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Comparative anatomical, histological and histochemical study of the pancreas in two species of birds

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ABSTRACT

The purpose of this study was to determine the anatomical characteristics and histological structure as well assome histochemical aspects of the pancreas of two species of birds, which differ in theirclassification, habitat, nutrition and activity by using a light microscope. The birds were omnivorous Guineafowl *Numida meleagris* and carnivorous Common gull *Laruscanus*. The results showed that the pancreas of the two species of birds was a bilobed organ, it differed in size, color and appearance. The pancreas tissue of the two birds was consisted of exocrine and endocrine part. The exocrine part in the both birds arecomposed of acini, ducts and it occupied a larger area of the pancreas. The aciniare varied in shapes in the both birds. These aciniconsisted of a single layer of variable number of different shapes cells that are differing in their thickness between the two species. The ductal system of the exocrine part of the pancreas in both birds included the intercalated ducts, intralobular ductsand interlobular ducts thatdiffered in their number, size and distribution between the two species. The endocrine part of the both birds areconsisted of various shapes and sizes of the islets thatwere scattered in the exocrine part, these islets are composed from alpha (A) and beta (B) cells. The mixed islets that consist of A and B cells, alpha islets and beta isletsare observed in Guineafowl whereas,in the Common gull, the mixed islets were the only type observed and the islets in this bird are smallerand are less densely populatedthan the islets inGuineafowl. In conclusion the anatomicalfeaturesand histological structure of Guineafowl and Common gullpancreas was found to besimilar to that of other avian species with the exception of some secondary characteristics.

KEYWORDS

Pancreas;
Guinea fowl;
Common gull;
Pancreatic acini;
Pancreatic islets.
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INTRODUCTION

It is generally known that the pancreas of vertebrates is subdivided into two regions, one is an exocrine portion where digestive enzymes are released and the other is an endocrine portion where regulatory hormones such as insulin, glucagon and somatostatin are released into blood vessels[1,2]. The avian pancreas is located on the right side of the abdominal cavity in all birds. It is tightly bound by mesentery and the blood

vessels positioned between the descending and the ascending duodenal loops[3,4]. The avian pancreas is generallycomposed of two lobeswhichextend from the apex of the duodenal loop tothe point where the pancreatic ducts enter thedistal duodenum[2,3,5], but many reports indicated that it had four lobes: dorsal, ventral, splenicand third[2,3,6].

Dorsal and ventral lobe extend in a duodenal loop from apex to distal duodenum. Splenic lobe extends from the head of pancreas towards the spleen. Mikami and Ono (1962) state that the third lobe of chicken pancreas is a part of the ventral lobe so it divides into the ventral lobe properly and third lobe on the basis of an independent form of the latter, but in some other studies dorsal lobe is divided to the additional third lobe[6-8].

The exocrine pancreas is formed by acinar cells and excretory ducts with small and largediameter. The cytoplasm of the acinar cells is rich in the secretory zymogene granules[7,8]. Whereas, the endocrine parts of the avian pancreas are scattered singly or in small groups in variousshapes and sizesin the interstitial section of the exocrine part. They can be observed as two typesof islets, the dark A islet and the light B islets[8].

The anatomical characteristics and histological structureof the pancreas, as well as, the Pancreatic ducthas been investigated in a some avian species[2-5,8-15], these studies show that there are some differences in structure of the avian pancreas in the division of lobes, distribution of the islets in lobes, the form and frequency of endocrine cells in islets and some special features in structure of the ducts[8]. Therefore, this study was conducted to compare the anatomicalfeaturesand histological structure, as well as some histochemical aspects of the pancreas intwo species of birdsusing a light microscope,that differ in their nutrition, habitat and taxonomy. The two species are the omnivorous Guineafowl *Numidameleagris* (Galliformes, Linnaeus 1766) and carnivorousCommon gull *Laruscanus* (Charadriiformes, Linnaeus 1758).

MATERIALS AND METHODS

Sixadult from each Helmeted Guineafowl and Common Gull from both sexes obtained from different areas in Mosul city, Iraq were used in the current study. After anesthesia, a mid-ventral line incision was made to expose the digestive organs, the pancreas was immediately dissected out and washed with saline solution to remove blood and any other adhering debris, different morphological features of pancreas were examined, recorded, and photographed by using digital Sony camera (DSC-W530). Tissue pieces of (1cm) thickness were fixed at8% buffered formalin solution and then routinely processed for embedding in paraffin. Tissue blocks were cut by a microtome into 6μm sections[16,17]. The histological and histochemical methods of staining were employed as follows: DelafiedsHaematoxylin and Eosin (HE), Mallory's triple (TS), Toluidin blue (TB) and Azan (AZ) Stains[18,19] for general histological purposes, Periodic Acid-Schiff technique (PAS) and Alcian Blue (AB) (pH 2.5) for carbohydrates, bromophenol blue (PB) for proteins and Sudan Black B (SB) for lipids[20,21]. All stained slides were viewed for Histological examination under a light microscope (Reichert Neovar Type 300422) and photographed by using MDCE- 5A digital camera.

RESULT

Anatomical features

Thepancreas of the both birds is located between the ascending and descending arms of the duodenum and enclosed with thepancreatic duodenal ligament and it is composed of two lobes the dorsal and the ventral lobe, well-developed interlobar connectionsmade it difficult to distinguish between the two lobes in the both birds. The dorsal and ventral lobes are drained bya separate duct which opened into the distal part ofthe duodenum (Figure 1a,b). The Guineafowl pancreasis pale yellow organ and its length is about 8.2 cm and 3.1cm width. Its thickness is almost equal with the edges of duodenum and its external surface is curly. It is related to the duodenum by connective tissue which means thatis not directly related to a wall ofduodenum (Figure 1a). Whereas, the Common gullpancreasis pale pink organ and its length is about 5.1 cm and 3.4cm width. Its lobes are connected with each other in the coiling portion of duodenum. Its thickness is also equal with the edges of duodenum and its external surface is smooth and free of wrinkles that have appeared in the Guinea fowl pancreas. On the contrary of the Guinea fowl pancreas, the Common gullp ancreas is related directly to a wall of duodenum without of connective tissue (Figure 1b).

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Histological structure

The pancreas of both birds is covered with a thin connective tissue capsulewhich consisted of collagen, elastic and reticular fibers. Blood and lymph vessels, nerves and excretory ducts appear in some areas of this capsule in both birds. This capsule appears to be thicker in Common gull than in Guineafowl. From thecapsule of Guineafowl extends a number of septula to the parenchyma of the pancreas which don't appear inCommon gull (Figure 2a,b). The parenchyma of pancreas of both birds isconsisted of exocrine and endocrine parts and is supported by a thin reticular stroma (Figure 3a,b).

The exocrine part in the both birds are composed of acini and ducts. The exocrine part of the Guinea fowl pancreas is arranged in the form of serous tubuloacinar glands and occupied a larger area of pancreas. The secretory acinivary in shapesas globoid,oval elongated and amorphous, its thickness average (46.588 ± 6.18μm). These acini are consisted of a single layer of variable number of pyramidal to tall columnar and/or quadrate cells basing on the plane of the section having basophilic base and numerous apical acidophilic zymogen granules, its thickness average (6.584± 0.44μm). Each acinar cell contains a round, large and basally situated nucleus, its diameter average (3.415 ± 0.21μm) with a prominent dark nucleolus. Moreover,these acinihave no centroacinar cell (Figure 4a,b). The exocrine portion of Common gullpancreas isalso arranged in the form of serous tubuloacinar glands and occupied a larger area of the pancreas. The secretory acinivary in shapes as in Guinea fowl pancreas but the most common shape is the elongated, its thickness average (29.754 ± 4.72μm). The acini are consisted of pyramidaltotall columnar and/or quadrate cells as in Guinea fowl pancrea shaving numerous apical zymogen granules, its thickness average (5.731± 0.41μm). Each acinar cell contains a round, large and basally to centrally situated nucleus, its diameter average (4.268 ± 0.27μm) with a prominent dark nucleolus contains paracentric heterochromatin. The centroacinar cell is also absent in the center of acinias in Guinea fowl (Figure 5a,b).

The ductal system of the exocrine part of the pancreas in both birds includes the intercalated ducts, intralobular ductsand interlobular ducts as well as two main ducts the dorsal and ventral pancreatic ducts that extending from the pancreas to thepoint where it empty its contents into theduodenum. Intercalated ducts are circular in almost and are lined with a simple flattened epitheliumin the both birds but it look more regular in Guinea fowlthan Common gull, its average diameters in Guinea fowland in Common gull (8.253 ± 0.93μm) and (12.145 ± 1.69μm) respectively (Figure 2a,b,6a). The

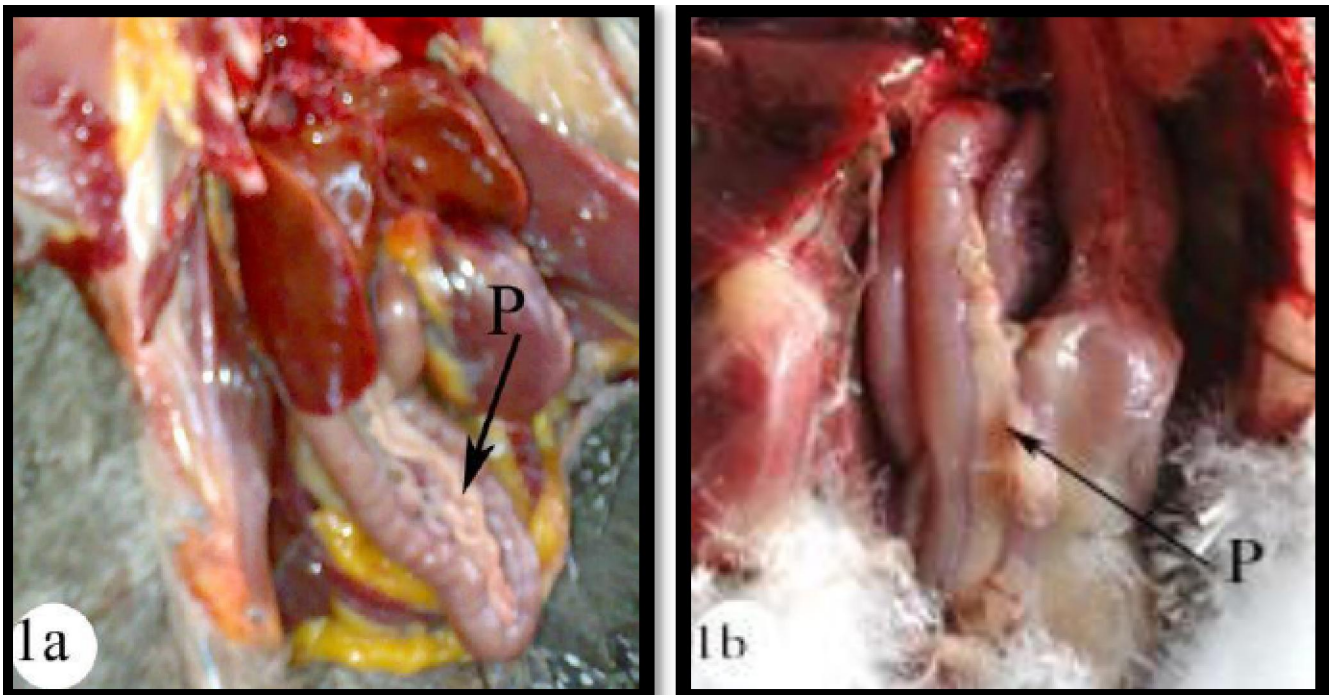

Figure 1 : Photograph of the Pancreas (P) of Guinea fowl (a) and Common gull (b).

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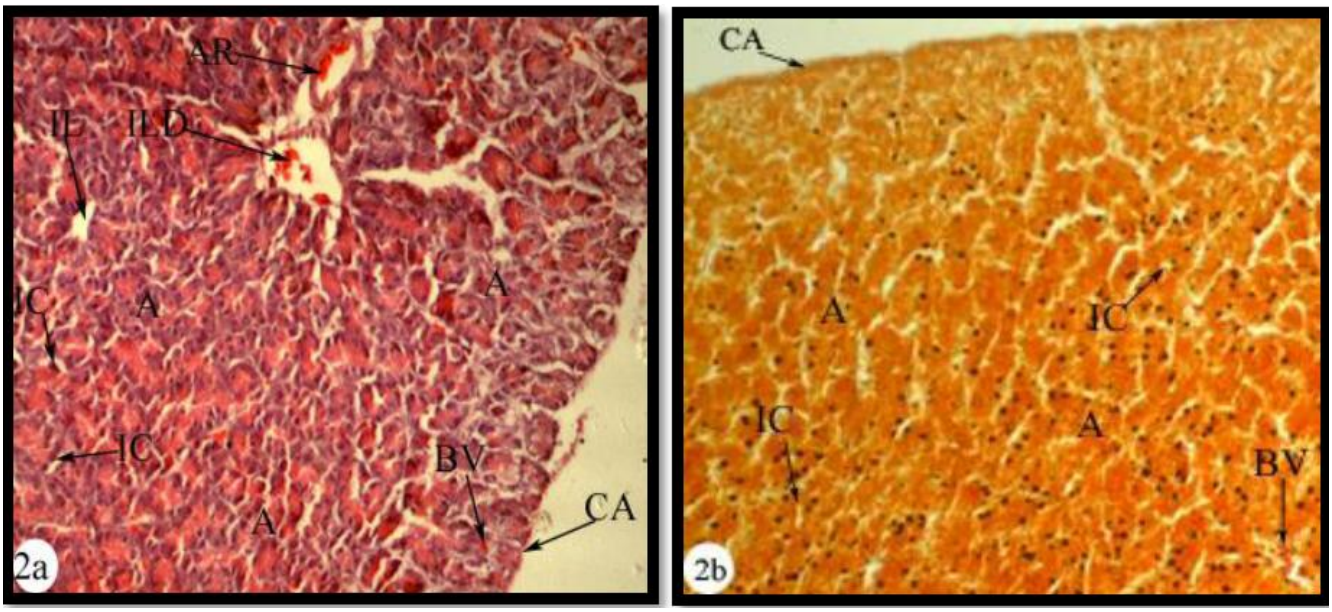
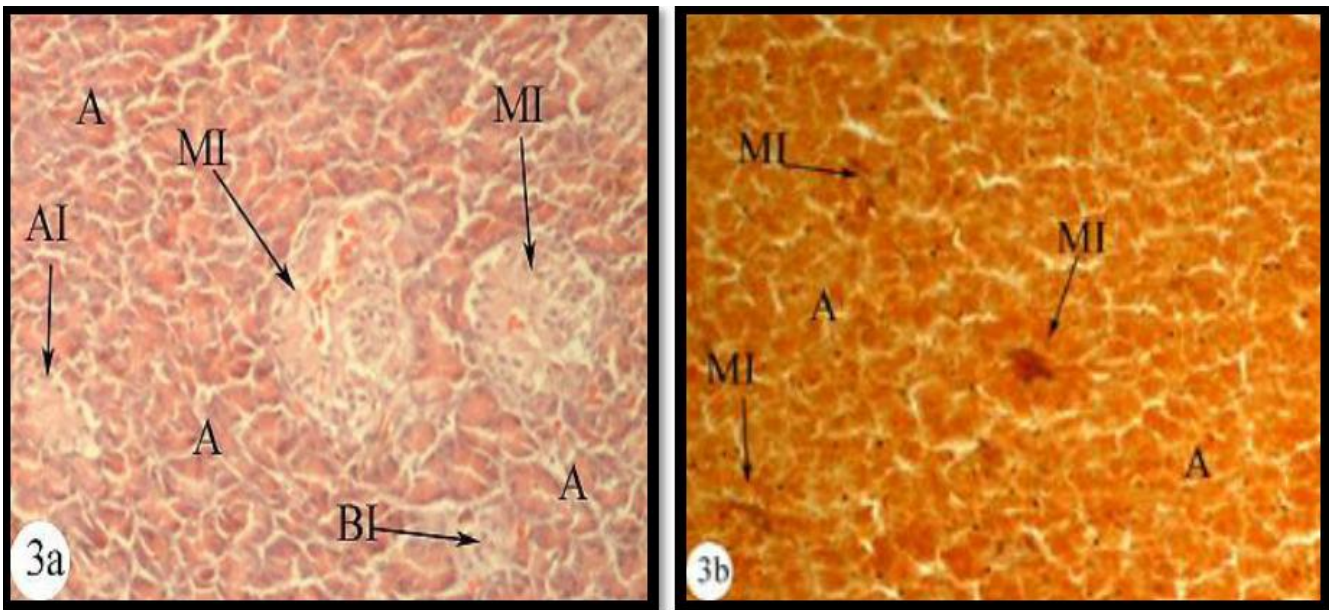


Figure 2 : Histological picture of the Pancreas (P) of (a) Guinea fowl (HE-10X) and (b) Common gull (AZ-10X).



A, acinus; AI, alpha islet;AR, Artery; BI, Beta islet; BV, blood vessel; CA, Capsule;ILD, interlobar duct; IL, Intralobar duct; IC,intercalated duct; MI, mixed islet.

Figure 3 : Histological picture of the Pancreas (P) of (a) Guinea fowl (HE-10X) and (b) Common gull (AZ-10X).

intralobular ductsare scattered through the pancreas parenchyma of the two species of birds without a defined arrangement, these ducts have mostly circular shape and are lined by the same epithelial cell that lined the intercalated ducts, its average diameter in Guinea fowl (25.889 ± 4.07μm) and in Common gull (39.312 ± 5.94μm), and they are surrounded by secretory acini (Figure 2a,6a,6b). The interlobular ducts have a circular to oval shape and its average diameter in Guinea fowl (96.577 ± 12.92μm) and in Common gull (99.477 ± 13.76μm). These ducts are composed of an epithelium, that appear simple squamous to cuboidal in Guinea fowl and simple flattened epithelium, which lines up inside the ducts andconnective tissue surrounding outside theducts. Furthermore, interlobular ducts have thin muscle layers surrounding the connective tissue in the Guinea fowl and absent in Common gull(Figure 6a,b). Also the blood and lymph vessels, nerves and excretory ducts run within the connective tissue septa in the both birds (Figure 2b,3a,b,6b).

The endocrine part of the both birds are consisted of various shapes and sizes of islets which is called

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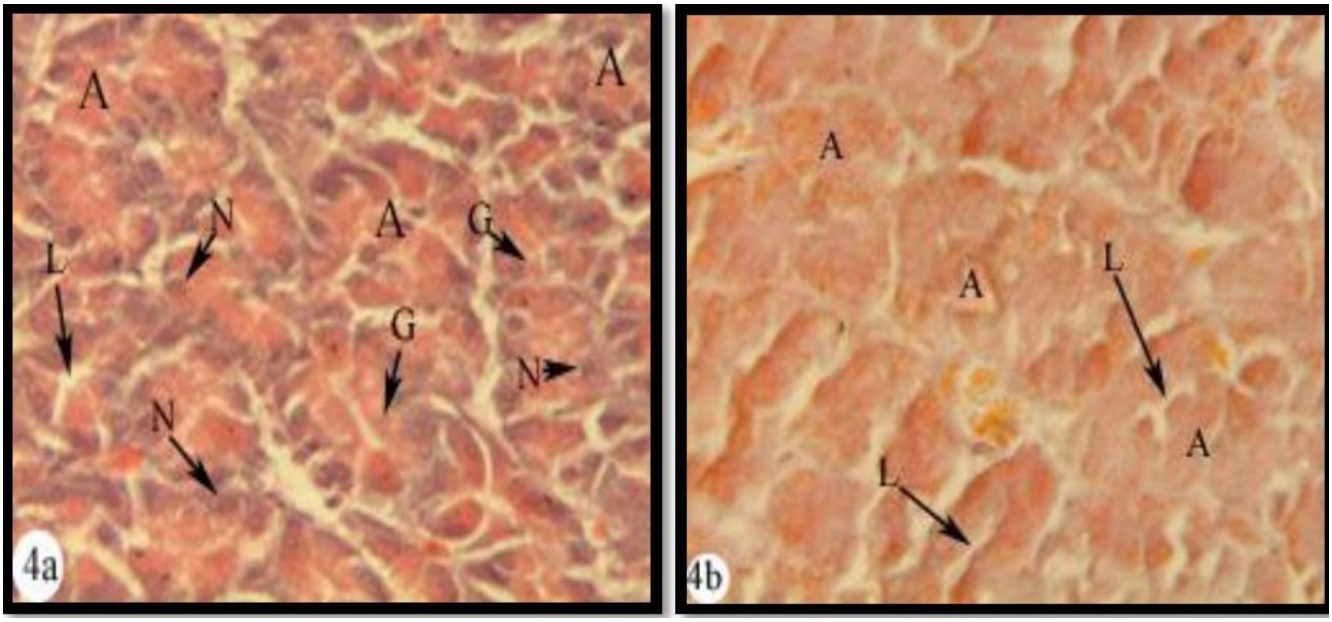
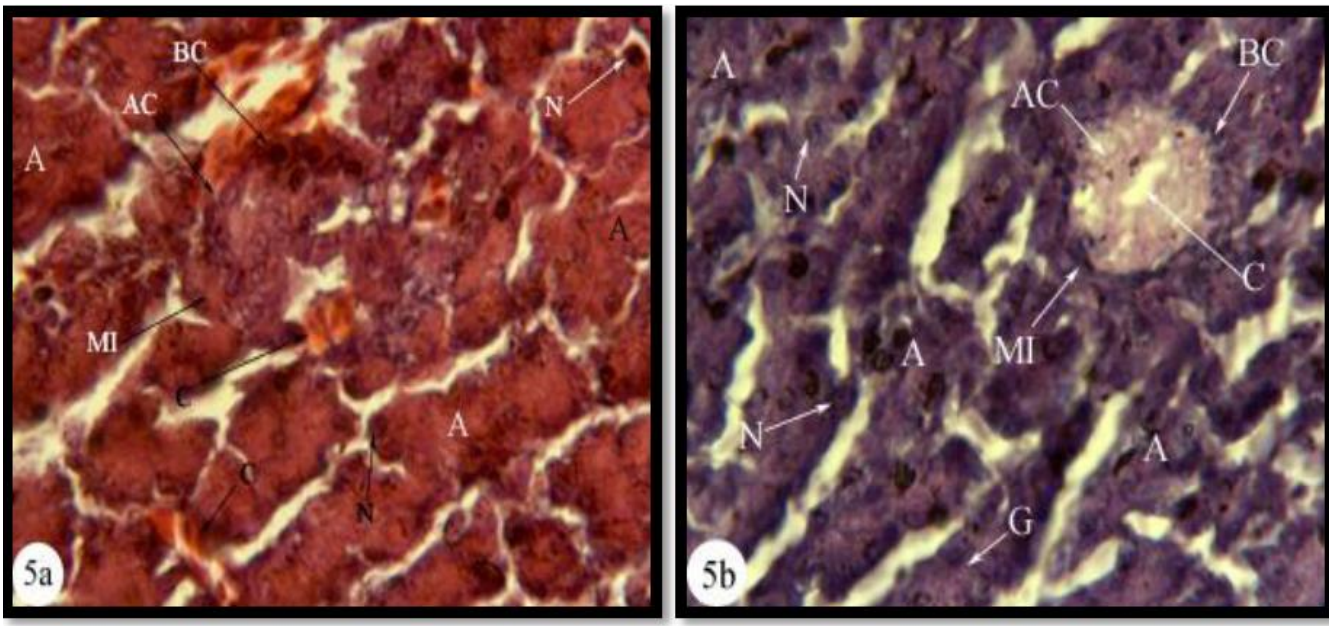


Figure 4 : Histological picture of the Pancreatic acini in Guinea fowl (a) (HE-40X) and (b) (AZ-40X).



A, acinus; AC, A cell; BC, B cell; C, capillary; G, granules; L, acinus lumen, MI, mixed islet; N, nucleus.

Figure 5 : Histological picture of the Pancreatic acini and mixed islet in Common gull(a) (HE-40X) and (b) (PAS-40X).

Langerhans islets that is scattered in the exocrine part, these islets are composed from alpha (A) and beta (B) cells while the Delta cells don't noticed in the both birds. These islets don't have distinct borders with the exocrine part in the both birds (Figure 3a,b). In the endocrine part of Guinea fowl, the mixed islets that consist of A and B cells are observed and also there are islets that contain only A cells which known as alpha islets and beta islets that contain only B cells, the alpha islets were larger than the beta and are more densely populated A cells are more demonstrated than B cells and the nucleus in both cell types are seemedsimilar and is round to oval in shape with a prominent nucleolus, its average diameter in both cells (5.489 ± 0.87μm) (Figure 7a,b). Whereas, inthe endocrine part of Common gull, the mixed islets that consist of A and B cells are also observed and they are the only type, that mean thealpha islets and beta islets that appeared in the Guinea fowl are absent in this bird. The islets in this bird are smaller and are less densely populated than the islets inGuineafowl (Figure 3a,b), but the islets borders in this bird are more pronounced than in Guineafowl, they are

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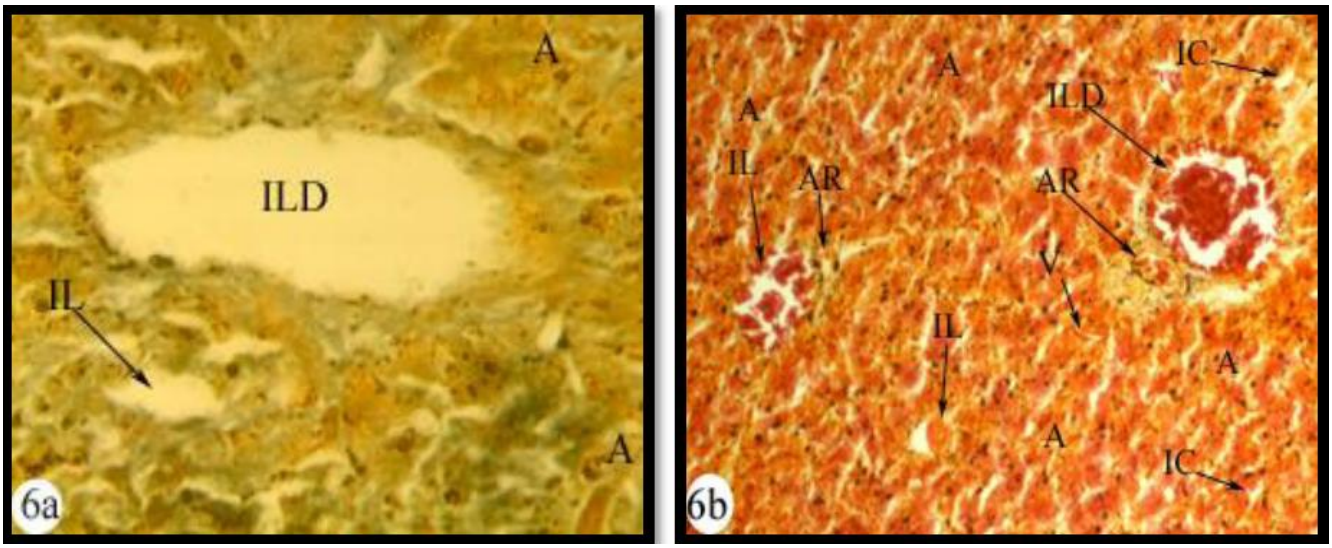


A, acinus; AR, Artery; ILD, interlobar duct; IL, Intralobar duct; IC,intercalated duct; V, vein.

Figure 6 : Histological picture of the Pancreatic ductal systemof (a) Guinea fowl (TS-10X) and (b) Common gull (TS-10X).

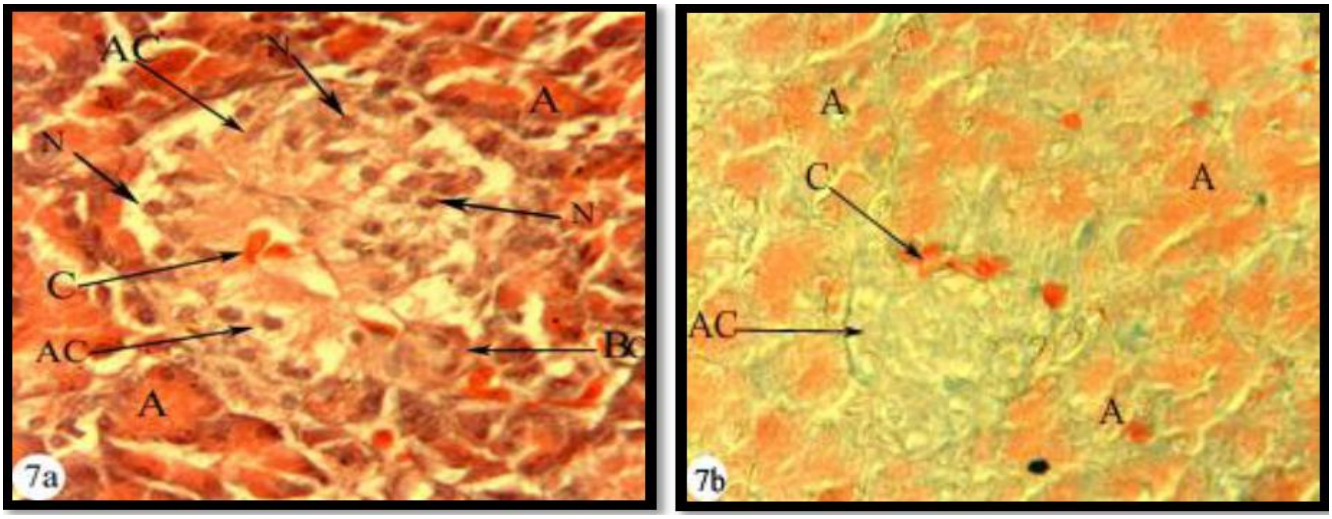
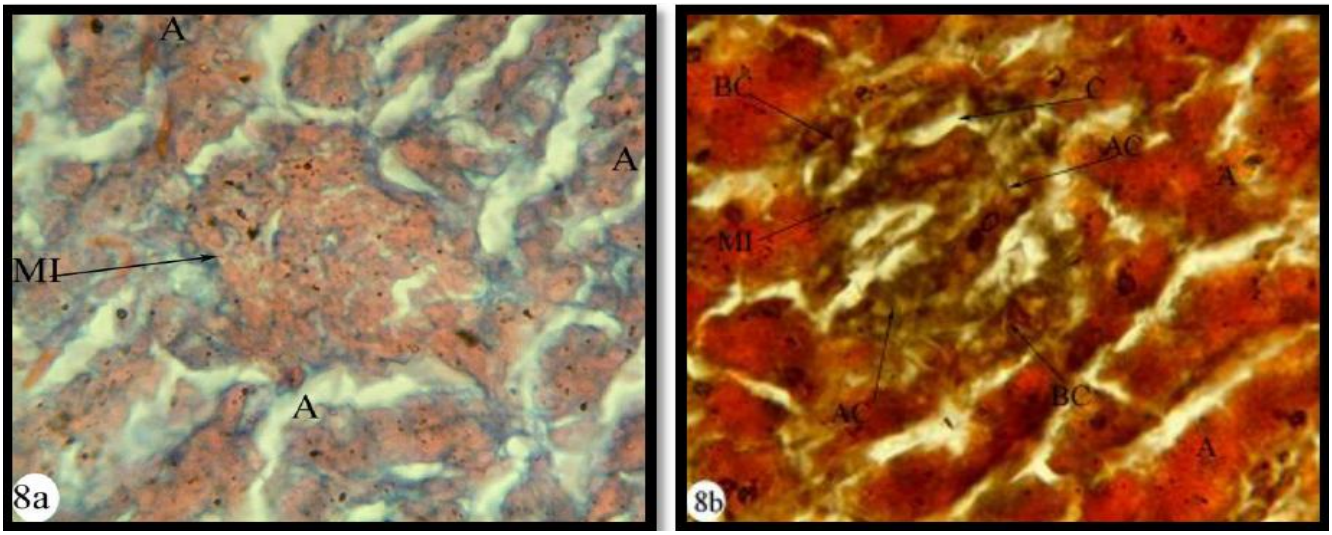


Figure 7 : Histological picture of the Pancreatic islets in Guinea fowl (a) (HE-40X) and (b) (AB-40X).



A, acinus; AC, A cell; BC, B cell; C, capillary; MI, mixed islet; N, nucleus.

Figure 8 : Histological picture of the Pancreatic islets in Common gull (a) (AB-40X) and (b) (TS-40X).

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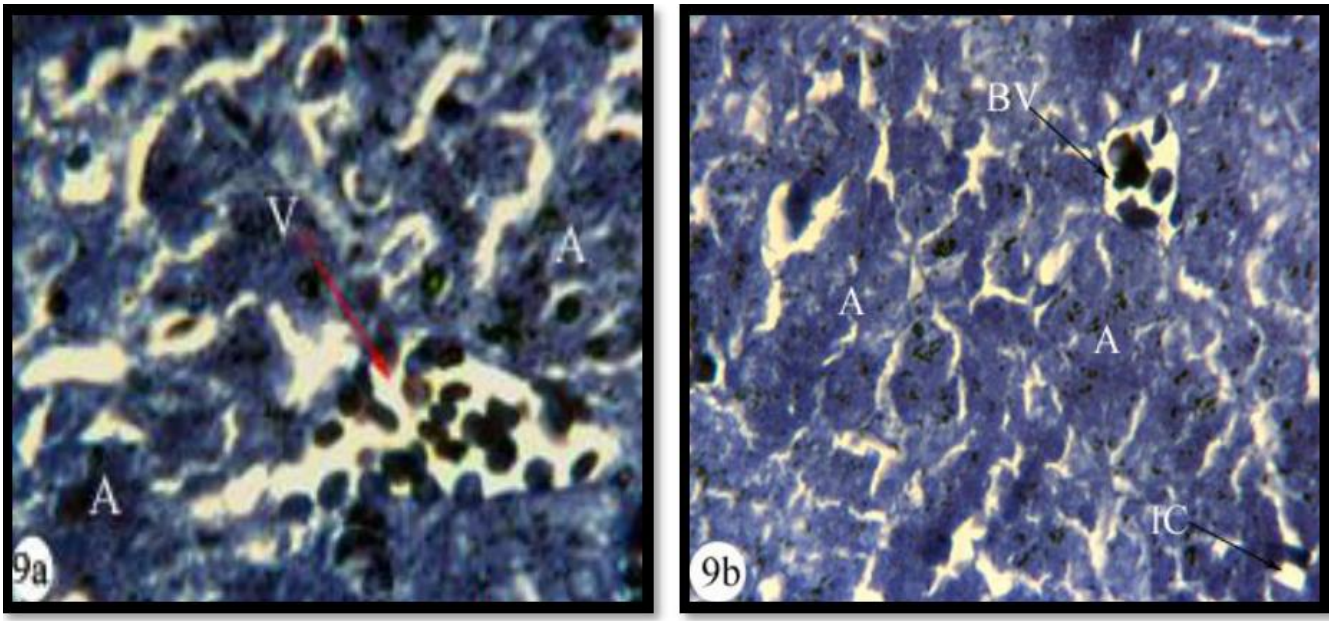
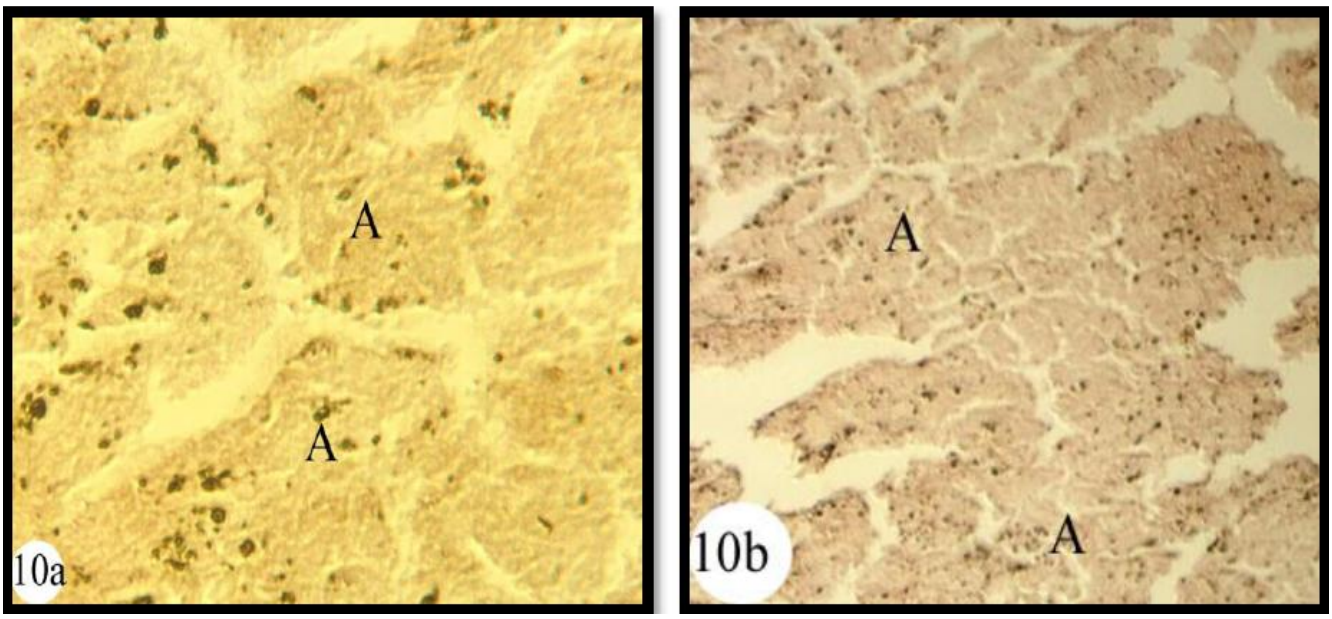


Figure 9 : Histological picture of the Pancreatic acini in Guinea fowl (a) (PB-40X) and (b) Common gull (PB-40X).



A, acinus; BV, blood vessel;IC,intercalated duct; V, vein.

Figure 10 : Histological picture of the Pancreatic acini in Guinea fowl (a) (SB-40X)and (b) Common gull (SB-10X).

delineated from surrounding secretory acini by delicate collagenous fibres, also there were few fibres within islets tissue. A cells B cells are more demonstrated than in Guinea fowl and the nucleus in both cell types is similar to that in Guinea fowl on shape and its nucleolus, its average diameter in both cells (4.268 ± 0.83m) and the cell membrane are usually more distinguished in B cells (Figure 5a,b,8a,b).

The islets of the both birds are very rich in blood capillaries, that appear more in Guinea fowl than in Common gull (Figure 5a,b,7a,b,9a,b).

Histochemical aspects

The pancreatic cells, whether the acinar cells orislets cells are moderately positive for PAS and Alcian Blue (pH 2.5) reactions in the both species of birds but the interaction is stronger in Guinea fowl than in Common gull and in the acinar cells than in the islets cells (Figure 5b,7b,8a). The carbohydrates appear as pink-color deposits as identified throughout the cellular parenchyma of the pancreas especially in Guinea fowl. The pancreatic cells in the two species of birds show moderate to weak negative reaction

with PB stain for the proteins which indicates the lack of aggregations of protein inside the pancreatic cells, whether the acinar cells or islets cells (Figure 9a,b,). On the other hand, the pancreatic cells of the two species show a negative reaction with SB stain for the lipids (Figure 10a,b,).

DISCUSSION

In the present study, the pancreas of the both birds fill the gap between the duodenal limbs, this finding is in agreement with the result of[8,13,15,22] while in the ostrich, duck and goose, thepancreas is short to reach the end of these limbs[5,11,14,23]. According to suggestions by Gussekloo and London (2006) on chicken and otherbirds that feed on grains and seed, they need moreenzymatic activity to compensate for their lack of teeth and hydrolytic enzymes in their saliva[22,24]. There are two lobes distinguished in the two species of birds. This design appeared also incaptive bustards[5] and Ostrich[11], but many otherbirds as Mynah has three lobes[13] and duck[25], goose[10], red jungle fowl[14], Palam Dove[8] and Pigeon[15] have four lobes.

Histologically, the pancreas of both birds is covered with a thin connective tissue capsule which correlate with the findings of[26] in duck[14] in red jungle fowl[4] in Goose and[15] in Pigeon. The parenchyma of pancreas of both birds is consisted of exocrine and endocrine parts. This arrangement also appears in most other birds[2,8,10,11,13,14,15] and vertebrates[27,28], whereas in some fishes the pancreatic cells founded within the liver tissue which also called the hepatopancreas[29,30,31,32] and even in some mammals[33], these cells separated from hepatic parenchyma bya thin layer of connective tissue[31]. In fact, the avian pancreas is mainly composed of exocrine glandsabout 99 %[4].

The current study also shows that the exocrine part in the both birds are composed of serous tubuloacinar glands and occupied a larger area of the pancreas. The shape of secretory acini varied from in shapes as globoid, oval elongated and amorphous. These shapes are based on the plane of section as documented in other birds[2,3,14,15,26]. These acini in the both birds consist of a single layer of variable numbercells, this finding is in agreement with the result of[4,8,13,15]. One of the striking findings in this study is the absence of the centroacinar cellin the center of aciniin the two species of birds, these cells are also absent in Mynah[13], but they are present in the central lumen of acini in many others vertebrates species[4,8,14,15,29,32,34].

Also results of the present study show that the structure of the ducts in the two birds pancreas was found to be similar to that described in previous studies[3,4,11,14,15,32]. Gulmez (2003) founded glands inside the connective tissue of the ductsstarting from the interlobular ducts to the point where the pancreas emptied its contentsinto the duodenum as well as inside themuscular layer of the pancreatic ducts. Those glands contained Centro-acinar cells and also had the same staining features as the acinus[3].

Our results showed that the endocrine part of the both birds areconsisted of various shapes and sizes of islets thatis scattered in the exocrine part, this islets, as in goose pancreas, its exact border between the endocrine part and endocrine part are not always distinguished[4,10]. These islets are composed from alpha (A) and beta (B) cells while the Delta cells don't noticed in the both birds,this finding is in agreement with the result of[4,11] in ostrich and goose while in other birds, the islets contained alpha (A), beta (B) and delta (D) cells[15,32,35,36]. These cells are distributed inalpha islets, beta isletsandmixed islets in Guineafowl,these results also documented in[15], whereas the mixed islets are the only type in Common gull, in general the avian pancreas is divided into light, dark and mixed islets[4,26,37], the mixed islets didn't appear in in goose and Mynah[4,10,13]. The islets of the both birds are very rich in blood capillaries, these also appeared in other birds[4,8,10,11,13-15].

Histochemically, current results showed that the pancreatic cellsin the both species of birds, whether the acinar cells or islets cells are moderately positive for PAS and Alcian Blue reactions and moderate to weak negative reaction to PB stain and negative reaction with SB stain, which indicate to the importance of the panceras in the metabolism of the birds and this also are comely with the results of previous studiesas[2,11,14].

In conclusion the anatomical features and histological structure of the pancreas in the Guinea fowl *Numida meleagris* and the Common gull *Laruscanus* was found to be similar to that of other avian species with the exception of some secondary characteristics.

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