

Original Research Article

Histological, Histochemical, and Immunohistochemical Study of the Pancreas in the Gull (*Chroicocephalus Genei*)

Hadeel Kamil Khaleel^{1*}, Ruqaa Badr Reda², Ahmed Mustafa Ahmed¹

¹Department of Basic Science, College of Dentistry, Al-Iraqia University, Baghdad, Iraq

²Second Karkh Education Directorate, Ministry of Education, Baghdad, Iraq

***Corresponding Author:** Hadeel Kamil Khaleel

Department of Basic Science, College of Dentistry, Al-Iraqia University, Baghdad, Iraq

Article History

Received: 19.10.2025

Accepted: 26.12.2025

Published: 14.01.2026

Abstract: The study set out to examine the pancreas' histological structure and some of its histochemical, immune-histochemical characteristics in the Gull *Chroicocephalus Genei*. Eight mature Gulls were bought from Baghdad's Al-Gazal market. The animals were sacrificed, and the pancreas isolated, and prepared the general histological components were demonstrated by staining with Harris Hematoxylin and Eosin(H&E). Some special histochemical stains were used, Mallory Trichrome (MT), Azan (Az), Periodic acid Schiff (Pas), Alcian blue (Ab), and Sudan black (Sb). In addition, some immunohistochemical stains were used, including polyclonal guinea pig anti-insulin, polyclonal guinea pig anti-glucagon, and polyclonal rabbit anti-somatostatin for microscopic examination. The results showed that the pancreas is surrounded by thin-connective tissue capsule, around the pancreatic-acini, which are exocrine components that contain neutral muco-poly-saccharides, and have intra-lobular, interlobular, and main ducts which lined by simple low cuboidal to low columnar epithelium which haven't neutral muco-poly-saccharides, blood vessels, and islets of Langerhans, known as endocrine parts that contain acidic muco-poly-saccharides. The pancreas has a moderate amount of the collagen, reticular fibers, and lacks lipids. The pancreatic endocrine components have different-sized cells, including glucagon-generating alpha-cells, insulin-generating beta-cells, and somatostatin-generating delta-cells. The study concluded that there are different fine structural details of the gull's pancreas of other birds.

Keywords: Anti-Insulin, Anti-Glucagon, Anti-Somatostatin, Alcian Blue, Mallory.

1. INTRODUCTION

The structure of the pancreas in birds varies in certain ways. The most significant variations are lobe division, islet distribution within lobes, the kind and number of endocrine cells in islets, and certain unique duct structural characteristics [1]. All vertebrates have a pancreas, which is a small, semi-wide organ situated between the branches of the duodenum. It has a light to white, pinkish color [2]. The most common knowledge is that it is divided into two sections: the exocrine component releases digesting enzymes, while the endocrine portion releases regulating hormones into blood vessels, like glucagon, somatostatin, and insulin[3]. All birds have the avian pancreas on the right side of their abdomen. Besides the blood vessels situated between the ascending and descending duodenal loops and the mesentery, firmly bind it [4]. As well as the avian pancreas is typically made up of two lobes that stretch from the point where the pancreatic ducts enter the distal duodenum to the apex of the duodenal loop[5]. Connective tissue septa separated the pancreatic parenchyma into a large number of lobules, which protruded from the capsule of the pancreas [6]. Yet, other studies claimed that it was made up of four lobes: the dorsal, ventral, splenic, and third in red jungle fowle [7]. Some studies referred to there being a serous complex tubule-acinar gland in the exocrine component of the pancreas of the mature male ducks and turkeys in the area. This gland took up a larger area in the pancreatic parenchyma than the endocrin portion. The acini, or secretory units and excretory ducts with varying widths, comprised the exocrin portion [8]. In addition, a single spherical mass made up of several serous cells encircling a small lumen was the secretory acinus unit [9]. Simple to stratified cuboidal epithelium surrounded by a muscle layer and devoid of glands was seen in the exocrine region of the ducts[10]. Both large and small

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Citation: Hadeel Kamil Khaleel, Ruqaa Badr Reda, Ahmed Mustafa Ahmed (2026). Histological, Histochemical, and Immunohistochemical Study of the Pancreas in the Gull (*Chroicocephalus Genei*). *South Asian Res J Bio Appl Biosci*, 8(1), 10-18.

islets, commonly referred to as Langerhans islets, make up the pancreatic endocrine component; those islets have beta, alpha, and delta cells [11, 12]. Furthermore, local lymph follicles were seen in the connective tissue section of the goose pancreas [13]. Thus, the study was utilized to determine the histomorphological, histochemical, and immuno_histochemical properties of the pancreas in the gull (*Chroicocephalus Genei*) as well as to investigate the histological anatomy of the pancreatic acini and Langerhans islets.

2. MATERIALS AND METHODS

2.1 Ethical Approval

According to the ethical code number (ESA&HER-08-21-06-2025) of the scientific committee of the Department of Basic Science, College of Dentistry, Al-Iraqia University, Iraq, the animals and evaluations were allowed.

2.2 Animals

The current study used 8 adult Gull *Chroicocephalus Genei*, irrespective of gender. The birds used in the study were obtained from the specialized laboratories in Baghdad. After being monitored under controlled conditions to select the healthy group of birds, the birds were sedated with chloroform and autopsied right away. After being removed from its organ and thoroughly cleaned with normal saline solution, the pancreas was preserved for 72 hours at room temperature by immersing it in 10% formalin [14].

2.3 Histological Preparation

Samples that underwent standard histology processing. The samples were sectioned to 5 μm and embedded in paraffin wax [15]. Following that, the general histological components were demonstrated by staining with Harris Hematoxylin and Eosin (H&E) [16]. Some special histochemical stains were used, Mallory Trichrome (MT), Azan (Az), Periodic acid Schiff (Pas), Alcian blue (Ab), and Sudan black (Sb). In addition, some immunohistochemical stains were used, including polyclonal guinea pig anti-insulin, polyclonal guinea pig anti-glucagon, and polyclonal rabbit anti-somatostatin [17]. Sections underwent examination with a compound microscope and digital camera [18].

2.4 Statistical Analysis: Excel software 2013 was used for statistical-analysis of the data including mean \pm SE [19].

3. RESULTS

Depending to the histology observation, the pancreas in the Slender-Billed Gull was separated into two sections: the endocrine section (islets of Langerhans), and the exocrine section (pancreatic acini and ducts). The thin capsule of connective tissue that covers a bird's pancreas was made up of reticular, collagen, and elastic fibers also between acini, the average capsule thickness was $(18.09 \pm 0.66) \mu\text{m}$ (Table 1). Pancreatic acini were a flat pyramidal form to cuboidal cells with acidophilic granules in the middle of a dark, spherical nucleus (Figure 1). The pancreatic parenchyma was divided into numerous lobules by the connective tissue septum that extended from the capsule into it. Blood vessels were dispersed throughout the pancreas by some of these extremely thin septa and diverse in size. The duct system was constructed up of interlobular, intra-lobular portions and connected with the main duct. In intra-lobular ducts, the epithelial lining was flattened to low cuboidal; in interlobular ducts (Figure 2), it progressively transformed from cuboidal to low columnar in the main duct (Figure 3). The diameters and sizes of these ducts differ from each other, as the main duct has $576.69 \pm 8.46 \mu\text{m}$, other duct's mean diameter were $144.90 \pm 13.67 \mu\text{m}$ for interlobular ducts and $42.86 \pm 4.56 \mu\text{m}$ for the intra-lobular ducts (Table 1). The endocrine section of the Slender-Billed Gull's pancreatic parenchyma was another composition, and it was the smallest portion. It is represented by the islets of Langerhans, which are scattered over the glandular basic structure's units and come in a variety of sizes and shapes (Figure 4).

Table 1: Histological pancreatic measurements in the Slender-Billed Gull:

Pancreatic measurements	μm (mean \pm SE)
Capsule Thickness	18.09 ± 0.66
Main duct	576.69 ± 8.46
Inter-lobular duct diameter	144.90 ± 13.67
Intra-lobular duct diameter	42.86 ± 4.56

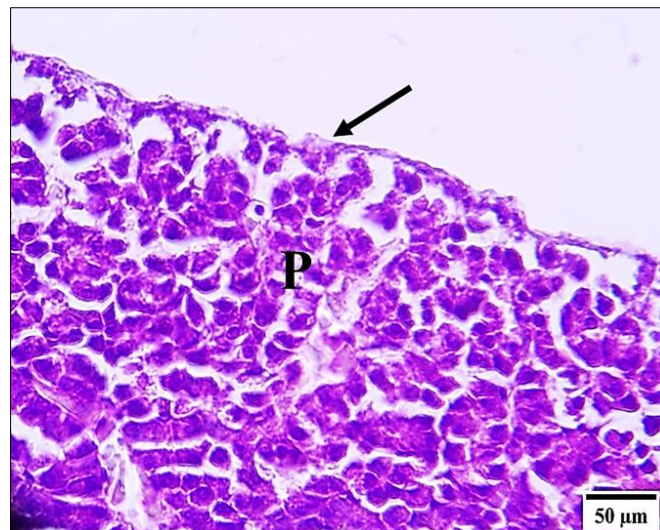


Figure 1: Micrograph in pancreas of Gull *Chroicocephalus Genei*: shows capsule (arrow), and pancreatic acini (P), (H&E)

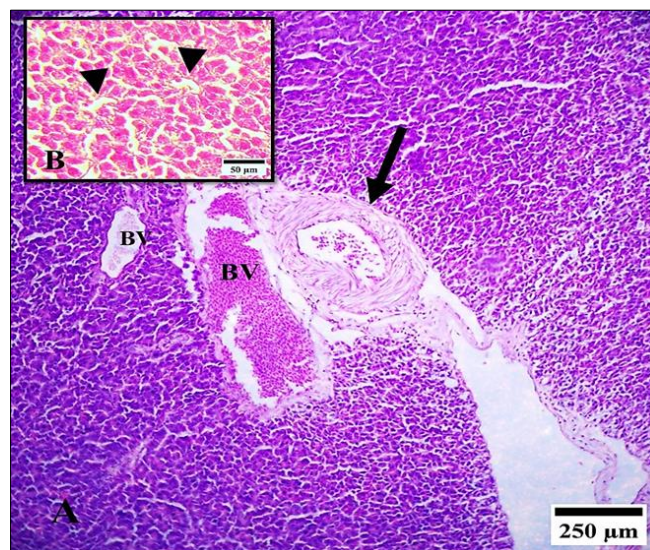


Figure 2: Micrograph in pancreas of Gull *Chroicocephalus Genei*: shows A: interlobular duct (arrow) between pancreatic lobes and blood vessels (BV). B: Intra-lobular duct (arrowheads) (H&E)

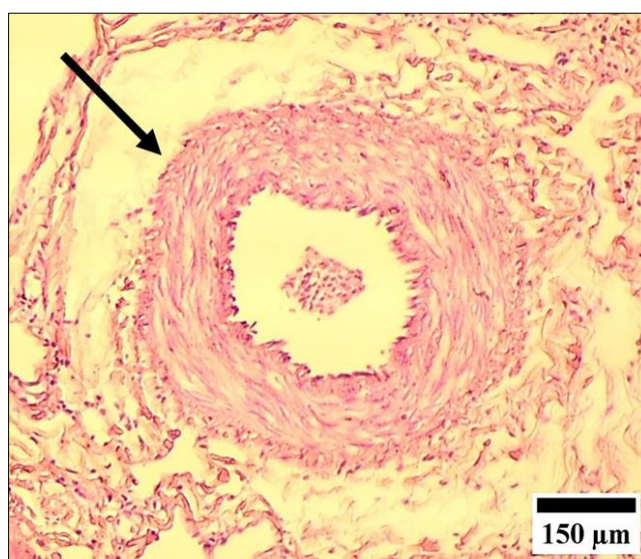


Figure 3: Micrograph in pancreas of Gull *Chroicocephalus Genei*: shows main duct (arrow) (H&E).

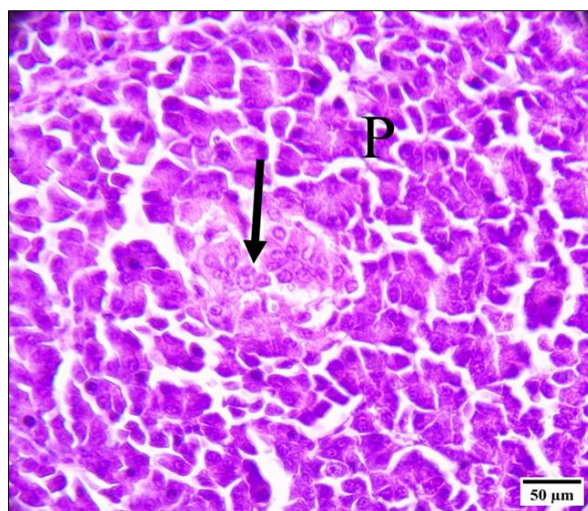


Figure 4: Micrograph in pancreas of Gull *Chroicocephalus Genei*: shows islets of Langerhans (arrow) and exocrine pancreatic acini (P), (H&E).

According to histochemical data, there is a moderate amount of neutral muco-poly-saccharides present because of the positive responses of periodic acid_Schiff (PAS) staining in the pancreatic secretion acini (Figure 5). On the other hand, the secretory unit reacts negatively with Alcian Blue (AB), suggesting that it lacks acidic muco-poly-saccharides, or mucin (Figure 6). The duct's histochemical results revealed a negative reactivity with AB, showing a lack of acidity muco-poly-saccharides in the ducts (Figure 6). At the same time, the negative reaction of PAS indicates the lack of neutral muco-poly-saccharides (Figure 5).

The histochemical analysis of the islets of Langerhans reveals that cells exhibit a negative reaction to PAS staining (Figure 7) and a positive reaction to AB, indicating the lack of neutral muco-poly-saccharides and the presence of acidity muco-poly-sacchrides (Figure 8).

Collagen fibers are distributed in the capsule and surrounding the blood vessels, pancreatic acini, and the duct system when the tissue is stained with Mallory trichrome (Figure 9).

Histochemical Azan stain findings, the pancreas contains reticular and collagen fibers in the connective tissue capsule, between pancreatic acini and the walls of the duct system (Figure 10).

When Sudan stain is applied to the lipids, the Slender-Billed Gull's pancreatic cells, and ducts respond negatively (Figure 11).

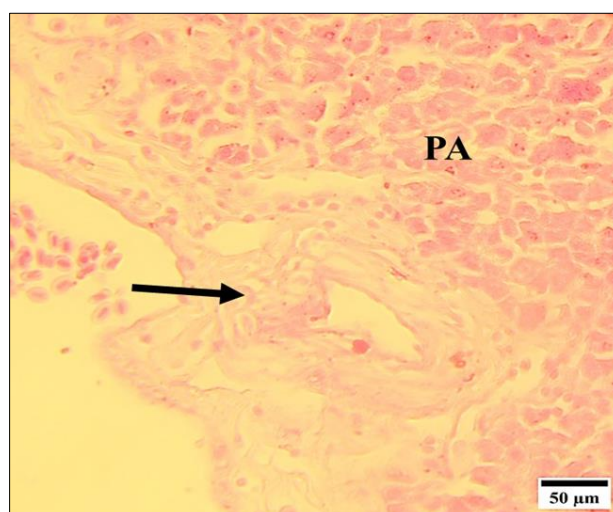


Figure 5: Histochemical micrograph in pancreas of Gull *Chroicocephalus Genei*: demonstrates the presence of neutral muco-poly-saccharides due to the moderate responses of the pancreatic secretion acini (PA) to periodic acid_Schiff staining, and negative reaction in the ducts. (PAS)

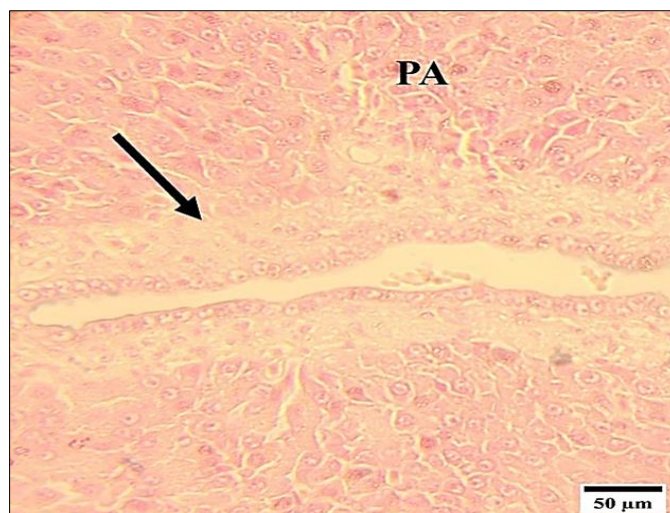


Figure 6: Histochemical micrograph in the pancreas of Gull *Chroicocephalus Genei*: demonstrates a lack of acidic muco-polysaccharides due to the negative responses of the pancreatic secretion acini (PA) to Alcian Blue staining, and a negative reaction in the ducts(AB).

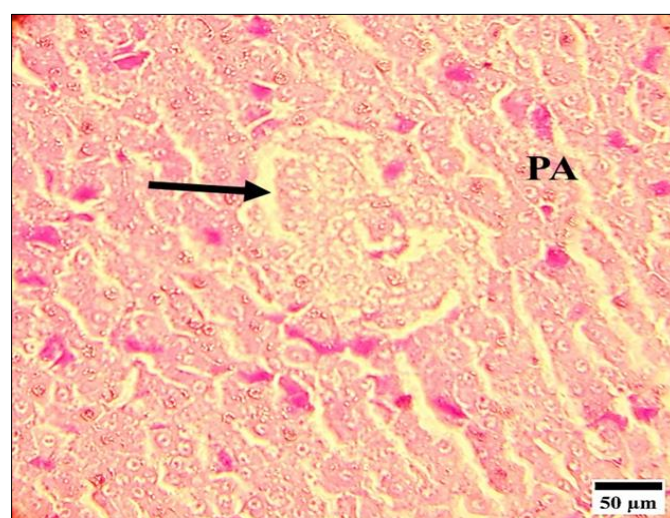


Figure 7: Histochemical micrograph in the pancreas of Gull *Chroicocephalus Genei*: The islets of Langerhans reveals that cells exhibit a negative reaction (arrow) and positive reaction to pancreatic acini (PA) (PAS).

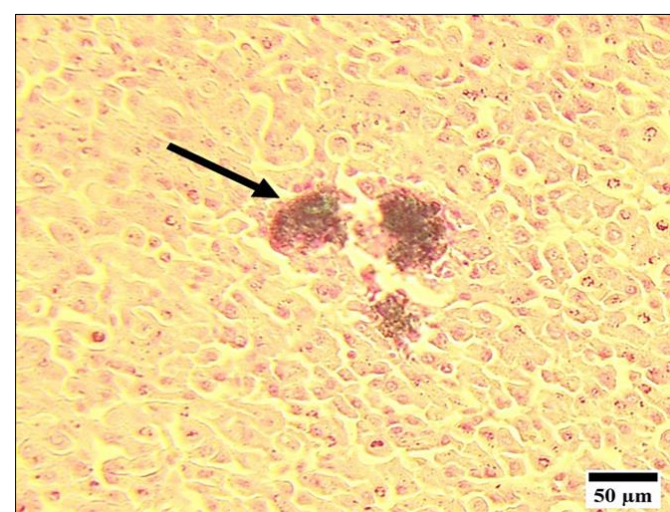


Figure 8: Histochemical micrograph in the pancreas of Gull *Chroicocephalus Genei*: The islets of Langerhans reveal that cells exhibit a positive reaction (arrow) (AB)

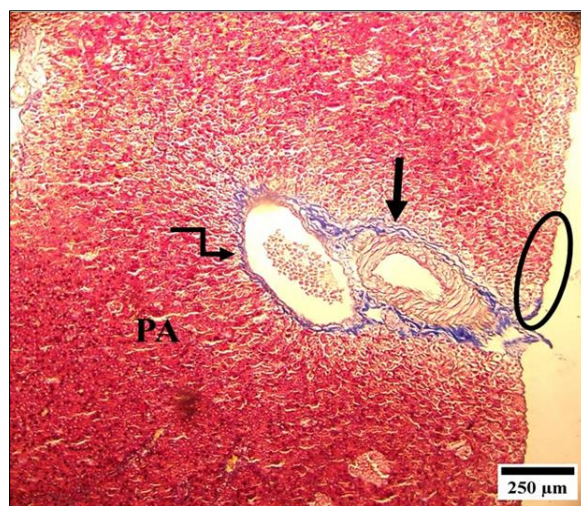


Figure 9: Histochemical micrograph in the pancreas of Gull *Chroicocephalus Genei*: Collagen fibers are distributed in the surrounding the blood vessels (elbow arrow), pancreatic acini (PA), the duct system (arrow), and the capsule (oval) (MT).

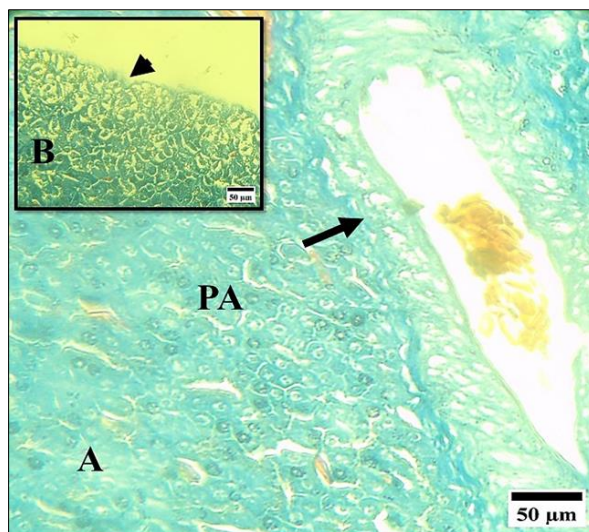


Figure 10: Histochemical micrograph in the pancreas of Gull *Chroicocephalus Genei* showed: A: reticular and collagen fibers between pancreatic acini (PA), and the walls of the duct system (arrow), B: and in the connective tissue capsule (arrowhead),(Az).

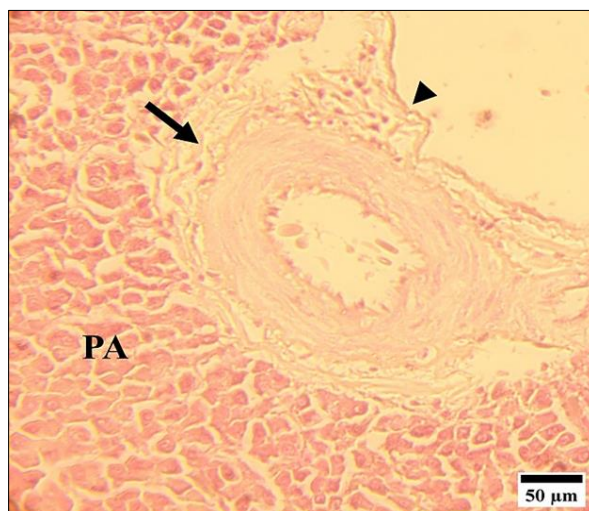


Figure 11: Histochemical micrograph in the pancreas of Gull *Chroicocephalus Genei*: showed lack to adipose tissue due to a negative reaction of the pancreatic secretion acini (PA), capsule (arrowhead), and the duct (arrow) (SB)

In the pancreatic islets, antisera against somatostatin, glucagon, and insulin were used to identify the category of endocrine cells. In the Gull, there was evidence of anti-somatostatin antibody positive cross-reactivity, which showed that the islets were rich in Delta cells, as depicted in (Figure 12). The pancreatic islets in the Gull were shown to exhibit a positive reaction to the anti-glucagon antibody, and there was evidence that they contain alpha cells (Figure 13).

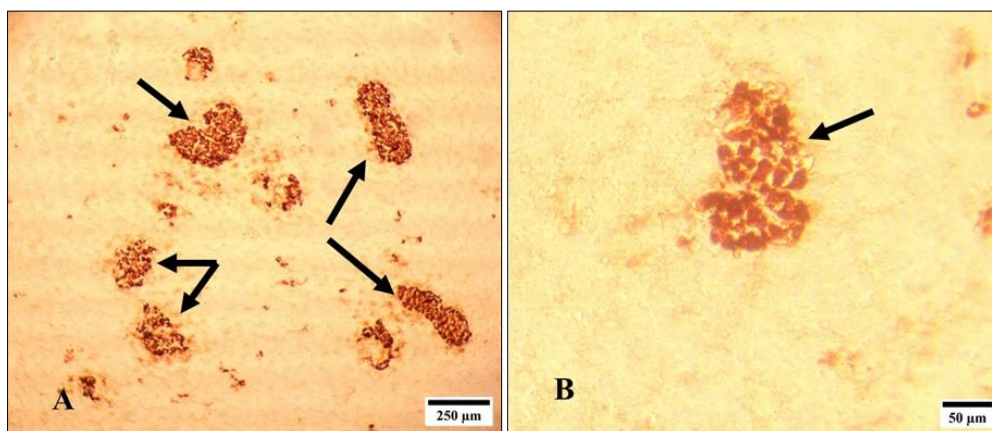


Figure 12: Immunohistochemical micrograph in the pancreas of Gull *Chroicocephalus Genei*: shows A: distribution of Delta cells in the islets of Langerhans between pancreatic acini (arrows), B: different shapes and sizes of Delta cells (arrow), positive reaction to anti-somatostatin antibody.

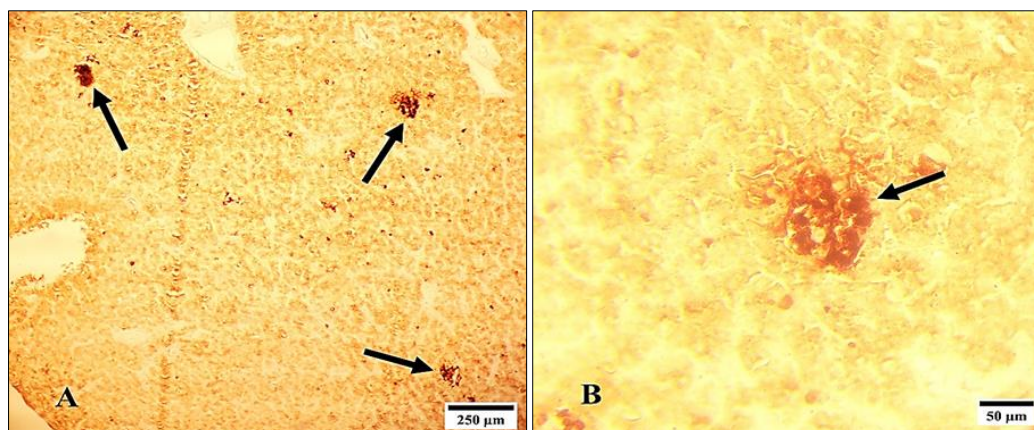


Figure 13: Immunohistochemical micrograph in the pancreas of Gull *Chroicocephalus Genei*: shows A: distribution of alpha cells in the islets of Langerhans between pancreatic acini (arrows), B: different shapes and sizes of alpha cells (arrow), positive reaction to anti-glucagon antibody.

There was proof that the Slender-Billed Gull's pancreatic islets contain Beta cells, and they demonstrated an impressive response to the anti-insulin antibody (Figure 14).

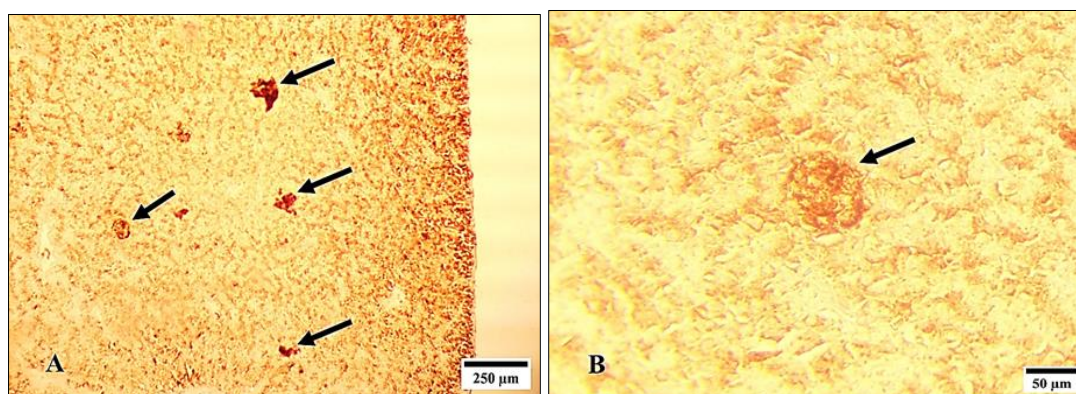


Figure 14: Immunohistochemical micrograph in the pancreas of Gull *Chroicocephalus Genei*: shows A: distribution of Beta cells in the islets of Langerhans between pancreatic acini (arrows), B: different shapes and sizes of Beta cells (arrow), positive reaction to anti-insulin antibody.

4. DISCUSSION

The pancreas of the Slender-Bill-Gull revealed that it is surrounded by a thin connective tissue layer and consistent with [5-9] on the kestrel's and goose pancreas capsule. It contains exocrine and endocrine components, like the geese and hens [20, 21], respectively. The secretory acini were exocrine components like flat to cuboidal cells that contained different-sized ducts, including intra-lobular, interlobular ducts [22]. These ducts were padded by a single layer of squamous to cuboidal epithelium, and the main duct was padded by cuboidal to low columnar epithelium, so in the bulbul and ostrich [23, 24]. The islets of Langerhans are endocrine components that contain different-sized cells, including alpha, beta, and delta cells, which are embedded between pancreatic acini as seen in bulbul [23]. Neutral muco-poly-saccharides were found in the pancreatic acini, whereas lacking in islets of Langerhans and ducts [24]. The acidic muco-poly saccharides were absent in the pancreatic acini, and the ducts, but they were found in the islets of Langerhans; however, they were present in the goose and moorhen [13-25]. Collagen fibers are found in the capsule, around the pancreatic acini, the ducts, and blood vessels, as observed in the goose and local partridge [9,11]. Reticular fibers are distributed between the pancreatic acini, the ducts, the capsule, and lacked lipids as in Guinea-fowl [3]. Endocrine pancreatic cells in the islets of Langerhans have different-sized cells, including glucagon-generating alpha, insulin-generating beta, and somatostatin-generating delta cells are seen after applying immune-histochemical stains, resembling those of the goose and the raptors [26, 27].

5. CONCLUSION

The histologic pancreas features of Slender-Billed-Gull have a connective tissue capsule that contains collagen and reticular fibers surrounding the internal structures, including exocrine and endocrine components. Pancreatic acini are pyramidal and contain moderate amounts of fibers. The duct system consists of intra-lobular and interlobular ducts that connect with the main duct. The pancreatic acini contain neutral muco-poly-saccharides, whereas the other birds contain acidic muco-poly-saccharides. The endocrine structure has islets of Langerhans, involved alpha, beta, and delta cells. Their cells contain acidic muco-poly-saccharides only. There are different details of the gull's pancreas with other birds.

Acknowledgment: So grateful for the Department of Basic Science, College of Dentistry, Al-Iraqia University, for facilitating the work.

Credit Authorship Contribution Statement:

The authors attest to the following contributions they made to the review: Hadeel Kamil Khaleel and Ruqaa Badr Reda contributed to the ideation of the study, procedure, and writing of the manuscript. The general concept of the research, text editing, and proofreading were all done by Ahmed Mustafa Ahmed.

Data Availability Statement: The corresponding author can provide the data created and examined during the work upon adequate request.

Declaration of Competing Interest: Not found

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