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MICROSCOPIC STUDY OF THE INTRAPANCREATIC GANGLIA AND ITS EFFECT ON THE ENDOCRINE AND EXOCRINE PART OF RAT PANCREAS

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ABSTRACT

Pancreas is an important endocrine and exocrine organ which plays a mandatory role in nutritional hemostasis. The intrapancreatic ganglia is a very important structure which works like a small brain for a rapid control of diet metabolism and any defect in the intrapancreatic ganglia may lead to hormonal imbalance and malfunctioning of the pancreas leading to many diseases. The purpose of this study is to point out the normal histology and location of the intrapancreatic ganglia and its critical function in controlling both the exocrine and ductless parts of the pancreas. So, the main objectives of this study are as follows; i) To conduct a detailed microscopic structure by the examination of intrapancreatic ganglia within the rat pancreas…ii) To determine the location and changes in the intrapancreatic ganglia after surgery and their effects on the endocrine and exocrine parts of the pancreas…iii) To explain the potential role of the intrapancreatic ganglia as a reflex center in regulating metabolic changes in the pancreas. In this study, twelve adult albino rats of both sexes were used. Divided into control and experimental groups. the location and normal histology of the intrapancreatic ganglia were studied in the control and the experimental groups which had sympathectomy and vagotomy. Measurements of the changes in the fasting blood glucose levels and glucose tolerance tests in the control and experimental animals were recorded three weeks after vagotomy and sympathectomy. Degenerative changes were observed in the intrapancreatic ganglia after surgery. Also, the blood glucose level after surgery were increased significantly.

KEYWORDS: Rat pancreas; Intrapancreatic ganglia; Microscopic study; Vagotomy and Sympathectomy.

INTRODUCTION

Pancreas is an abdominal organ that receives innervation from the higher center.[1] Through this innervation and with the effect of hormonal changes, the pancreas plays a major role in nutritional hemostasis.[2] The pancreas is made up of two major parts, endocrine and exocrine. The endocrine part secretes hormones like insulin, glucagon, somatostatin, and polypeptide,[3] while the exocrine part releases digestive enzymes.[4] There are many similarities in the exocrine and endocrine parts between a rat and human pancreas.[5,6,7,8,9] and this make the rats more preferred than the other animals in our experimental studies. Thus, the anatomy and histology of the pancreas of a rat is significant in achieving the reliability of the results. The pancreas innervation has been known since the 19th century.[10] In 1869, Langerhans first described the intrapancreatic ganglia and then many studies were done over many decades.[11,12,13,14,15,16,17,18,19]

The intrapancreatic ganglia is viewed as a simple parasympathetic relay, but emerging evidence portrays its similarity to the enteric nervous system.[20] Intrapancreatic ganglia entails comprehensive information-processing center which contains neurotransmitters and form an endogenous neural network.[20,19,21] Despite the influence of extra pancreatic nerves, particularly in autonomic nerves, the pancreatic endocrine function has been extensively studied, the function of intrapancreatic ganglia on endocrine and exocrine functions has less not been given attention.[14,22,20,1] Sensory information from the pancreas is transmitted to the central nervous system,[23,4] Pancreatic vagal afferent through the parasympathetic afferent neurons transfers sensory information to the brain from the pancreas, these neurons emerge in nodose ganglia,[24] the primary target of these neurons is the solitary tract nucleus,[25,5] Sympathetic afferent neurons ascend up through dorsal root ganglia T6-L2,[22,1,5] the neurons synapse with interneurons in the spinal cord in laminae I and IV,[26,23,4] On the other hand, nerve input of the pancreas emerges both sympathetic and parasympathetic neurons. The sympathetic innervation of

the pancreas originates from the sympathetic preganglionic neurons in the lateral horn of the lower thoracic and upper lumber segments of the spinal cord.[27] These neurons give branches to Islets, blood vessels, ducts, acini, and intrapancreatic ganglia.[29,30]

Most parasympathetic preganglionic fibers supply the pancreas from the dorsal motor nucleus of the vagus and some may originate from a nucleus ambiguous in the brainstem.[22,31,1] The vagus nerve fibers enter the abdomen through esophageal hiatus and give the ventral and dorsal vagal trunk and their branches. Concerning the pancreas, a number of fibers enter directly the pancreas and supply the intrapancreatic ganglia.[32] Some of the vagal parasympathetic fibers supply the pancreas indirectly through the myenteric plexus which lies in the duodenum, which in turn sends a number of fibers distributes to the endocrine part (islets of Langerhans), exocrine parts (acini) and intrapancreatic ganglia.[2,4] However, innervation of the pancreas needs more research because one of the causes of failure of pancreas transplant is the innervation.

In a previous study done by Al-Muhtaseb.[33] the effect of denervation on the pancreas result in the degeneration of the endocrine and exocrine tissue of the rat pancreas. Measurements of the changes in fasting blood glucose levels after denervation were done and compared with the control group. Both showed significant elevation in blood glucose after denervation. Therefore, the aim of this study is to bring out the histology and location of the intrapancreatic ganglia and to investigate the effect of vagotomy and sympathectomy on the metabolic function of the pancreas, by observing the histological changes on the intrapancreatic ganglia after surgery, and measuring the changes in glucose concentration after the procedure.

MATERIALS AND METHODS

Twelve adult albino rats from both sexes with a body weight ranging from 250-500g were used in this study. The animals were taken from the Animal House of the institute but under the NIH guidelines. The animals were divided into three groups, the first group of 4 animals was the control group, to study the histology of intrapancreatic ganglia, the second group of 4 were allowed to survive three weeks after sympathectomy, while the last group was allowed to survive three weeks after vagotomy, to see any changes in the structure of the intrapancreatic ganglia. The animals were anesthetized by intraperitoneal injections with Sodium Pentobarbital (25mg/kg). This was purposed to ensure that the animals were in a stable and non-responsive state during the procedures. Following the anesthesia, the rats were sacrificed by perfusion. Perfusion strives to flush the circulatory system to remove blood. The pancreas was dissected and prepared for the light microscopy examination. The dissected pancreas was cut into small pieces and placed in a fresh fixative for 48 hours.[33] Tissues from the dissected pancreas were fixed in 10% formaldehyde for 48 hours, dehydrated in alcohol, cleared in xylene and then embedded in paraffin wax.[33] Tissue blocks were sectioned into 10 µm thick slices, rehydrated, stained with hematoxylin and eosin. Truncal vagotomy was performed by cutting the anterior and posterior vagal trunks around the esophagus below diaphragm, each trunk was isolated and then a piece of 1 cm was cut and removed between two ligatures.[33]

On the other hand, the sympathectomy was performed by cutting the splanchnic nerve fibers projecting to the pancreas, all nerve fibers around the celiac trunk, the right and left gastric arteries, and the nerve fibers around the renal arteries.[33] Experimental animals were sacrificed 3 weeks after denervation. The stained tissue sections were examined under the light microscope equipped with a digital camera. The 40*objective lens was used to visualization in high resolution. Light microscopy allowed for the visualization of the intrapancreatic ganglia and their components, including nerve cells, axons and connective tissues. Multiple fields of view were randomly selected to ensure a representative sampling of the ganglia distribution within the pancreatic tissue. Nerve fiber density, ganglion size and the presence of any pathological changes were quantified using image analysis software. An experiences histologist blinded to the experimental groups conducted the examination to minimize bias. The examination provided critical insights into the structural characteristics and variations in the intrapancreatic ganglia. This aided in the assessment of their role and function in the pancreas. Images were captured to document the histology and location of the intrapancreatic ganglia. Measurement of fasting blood glucose levels in experimental animals three weeks after sympathectomy and vagotomy were done and compared with the control group. Also, glucose tolerance tests were conducted two hours after intravenous injection of 0.5 gm/kg body weight of glucose in the animals that has survived three weeks after vagotomy and sympathectomy.

RESULTS

The microscopic examination of the control group of rats' pancreas highlights that the intrapancreatic ganglia located in the connective tissue between the pancreatic acini. The ganglia entails of many neurons and axons. The intrapancreatic neurons vary in size and shape. The nerve cells are surrounded by a number of glial cells. The axons of the ganglia are surrounded by Schwann cells (Figures1 and 2). The effect of sympathectomy and truncal vagotomy three weeks after the surgery showed degenerative changes in the intrapancreatic ganglia, the nerve cells disappeared. The axons and glial cells showed degenerative changes and spaces as well, due to degeneration of the connective tissue around the ganglia (figure 3) and (figure 4a&4b). Measurement of the glucose blood levels and the glucose tolerance test of the control group were normal and it ranged between 3.3-4.5 mmol/liter (Table 1 and 2). On the other hand, measurement of the glucose tolerance test showed a vital

rise in blood glucose levels two hours after the intravenous injection of 0.5 gm /kg body weight of glucose in the surviving animals three weeks after denervation as a result of the degeneration of the intrapancreatic ganglia (Table 1 and 2).

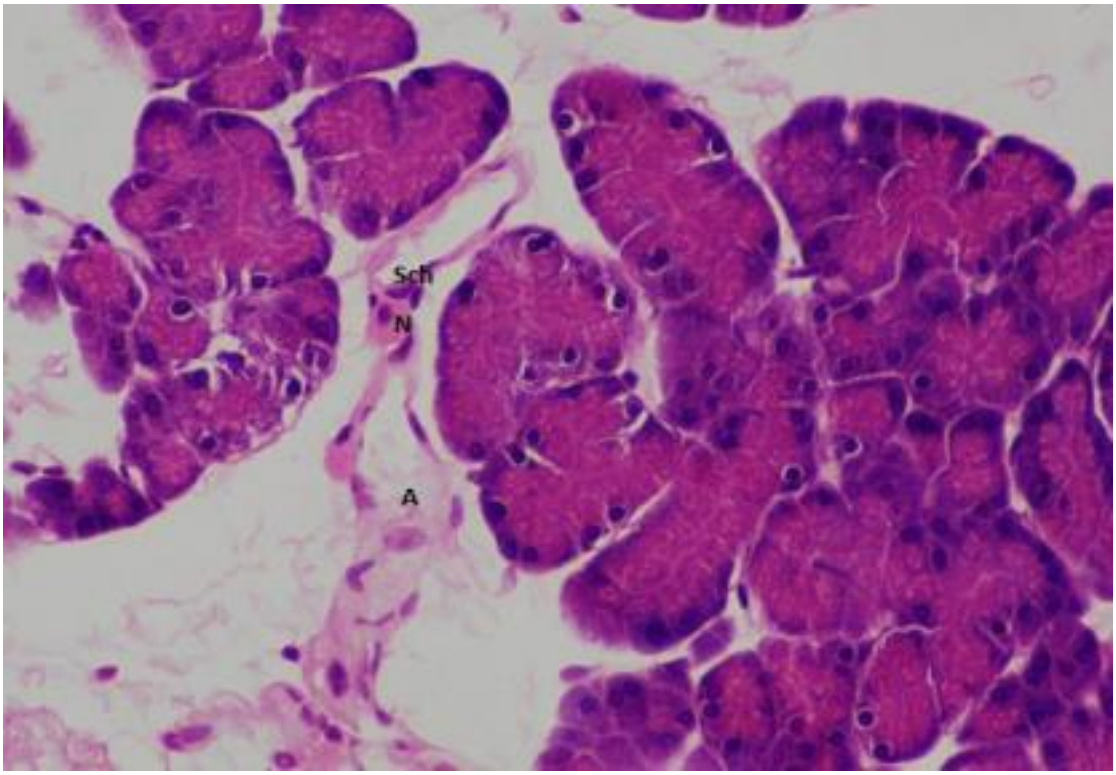

Figure (1): Light micrograph of a section in rat pancreas showing the normal intrapancreatic ganglia contains nerve cell nucleus (N), axons (A), Schwann cell (Sch) (H&Ex400).

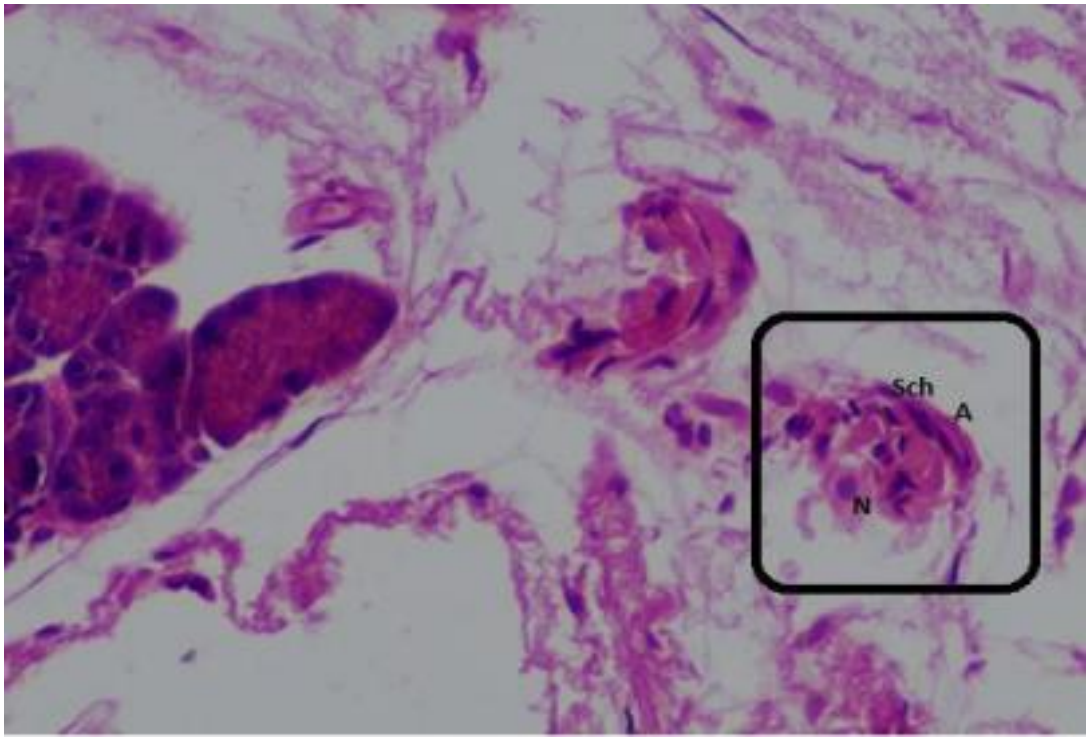

Figure (2): Inside the square, the normal intrapancreatic ganglia lies in connective tissue between the pancreatic acini, contains nerve cell (N), schwann cell (Sch) and axons (A) (H&Ex400).

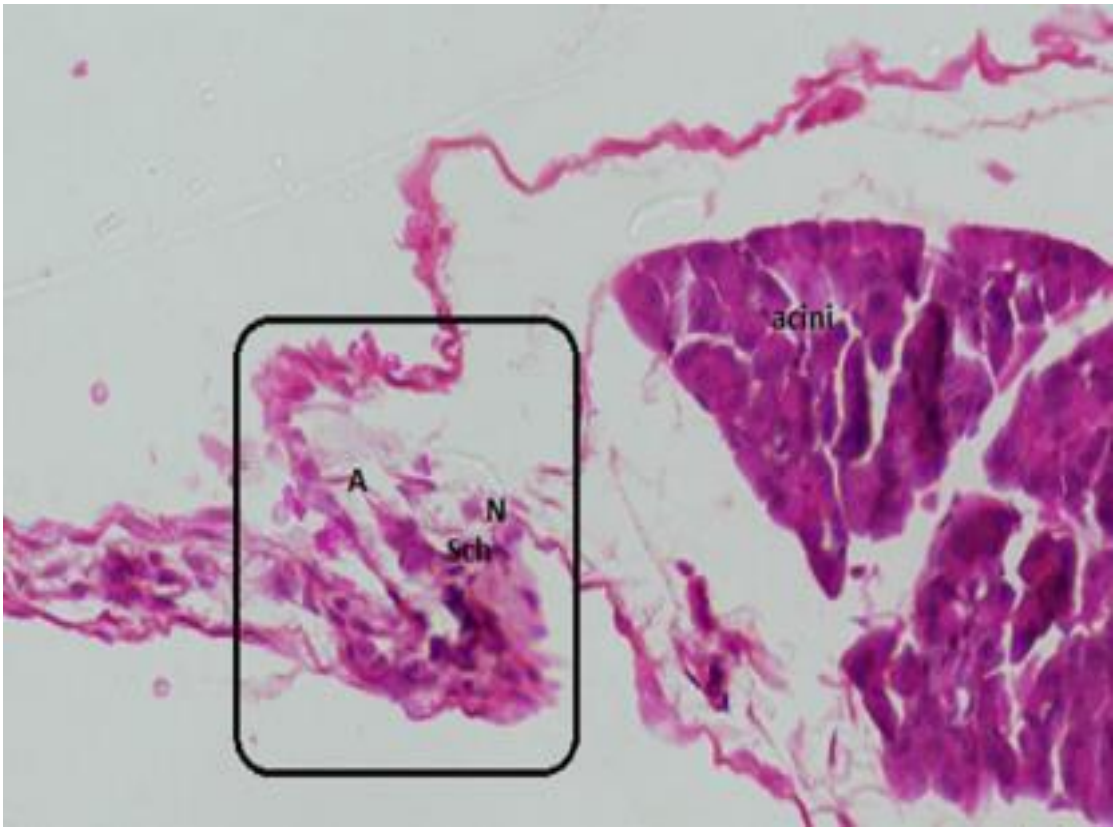

Figure (3): Inside the square, degenerative changes in the intrapancreatic ganglia and spaces in the connective tissue 3 weeks after vagotomy, nerve cell (N), schwann cell (Sch) and axons (A) (H&Ex200).

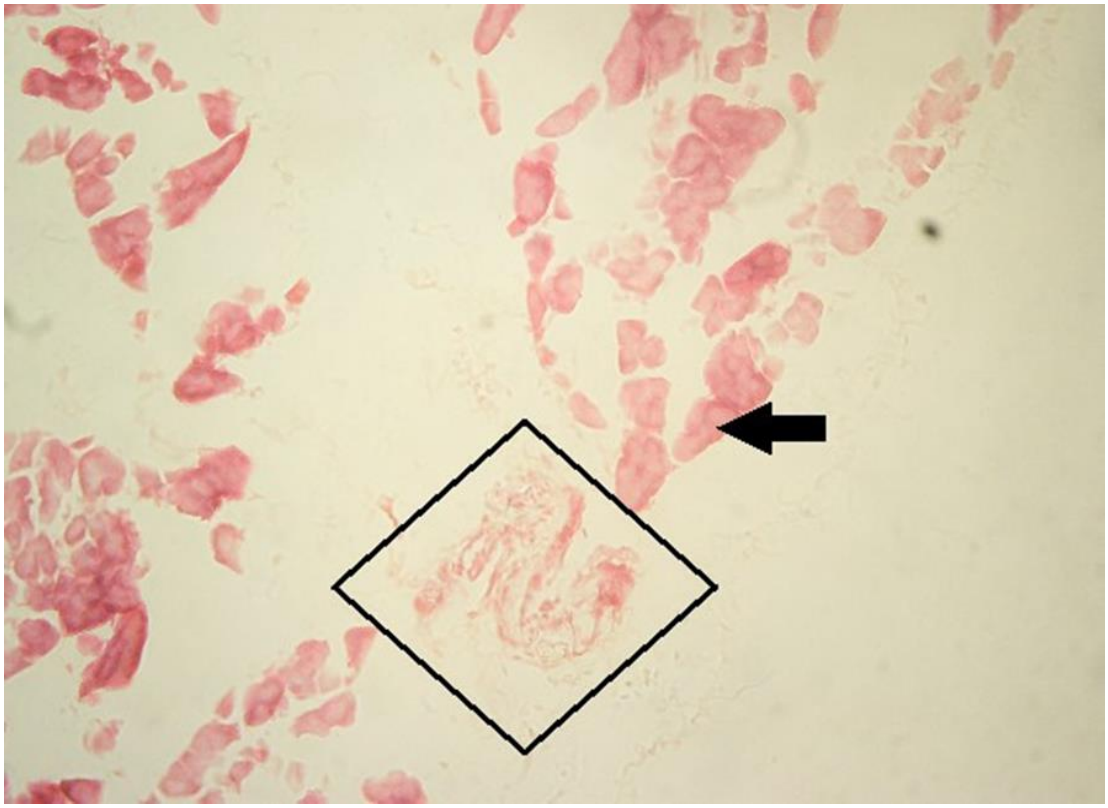

Figure (4a): Inside the square, degenerative changes in the intrapancreatic ganglia, nerve cells disappeared and degenerated axons 3 weeks after sympathectomy. The arrow on the exocrine part shows degenerated pancreatic acini (H&Ex200).

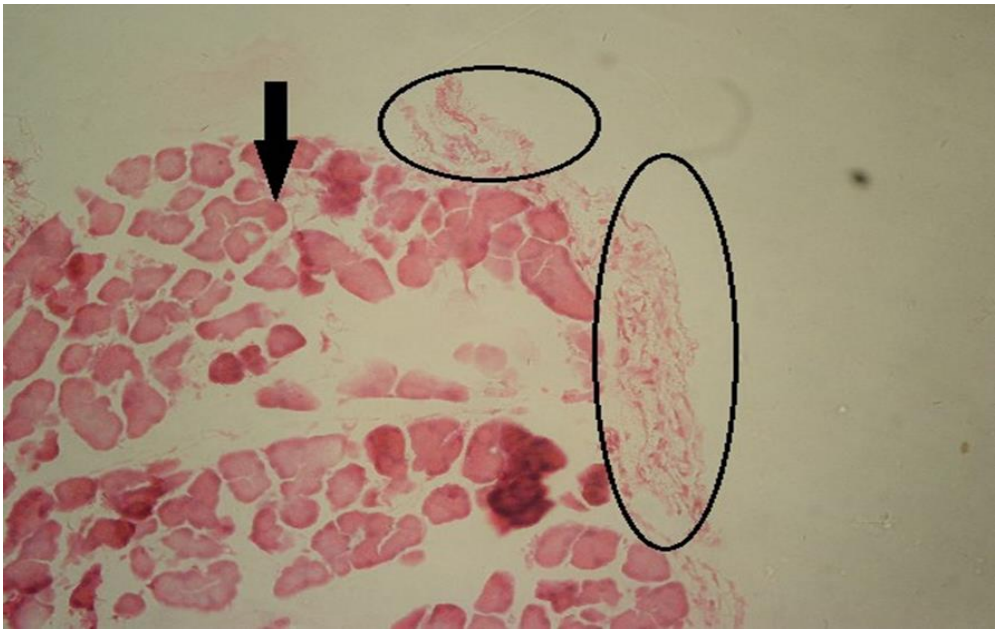

Figure (4b): Inside the circles, degenerative changes in the intrapancreatic ganglia 3 weeks after sympathectomy, nerve cells disappeared and degenerated axons. The arrows showed the exocrine part with degenerative pancreatic acini (H&Ex200).

Table (1): Fasting Blood Glucose Levels in mmol/L.

	Control	3 weeks
After Truncal Vagotomy	3.3-4.4 mmol/L	8.5-10.2 mmol/L
After Sympathectomy	3.5-4.5 mmol/L	8.7-10.5 mmol/L

Table (2): Glucose Tolerance Test Levels in mmol/L.

	Control	3 weeks
After Truncal Vagotomy	4.2-4.9 mmol/L	10.4-14.2 mmol/L
After Sympathectomy	4.0-4.5 mmol/L	8.9-12.6 mmol/L

DISCUSSION

The innervation of the pancreas in different animals has already been assessed through the classical light microscope.[34,35,36,37,38,39] Their results highlight significant information of the innervation pattern of the pancreas. In this study, the histology, location and correlation between the intrapancreatic ganglia with the parts of the pancreas are demonstrated. We believe that the pathway of intrapancreatic ganglia is very rapid and short, this explains the rapid reflex from the pancreas by its secretions after each meal. So, any metabolic changes that affect the glucose level explains the rapid hemostasis of glucose level. Existing research on the pancreas of the mouse,[40] showed that intrapancreatic ganglia contain terminal nerves which create a web of unmyelinated nerve fibers. Another study conducted at China Medical University,[41] it was found that the endogenous neural networks and neurotransmitters exist in the intrapancreatic ganglia. These results are in agreement with our microscopic findings that showed the normal histology and location of the intrapancreatic ganglia. The intrapancreatic ganglia transmit the nerve fibers directly to the islets as demonstrated by tract tracing of the ganglionic neurons,[42] This association with the ganglion-islet portrays a three-dimensional histological study,[21] The activity of the neurons of intrapancreatic ganglionic encourages the secretion of insulin, which results to blockage of the activity that gets rid of modulation.[14] These results are in agreement with the results of our microscopic findings and AL-Muhtaseb study.[33] which showed the location and changes in the intrapancreatic ganglia after surgery and elevation in glucose levels after denervation.

Moreover, several studies have been conducted to investigate the changes in the pancreatic tissue after truncal vagotomy.[43] It is well known that the cholinergic parasympathetic postganglionic nerve fibers have a direct effect on the muscarine receptors of the beta cells, thus stimulating them to release insulin.[44] Also, Al Muhtaseb et al. (2020) showed that there are degenerative changes in the pancreatic tissue after denervation. The effect of vagotomy and sympathectomy causes a vital elevation in blood glucose levels in the pancreas of the rat. The results of this study emphasize that vagal innervations of the pancreas has a direct and indirect impact on the intrapancreatic ganglia. According to Rossi et al, (2005), the parasympathetic fibers that innervate the endocrine part emerge from intrapancreatic ganglia, which are connected with the vagus nerve fibers.

In conclusion, the parasympathetic fibers pass to the enteric plexus of nerves and other nerves to the intrapancreatic ganglia. This reveals that the intrapancreatic ganglia control the function of the endocrine and exocrine parts of the pancreas by direct and indirect connection. This is manifested by the histological changes in the intrapancreatic ganglia and the rise in blood glucose levels after vagotomy and sympathectomy.[33]

The pattern of innervation of the rats' pancreas was found very similar to other organs such as the heart. According to Mitchell 1956, sympathetic and parasympathetic nerve fibers join the cardiac plexus around the trachea, then the cardiac plexus innervate the heart directly or indirectly through the intracardiac ganglia.[45] Therefore, we can consider the intrapancreatic ganglia as a pacemaker of the pancreas. In the future, more studies are needed to be conducted using histochemistry as that might add more to the findings of the current study.

CONCLUSION

The morphology and location of the intrapancreatic ganglia was identified in the rat pancreas. Also, degenerative changes of intrapancreatic ganglia were observed after denervation and this affect the exocrine and endocrine function of the pancreas. This was approved by a high glucose level both in fasting blood and the glucose tolerance tests, which is consistent with the histological results. Therefore, the intrapancreatic ganglia control any metabolic changes through direct or indirect pathways and any disease that affect the ganglia may affect the exocrine or endocrine function of the pancreas for example it may cause diabetes mellitus.

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