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Histological and immunohistochemical study of the endocrine pancreas of duck (Anas platyrhnchos)

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Abstract---Because ducks are one of the important poultry in Iraq and due to the important role of the pancreas in the digestive process, this study was conducted to obtain the histological structure of the duck endocrine pancreas with its immunohistochemical study. The pancreas samples were taken from 10 indigenous adult male ducks. The histological study used two stains. Primary and secondary antibodies to the glucagon and insulin hormones were used for the immunohistochemical study. The result of the current study revealed three types of islets (alpha, beta, and mixed islets) in the duck pancreas. Alpha islets consisted of mainly alpha cells with few delta cells, and beta islets contained beta cells and a few delta cells. Also, alpha and beta cells with numerous delta cells were presented in mixed islets. The pancreatic islets are concentrated in the splenic lobe. The immunohistochemical result found the average glucagon intensity in alpha and mixed islets of duck pancreas was (295.3 ± 8.32 megapixel, and 110.2 ± 4.35 megapixel) respectively, and the average insulin intensity in beta and mixed islets of ducks was (265.4 ± 5.14 megapixels, 122.1 ± 3.65 megapixel) respectively. The glucagon immunopositively cells were more in the islets of the duck pancreas than the insulin immunopositively in these islets. The present study concludes that the duck's pancreas needs a higher amount of glucagon and lower insulin depending on the feeding of ducks and the nature of their living in the aquatic environment.

Keywords---pancreas, alpha islets, beta islets, mixed islets.

Introduction

The duck is a poultry fowl characterized by its aquatic life (Carboneras and Carlos,1992). The avian pancreas is one of the digestive organs that have numerous structural differences in avian species (Gülmez *et al.,* 2004).The exocrine portion of the pancreas is a compound of tubuloacinar glands able to producedigestive enzymes and then transport them to the gut by ducts (Evans, 1996).The pancreas contains also an endocrine portion, which is referred islets of Langerhans. This portion releases metabolic regulatory hormones in the birds' body such as insulin, glucagon, and somatostatin (Aughey and Frye, 2001). The endocrine tissue is occupied a further parenchyma mass in the avian pancreas than in mammals, as well as the endocrine cell types are distributed in different forms (Pilny, 2008).The pancreas comprises mainly two lobes or more with two or three types of islets according to the bird groups (Gülmez*et al.,* 2004; Steiner *et al.*, 2010).These islets are alpha islets (α islets), these islets are larger than other islets,beta islets (βislets), which are smaller than other islets, and mixed islets, these islets were found in some birds which scattered randomly (Do Prado *et al.,*1989; Steiner *et al.*, 2010). The islets of Langerhans contain endocrine cells, these cells include beta (β) (insulin-secreting cells) cells, alpha (α) cells (glucagon secreting cells), delta (δ) cells (somatostatin secreting cells), and (PP) cells pancreatic polypeptide secretion cells (Rawdon, 1988).Beta cells of the rufous' collared sparrow are found mostly in the central region of the islet, the alpha and delta cells are located inside the pancreatic islets, although predominantly found in the peripheral area of islets.Pancreatic polypeptide cells are exactly in the peripheral region of islets (Nascimento *et al.*, 2007).This study was designed to discover the endocrine tissue of the pancreas histologically and immunohistochemistry in ducks.

Materials and Methods

The specimens of the pancreas were collected from 10 indigenous male adult ducks (*Anas platyranchos*). All experimental birds were slaughtered and the pancreas was removed from them, then the lobes were separated from each other.The specimenswere washed in 0.9% normal salineand then fixed in 10% formalin for 24h. Then washed anddehydrated the specimens witha series of concentration gradients of ethanol. Then clearing the specimens by xylene and infiltration and embedding in paraffin wax. The specimens weresectioned in 5 µm. The specimens were stained by H&E stain and Gomori's method for pancreatic islets.All the slides scanning by using a light microscope and then used a digital camera to take photographs of the sections. For the immunohistochemical study, the sections dewax withxylene and hydrated with ethanol gradients.Then Peroxidase Block was used to incubate the sections. After that, heating slides in an oven (100 ºC) with citrate buffer. Then Phosphate buffer saline (PBS) was used to wash the slide three times. The sections with protein blocking solution are incubated and then incubated with anti-glucagon and anti-insulin antibodies raised from rabbit for 20-30 minand can be used with the dilution buffer of the primary antibody supplied as a negative control. Rinse the slide in PBS and distilled water. Then, incubate the sections with the polymer of One-Step HRP. After washing the slides in PBS,a few drops were added of DAB reagent on the slides, and repeat they wash with PBS and distilled water. Thenhematoxylin was

incubated with a section for 30–60 seconds. All the steps were conductedat room temperature. The statistical analysis of the intensity of glucagon and insulin hormones was measured after all the immunostained sections were photographed with a digital camera installed on the light microscope, they were analyzed on the computer by using the Image J program.

Results and Discussions

Histological research

The present study in the pancreas of indigenous male adult ducks observed the pancreas had four lobes ventral lobe, dorsal lobe, accessory lobe, and splenic lobe. In all lobes, an endocrine portion is scattered through the exocrine portion, which included a small amount of parenchyma. The pancreatic endocrine is found as the aggregation of cells, which form islets of different sizes and shapes(Figure 1)**.** This result is in line with Al-Shaeli (2010) in ducks and Al-Sharoot (2016) inEarly hatched geese.

The Structure of Pancreatic Islets

The microscopicscanning of the duck pancreas revealed that pancreatic islets were found in three types alpha, beta, and mixed islets(Figure 2). This result agrees with Simsek and Alabay (2008) in quails, Simsek *et al.* (2009) in the Falcon, Al-Shaeli (2010) in duck, and Helmy and Soliman (2018) in ostrich. Moreover, this result is in contrast with Al-Agele and Mohammed (2012) on golden eagle and Jaafar (2019) in Falcon, those researchers reported the islets are found in two types of darks and light islets in these birds. Furthermore, this result is not similar to the finding of Hamodi *et al.* (2013) reported that the Common gull contained only mixed islets.

The alpha islets in ducks had an irregular outline and form discontinuous cords, which varied in shape and some of these had branches, The cells in alpha islets of ducks form nonpersistent cords. The beta islets in ducks had regular borders, these appear as small globular and oval islets, the cells in beta islets of ducks arrange in irregular ropes or plates. The mixed islets in ducks were irregular and similar in form to the alpha islets, they were difficult to recognize between them. The alpha islets of ducks contained alpha cells with few delta cells, and beta islets consisted of beta cells and a few delta cells. Also, mixed islets had alpha and beta cells with higher numbers of the delta cells. The pancreatic islet types in the duck pancreas contained more capillaries (Figures 3,4,5) for an alpha, beta, and mixed islets respectively. The result of the present study on the shape of endocrine islets is similar to that described by Mikami *et al.* (1985) in Japanese quail, but this result of shapes of endocrine islets in disagreement with Simsek *et al.* (2009) found the pancreatic islets of falcon form irregular and global shapes.Furthermore, The result of islet cells in pancreatic islets is not in line with Mobini (2011) in goose, and Mobini (2013) in pigeon stated the alpha islets consist of alpha and beta cells. But the beta islets had beta and delta cells. On other hand, is not similar to the finding ofHelmy and Soliman (2018) mentioned that the alpha islets in ostrich consist of a large number of only alpha cells or with few beta and delta cells, the beta islets in ostrich had numerous beta cells

with a small number of alpha and delta cells, and the mixed islets contain an even number of alpha, beta, and delta cells. The alpha and mixed islets in ducks had a branch that due to is found sometimes exocrine tissue within islets (McClish and Eglitis, 1969).

The mean diameters of pancreatic islets of indigenous male adult ducks were (121.46 ± 9.77 µm, 85.97 ± 7.52 µm, and 119.15 ± 8.52 µm) in alpha, beta, and mixed islets respectively, as shown in (Table 1). There were no significantly different between the alpha and mixed islets in ducks, but there weresignificant differences between alpha and beta islets as well as between beta and mixed islets in duck pancreas at ($p \leq 0.05$).This finding indicated the alpha islets in duck pancreas had a diameter larger than the diameter of beta islets. This result is similar to that reported by Ku *et al.* (2000) in the chicken embryo, and Gülmez *et al.* (2004) in goose. Themean ratio (%) of distribution of alpha, beta, and mixed islets in male adult indigenous duck pancreas were (38.99 ± 3.31%, 51,77 ± 1.7%, and 10.53 ± 1.21%) respectively, as noted in (Table 1) These results were obtained refers that the beta islets in duck pancreas are more abundant than the dark islets. This result concords with the observation of Rawdon (1998) in the chick embryo,and Gülmez *et al.* (2004) in goose.

The observation of the current study detected that the islets in the duck pancreas were more concentrated in the spleniclobe than in the other lobes, which are found as a big group of all types of islets, as noted in (Figure 6). The finding of the present study agrees with Kara *et al.* (2014) in the Sparrowhawk, but this result disagreement with Rawdon (1998) in the chicken, Gülmez *et al.* (2004) in the goose, those researchers stated that the endocrine tissue is more concentrated in the third and splenic lobes. Moreover, This result disagrees with what Saadatfar *et al.* (2011) reported in their research that pancreatic islets were concentrated in the central portion of the ventral lobe. However, the splenic lobe consisted of a small number of pancreatic islets in the Palam Dovepancreas.

Table 1
Measurements of the diameters and percent distribution of the pancreatic islet typesin the pancreas of indigenous male adult ducks, displayed as (average ± SD)

Parameters	Alpha islets	Beta islets	Mixed islets
Diameters\ (µm)	121.46 ± 9.77 ^{A}µm	85.97 ± 7.52^{B} µm	119.15 ± 8.52^{A} µm
Distribution \ (%)	38.99 ± 3.31%	51,77 ± 1.7%	10.53 ± 1.21%

***** The different letters (A, B) are the one raw denoted the significant differences between islets at ($p \leq 0.05$)**.**

The Pancreatic Islet Cells

Histologically, there were three types of cells were found in pancreatic isletsof ducks in the present study, these cells recognized based on their shapes and staining of cells. These cells include alpha, beta, and delta cells, these cells were foundas a following: *The alpha cells* were polygonal and some of these were oval with prominent oval or spherical nuclei and distinct nucleoli. Alpha cells had

granules stained red color with the Gomori's stain of islets of Langerhans. These cells were found in most regions of alpha islets and peripheral mixed islets of duck pancreas but were not found in beta islets.*The beta cells* wererounded and sometimes rectangular with oval or round nuclei. Beta cells contained a bluish-gray color with the Gomori's stain of islets of Langerhans. These cells in ducks are located in the central area of beta islets, which occupied most regions of these islets and accumulated centrally in mixed islets but were not found in alpha islets of ducks.*The delta cells* had an oval shape with oval or round nuclei. Delta cells contained a few granules. These cells had a red to pink stain with Gomori's stain of islets of Langerhans and appear the same color as alpha cells. These cells diffused randomly in alpha and mixed islets of duck pancreas, which were located in the central and peripheral of these islets. While beta islets were located peripherally, as shown in (Figures 3,4,5) for an alpha, beta, and mixed islets respectively.

The observation of alpha cells agrees with Saadatfar *et al.* (2011) in Palam Dove. Moreover, This result is in line with Mikami *et al.* (1985) in Japanese quail, but differs in the shape of the alpha cells, they stated the alpha cells had a columnar shape. The result in the present study of beta cells is in line with the result of Helmy and Soliman (2018) in ostrich.The observation of delta cells in this study is in line with Al-Shaeli (2010) in duck pancreas, Furthermore, some researchers recorded that delta cells were not found in pancreatic islets of some birds such as Stornelli *et al.* (2006) in ostrich and Mobini (2011) in goose.

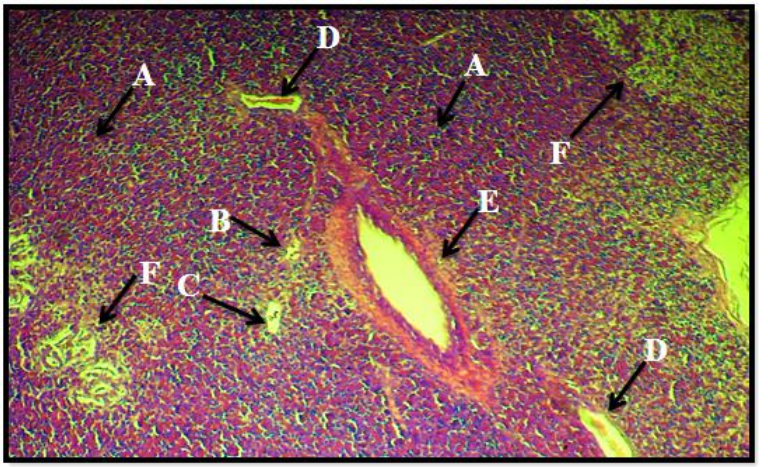

Figure 1. Photomicrographof the duck pancreas illustrates the pancreatic parenchyma, which includes:1- Exocrine portion (A- Acini tissue. B- Intercalated ducts. C- Intralobular ducts. D- Interlobular ducts. E- Main ducts). 2- Endocrine portion (F- Pancreatic islet). H&E stain. (40X).

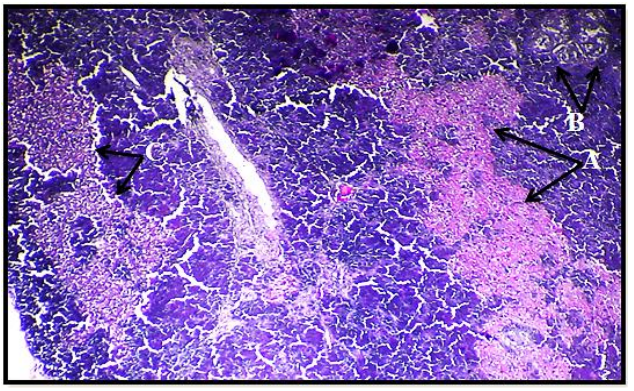

Figure 2. Photomicrographof the duck pancreas shows the islets of Langerhans. A- Alpha islets. B- Beta islets. C- Mixed islets. Gomori's stain. (40X)

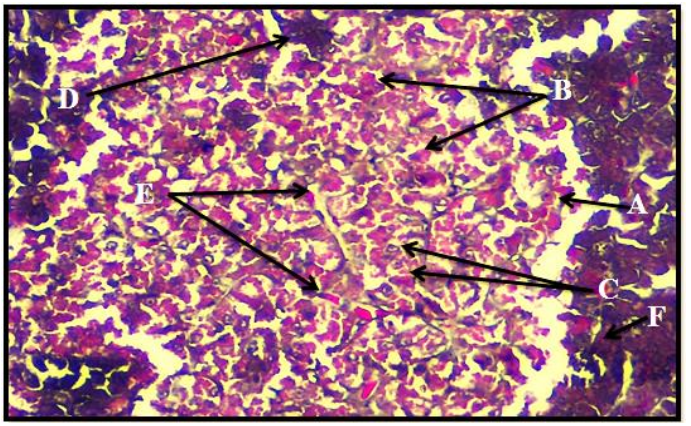

Figure 3. Photomicrographof duck pancreas illustrates the structure and islets cells of the alpha islet. A- Alpha islet. B- Alpha cells. C- Delta cells. D- Acinitissue within alpha islets. E- capillary. F- Acini tissue. Gomori's stain. (200X)

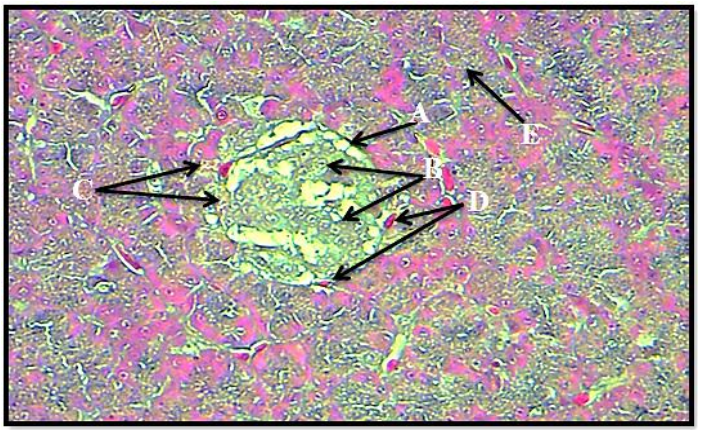

Figure 4. Photomicrograph of duck pancreas exhibits the structureand islets cells of the beta islet. A-Beta islet. B- Beta cells. C- Delta cells. D- capillary. E- Acini tissue. Gomori's stain. (200X)

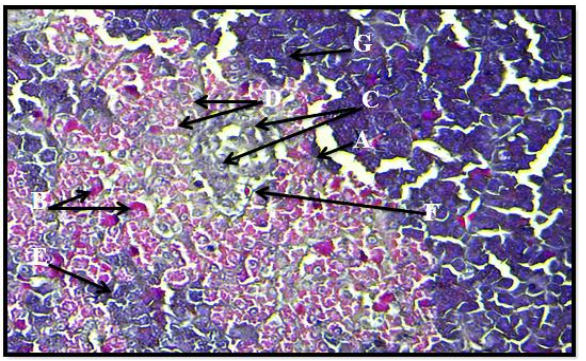

Figure 5. Photomicrograph of duck pancreas illustratesthe shape, structure, and islets cells of the mixed islet. A- Mixed islet. B- Alpha cells. C- Beta cells. D- Delta cells. E- Acini tissue within islets. F- Blood capillary. G- Acini tissue. Gomori's stain. (200X)

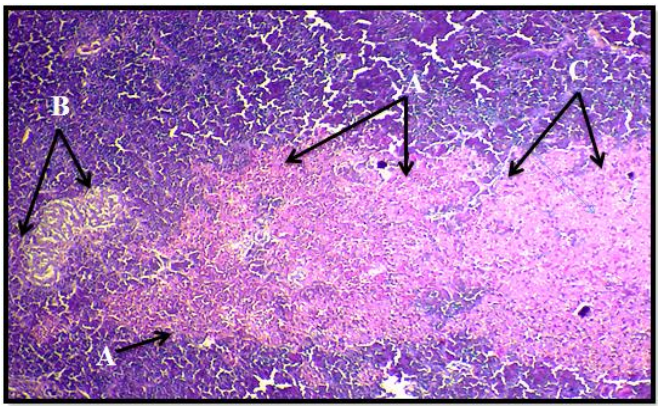

Figure 6. Photomicrographof the splenic lobe in the duck pancreas. A- Group of alpha islets. B- Group of beta islets. C- Group of mixed islets. Gomori's stain. (40X)

Immunohistochemical Research

Immunohistochemical Research for Glucagon

The technique of immunohistochemistry was conducted for islets of Langerhans in the pancreas of indigenous male adult ducks by using glucagon antibody revealedthe immunopositive reaction was presented in alpha and mixed islets, but the beta islets were not had any glucagon immunoreactivity. The alpha islets in the duck pancreas showed strong immune reactivity with glucagon polyclonal antibodies, which appeared large and irregular in shape, and contained dark brown color when incubated with glucagon antibodies, where the immune reactive cells occupied most of the alpha islets, as illustrated in (Figure 7). This result was agreed with the fact of Simsek *et al.* (2008) in quails. On the other hand, slightly periphery immune reactive cells with glucagon antibody was observed in the mixed islet of the duck pancreas.

The mixed islets exhibited a large irregular shape. Also, these islets were stained with brown color in the peripheral region of islets when reacted with glucagon polyclonal antibody, as shown in (Figure 8). This result is similar to Kara*et al.* (2014) in Sparrowhawk. The average glucagon intensity in endocrine islets of duck pancreas was documented by application of image analysis. The average glucagon intensity in alpha and mixed islets of duck pancreas was (295.3 ± 8.32 megapixel, and 110.2 ± 4.35 megapixel) respectively, as noted in (Table2).

Table 2
Illustrates the average intensity of glucagon and insulin in islet types of duck pancreas, expressed as (average ± SD)

Intensity\ megapixel	Alpha islets	Beta islets	Mixed islets
Glucagon	295.3 ± 8.32megapixel	0	110.2 ± 4.35megapixel
Insulin	0	265.4± 5.14 megapixel	122.1 ± 3.65 megapixel

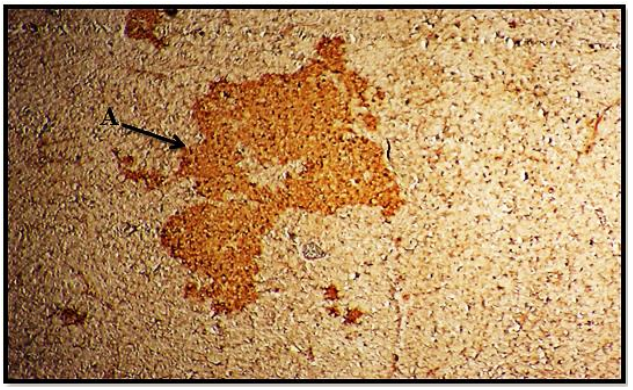

Figure 7. A photomicrographof the glucagon Immune-reactive staining in the alpha islet of duck pancreas. A- Alpha islet. (40X)

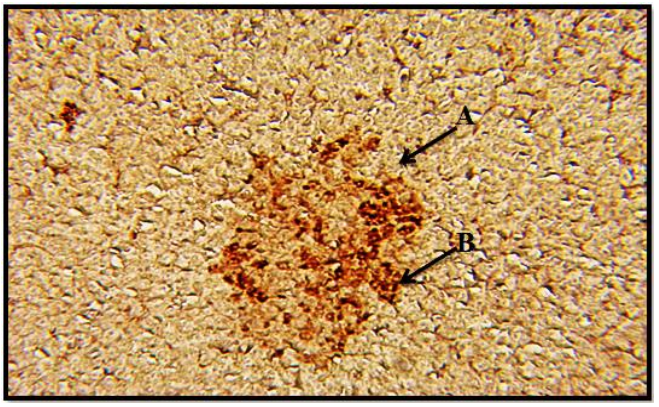

Figure 8. A photomicrograph of theglucagon Immune-reactive staining in the mixed islet of duck pancreas. A- Mixed islet. B- Glucagon immunoreactive cell. (100X)

Immunohistochemical Research for Insulin

Through the immunohistochemical scanning observed the immunereactivity was found in beta and mixed islets but was not presented in alpha islets.The beta islets seemed round or oval and smaller than other islets in the duck pancreas, which contained an abundance of immune-reactive cells, which occupied a wide area of islets and accumulated centrally. These islets exhibited intense to moderate immune reactivity with insulin antibody. The beta islets exposed brown color when incubated with insulin antibody, as noted in (Figure 9). This result correlated with the observation of Cardoso *et al.* (1996) in Tangara seledon pancreas and Nascimento*et al.* (2007) in Brazilian sparrow. Furthermore, the immunohistochemical examination by using insulin showed the mixed islets. These islets appeared in large irregular shapes and contain a few immunoreactive cells, which diffuse in the central region of islets. Mixed islets displayed weakly immunoreactive with insulin antibody, which had a few brown colors in the central area of islets as noted in (Figure 10). This result agreement with Şimşek *et al.* (2009) in falcon, and Kara*et al.* (2014) in Sparrowhawk.

The average insulin intensity was recorded via image analysis in the pancreatic islets of the duck pancreas. The average insulin intensity in beta and mixed islets of ducks was (265.4 ± 5.14 megapixels, 122.1 ± 3.65 megapixel) respectively, as illustrated in (Table2). Finally, the result of the immunohistochemical technique showed the glucagon immunopositive cells were more abundant in the islets of duck pancreas than the insulinimmunopositive cells in the pancreatic islets of ducks. The result of the current study is in line with Falkmer and Noorden (1983) and Hazelwood (1984) those researchers reported that glucagon is a great metabolic hormone in the avian pancreas.

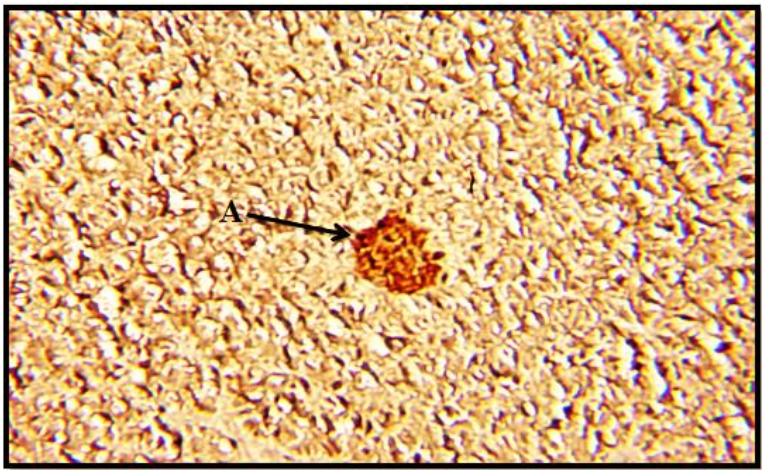

Figure 9. A photomicrograph of insulin Immune-reactive staining in the beta islet of duck pancreas. A-Beta islet. (100X).

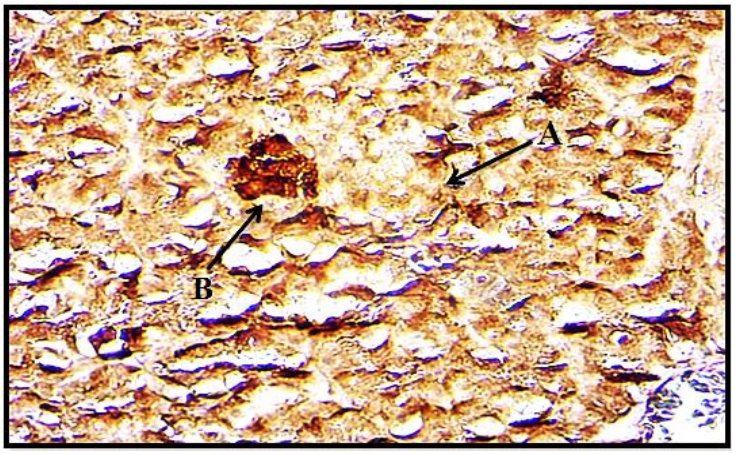

Figure 10. A photomicrograph of insulin Immune-reactive staining in the mixed islet of duck pancreas. A- Mixed islet. B- Insulin immunoreactive cell. (200X)

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