



## Histoarchitecture of Islet's of Langerhans and Their Related Hormones Analysis in the Pancreas of Adult Guinea Pig (*Cavia porcellus*)

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### ABSTRACT

The present study was conducted to study the endocrine portion of the pancreas represented by Islets of Langerhans in the adult males and females guinea pigs. Eight animals of each sex were collected to conduct these aims. Histological sections prepared from the specimens which were collected from three identified lobes of the pancreas and subsequently fixed by 10% neutral buffered formalin. Post conducting the routine histological procedure on specimens, three stains were used such as hematoxylin and eosin, Masson's trichrome and Gomori's Aldehyde trichrome for identification of cellular composition of the pancreatic Islet's of Langerhans.

The endocrine islets showed predominantly  $\beta$ , and for a lesser extent  $\alpha$  cells. The  $\delta$  cells were rarely detected. The percentage of  $\beta$  cells in the islets of male pancreas was higher than those in females. On contrary, the percentage of  $\alpha$  cells was higher in the islets of females than those in the males. Such findings were proportional to hormones analysis of insulin and glucagon which were corresponding products to these cells. In conclusion, Percentage of  $\beta$  cells in the islets of male pancreas was higher than that in females. On the contrary, the percentage of  $\alpha$  cells was higher in the islets of females than that in the males. Such observations were parallel positively with hormones analysis of insulin and glucagon which were corresponding products to these cells.

### INTRODUCTION

Domestic guinea pig (*Cavia porcellus*) is a descendant of the wild cavy (*Cavia aperea*) which is one of the common rodents who lived in South America. It is herbivorous rodent characterized by its stocky body, short neck, and limbs and it is more closely related to porcupine than mouse and rat (North, 1999; Kunzl and Sachser, 1999). Guinea pigs are now widely distributed because of their popularity as a pet and a food source.

The endocrine portion of the pancreas is an important structure required special attention from a medical aspect as it is the target of two important diseases that are pancreatic cancer and diabetes. Accordingly a better understanding is required to be focused on the morphology and histology of this organ. Such events certainly will contribute to the development of novel therapies and strategies for the treatment of either or both of the above two diseases (Seymour *et al.*, 2004).

Usually, the mature pancreatic islets of Langerhans constitute 1% - 2% of the total mass of the adult pancreas. They are composed of four principal endocrine cell types, namely alpha ( $\alpha$ ), beta ( $\beta$ ), delta ( $\delta$ ) and polypeptide (PP) cells that produced glucagon, insulin, somatostatin and pancreatic polypeptide hormones, respectively. The  $\beta$  cells are the most abundant type, comprising ~65% - 80% of the total number of endocrine cells and localize to the islet core. The remaining endocrine cells tend to be distributed at the periphery of the islet (Lucas-Clerc *et al.*, 1993; Halban *et al.*, 1982; Orci, 1982; Pipeleers *et al.*, 1982).

Up to date there is no research in the current literatures conducted to study the morphology and histology of the endocrine portion of the pancreas in the adult guinea pig and there is paucity of work focused only on the pathological aspect and concerned diseases of this organ in dogs (De Cock *et al.*, 2007), cats (Louwerens *et al.*, 2005), rats (Kara, 2005), rabbits (Saluja, *et al.*, 1989) and other rodent species.

The current study is realized that the data obtained will provide basic scientific information on the islet of Langerhans the representative endocrine portion of pancreas to conduct physiological and pharmaceutical researches that are related to the diseases of pancreas. Certainly the obtained data will provide good animal models for both veterinary fields in animals and public health in humans.

## **MATERIALS AND METHODS**

### **Animal Collection and Study Design:**

Clinically healthy sixteen adult guinea pigs (eight of both males and females) were bought from local farms at Diyala province and they were caged in the animal house till their euthanasia and dissection to obtain their pancreas and duodenum.

Each selected animal was euthanized prior to its dissection by intra-venous injection of an overdose of 140 mg/kg of sodium phenobarbital (Euthasol; Delmarva Laboratories, Midlothian, VA) (Eifler *et al.*, 2009). After that, the animal was dissected on a dissecting board. The abdominal wall was opened to view the abdominal viscera, and then the pancreas lobes were recognized and dissected out. Representative specimens were cut from each lobe for subsequent histological approach.

### **Histological Techniques:**

Specimens were immersed in 10% neutral buffered formalin for 72 hrs and some specimens in Bouin's solution for 16 hrs. Specimens were dehydrated through ascending series of ethyl alcohol (70%, 80%, 90%, and 100%) each for 2hrs, and then cleared with xylene for ½ hr. Processed specimens were infiltrated with paraffin wax on 58 °C then embedded with paraffin wax to obtain blocks of paraffin. Sections of 6  $\mu$ m were obtained by using rotary microtome. The sections were selected from right, body and left lobes of the pancreas. The sections were stained with either one of the following stains: Hematoxylin and Eosin were conducted to describe the general histological features. Masson trichrome stain was conducted to identify the collagenous connective tissue fibers. Gomori's chrome alum hematoxylin and phloxine were conducted for the demonstration of  $\alpha$ ,  $\beta$  and  $\delta$  cells of the islets of Langerhans. The tissue sections were examined using Olympus light microscope. Sections were photographed and analyzed by Dino-eye piece camera provided with Image software.

### **Micromorphometric Measurements:**

The following measurements were conducted:

1. Percentages of  $\alpha$ ,  $\beta$  &  $\delta$  cells of Islet's of Langerhans

2. Number of different sized Islet's of Langerhans distributed in the pancreatic lobes

#### **Hormones Analysis:**

Blood samples were collected directly from the heart and subsequently, sera were prepared and kept well under frozen condition till their sent to the lab for insulin and glucagon analysis.

#### **Statistical Analysis:**

Statistical calculations were carried out with the SPSS 15.0 for windows software package. All numerical values were expressed as the mean  $\pm$  standard error (SE). For comparisons parametric differences between the two genders and the statistical significance were assessed by Student *t*-test. The significance level was set at  $p < 0.05$ .

### **RESULTS**

The light microscopy of serially sectioned pancreatic lobes revealed the presence of the smaller endocrine portion represented by the Islet's of Langerhans embedded in the larger exocrine portion represented by different numerous pancreatic acini (Fig. 1).

Islets of Langerhans were different sized-rounded or oval-shaped structures that were distributed irregularly within the exocrine tissue of the pancreas. These Islets were either small in diameter (less than 50  $\mu\text{m}$ ) or medium (between 50 and 100  $\mu\text{m}$ ) or large islets (more than 100  $\mu\text{m}$ ). The small islets were embedded between the groups of acini and sometimes present as few gathered cells (5-9 cells) (Figs. 2 & 3). The shapes of medium and large islets were rounded or oval out of which the large islets may fill most of the pancreatic lobules and tend to be positioned at the periphery of them, whereas, the small-sized Islets were usually positioned in the interior of lobules. In general, each islet was constructed of irregular anastomosing cellular cords separated by scanty connective tissue fibers accompanied by many blood

capillaries. In fact, many blood capillaries were detected at the periphery of each islet's of Langerhans (Fig. 4).

Post-staining with Gomori's trichrome stain, the Islet's of Langerhans were showed three types of endocrine cells, that were  $\beta$ ,  $\alpha$ , and  $\delta$ . The  $\beta$  cells were the abundant cellular type constituting the bulk of islets of Langerhans. They were larger than  $\alpha$  cells but smaller than  $\delta$  in size. The  $\beta$  cells stained dark blue in color characterized by the presence of nuclei and nucleoli. They were distributed irregularly in all areas of the islets. Those of red-colored cells which represented  $\alpha$  cell were smaller in size than  $\beta$  and were also distributed irregularly within the interior of the islets. The pink-colored cells i.e.  $\delta$  cells were distributed singly as large cells with one or two nuclei. Mostly, these cells were located in the periphery and sometimes were situated in interior of islets (Figs. 5, 6, 7 & 8).

Micro-morphometric dimensions concerned these Islets were listed in table 1. The findings showed that the percentages of  $\beta$ ,  $\alpha$  and  $\delta$  cells in the islets of Langerhans in female pancreas were 75.55%, 22.17%, and 2.28%, respectively whereas, the similar measurements in the male individuals were 83.60%, 15.11%, and 2.29%, respectively (Table 1). These measurements showed higher percentage of  $\beta$  cells in the pancreas of males than those in females. On contrary, the percentage of  $\alpha$  cells was higher in females than those in males. Statistical analysis revealed the above differences in the percentages of  $\alpha$ ,  $\beta$  cells were significant between female and male pancreases.

The total means of the small, medium and large Islets of Langerhans / $\text{mm}^2$  of the female pancreas were 17, 47 and 159, respectively and so that total islets were 223 / $\text{mm}^2$  in the whole pancreas. Similarly, the total means of the small, medium and large islets of Langerhans / $\text{mm}^2$  in male pancreas

were 16, 46 and 154, respectively and so that total islets was 216 /mm<sup>2</sup>. Statistical analysis revealed the above differences in the total number of islets of Langerhans /mm<sup>2</sup> in female and male pancreas was not significant (Table 2).

### Results of pancreatic hormones

Post using immunohistochemistry, hormones assay of insulin and glucagon in the sera of studied female and male guinea pigs showed differences listed in table 3. The Means  $\pm$  SE of insulin hormone was 34.82 $\pm$ 1.76 pg/ml, whereas in males estimated as 43.07 $\pm$ 1.71 pg/ml.

**Table 1.** Percentages of  $\alpha$ ,  $\beta$  &  $\delta$  cells of Islet's of Langerhans

Sexes	Percentage of $\alpha$ to sum of $\alpha$ , $\beta$ & $\delta$	Percentage of $\beta$ to sum of $\alpha$ , $\beta$ & $\delta$	Percentage of $\delta$ to sum of $\alpha$ , $\beta$ & $\delta$
Female	22.17%	75.55%	2.28%
Male	15.11%	83.60% *	2.29%

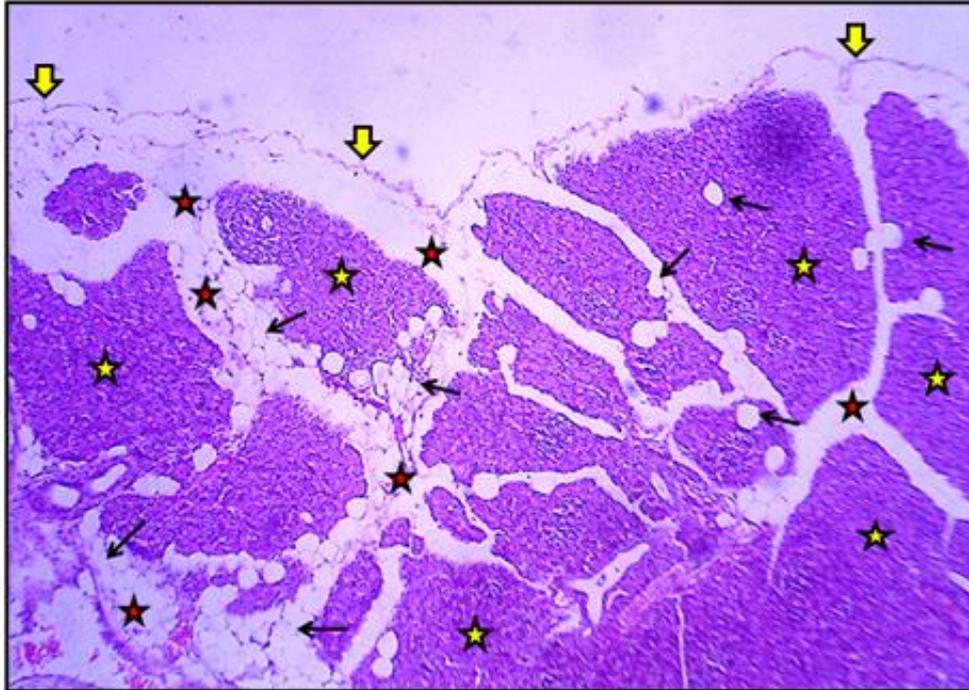
\* Significant differences between the two sexes.

**Table 2.** Number of different sized Islet's of Langerhans distributed in the pancreatic lobes

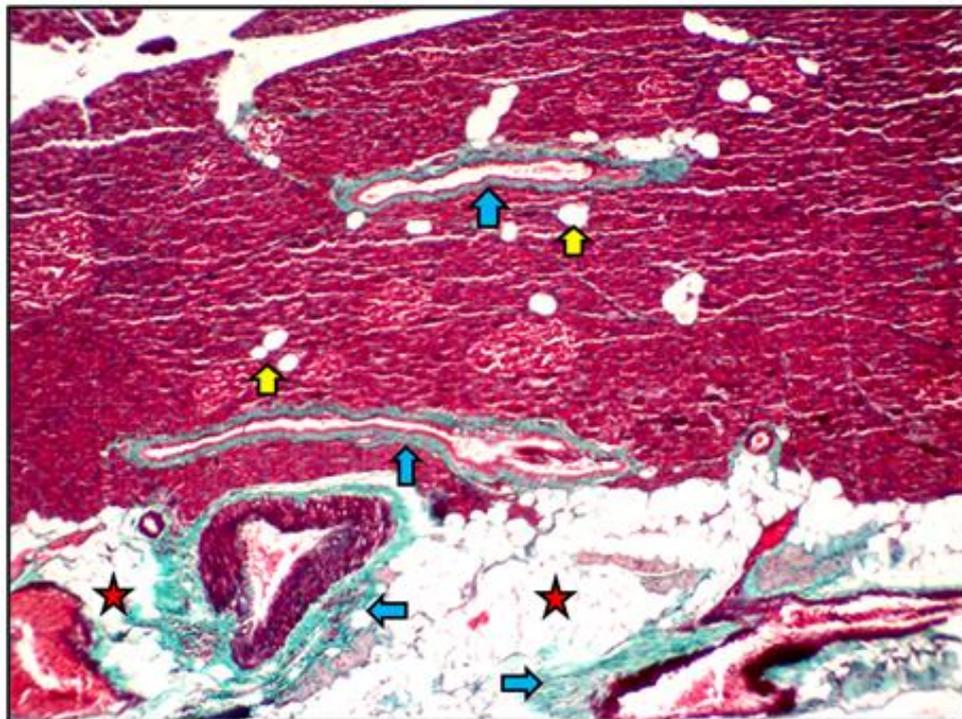
Sexes	Type of islets	Means of islets number /mm <sup>2</sup>			Total of islets number /mm <sup>2</sup> of whole lobes
		Right lobe	Body lobe	Left lobe	
Females	Small	5	6	6	17
	Medium	18	14	15	47
	Large	11	74	74	159
	Total	38	99	98	223
Males	Small	5	5	6	16
	Medium	19	13	14	46
	Large	14	69	71	154
	Total	51	66	82	216

**Table 3.** Pancreatic hormones concentrations in sera of both sexes

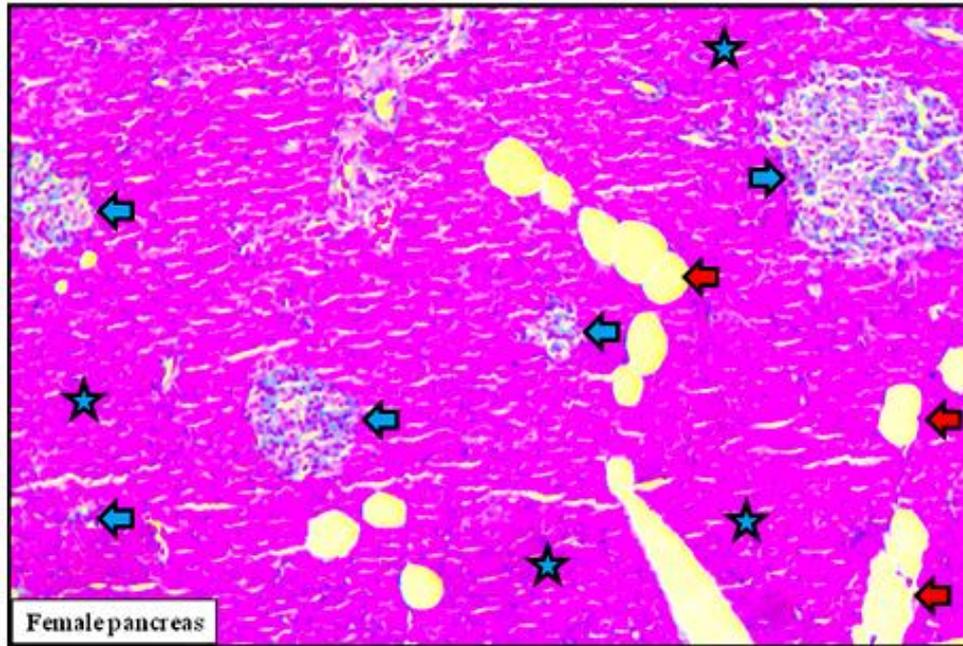
Hormones	sexes	Concentrations pg/ml	Means $\pm$ SE
Insulin	Males	41.20	43.07 $\pm$ 1.71 ↑
		40.14	
		51.20	
		42.11	
		40.14	
		43.62	
	Females	32.89	34.82 $\pm$ 1.76 ↓
		31.80	
		43.20	
		33.83	
		31.80	
		35.39	
Glucagon	Males	79.77	76.78 $\pm$ 3.20 ↓
		74.59	
		73.00	
		77.38	
		89.73	
		66.23	
	Females	87.10	84.12 $\pm$ 3.20 ↑
		81.92	
		80.33	
		84.71	
		97.06	
		73.56	



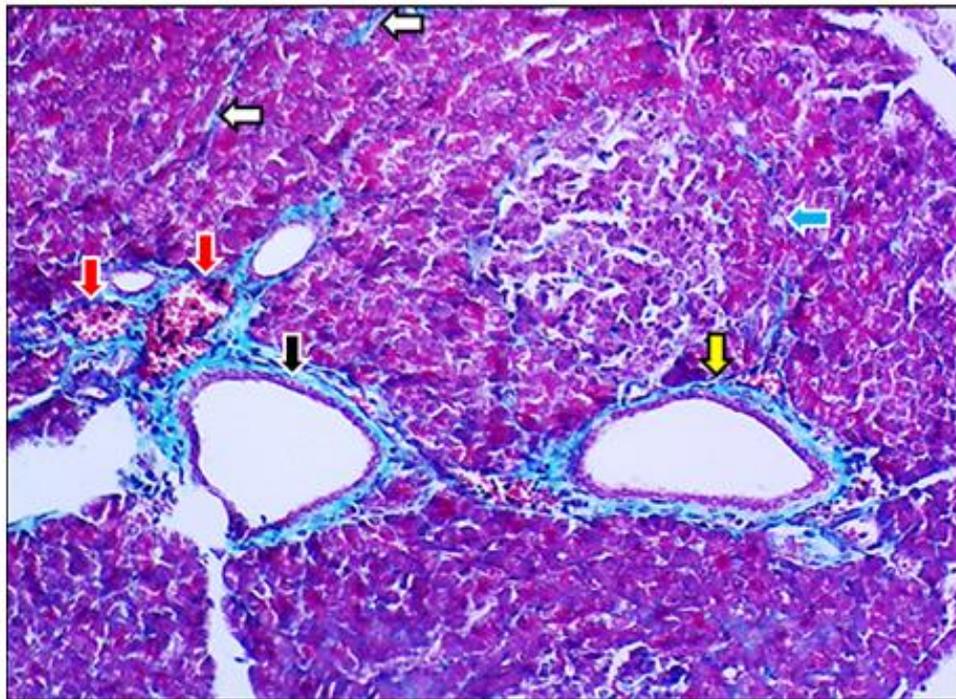
**Fig. 1.** Capsule (yellow arrows), septae (red stars), pancreatic lobules (yellow stars), fat cells or tissue (black arrows). H&E, X40



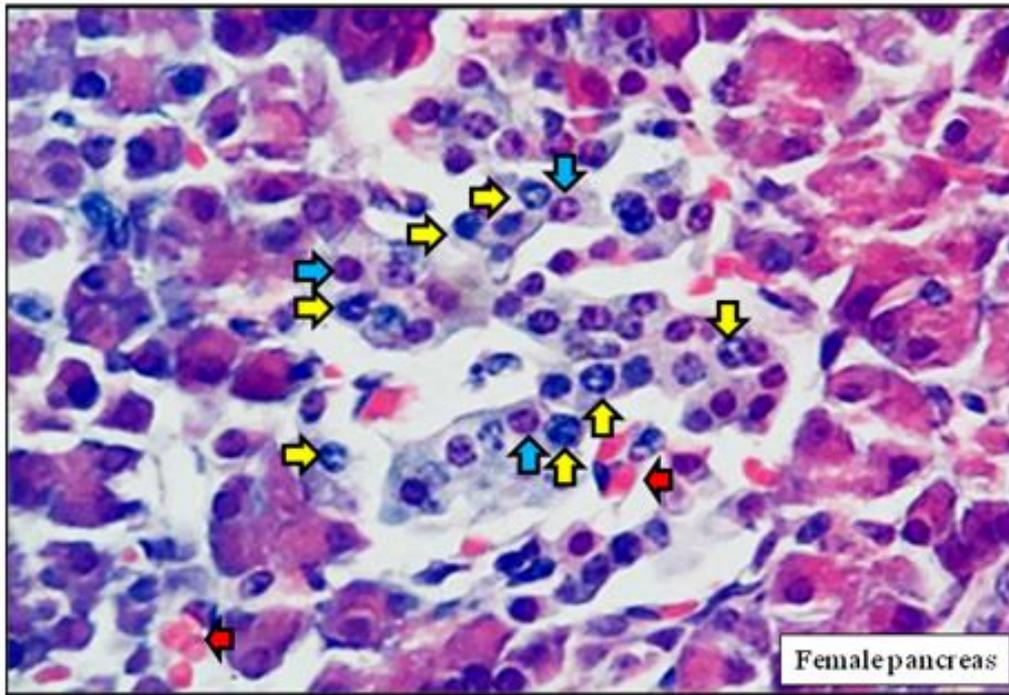
**Fig.2.** Interlobular septa of loose connective tissue filled with adipose tissue (red stars) and scanty collagen fibers condensed around the blood vessels and the intralobular ducts (blue arrows). Few fat cells present in the pancreatic lobules (yellow arrows). MTC, X100



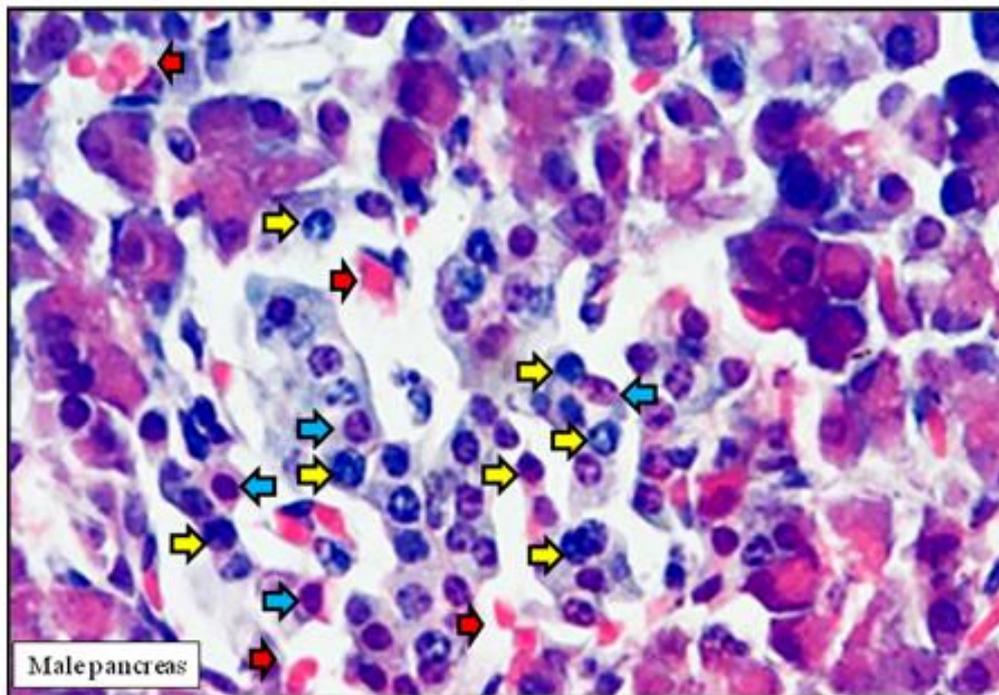
**Fig. 3.** Different sized islet's of Langerhans (blue arrows) were distributed in pancreatic lobules (blue stars), interlobular connective tissue showed fat cells (red arrows), Gomori's Aldehyde Trichrome, X100



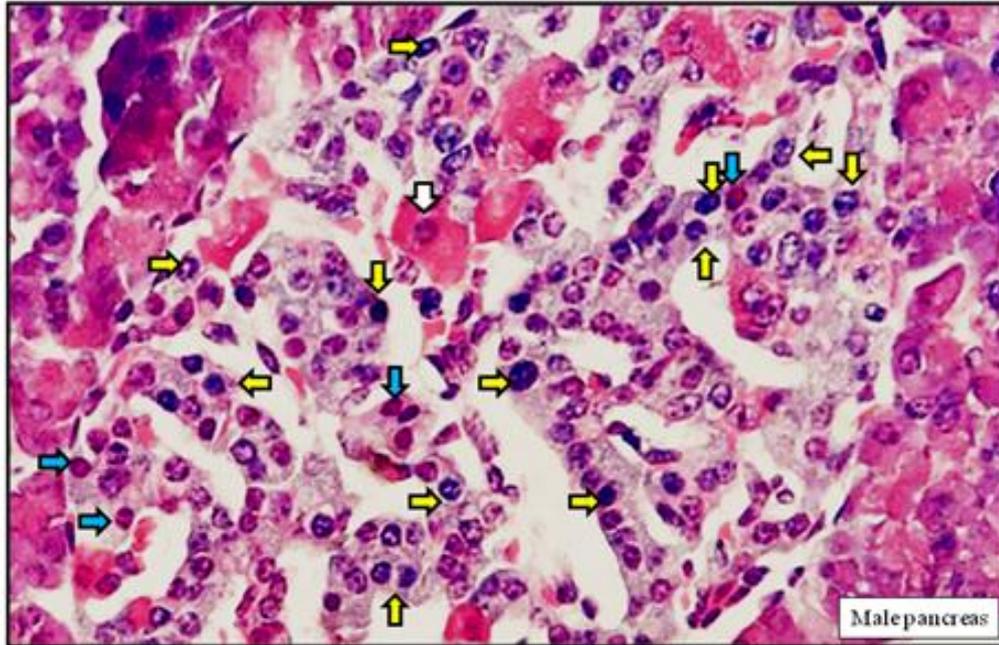
**Fig.4.** Distribution of connective tissue stroma around blood vessels (red arrows), intralobular (yellow arrow) and interlobular (black arrow) ducts (black arrows) with scanty fibers around acini (white arrows) and islets of Langerhans (blue arrow). MTC, X100



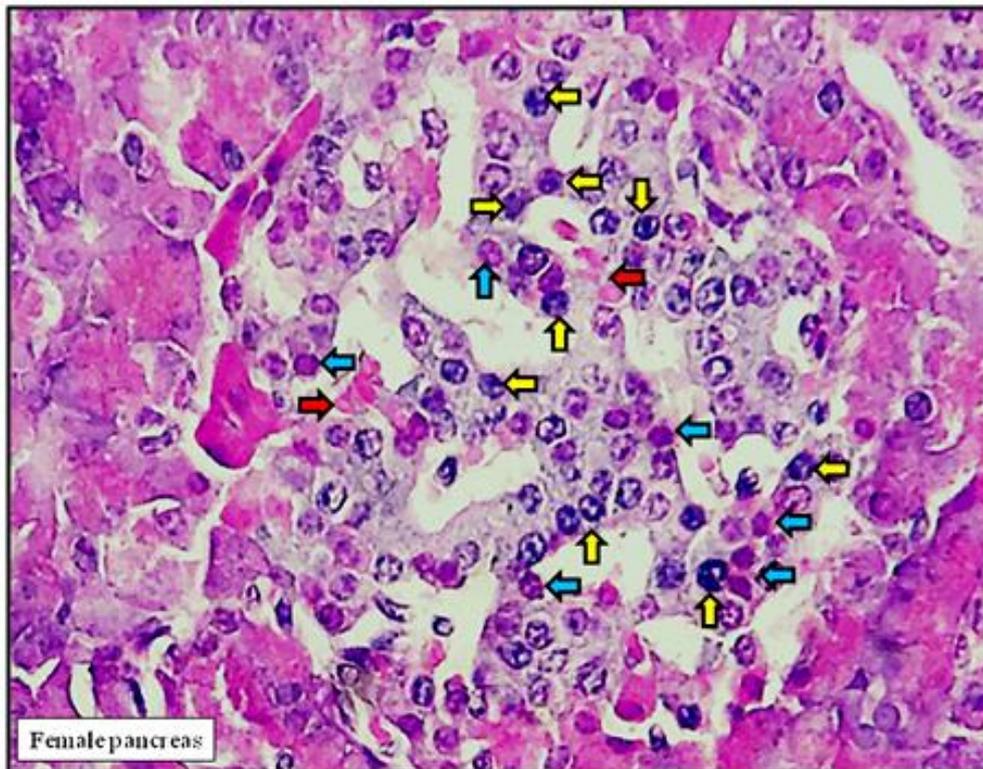
**Fig. 5.** Islet's of Langerhans showed  $\alpha$  (blue arrows),  $\beta$  (yellow arrows), and blood Capillaries (red arrows). Gomori's Aldehyde Trichrome, X400



**Fig. 6.** Islet's of Langerhans showed  $\alpha$  (blue arrows),  $\beta$  (yellow arrows), and blood Capillaries (red arrows). Gomori's Aldehyde Trichrome, X400



**Fig. 7.** Islet's of Langerhans showed  $\alpha$  (blue arrows),  $\beta$  (yellow arrows) and  $\delta$  (white arrows). Gomori's Aldehyde Trichrome, X400



**Fig. 8.** Islet's of Langerhans showed  $\alpha$  (blue arrows),  $\beta$  (yellow arrows) and blood Capillaries (red arrows). Gomori's Aldehyde Trichrome, X400

## DISCUSSION

In the pancreas of the guinea pig, the current study showed numerous islets of Langerhans embedded within the exocrine pancreatic tissue of different sizes. These Islets appeared formed from a group of cells that were arranged in irregular branches and anastomosing cellular cords separated by scanty connective tissue fibers associated with richly blood capillaries. Similarly, previous records were documented by Fattah (2008) in the pancreas of rats. They were small in diameter (less than 50  $\mu\text{m}$ ) intermingled among acini units whereas, the medium (between 50 and 100  $\mu\text{m}$ ) and large islets (more than 100  $\mu\text{m}$ ) were usually rounded or oval tend to be positioned at the interior and periphery of pancreatic lobules respectively. Similar findings were demonstrated previously in the pancreas of rats by (Chumassova *et al.*, 2012), who detected extensive variance in islets size (from 50 to 300  $\mu\text{m}$ ) and distribution density in different areas was clearly visible in the pancreatic tissue lobules.

The present study detected three types of endocrine cells, that were  $\beta$ ,  $\alpha$ , and  $\delta$ . The  $\beta$  cells were represented the predominant cell type and constituted the most portion of the islets of Langerhans. They were larger than  $\alpha$  cells but smaller than  $\delta$  in size. The Percentage of  $\beta$  to the sum of  $\alpha$  &  $\delta$  was 75.55% and 83.60% in females and males respectively. The  $\alpha$  cells were slightly smaller than  $\beta$  cells and they were disseminated irregularly at the intermediate portion. They were occupied by about 22.17% and 15.11% from the total islet cells of both sexes respectively. Regarding  $\delta$  cells, they were seen as singly as large cells. These cells were scattered periphery and occasionally in the interior of the islets comprised of 2.28% and 2.29% from the total islet cells of the same species respectively. These features were similarly described in humans by

(Cabrera *et al.*, 2006; Kim *et al.*, 2009) and cats (Al-Saffar and Al-Zuhairy 2017). In conclusion, percentage of  $\beta$  cells in the islets of male pancreas was higher than that in females. On the contrary, the percentage of  $\alpha$  cells was higher in the islets of females than that in the males. Such observations were parallel positively with hormones analysis of insulin and glucagon which were corresponding products to these cells.

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