia (Figures 9(A, B)).

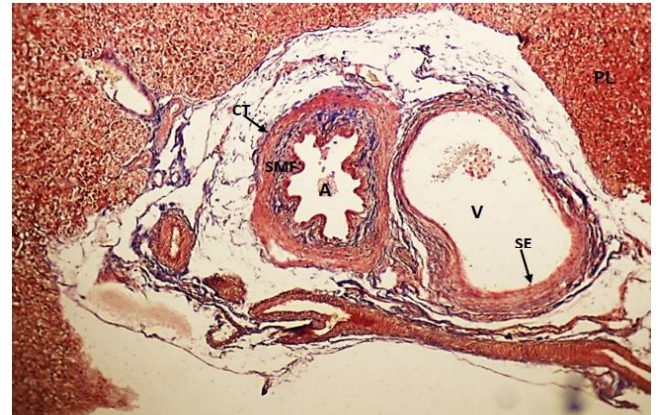


Figure 8. Cross section in the ventral lobe of the Indian Roller pancreas passing through the arteries and veins of the pancreas showing: Arterioles (A), Venues (BV), squamous epithelial tissue (SE), Smooth muscle fiber (SMF), Connective tissue (CT) (MT stain, 10x)

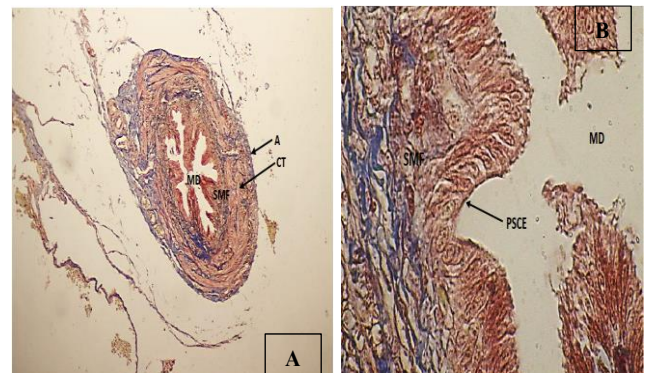


Figure 9. Cross section in the ventral lobe of the Indian Roller pancreas showing: Main duct (MD), Venues (BV), Smooth muscle fiber (SMF), Connective tissue (CT), Adventitia (A), Pseudostratified columnar epithelium (PSCE), (A, 10x, B, 40x, MT stain)

The endocrine part is a cluster that is scattered within the units of exocrine and is not separated from the units of external secretion by any connective tissue. They take the form of irregular strands of cells separated by a network of capillaries. The islets vary in size and shape, including small, medium, and giant islets. Giant islets have been observed in the dorsal lobe and splenic lobe (Figure 10), and the islets can have different shapes in three types of pancreatic lobes, including oval or round or extended, or irregular shapes (Figure 11).

The islets contain cells of different shapes and occupy different positions on the island. It was found that there are three types of cells within the islets of the pancreas, represented by spindle-shaped cells with small nuclei and oval central location near the periphery of the islet known as alpha cells, that secrete the hormone glucagon, and oval cells with small spherical nuclei and central location in the center of the islet known as beta cells, that secrete the hormone insulin, and the presence of irregularly shaped cells was observed. These

cells are located between the alpha and beta cells and are known as delta cells, which secrete the hormone somatostatin.

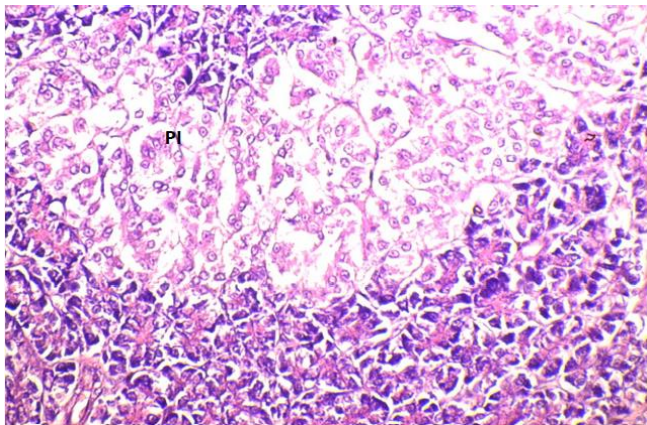


Figure 10. Cross section in the ventral lobe of the Indian Roller pancreas showing: Pancreatic islets (PSCE) (H&E stain, 40x)

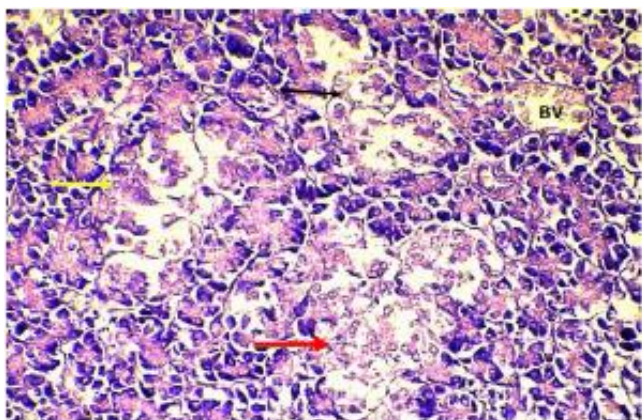


Figure 11. Cross section in the splenic lobe of the Indian Roller pancreas showing: Oval (black arrow), Round (yellow arrow), Irregular shape (red arrow) pancreatic islets, Blood vessels (BV) (H&E stain, 40x)

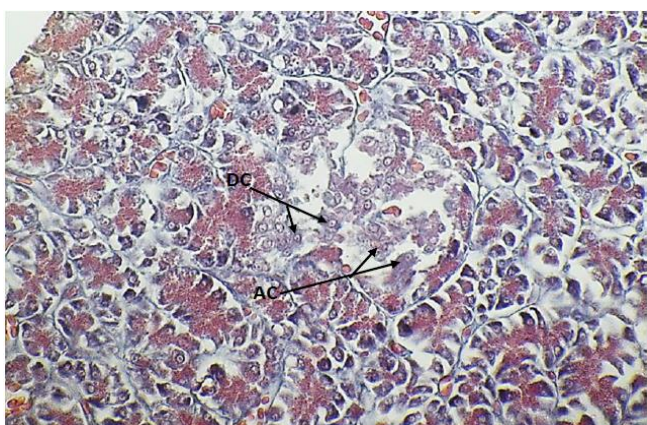


Figure 12. Cross section in the ventral lobe of the Indian Roller pancreas showing: Alpha islet (AC) contains delta cells (DC) (Mallory Heidenhain-Azan stain, 40x)

It was found that there is a variation in the density and distribution of these cell types between islets and this distribution determines the type of islets, so the study found that three types of islets are represented: Alpha islets, which

are characterized by containing a large number of alpha cells at the periphery and a few delta cells (Figure 12). Beta islets contain a large number of beta cells and a small number of delta cells (Figure 13). The third type of large islets, the so-called mixed islets, which contain alpha, beta, and delta cells, was also observed (Figure 14).

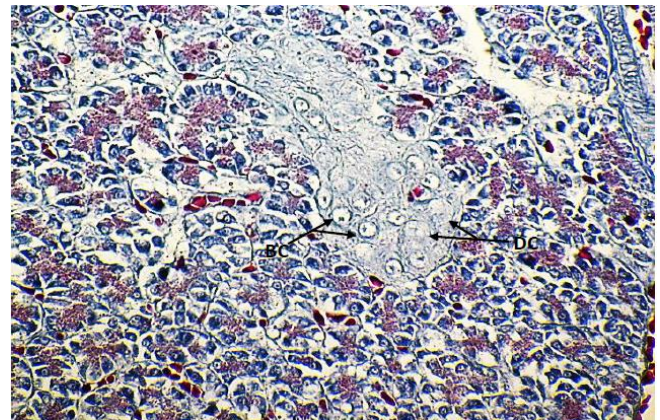


Figure 13. Cross section in the ventral lobe of the Indian Roller pancreas showing: Beta islet (BC) contains delta cells (DC) (Mallory Heidenhain-Azan stain, 40x)

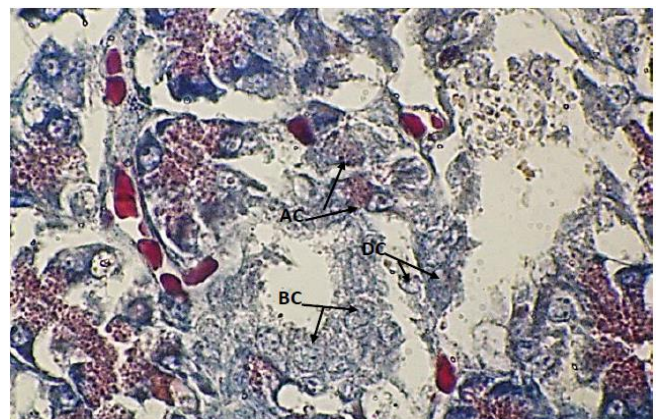


Figure 14. Cross section in the ventral lobe of the Indian Roller pancreas showing: Mixed islet contains alpha (AC), Beta (BC), and delta cells (DC) (Mallory Heidenhain-Azan stain, 100x)

3.3 Immunohistochemical study

Immunohistochemistry techniques in the current study showed that the islets of Langerhans in the pancreas of the Indian Roller bird, using antibodies to glucagon and insulin, and the study revealed a difference in the intensity of the immune reaction in the islets of the pancreas using antibodies to glucagon and insulin. Through the immunochemical reaction of glucagon and insulin, the results demonstrated the presence of three different types of islets in the Indian Roller bird's pancreas: mixed islets, beta islets (light), and alpha islets (dark). The three pancreatic lobes had alpha and mixed islets, but only the ventral lobe contained beta islets (Figure 15).

The results of the reaction of alpha (dark) islets with glucagon antibodies in the Indian Roller bird showed a strong immune response to the glucagon antibodies, alpha islets were observed in the three pancreatic lobes, where the immune cells of the islets were large and round, oval or irregular in shape with a dark brown color. Glucagon-immune cells are found in

large numbers throughout the islets, and a small number of alpha cells have been observed scattered in the exocrine pancreas, as shown in Figure 16.

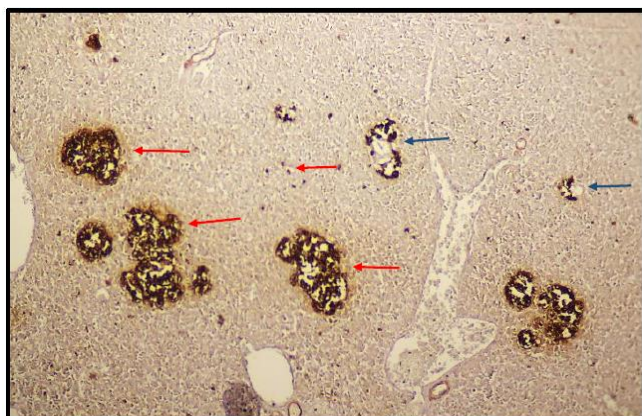


Figure 15. A cross-section in the dorsal lobe showing the pancreas of the Indian Roller bird by using antibodies immune reaction of glucagon, alpha (dark) islets (red arrow), mixed islets (blue arrow), 10x

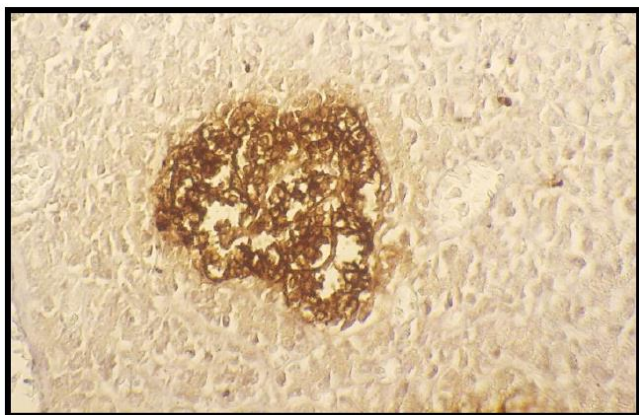


Figure 16. A cross-section in the dorsal lobe showing alpha islets in the Indian Roller bird by using antibodies immune reaction of glucagon, alpha islets (dark), 40x

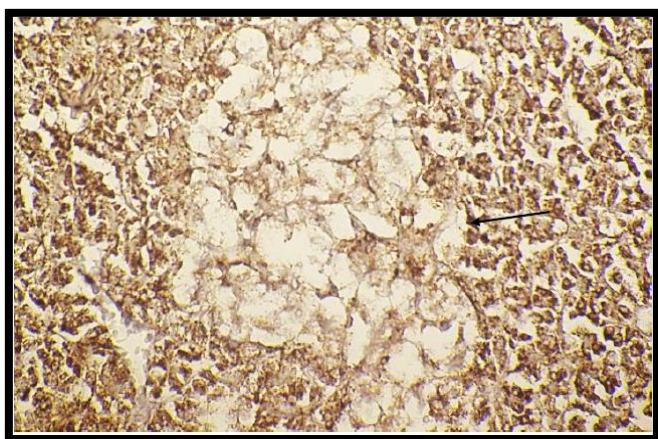


Figure 17. A cross-section in the splenic lobe showing alpha islets in the Indian Roller bird by using antibodies immune reaction of insulin, alpha islets (black arrow), 40x

The results of immunohistochemistry of the current study showed that when alpha islets were examined with an insulin

antibody, the cells showed no response when exposed to insulin antibodies, as shown in (Figure 17); because of alpha islets contain only alpha cells and not beta cells, which are immune cells that react to an insulin antibody.

The results of the current study showed that the beta islets (light) in the Indian Roller bird pancreas are small and round without any immune reaction with the glucagon antibodies and it was difficult to observe them in the pancreas and it was observed in the ventral lobe only. These islets contain only beta cells that do not show an immune reaction with glucagon as shown in (Figure 18).

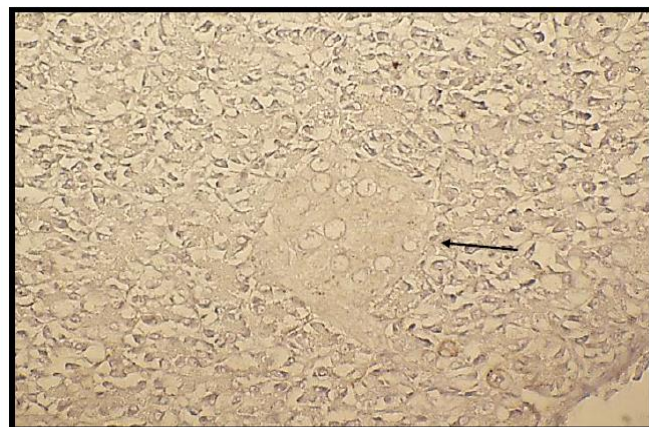


Figure 18. A cross-section in the ventral lobe showing beta islets (black arrow) in an Indian Roller by using antibodies immune reaction of glucagon, 40x

Beta islets showed a strong immune response with the insulin antibody when treated with it, and appeared mildly brown, as shown in Figure 19.

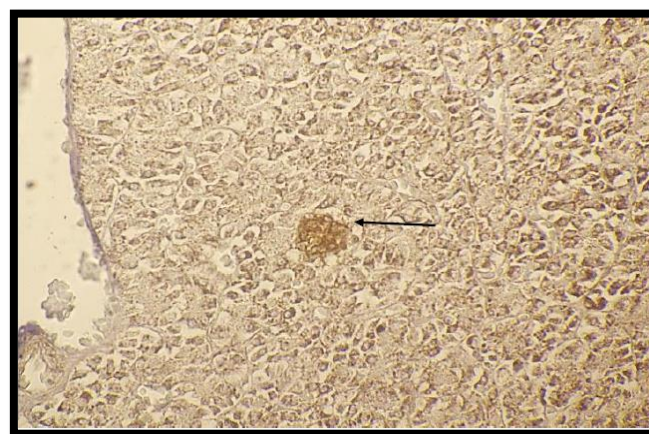


Figure 19. A cross-section in the ventral lobe showing beta islets (black arrow) in the Indian Roller bird by using antibodies immune reaction of insulin, 40x

The results of the current study showed the presence of an intensive immune reaction at the mixed islets in the Indian Roller bird when exposed to the glucagon antibody. These islands were present in the three pancreatic lobes in the form of round or irregularly shaped and colored brown in the peripheral part of the mixed islands when reacting with the glucagon antibody, where immune cells were localized in the peripheral area, as shown in Figure 20.

While the immune reaction with the insulin antibody in the

mixed islands showed the concentration of immune cells localized in the center of the islets and became mildly brown in color as a result of the use of the insulin antibody, as shown in Figure 21.



Figure 20. A cross-section in the dorsal lobe showing mixed islets (black arrow) in the Indian Roller bird by using antibodies immune reaction of glucagon, alpha cells (A), beta cells (B), 40x

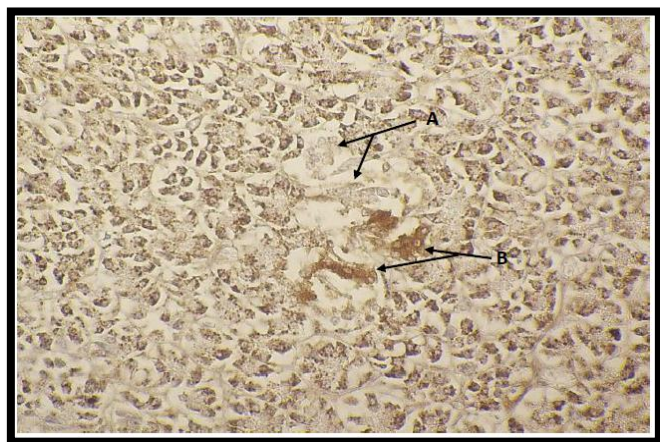


Figure 21. A cross-section in the ventral lobe showing mixed islets (black arrow) in the Indian Roller bird by using antibodies immune reaction of insulin, alpha cells (A), beta cells (B), 40x

4. DISCUSSION

The results show variation in the presence of cells in the pancreatic islets, where there is a high secretory activity of the hormone glucagon produced by alpha cells, without diminishing the importance of the role of insulin, which is secreted by beta cells. The density of cells producing glucagon and insulin varies among different bird species, reflecting the functional adaptations of the bird to its environment and metabolic needs [22].

The current study has shown that the pancreas of the Indian Roller bird consists of two main portions: the exocrine pancreas, represented by the acini and ductal system, and the endocrine part, represented by the islets of Langerhans or pancreatic islets. This finding is consistent with the results of some studies that have looked at the pancreas in various

vertebrate species, including birds, such as the Chabero chicken [23] and *C. livia* [24].

The results of the current study also show that the pancreas is surrounded by thin connective tissue. The structure of the capsule in the current study is similar to that of Yehia et al. [25] in their study on *Coturnix coturnix japonica* and *Bubulcus ibis*, as well as consistent with what Mahmood et al. [26] pointed out in their study on the pancreas of *Anas platyrhynchos* and the study by Khuluf [27] in *Pterocoles alchata*, while the structure of the capsule in the current study by Beheiry et al. [28] study, which found that the capsule of goose pancreas consists only of collagen fibers and there are no elastic and reticular fibers, which is not consistent with the results of the current study.

The exocrine pancreas in the Indian Roller is represented by a compound tubuloacinar gland composed of many serous acini with the ductal system, and the exocrine pancreas may occupy the largest area of the gland, and this is consistent with many studies related to the exocrine pancreas in different bird species, including the study by Mahmood et al. [26] in *Anas platyrhynchos* and the study by Bargooth and Jasim [29] in the domestic fowl (*Gallus gallus domesticus*) and the study by Naser et al. [30] on the black goose (*Melanopendix niger*).

Acini appear in the exocrine pancreas of the current study in various sizes and shapes, including small and large, spherical and oval, as well as elongated acini, and the lining of each acinar in a series of pyramidal cells to columnar long shape, and each cell contains a round nucleus base site on the basis of a thin basement membrane, and this is compatible with the results of Beheiry et al. [28] in goose and Abd and Faris [24] in *C. livia*, while the result of the current study differs from the results of Hussein and Bargooth [22] in Turkey (*Meleagris gallopavo*) and ducks (*Anas platyrhynchos*), as these results showed that the acinar cells appear in a triangular or multigonal shape and do not resemble the study of Mahdi and Bargooth [31] in *Numida meleagris*, which showed that the acinar cells have a square to cylindrical shape.

The results of the current study have also shown that the centroacinar cells represent the beginning of the ductal system and protrude into the acoustic cavity resulting from the stacking of acinar cells among themselves, and that these cells are connected to the intercalated duct lined with simple squamous epithelial tissue, and this is consistent with the results of Abd and Faris [24] in *C. livia* and the study by Yehia et al. [25] in *Coturnix coturnix japonica* and *Bubulcus ibis*, while the current result differs from the findings of Al-Khakani et al. [32] in *Pycnonotus leucotis* and Beheiry et al. [28] in Goose, as they showed in their study that the intercalated duct is lined with simple cuboidal epithelial tissue.

This finding is consistent with Al-Shaeli [33] in the study of the pancreas of ducks and Beheiry et al. [28] in geese, while it differs from the study by Mahdi and Bargooth [31] in the pancreas of the African turkey (*Numida meleagris*), which showed that the intralobular duct is lined with simple squamous epithelial tissue. The intralobular duct is, in turn, connected to the interlobular duct, which resembles it in its histological structure but has a larger diameter. This result is similar to the findings of Al-Agele and Mohammed [34], who found that the interlobular duct in the pancreas of the golden eagle (*Aquila chrysaetos*) is lined with a layer of low columnar epithelial tissue.

It is consistent with the results of Hamodi et al. [35] in the African turkey (*Numida meleagris*) and the Mew Gull (*Larus canus*), and Al-Khakani et al. [32] in the white-eared Bulbul

(*Pycnonotus leucotis*), who showed in their study that the interlobular duct is lined with simple cuboidal epithelial tissue, while the current result differs from the results of the study by Bargooth and Jasim [29] in the domestic chicken (*Gallus gallus domesticus*), who found in their study that the interlobular duct is lined with simple columnar epithelial tissue. The interlobular duct is connected to the main pancreatic duct, which is lined with pseudostratified columnar epithelial tissue characterised by its cells having basal round nuclei. This result is consistent with the study by Al-Sharoot [36] in the goose (*Anser anser*).

Although the results of the current study differ from the results of Al-Khakani et al. [32] in their study of *Pycnonotus leucotis* and Suri et al. [37] in their study of the pancreas of Turkish birds, which showed that the main duct is lined with simple columnar epithelial tissue and this is not consistent with the current study, and the current result differs from the results of Saad and Dle [38] in the study on swan geese and the results of Al-Haak [39] in *Falco tinnunculus*, which indicated that the pancreas consists of the intralobular duct, the interlobular duct and the main pancreatic duct, but there is no intercalated duct.

With regard to the endocrine part represented by the islets of Langerhans or pancreatic islets, the results of the current study showed that the islets of Langerhans are scattered within the units of external secretion and there is no connective tissue separating them from the units of external secretion and consist of irregular cellular cords wrapped around each other and penetrate between them capillaries, the islets appear in different sizes and shapes, including small, medium and giant was observed. The giant islets in the dorsal lobe and splenic lobe can appear in various shapes, including oval, round, expanded, or irregularly shaped, in the three types of lobes. This finding is consistent with the results of Saad and Dle [38] in swan geese and Abboud and Bargooth [15] in *Coturnix coturnix*.

The study of Awad [40] in *C. coturnix*, *P. alchata* and Abd and Faris [24] in *C. livia* indicated the presence of three types of pancreatic islets represented by the alpha islets, which consist of a large number of alpha cells widely distributed in the islet, with a small number of delta cells. The beta islets contain a large number of beta cells and a few delta cells in their center, while the mixed islets consist mainly of alpha cells, beta cells, and delta cells. The alpha cells are located in the vicinity of the islets, while the beta cells are located in the central area of the islets, and the delta cells are located between the alpha cells and the beta cells, which is consistent with the result of the current study.

In a comparative study of the African turkey pancreas and the gull pancreas, Hamodi et al. [35] found that the pancreatic islets of the African turkey are divided into three types: Alpha, Beta and mixed islets, which is consistent with the current result, while in *Larus canus* there are only Alpha and Beta islets and no mixed islets, which is not consistent with the result of the current study. In the pancreas of the swan goose, it was found that there are only alpha and beta cells and no delta cells [38], as well as in the pancreas of *Falco tinnunculus*, in which only two types of islets were found, namely alpha and beta islets, and there are no mixed islets [38], and the study by Akhtar et al. [41] in chicks confirmed that beta islets have many beta cells with a small number of alpha and delta cells.

It was found that there are differences in the histological structure of the two portions of the pancreas (the exocrine pancreas and the endocrine part) in the different bird species compared to the results of the current study. Perhaps the reason

for this difference between bird species is the living environment and the variation in their dispersal methods, the type and nature of food, metabolic requirements, and their locations. This has had an impact on the great diversity in both structure and cell content, which is reflected in function and in turn affects their composition and the presence and distribution of islets of Langerhans, e.g. birds that feed on meat require a pancreas that secretes greater amounts of insulin due to their need for high energy from proteins and fats. While birds that feed on grasses or grains require a pancreas that secretes various enzymes to help digest starch, the pancreas in migratory birds faced with periods of food shortage evolves in adaptive ways to effectively regulate glucose levels during long flights, and these adaptations in relation to diet and physical activity led to diversity in the structure of the islets of Langerhans, as the density of insulin-producing cells and glucagon varies between bird species, reflecting the bird's physiological adaptations to its environment and metabolic needs [27].

This study is consistent with the study of Şimşek et al. [42] in *Coturnix coturnix japonica*, Kara et al. [43] in *Accipiter nisus*, Helmy and Soliman [44] in ostriches (*Struthio camelus*), and with study of Hussein and Bargooth [45] in ducks *Anas platyrhynchos* while the results of the current study do not correspond to the results of Gülmez et al. [46] in *Anser anser* and Bayrakdar et al. [47] in the long-legged eagle buzzard (*Buteo rufinus*), where they found only two types of pancreatic islets (alpha and beta islets). As well as it did not agree with the results of Abou-Zaid et al. [13] in pigeons, who concluded that the pancreas consists of alpha (dark) islets and mixed islets. It does not match the results of Yadav et al. [48] in chabro chickens that indicated that the Langerhans islets consist of beta and mixed islets. They mentioned that the pancreas contains dark islets and light-colored ones.

The results of the present study agree with Şimşek et al. [42] study in *Coturnix coturnix japonica* quail; Kara et al. [43] in *Accipiter nisus*; and with the results of Hussein and Bargooth [45] in *Anas platyrhynchos* and Mahdi and Bargooth [49] in *Numida meleagris*. While this result differs from the results of Tarakci et al. [50] in the ostrich (*Struthio camelus*), which observed that the immune cells of glucagon are located in the periphery of the pancreatic islets.

These results are consistent with Gülmez et al. [46] in *Anser anser* and Hussein and Bargooth [45] in ducks (*Anas platyrhynchos*) and Mahdi and Bargooth [49] in *Numida meleagris*, who reported that no immune cells were reactive with insulin in dark islets. It disagrees with the results of Tarakci et al. [50] in *Struthio camelus*, Şimşek et al. [42] in *Falco anaumanni*, where they discovered a small number of insulin reactive cells in dark islets.

This result is consistent with Abboud and Bargooth [15] in common quail (*Coturnix coturnix*). Beta (light) islets contain reactive immune cells that occupy all parts of the islets.

The present result is consistent with the results of Ku et al. [51] in chickens; with the study of Gülmez et al. [46] in *Anser anser* and Tarakci et al. [50] in *Struthio camelus*; the study of Şimşek et al. [42] in quail *Coturnix coturnix japonica*, and the study of Hussein and Bargooth [45] in *Anas platyrhynchos* and Jasim and Bargooth [52] in domestic chickens *Gallus gallus domesticus* all they found that light islets contain a small number of peripheral glucagon cells which positivity of the immune reaction.

The mixed islands were present in the three pancreatic lobes in the form of round or irregularly shaped and colored brown

in the peripheral part of the mixed islands when reacting with the glucagon antibody, where immune cells were localized in the peripheral area. This finding is consistent with Kara et al. [43] in *Accipiter nisus* and Mahdi and Bargooth [49] in *Numida meleagris*, who found that glucagon-immune cells were localized in the periphery.

Mensah-Brown et al. [53] in *Chlamydotis undulata* and Tarakci et al. [50] in *Struthio camelus*, Şimşek et al. [42] in quail *Coturnix coturnix japonica*, and Abou-Zaid et al. [13] in pigeons were consistent with this study, which found that immune cells were in the center of the beta islands.

5. CONCLUSION

The study is the first report of the pancreas in the *Coracias benghalensis* species in Iraq. It has been studied for its internal organs in avian, particularly the pancreas. The study revealed that alpha islets are present in the dorsal, ventral, and splenic lobes, while beta islets are present in the ventral and splenic lobes. Mixed islets are present in all lobes, and the percentage of alpha, beta, and mixed islets is high across all pancreatic lobes. The pancreas is a mixed gland with an exocrine and endocrine part, surrounded by a thin capsule. Immunohistochemistry results show three types of pancreas: dark islands containing alpha and delta cells, light islands containing beta cells, and mixed islands containing alpha, beta, and delta cells. Therefore, this study suggested that an avian model is the best for diabetes research.

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