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COMPARATIVE HISTOCHEMICAL STUDY OF PANCREAS IN EURASIAN COLLARED DOVE (*STREPTOPELIA DECAOCTO*) AND BUZZARD (*BEUTEO BEUTEO VULPINUS*)

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ABSTRACT

This study aimed to investigate and compare the histochemical structure of pancreatic tissue in healthy adult Collared Doves (*Streptopelia decaocto*) and Buzzards (*Beuteo beuteo vulpinus*) divided by their diet habitats. The birds were anesthetized with Ketamine, the pancreas was dissected, fixed with paraformaldehyde 10%, processed for paraffin embedding and histological sections were stained with hematoxylin and eosin, Masson trichrome and Gomori stain. The buzzards' pancreas is composed of capsule and parenchyma, which contains acini, ducts, along with blood vessels surrounded by connective tissue. Among the pancreatic acini, tiny irregular islets, single endocrine cells, and the intercalated duct could be identified. Magnified sections reveal the tiny irregular islets, Langerhans islet, interlobular connective tissue, and intercalated duct. Endocrine and exocrine parts, parenchyma, interlobular and interacinar connective tissue, alpha and beta cells, and blood capillaries under high magnification. The dove's pancreas (*Streptopelia decaocto*) was tiny and extended between the duodenum. Dove pancreas histology exhibited Interlobular connective tissue septa, interlobular duct, pancreatic islets, and acini containing zygomatic cells were found in dove pancreases. Myoepithelial cells, massive alpha and beta cell islets, and a connective tissue capsule surround the main duct and lumen. In conclusion, the histological structures of the pancreas in both birds were largely similar, with minor differences observed in the width of the pancreatic ducts and the number of alpha and beta islets. The histochemical study revealed variations in the intensity of different pancreatic components.

Keywords: Pancreas, Collared Dove, Buzzard, (*Beuteo vulpinus*), Sterptopelia decaota.

INTRODUCTION

The common buzzard (*Buteo buteo*), belongs to *Buteo Accipitridae*, is a large ubiquitous bird of prey. This species breeds over the Palearctic, western Siberia, Mongolia, and northwest China (Tian Shan) (Bowler, 2018). It dwells there in winter. Buzzards from the eastern part of their range and elsewhere in the northern hemisphere typically move south for the winter, some reaching South Africa (Winkler *et al*., 2017).

In 1838, a Hungarian naturalist named Imre Frivaldszky reported a bird that would later be given the scientific name "Eurasian collared Dove"(*Columba decaocto*) (Frivaldszky, 1838). Bulgaria Plovdiv serves as

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the type locality. Charles Lucien Bonaparte, a French ornithologist, reclassified it in 1855 as a member of the genus Streptopelia (Bonaparte, 1855). The Burmese collared Dove (*S. xanthocycla*) was previously thought to be a subspecies of the Eurasian Collared Dove; however, in 2021, the IOC officially recognized it as a separate species. *Streptopelia decaocto stoliczkae* of central Asia, and *S. d. intercedens* of southern India and Sri Lanka, were historically sometimes considered as separate subspecies. Nominal subspecies *S. d. decaocto* now has senior synonyms for both of them (van Grouw, 2022). The Eurasian Collared Dove forms a super species with its close relatives, the Sunda collared Dove of Southeast Asia, and the African Collared Dove of Sub-Saharan Africa. Although the African Collared Dove is slightly smaller and darker than the Eurasian variety, the two species may be easily distinguished from one another thanks to their distinctive calls: the quiet purring of the African species, in contrast to the cooing of the Eurasian variety.

Since alpha and beta cells of the pancreas are separated into two islets, the avian pancreas is a good model for studying changes in its morphology and function (Gannon *et al*., 2018). The avian pancreas is lobular, unlike rat, squirrel, rabbit, and other lagomorph pancreatic tissue. An adult chicken's pancreas 10-15 cm length and 2-3 cm breadth fit snugly in the duodenal U-loop (Theodory *et al*., 2022). The tongue-shaped organ is suspended and invested by a complex circulatory system that creates a network of vessels between the duodenal loop and the pancreatic organ (Hörnblad *et al*., 2016). The pancreas has two parts: exocrine (pancreatic acini and duct system) and endocrine (islets of Langerhans). Thin connective tissue capsules with tiny collagen fibers encased the pancreas and in between the acini (Mahadevan, 2019). The pancreas is a large dual-function gland, having both exocrine and endocrine components. It plays an important role in the digestive and endocrine systems of vertebrates. The main bulk of the gland is formed of exocrine component. It secretes an alkaline fluid into the duodenum through the pancreatic ducts which is rich in enzymes Beheiry *et al*. (2018). The endocrine part which although forms very little part of the gland, secretes several imperative proteinaceous hormones including insulin, glucagon and somatostatin into the circulation (Akhtar *et al*., 2020). This study aimed to investigate and compare the histochemical structure of pancreatic tissue in adult Collared Doves (*Streptopelia decaocto*) and Buzzards (*Beuteo beuteo vulpinus*) as representative of two different diet habitats.

MATERIALS AND METHODS

Ten healthy adult Collared Doves (*Streptopelia decaocto*) and Buzzard (*Beuteo beuteo vulpinus*), five of each species, based on food type were employed in the histological investigation. Local vendors in Babylon province sold all kinds of birds. Ketamine was injected intravenously at 15 milligrams per kilogram of body weight after the birds were weighed on a digital scale (Sartorius, Germany) (Schmidt, 2024). After an abdominal laparotomy and sternum cranial displacement, the pancreas was carefully dissected and washed in normal saline to remove any blood or debris. The procedure to acquire these sections is detailed in research by Kong *et al*., (2018). Tissue samples were fixed in 10% neutral buffered formalin solution for 72 hours. After the fixation of tissue samples, the specimens were washed in PBS, dehydrated in alcohol, cleared in xylene and embedded in paraffin. Paraffin blocks were cut at 5 µm thickness and the sections were stained with hematoxylin and eosin for general structures (Suvarna, Layton et al. 2018; Ahmed 2016), and Masson trichrome stain for identification of elastic and collagen fibers (Muhson and Dawood 2023), and Gomori stain to bring attention to collagen and muscle fibers (Bacha Jr and Bacha 2012). The sections were examined using Olympus Light microscope with the digital camera connected to the computer.

RESULTS

In this study, the histological section of pancreas of buzzard showed a thin capsule, acini and duct (Figure 1). The pancreas parenchyma contained blood vessels within the interlobular connective tissue and among pancreatic acini and intercalated duct (Figure 2). The magnified pictures showed pancreatic acini, small irregular islets, solitary endocrine cells and intercalated duct, interlobular connective tissue (Figure 3). Masson trichrome stain showed the interlobular and interacinar connective tissue, parenchyma including endocrine portion (light stained) and exocrine portion (dark stained) (Figure 4). The high magnification showed islet (alpha cells, beta cell) and blood capillaries (Figure 4). The mean difference of the diameter between the interlobular duct, intra-lobulated, intercalated of birds' pancreas were investigated. There was statistically significant difference between them. The mean diameter of Inter interlobular duct of pancreas dove was (0.0012±0.0001) µm decrease than the mean of interlobular duct of pancreas buzzard was (0.0019±0.0002). In addition, the mean of interlobular duct of pancreas dove was (0.0041±0.0012) decrease than the mean of Intra-lobulated of pancreas buzzard was (0.0058±0.0014). However, the mean of Intercalated of pancreas dove was (0.0201±0.004) decrease the mean of Intercalated of pancreas buzzard was (0.0259±0.002) Table (1).

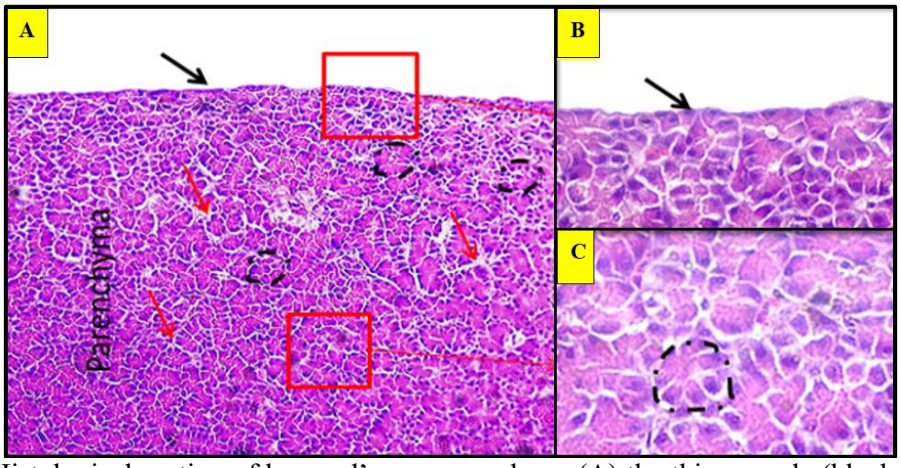

Figure (1): Histological section of buzzard's pancreas show: (A) the thin capsule (black arrow), acini (black circle), and duct (red arrow). H&E stain. 10X. (B) magnified section show capsule (arrow). H&E stain.40x. (C) Magnified section show the acini (circle). H&E stain.40X.

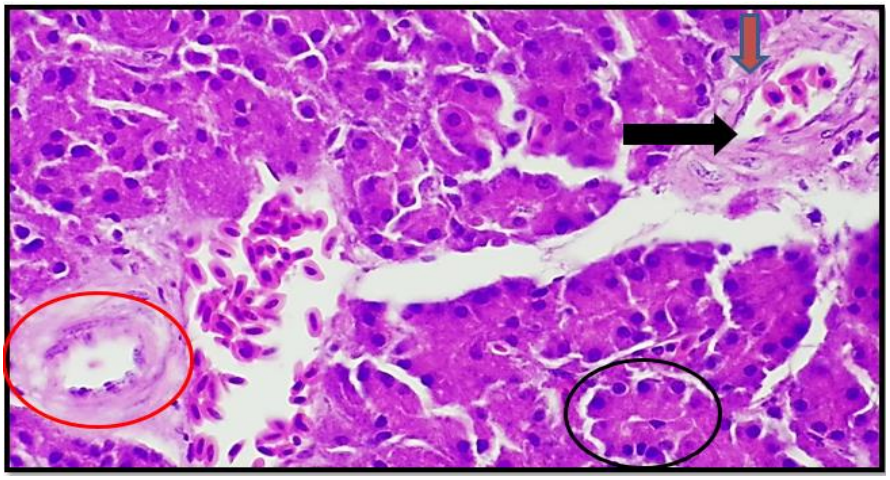

Figure (2): Histological section of pancreas of buzzard shown parenchyma of the pancreas with blood vessels (black arrows) within the connective tissue around it (red arrow) among pancreatic acini (black cercle) intercalated duct (red circle). H&E buzzard 40X.

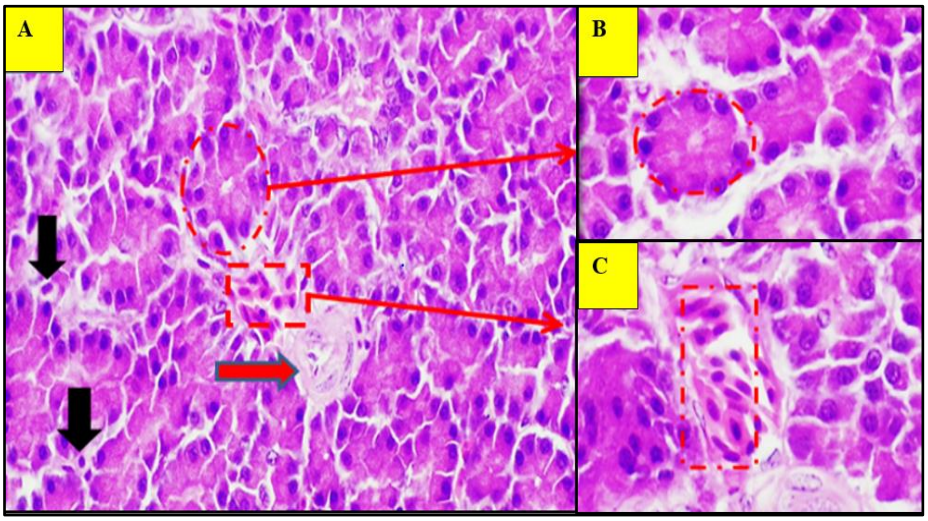

Figure (3): Histological section of buzzard's pancreas shows: (A) pancreatic acini (red circle), small irregular islets (red square). Interlobular connective tissue cells (black arrow), intercalated duct (red arrow) H&E. stain. 40X. (B) Acinus (red circle) H&E stain. 100X, (C) magnified section show blood vessel contains RBCs (red square), H&E stain. 100X.

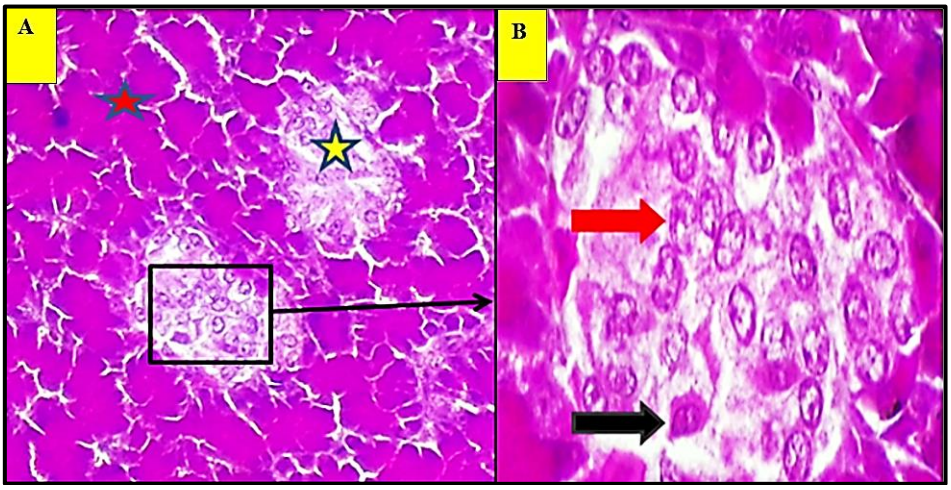

Figure (3): Histological section of buzzard pancreas showing: (A) Endocrine pancreas islet of Langerhans (black square & yellow star), Exocrine pancreas (red star), (B) high magnified section of islet show Alpha cell (red arrow), Beta cell (black arrow) Gomori stain (A) 40X,(B) 100X.

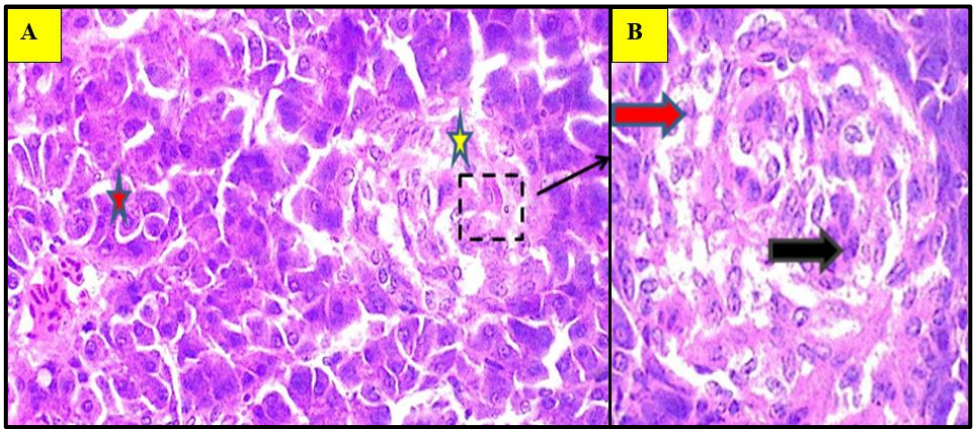

Figure (4): Histological section of buzzard pancreas show (A) endocrine portion (yellow star) and exocrine portion (red star) (B) high magnification section show islet with their alpha cells (red arrow), beta cell (black arrow) and blood capillaries, H&E stain 40X.

In this study, the histological section of pancreas in Dove showed interlobular connective tissue septa, interlobular duct, pancreatic islet and their cells acini with zygomatic cells (Figure 5). so, histological section of pancreas in dove showed main duct and the lumen of duct that surrounded by myoepithelial cell (Figure 6), large islets Alpha cells and Beta cells and connective tissue capsule (Figure 7), in addition, the histological section of pancreas in Dove show acini with Inter lobar septa, intercalated duct and blood vessels (Figure 8), small irregular islets, pancreatic acini with Centro acinar cells in the center of acini (Figure 9). Figure (10,11) duct system, connective tissue septa, acinus and acinus lumen, and the mean difference of diameter of Alfa, Beta and acini in island of pancreas birds were investigated, the results showed that, there were statistically significant difference between diameters of them. The mean of diameter of Alfa in island of pancreas dove was 0.0029±0.0004 µm and the mean of diameter of Alfa in island of pancreas buzzard was 0.0036±0.0003 µm. In addition, the mean of diameter of beta in island of pancreas dove was (0.0342±0.009) and the mean of diameter of beta in island of pancreas buzzard was 0.0483±0.0051 µm. However, the mean of diameter of acini of pancreas dove was 0.0101±0.0049 µm and the mean of diameter of acini of pancreas buzzard was 0.0214±0.0031 µm Table (2).

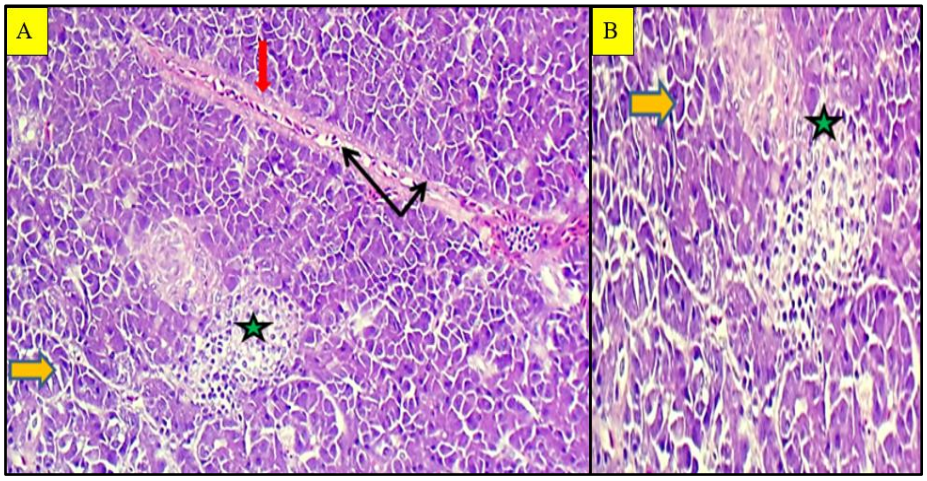

Figure (5): Histological sections of pancreas in dove show: long blood vessels (red arrow), contain RBCs (black arrow), pancreatic islet and their cells (green star) and pancreatic acini (yellow arrow) H&E stain (A) 20X. (B) 40X.

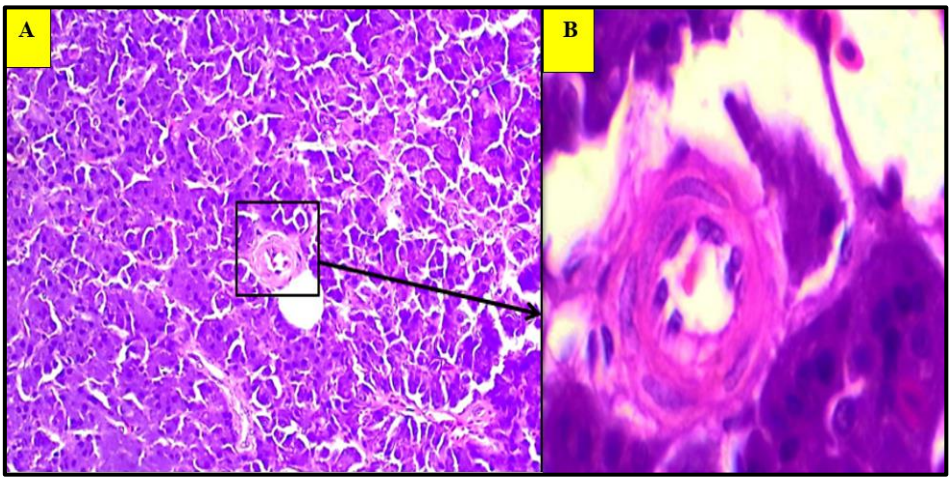

Figure (6): Histological section of pancreas in dove show: (A) main duct (square) H&E stain. 20X, (B) magnified section of main duct show the lumen of duct and surrounded by Myoepithelial cell. H&E stain 100X.

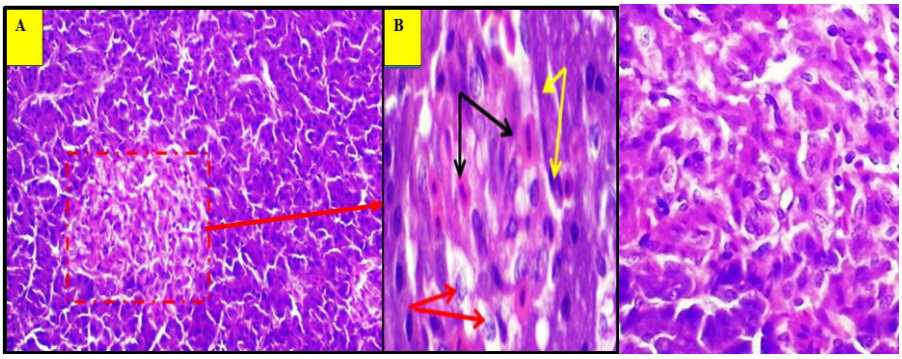

Figure (7): Histological section of pancreas in dove show (A) large islets (red square) H&E stain. 20X, (B) Alpha cells (black arrow), and Beta cells (red arrow), connective tissue capsule (yellow arrow). H&E stain 100X.

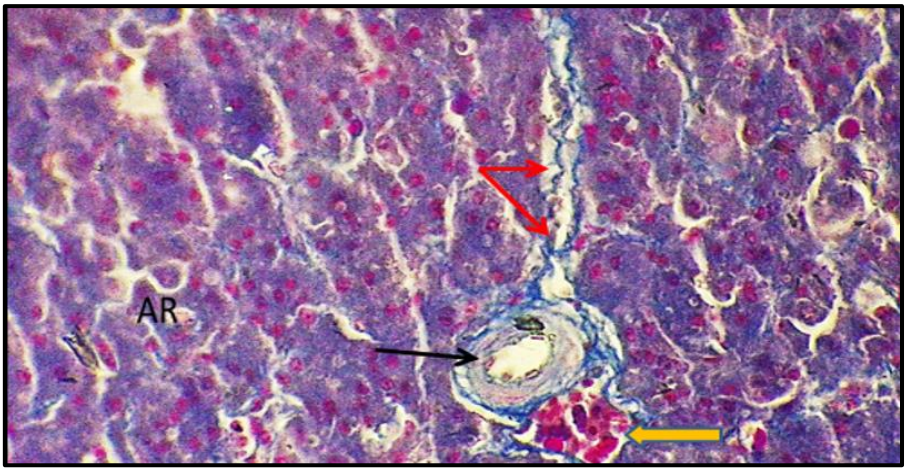

Figure (8): Histological section of pancreas in dove show acini with Interlobar septa (red arrow), intercalated duct (black arrow) and blood vessels (yellow arrow), acini region (AR) Masson trichrome stain 40X.

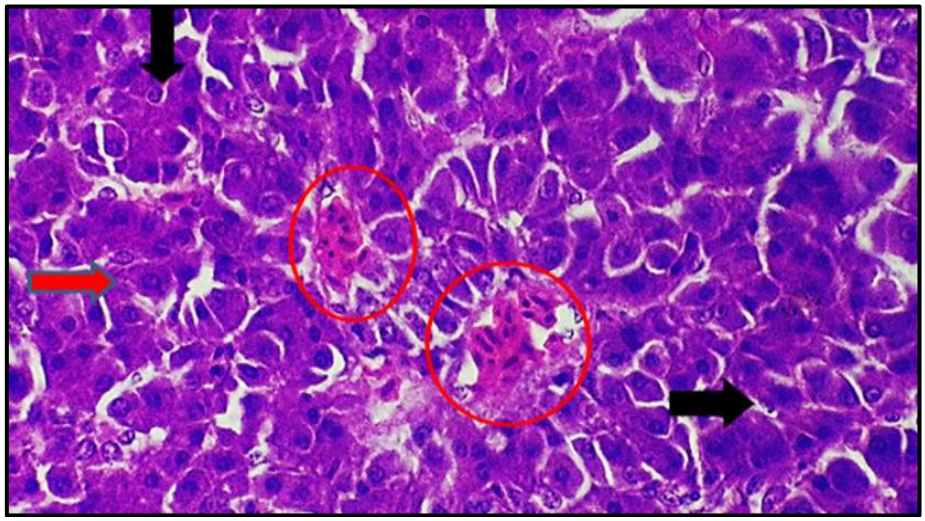

Figure (9): Histological section of pancreas in dove show small irregular islets (red circle), pancreatic acini (red arrow) with centroacinar cells (black arrows) in the center of acini, H&E stain, 40X.

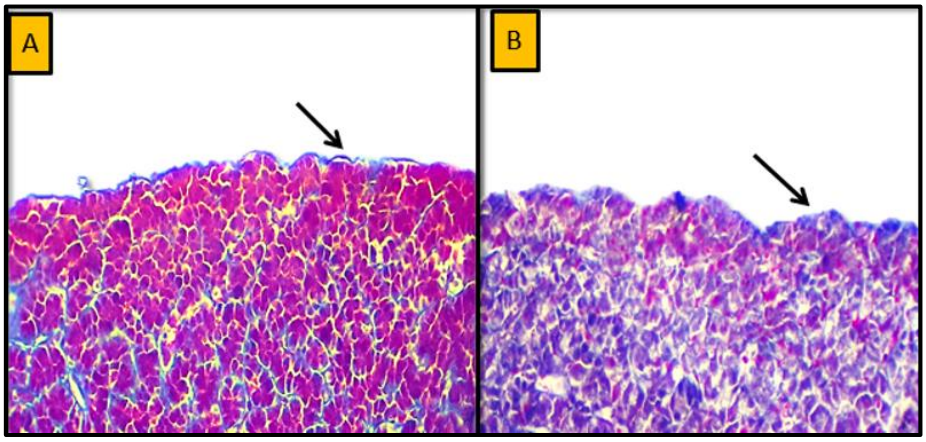

Figure (10): Cross-section of pancreas show: connective tissue capsule in buzzard and dove (black arrow) (A), (B) Masson trichrome 20X, with interlobar septa of connective septa.

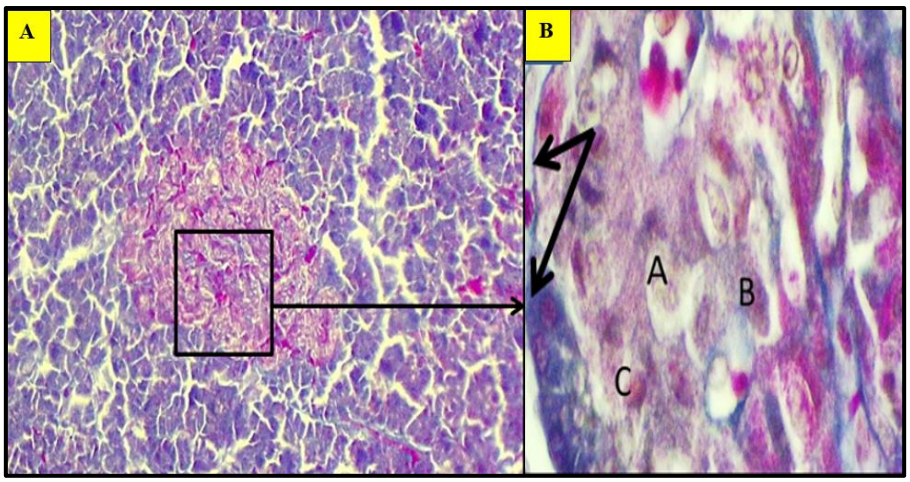

Figure (11): Histological section of pancreas in dove show: large irregular islet (black square) 20X. (B) Magnified section of pancreatic islet show Alpha cells (A) and Beta cells (B) with capillaries (C), connective tissue capsule (black arrow) Gomori trichrome stain. 100X.

Table 1: The mean diameter of interlobular, intralobulated, and intercalated ducts of pancreas of birds (µm) (Dove, Buzzard)

Groups / Diameter	Dove	Buzzard	P value
	Mean± SD		
Inter lobular duct	0.0012±0.0001	0.0019±0.0002	0.018(S)
Intra-lobulated	0.0041±0.0012	0.0058±0.0014	0.031(S)
Intercalated	0.0201±0.004	0.0259±0.002	0.023(S)

S: Significant difference at P<0.05

Table 2: The mean diameter of Alfa, Beta cells and acini in pancreas of birds (µm) (Dove, Buzzard)

Groups / Diameter	Dove	Buzzard	P value
	Mean± SD		
Alfa	0.0029±0.0004	0.0036±0.0003	0.039(S)
Beta	0.0342±0.009	0.0483±0.0051	0.020(S)
Acini	0.0101±0.0049	0.0214±0.0031	0.001(S)

S: Significant difference at P<0.05

DISCUSSION

This study focused on the histological description of the pancreas of doves and buzzards. The pancreas of doves contains a more developed endocrine system, characterized by a higher quantity of islets of Langerhans. These islets are responsible for producing insulin and glucagon, suggesting an enhanced functional capacity in regulating blood sugar levels (Yadav *et al.,* 2020).

The pancreatic tissue in buzzards showed the same histological architecture as that of doves but the size of islets showed some differences. The exocrine part of the gland with the presence of acini is also prominent in Dove to cover all their digestive requirements (Beheiry *et al*., 2020; Mahmood *et al*., 2022). Pancreatic ducts are present and pronounced in both pigeons and Dove; however, the width and the disposition of the ducts might be different. In examining doves and buzzards, we noticed interlobular ducts and myoepithelial cells adjacent to the ducts that are similar to the structure of buzzards's digestive system (Al-Khakani *et al*., 2019). The research further proposes that dove's endocrine to exocrine mass ratio may be higher than that of the buzzards. It could be due to their feeding patterns and their physiology may therefore require a tighter hormonal control of metabolism (Zhu *et al*., 2022). Conversely, due to being more carnivorous, buzzards may have had a more sophisticated pancreas to assist with the exocrine functions in digesting food. Doves have more, and larger, pancreatic islets than do buzzards. It might be due to the difference in the metabolic rate of these birds due to the feeding habits of these birds (Zhang *et al*., 2018).

The histochemical methods applied in the researched works include H&E and Masson trichrome, revealed visible discrepancies of the connective tissue surrounding acini and islets. It has been demonstrated that the connective tissue capsule of doves may be thicker around the islets than that of falcons, which can influence the functional aspects of the pancreas (Baskin 2015). Despite most similarities in histological analysis of the pancreas of both dove and pigeons, there are differences in the endocrine/exocrine ratios, further branching of the pancreatic duct and the morphological arrangement of the pancreatic islets (Isidoro-Ayza *et al*., 2017). These differences were possibly due to the differences in diet and their basal metabolic rate.

This study's findings were in line with those of TAŞÇI *et al*. (2018), who also discovered that histological sections of a buzzard's pancreas would most likely have features comparable to those seen in other birds or mammals. In addition to its endocrine and exocrine roles, the pancreas was a multi-function organ. The exocrine pancreas was made up of groups of acinar cells that produce digestive enzymes, according to research by Dolenšek *et al*. (2017). Connected to ducts, these cells were grouped in clusters like grapes. In addition, Ouyang & Wang (2016) found these acinar cell clusters with central lumens in a histological slice. The Islets of Langerhans, which are groups of endocrine cells, were discovered dispersed throughout the exocrine tissue. Based on research by (Da Silva Xavier, 2018), these islets house several cell types, such as alpha cells (which produce glucagon), beta cells (which produce insulin), delta cells (which produce somatostatin), and others. According to Bader *et al*. (2016), different cell types inside these islets would cause them to color differently in a histological slice. According to Urbas *et al*. (2021), histological slices of the pancreas might also show the connective tissue that supports the organ and the blood vessels that feed it. Histological sections may also reveal the nerve fibers that innervate the pancreas. Pancreatic acini were seen, which were groups of exocrine cells, mostly acinar cells that make digesting enzymes. These structures resemble grapes and have center lumens. Different staining methods may provide different results when used to these cells (Doneley, 2018). Connective tissue encircles the pancreatic acini and supports the glandular tissue (Campbell *et al*., 2021). The

nerve fibers, blood arteries, and other underlying components were all housed in this connective tissue. Blood vessels would be evident inside the connective tissue around the pancreatic acini. These blood arteries allow the pancreatic tissue to receive oxygen, nutrients, and remove the waste products. The appearance of the vessels may range from tiny capillaries to bigger ones like arterioles and venules, all depending on their size (Mahmood *et al*., 2022). Previous study of Wehrwein *et al*. (2016) found nerve fibers may also be present within the connective tissue, responsible for regulating the function of the pancreas through the autonomic nervous system. The connective tissue surrounding the pancreatic acini and blood vessels was collectively referred to as the stroma. It provides structural support to the glandular tissue and contains various cell types, including fibroblasts and immune cells (Maneshi *et al*., 2021). Buzzard islets of Langerhans and tiny clusters of endocrine cells were discovered in the histological section of the pancreas of a buzzard (Al-Haaik, 2019). They include a variety of cell types, such as beta and alpha cells. The Islets of Langerhans included a variety of cell types, one of which being beta cells. The hormone insulin, which controls blood sugar levels by encouraging glucose absorption into cells, was produced and secreted by these organs (Ornellas *et al*., 2020). One other kind of cell observed in the Langerhans islets was identified by Robertson, (2023) as alpha cells. In order to raise blood sugar levels, they release and create the hormone glucagon, which stimulates the liver to break down glycogen into glucose. Research suggests that the distinct physiological demands and evolutionary adaptations of buzzards and doves would be evident in the histological sections of their pancreas (Doneley, 2018). In contrast to doves, who mostly consume seeds, buzzards, which were once birds of prey, devour meat (Hyder *et al*., 2023). Buzzards secrete digestive enzymes to break down the proteins in meat, which means their pancreas can be smaller and have more exocrine tissue. Doves may have a more extensive pancreatic endocrine system, including more islets of Langerhans, a gland responsible for producing insulin and glucagon, and other hormones that help control blood sugar levels, (Abd & Faris, 2022).

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دراسة مقارنة للنسيج الكيميائي للبنكرياس في اليمام الأوراسي (Streptopelia decaocto) والصقر الحوام (Beuteo beuteo vulpinus)

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صممت هذه الدراسة لوصف مقارنة للنسيج الكيميائي للبنكرياس في اليمام الأوراسي (Streptopelia decaocto) والصقر الحوام (Beuteo beuteo vulpinus) أجريت الدراسة على 10 يمامات بالغة صحية (Streptopelia decaocto) و10 طيور من الصقر الحوام (Beuteo beuteo vulpinus) مقسمة حسب النظام الغذائي الهذه التجربة، وتم حقن الكيتامين عن طريق الوريد بجرعة 15 مليجرام لكل كيلوجرام من وزن الجسم بعد وزن الطيور على ميزان رقمي. بعد فتح البطن وإزاحة عظمة القص، تم تشريح البنكرياس وغسله بمحلول ملحي طبيعي لإزالة الدم والحطام. النتائج: يظهر بنكرياس الصقور مكونًا من كبسولة وحويصلات وقنوات ونسيج عضلي، إلى جانب الشرايين الدموية في النسيج الضام المحيط بالحويصلات والقناة المتداخلة. اظهرت المقاطع المكبرة عن الجزر الصغيرة غير المنتظمة وجزر لانجرهانز والنسيج الضام بين الفصيصات والقناة المتداخلة. الأجزاء الصماء والإفرازية، النسيج العضلي، النسيج الضام بين الفصيصات وبين الأسينات، الخلايا ألفا وبيتا، والشعيرات الدموية تحت تكبير عالٍ. كان بنكرياس اليمام (Streptopelia decaocto) صغيرًا وممتدًا بين الاثني عشر. أظهرت النتائج النسجية بنكرياس اليمام تتكون من حواجز نسيج ضام بين الفصيصات وقنوات بين الفصيصات وجزر بنكرياسية ونسيج عضلي يحتوي على خلايا العضلية في بنكرياس اليمام. الخلايا العضلية الظهارية، وجزر الخلايا ألفا وبيتا الضخمة، وكبسولة النسيج الضام تحيط بالقناة الرئيسية والتجويف. استنتجت الدراسة أن التركيب النسيجي للبنكرياس لدى كلا الطائرين كان متشابهًا، مع اختلافات طفيفة في قطر القناة البنكرياسية، وعدد جزر الخلايا ألفا وبيتا، وسجلت الدراسة النسيجية الكيميائية للبنكرياس اختلافات في بعض مكوناته.