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Histological and Immunohistochemical Investigation of Pancreatic Buffaloes

Muqdad Ahmed Shihab [1] *, Nasam Emad Daim [2]
[1, 2] Department of Anatomy, College of Medicine, University of Al-Qadisiyah, Al-Qadisiyah, Iraq
Emails: Muqdad.shihab@qu.edu.iq [1], nasam.alfoudi@qu.edu.iq [2]
* *Corresponding author*

Abstract
This study was done to describe distribution of endocrine and exocrine pancreatic cells in water buffalo. Hence, a total of 25 fresh pancreas samples were collected from slaughtered adult buffaloes in Al-Diwaniyah slaughterhouses (Al-Qadisiyah, Iraq), and then washed, processed, stained and examined. The findings revealed that there were a compound tubulo-acina and lobulated gland surrounded with thin connective tissue capsule, blood arteries, nerves, lymphatic vessels, adipose cells with collagen and elastic fibers. The endocrine tissue, islets of langerhans, displayed clear regular structure immersed within the acinar tissue and delineated by well define delicate connective tissue that contain blood vessels which give the islets spherical shape. The connective tissue involved in separating the endocrine tissue from exocrine. The abundant beta cells appeared normal, rounded to polygonal and stained blue. The smaller number of alpha cells took round to oval shape and almost stained red in color with distinct nuclei. The exocrine sections of the gland were the largest, and these glands were tubuloacinur glands, and each acinus consisted form single layer of large glandular pyramidal shaped epithelial cells resting on basement membrane, the acinar cells contained spherical nucleus basally near the basement membrane, and these cells had large, spherical and homogenous zymogens granules accumulate the cytoplasm in apex. Pancreatic endocrine cells were seen as groups, and separated from exocrine units by very thin layer of connective tissue that completely surrounded the islets. There was a large network of blood capillaries around the islets, and each islet has its own capillary network that is in communication with every other islet. Endocrine cells had an uneven branched cord-like structure that is mixed in with blood arteries. Immunohistichemical examination in the present finding elucidated the Langerhans islets in the pancreas of buffaloes via the using of glucagon and insulin antibody, and detected that the immune reaction varied in density in the islets of pancreas in their two lobes. The immunohistochemical reaction for glucagon and insulin was found three types of islets in the pancreas; alpha (dark), beta (light) and mixed islets. In conclusion, the studying of buffalo animal as it is one of the local economic animals, and comparing between the tissue structure of pancreas of all animals and relationships between them are recommended.

Keywords: *Bubalus bubalis*, Water buffaloe, Histology, Immunohistochemistry, Langerhans, Iraq

Introduction
The name of buffalo is applied to several different cud-chewing ruminants of the *Bovidae* family; however, the true Indian buffalo (*Bubalus bubalis*) known as water buffalo exists both as wild and domestic animal [1, 2]. Worldwide, buffalo considers as one of the most important agricultural animals that spread in various parts of Asian, African, South American and some Mediterranean countries [3]. In Iraq, water buffaloes are well adapted to swamps

and areas subject to flooding such as the marshes that located in the middle and southern parts of the country, and feed on the plants existed in marshes as well as on protein-rich concentrated food when available [4, 5]. This animal is primarily raised to produce milk, meat and other products, in addition to its use as for work on farms as in Egypt and Southeast Asia [6, 7].

Morphologically, buffaloes are large in size reaching 900 kg weight, 115.2-128 cm body length, 207.2-223.8 cm chest girth and having a black or gray color and sometimes irregular white spots on chest, legs and tail with presence of long and curved toward the back horns [8]. In digestive system, the stomach is composed mainly from four separate chambers like other ruminants; cattle, sheep and goats which responsible for fermentation, digestion and absorption [9]. The pancreas is an accessory composite organ in the digestive system located on the roof of the abdominal wall, and contains the tubular acini that having the exocrine and endocrine functions [10, 11]. While some digestive enzymes are secreted and stored by the exocrine, the endocrine system is made up of various scattered cell aggregates known as Langerhans islets that composed of five cell types are α -cells (glucagon-producing cells), β -cells (insulin-producing cells), δ-cells (somatostatin-producing cells), ε-cell (ghrelin-producing cell) and υ-cell [pancreatic polypeptide-producing cells (PP)] that makes up the majority of the endocrine cells in the mammalian pancreas [12, 13]. The different distribution of these cells is related to their regulatory mechanism, and the hormonal secretions of insulin and glucagon have a function on these interactions through positive-negative insulin feedback according to the need for these hormones in various species [14, 15]. Due to the low numbers of studies that described the different aspects of the animal in particular physiology, anatomy and clinics with the great economic importance of buffaloes in Iraq, this study was done to describe the histological and immunohistochemical distribution of endocrine and exocrine pancreatic cells.

Materials and methods

Samples

A total 25 fresh pancreas samples were collected from adult buffaloes of different ages and sexes, after buffaloes were slaughtered in Al-Diwaniyah slaughterhouses (Al-Qadisiyah, Iraq). The samples were washed with 0.9% normal saline to remove traces of blood and avoiding the postmortem changes, each organ was transferred to a large volume of fixative. The fixative was used 10% neutral buffered formalin.

Histology

Small pieces from each lobes of the pancreas were washed by 0.9% normal saline and then fixed in 10% formalin solution for 24 hours, then carefully rinsed with tap water for 4-5 hours to remove the fixative and dehydrated by passing it through ascending series of concentrations gradients of ethanol. Then the samples were cleared by immersing them in a solvent xylene, infiltrated, and embedded in paraffin wax. A 4-5 μm thick paraffin sections were obtained and were stained by Hematoxylin and Eosin (H & E) stain. Light microscope was served to scan all the slides, photos of the sections were then taken using a digital camera.

Immunohistochemistry

Sections were also prepared for routine immunohistochemistry procedures to identify the density of glucagon and insulin by using: glucagon and insulin primary antibodies, as directed by the manufacture (Bio vision). Tissue sections were dewaxed with xylene before being rehydrated in an alcohol gradient with distilled or deionized water. After incubating sections with peroxidase block for 5-10 minutes at room temperature, rinse 3 times by purified water.

At room temperature, incubate portions in protein blocking solution for 5-10 minutes. The slides are then washed three times with PBS. Then at room temperature incubate the sections with one- step HRP polymer for 20-30 minutes. A 1 ml reagent BS and 50 ml of reagent C (DAB chromogen) were well mixed in a test tube with adding few drops of ready to use DAB reagent to tissue slides that incubated for 6-10 minutes at room temperature. Then, the slides were washed with deionized or distilled water, and incubated for 30-60 seconds with a counter stain (hematoxylin compatible with DAB). Finally, the slides were wiped in deionized or distilled water, dried, examined microscopically and photographed using a digital camera [16].

Results

In the current study, the pancreas of buffalo was mixed gland which refers to its dual function as exocrine and endocrine. It was a compound tubulo-acina gland a lobulated gland surrounded with thin connective tissue capsule with blood arteries, nerves, lymphatic vessels, adipose cells with collagen, and elastic fibers which send the septa of connective tissue to divide the pancreas into lobules that appeared as regular tissues with clear organization of acinar cells. The cells possess clear basal nuclei with amphophilic cytoplasmic stain. The endocrine tissue (islets of langerhans) displayed clear regular structure immersed within the acinar tissue and delineated by well define delicate connective tissue that contain blood vessels which give the islets spherical shape. The connective tissue involved in separating the endocrine tissue from exocrine. The islets have regular shape and size with normal cellular pattern arrangement and cells number. The abundant beta cells appeared normal, rounded to polygonal and stained blue. The nuclei can be seen clearly throughout the islet tissue. The smaller number of alpha cells took round to oval shape and almost stained red in color with distinct nuclei. The exocrine sections of the gland were the largest, and these glands were tubuloacinur glands, the acinar adenomer was predominant than the tubular adenomer. Each acinus consisted form single layer of large glandular pyramidal shaped epithelial cells resting on basement membrane, the acinar cells contained spherical nucleus basally near the basement membrane, and these cells had large, spherical and homogenous zymogens granules accumulate the cytoplasm in apex. Pancreatic endocrine cells were grouped in a group in the pancreas gland, Pancreatic islets, also known as Langerhans islets, are a type of cell that has round, oval, and irregular shapes. The exocrine cell is distinguishable from the cells of the pancreatic islets by its whiter appearance. The pancreatic islets of Langerhans separated from exocrine units by very thin layer of connective tissue that completely surrounded the islets. There was a large network of blood capillaries around the islets, and each islet has its own capillary network that is in communication with every other islet. Endocrine cells had an uneven branched cord-like structure that is mixed in with blood

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arteries (Figures 1-3). Immunohistichemical examination in the present finding elucidated the Langerhans islets in the pancreas of buffaloes via the using of glucagon and insulin antibody, and detected that the immune reaction varied in density in the islets of pancreas in their two lobes. The immunohistochemical reaction for glucagon and insulin was found three types of islets in the pancreas; alpha (dark), beta (light) and mixed islets (Figure 4).

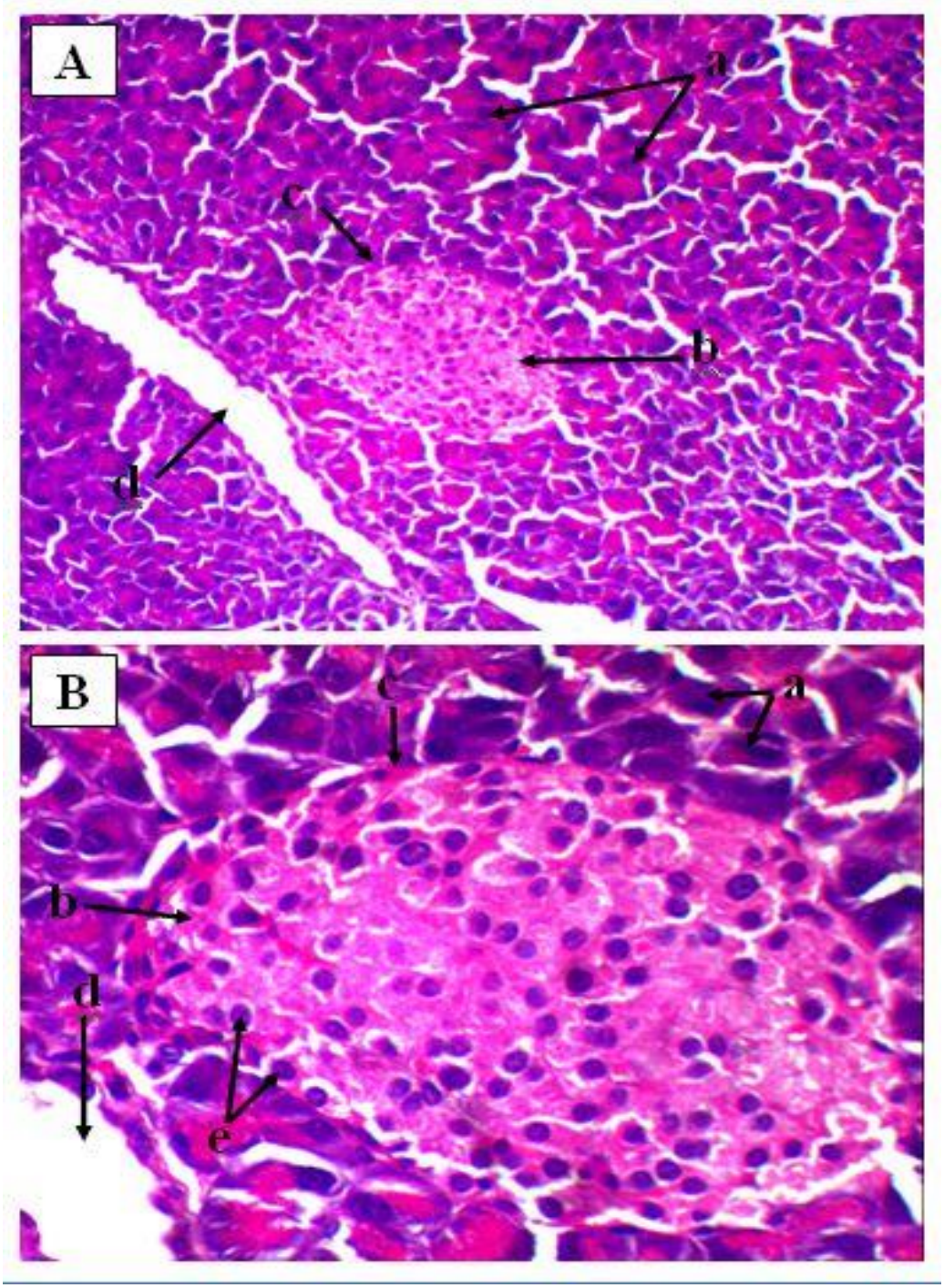

Figure (1): Histological structure of pancreas in buffalo; A (a): Exocrine acinar cells; (b): Islets of Langerhans; (c): Connective tissue fibers; (d): Pancreatic duct, 10X; (B) (a): Exocrine acinar cells; (b): Islets of Langerhans; (c): Connective tissue fibers; (d): Pancreatic duct; (e): Pancreatic endocrine cells. Images represent the histology of pancreas stained with H&E (40X).

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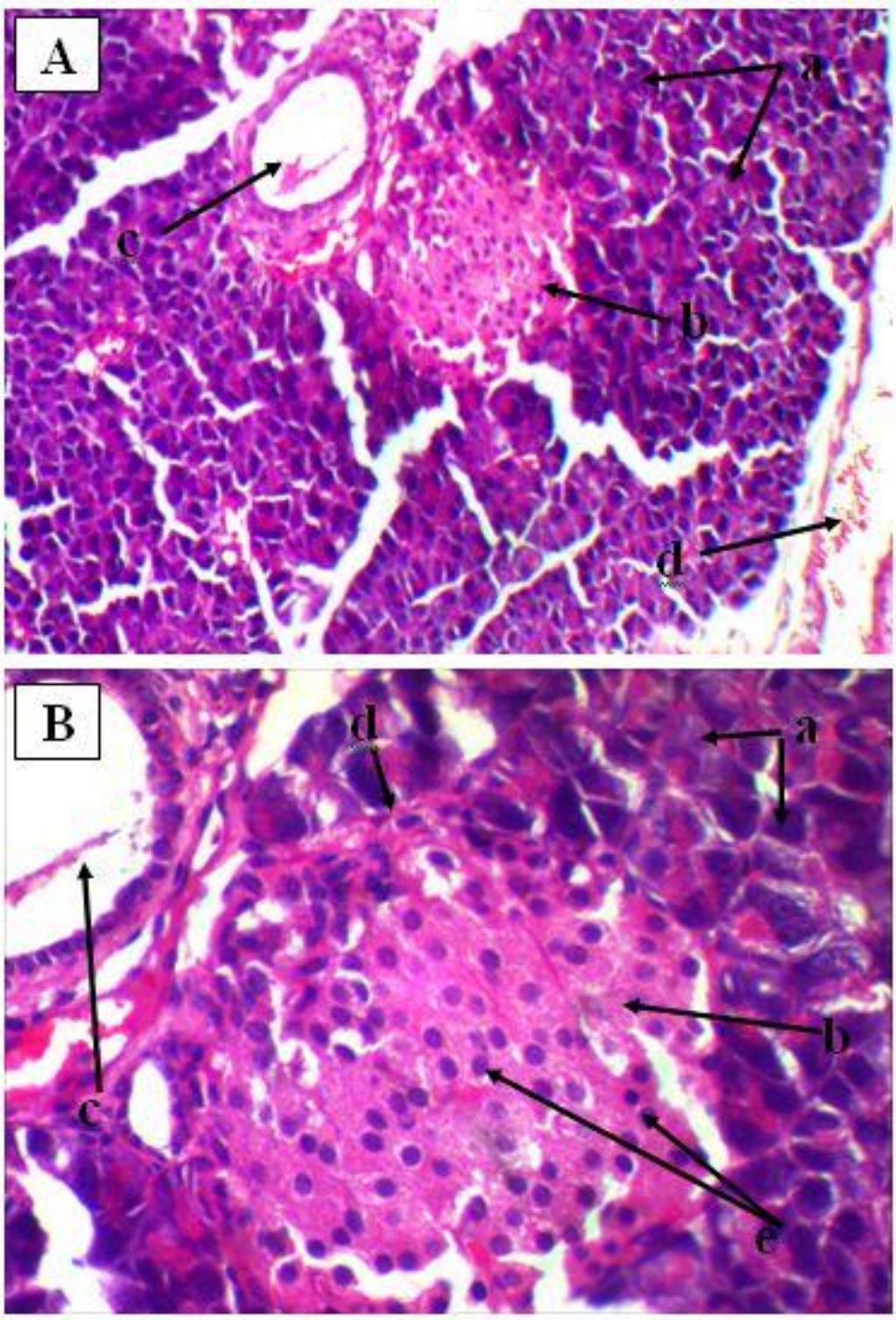

Figure (2): Histological structure of pancreas in buffalo; (A) (a): Acinar cells; (b): Islet of Langerhans; (c): Pancreatic duct; (d): Blood vessel bleeding, 10X. (B) (a): Acinar cells; (b): Islet of Langerhans; (c): Pancreatic duct; (d): Connective tissue covering the islet; (e): Endocrine cells. Images represent the histology of pancreas stained with H&E (40X).

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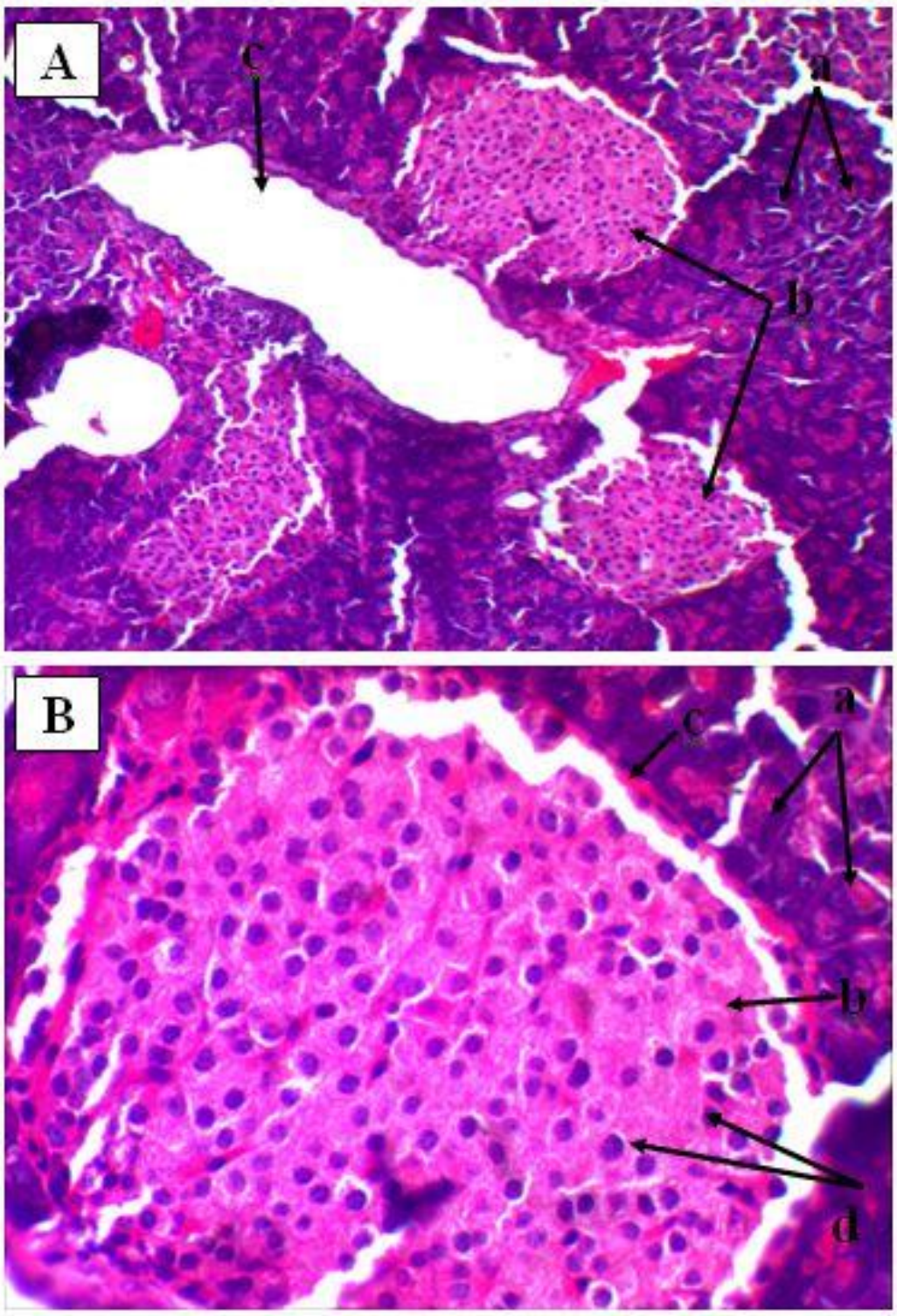

Figure (3): Histological structure of pancreas in buffalo; (A) (a): Acinar cells; (b): Islet of Langerhans; (c): Pancreatic duct, 10X. (B) (a): Acinar cells; (b): Islet of Langerhans; (c): Connective tissue covering the islet; (d): Endocrine cells. Images represent the histology of pancreas stained with H&E (40X).

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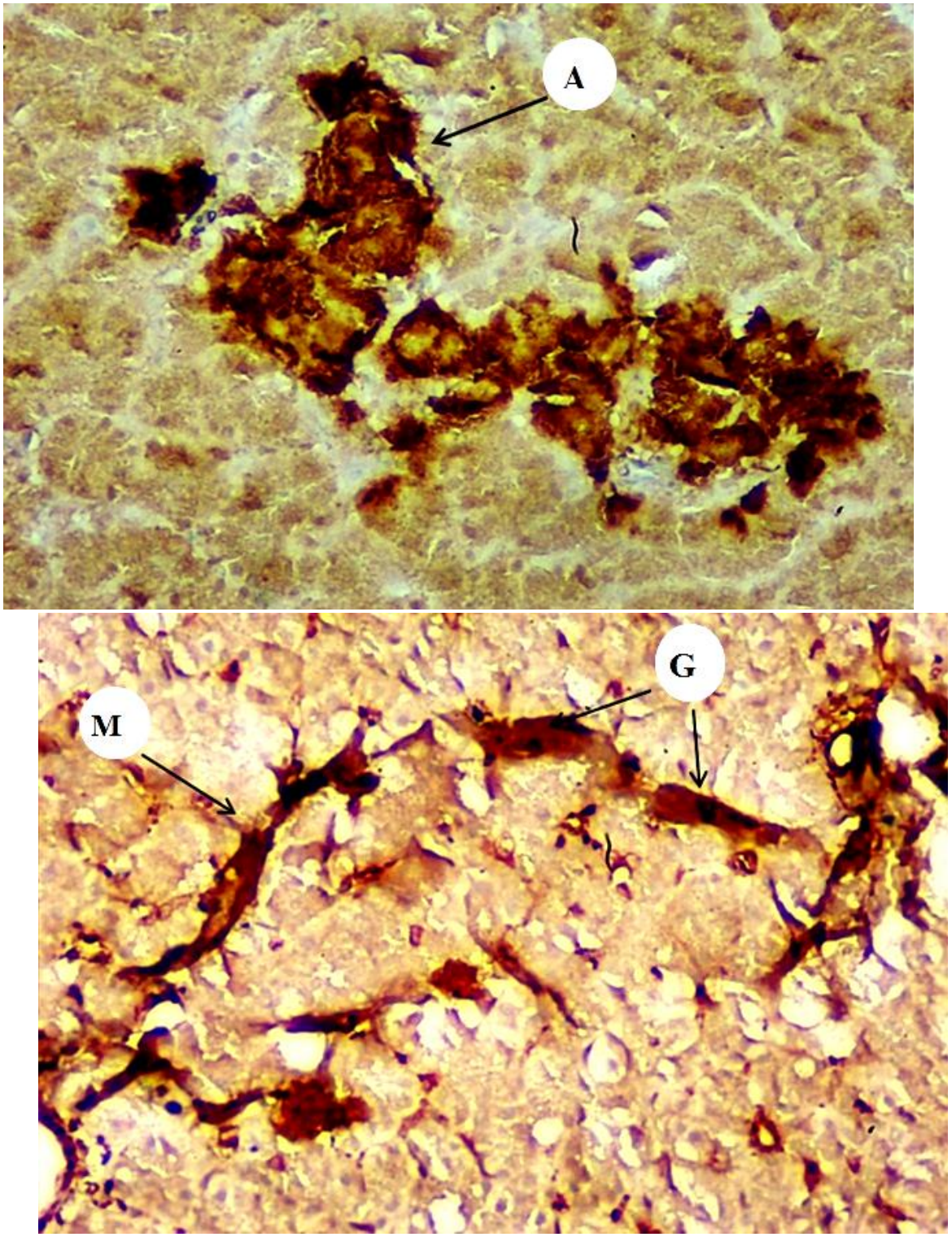

Figure (4): Immunohistochemical structure of pancreas in buffalo; (A): Alpha islets, (M): Mixed islets, and (G): Glucagon. Images represent the immunoreactive tissue stained with DAB and Mayer's Hematoxylin (200X).

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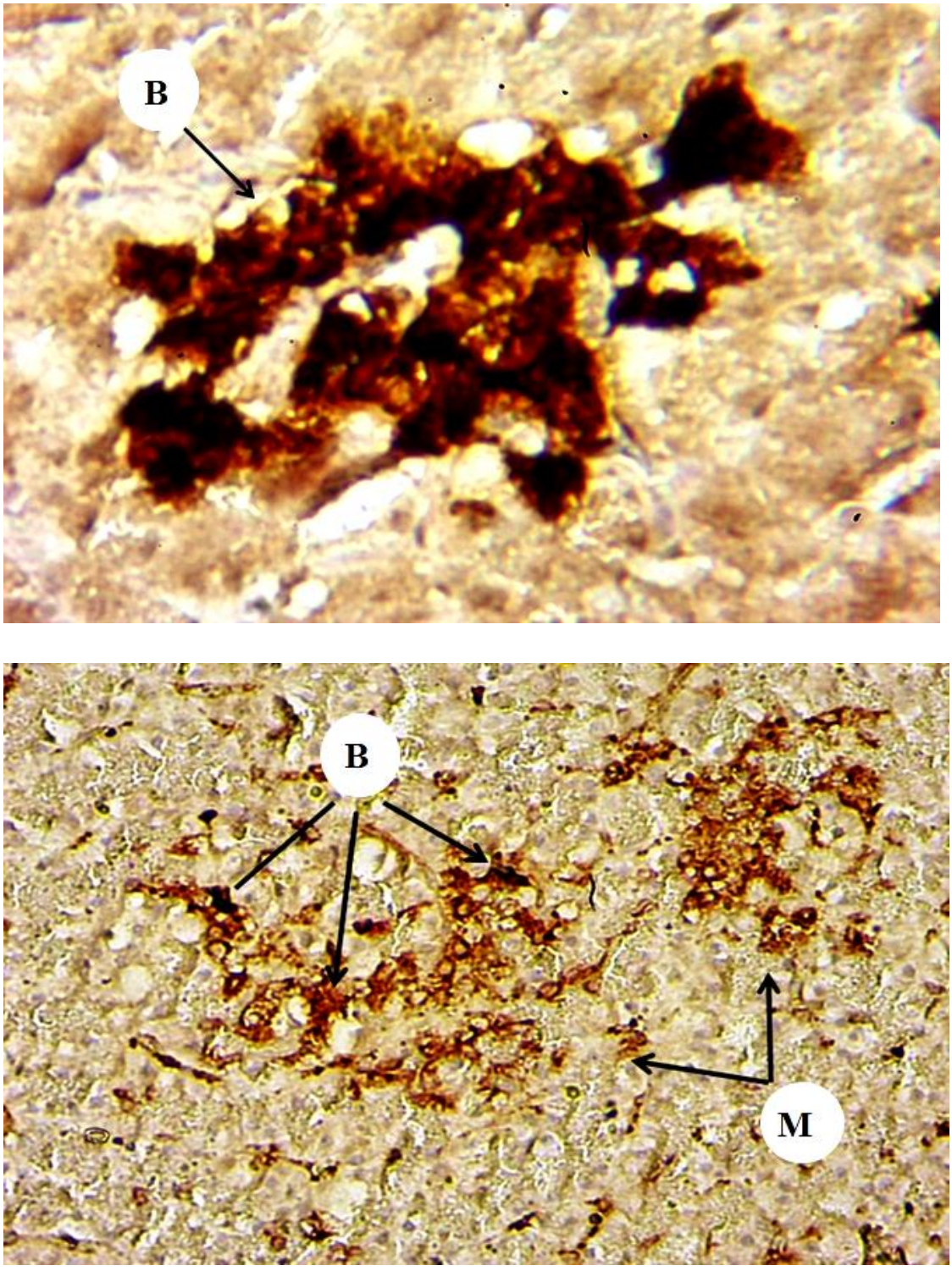

Figure (5): Immunohistochemical structure of pancreas in buffalo; (B): Light insulin islets, and **(M):** Mixed islets. Images represent the immunoreactive tissue stained with DAB and Mayer's Hematoxylin (200X).

Discussion

The pancreas is one of the most important organs of gastrointestinal tract, which produce and secrete a number of enzymes that participate in digestion process within intestinal tract in addition to it activity as part of an endocrine system through hormonal secretion within bloodstream for controlling the energy storage and metabolism [17, 18]. In comparison with the results of this study, a similar description has been given in buffalo [19], cattle and buffalo [20], and goat [21]. We believe that the irregular shape of Langerhans islets is due to the weakness or absence of the capsule surrounding the islets, and the researchers confirmed this fact in deccani sheep and bidri goat [22, 23]. Other study indicated pancreatic islets were partially encapsulated by few reticular fibers in both species which caused irregular shape of islets [24, 25]. Also, Gupta et al. [11] referred to the irregular shape of islets of Langerhans may be due to high cellular migration of part of islet cells from the endocrine to exocrine tissues axis and may be due to the absence of well developed capsule. Hafez and Zaghloul [26] mentioned that the parenchyma of the pancreas was separated into lobes which in turn divide into many lobules by a connective tissue stroma that hold up blood and lymph vessels, nerves, ganglia and different branches of exocrine duct system. Subsequently, each lobule consisted of numerous tubuloacinar secretory units, scattered among the secretory units were the functionally distinct pancreatic islets (islets of Langerhans) that endocrine structures.

The exocrine secretory areas were represented by the pancreatic acini, the basic secretory units, and their duct system. The glandular epithelial cells of the pancreatic acinus were pyramidal in shape with a spherical, more or less centrally located nucleus. The basal basophilic region of the acinar cells was visibly distinct from the apical eosinophilic zymogen granules [27, 28]. The outer part was compound acinus gland, the lobules of which were connected by lax blood vessels, nerve fibers, and lymphatic and secretory ducts [29]. The exocrine pancreas of a buffalo has an inferior tubular gland that makes the bulk of pancreatic tissue, and the roofs were of a single layer of pyramidal cells arranged around a narrow cavity. Acinar cells have two distinct regions: the apical portion filled with refractive zymogen granules that were acidophilic, orange and argyrophilic and the basal portion was highly base. Centroacinar cells were prominent in the pancreatic acini lumen. Dark and light angular cells filled with coarse orangophilic granules in association with secretory units either intralobular or intubular [30, 31]. Other studies mentioned that the exocrine secretor areas were the pancreatic acini, the basic secretory units, and their duct system. The glandular epithelial cells of the pancreatic acinus were pyramidal in shape with a spherical, more or less centrally located nucleus. The basal basophilic region of the acinar cells was visibly distinct from the apical eosinophilic zymogen granules [32-34]. The cells of each acinus were grouped around a small lumen which connected to an intercalated duct. Gastrointestinal endocrine cells are mainly clustered in the pancreas or islets, or scattered throughout the stomach and intestines [35]. The endocrine part consists of groups of endocrine cells in the form of oval or round structures of various sizes, and the islets make up 1-2% of the weight of the pancreas [36].

The hormonal component of the pancreas is the Langerhans system, which is rich in blood vessels and contains several types of endocrine cells that work together to maintain glucose homeostasis [23]. In domestic rabbits, it’s well documented that each Langerhans islet

consisted of α and β cells of different sizes, shapes and colors; while, islets are not fully developed at birth because their density and cell content change with age after birth [37]. In sheep, alpha cells are centrally located in the pancreatic islets, and their percentage is 10-22%, while most beta cells are peripheral and their percentage is 80-90%, while delta cells are located in different locations of the pancreatic islands and their percentage is 1%; while in the dog, alpha cells are peripheral, and their percentage is 25-27%, and β-cell locates at central part of pancreatic islets and their percentage is 73-75%, while delta cells are in different sites of the pancreatic islets and their percentage is 1% [11, 38]. Hafez et al. [39] showed that α and β-cells distributed in equine pancreatic islet in the center and surrounded by α-cells. In the case of camels, the hives were located in the center and at the ends of the islet. Alpha cells are mainly observed as clusters in the periphery. Few cell masses and many PP cells were found throughout the islet. In cattle, the hives were distributed throughout the island, and differences in endocrine cell distribution can lead to variations in hormone needs and interactions between species [40-42].

Conclusion

The endocrine part of the pancreas is composed of cell aggregates, the most important of which are beta cells and alpha cells, which differ in their shape, diameter and location. Studying the body of the buffalo animal as it is one of the local economic animals and everything related to it, whether from the histological or physiological point of view. We recommended to comparing between the tissue structure of pancreas of all animals and relationships between them.

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