Ministry of Higher Education and Scientific Research
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Immunohistochemical distribution of insulin in the pancreas of Iraqi catfish *(silurus triostegus)*

Graduation Research Submitted to the Department of Biotechnology as Part of Requirements for the Degree of Bachelor in Biotechnology Sciences

By

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بسم الله الرحمن الرحيم

فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَى إِلَيْكَ وَحْيُهُ وَقُلْ رَبِّ زِدْنِي عِلْمًا

صدق الله العظيم
(طه - 114)

الاهداء

الى صاحب القلب الكبير ...

الى من اعطاني وينتظر العطاء ...

الى من غرس في نفسي الأمل ...

والدي

الى من عشت في كنف حنانها وحبها ...

الى من رعتني حق رعاية وسهرت الليالي ...

والدتي

الى روافد الوفاء والأخلاص ...

اخوتي واخواتي

اليكم جميعاً اهدي ما وفقني اليه ربي ...

شـــكر وتقـــــــدير

الحمد لله رب العالمين والصلاة والسلام على سيدنا محمد وعلى آله واصحابه الطيبيين الطاهرين.

يسرني وقد انهيت دراستي هذه ان اقدم جزيل شكري وامتناني الى عمادة كلية العـــــلوم لدعمها لي أثناء فترة الدراسة.

كما يسرني ان اقدم شكري الجزيل الى رئيس قسم التقـــــانة الأحيـــائية الأستاذ المســـاعد الدكتـور عصام حامد حميد على رعايتـه الأخويـة.

كما اود أن اتقـــدم بفائـق الشـــكر والأحتـــرام والنقديـــرالـــى أستـاذي الفاضـــــل الدكتور رياض حميد نصيف لأقتراحـه خطـة الدراسـة لمشروع التخرج ولما قدمـه لي من نصائح وتوجيهات سـديدة طيلة فترة انجاز هذه الدراسة داعيا الباري عز وجل أن يحفظه ويوفقه في حياته العلميه والعملية.

ولا يفوتني في هذة اللحضات ان اعبر عن امتناني وشكري الجزبل الى أساتذتي في قسم التقانة الأحيائية الذين كانوا نعم الأخوة والسند لي خلال فترة الدراسة.

واخيراُ اتقدم بالشكر لكل من قدم لي يد العون والمساعدة.....

اقرار المشرف وترشيح رئاسة قسم التقانة الاحيائية

اشهد بان اعداد هذا البحث الموسوم بـ immunohistochemical distribution of insulin in the pancreas of Iraq catfish *(silurus triostegus)* الذي قدمته الطالبات/الطلبة (**رند محسن حسين، أسراء محمود نجم, اسامه توفيق قدوري, سجاد مجيد مهدي**) قد جرى تحت اشرافي في كلية العلوم/قسم التقانة الاحيائية/جامعة ديالى، وهو جزء من متطلبات نيل درجة البكالوريوس في علوم التقانة الاحيائية.

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التاريخ: 1 /7 /2020

بناء على التوصيات المتوافرة ارشح هذا البحث للمناقشة

التوقيع:
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التاريخ: / /2020

اقرار لجنة المناقشة

نشهد باننا اعضاء لجنة المناقشة، اطلعنا على هذا البحث الموسوم بـ
immunohistochemical distribution of insulin in the pancreas of Iraq catfish (silurus triostegus)
الذي قدمه كلا من الطالبات (**رند محسن حسين، أسراء محمود نجم, اسامه توفيق قدوري, سجاد مجيد مهدي**) في محتوياته وفيما لها علاقة به، ونعتقد بانهم جديرون بالقبول لنيل درجة البكالوريوس في علوم التقانة الاحيائية بتقدير ( ).

رئيس اللجنة
التوقيع:
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مصادقة رئاسة قسم التقانة الاحيائية

رئيس اللجنة
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المرتبة العلمية:
التاريخ: / /2020

Abstract

The purpose of this study is to describe the regional distribution, volume density, and relative frequency of some pancreatic endocrine cells in Iraqi catfish (*silurus triostegus*) were investigated via immunohistochemistry using specific mammalian anti-bodies. The pancreas is divided into exocrine and endocrine portions. Exocrine pancreatic tissues consist of scattered serous acini, and is observed in two forms: 1) disseminated in the spleen tissue, in mesentery around intestine and intestinal bulb, and 2) intra-hepatically, around the branches of the portal vein.

The exocrine portion formed up nearly the whole of the pancreas constructed of different sized lobules of well-formed parenchyma made by densely packed acinar units. The parenchyma provided with a well duct system in which most of the interlobular ducts were lined by simple columnar epithelium with goblet cells.

The pancreatic endocrine cells were examined, including the islets, exocrine pancreas, and pancreatic ducts. According to the immunohistochemistry,insulin-secreting beta (ß) cells were observed predominantly throughout the islets, and for a lesser extent α cells. The δ cells were rarely detected.

Contents

Chapter 1: Introduction

1. 1. Introduction

The pancreas is an organ of special interest from a medical viewpoint as it is the target of two major diseases that are diabetes mellitus and pancreatic cancer. It is to be hoped that a better understanding of the morphology and histology of this organ will eventually contribute to the development of novel therapies for the treatment of either or both of the above diseases (Seymour et al., 2004).

Generally known that the pancreas is an organ found in all vertebrates with two different functional components that are essential for the homeostatic regulation of glucose and food digestion. One is exocrine where the digestive enzymes are released and the other is endocrine, where regulatory hormones such as insulin, glucagon, somatostatin, and pancreatic polypeptide (PP) are released into the blood vessels (Orci, 1982).

The exocrine pancreas is composed of acinar and duct cells involved in the production and transport of digestive enzymes into the gut for the digestion of proteins, carbohydrates, lipids, and nucleotides, whereas the endocrine compartment regulates the production and secretion of peptide hormones into the bloodstream (Genten *et al*., 2009).

The hormonal expression is specific to each cell type: glucagon (α cells), insulin (β cells), somatostatin(δ cells), and pancreatic polypeptide (PP cells) (Ali, 1985). The appearance, regional distribution, and relative frequency of these regulatory hormones secreted by endocrine cells in the pancreas are well identified by histochemistry (Kobayashi and Ali, 1981), immunofluorescence (Orci,1982) and immunohistochemistry (Sternberger *et al*., 1970).

Iraqi catfish (silurus triostegus) were considered belong to:

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Siluriformes

Family: Siluridae

Genus: Silurus

Species: S. soldatovi

Up to date, there is no research in the current literature conducted to compare the morphology and histology of the pancreases of the Iraqi catfish (silurus triostegus), and there is a paucity of work focused only on the pathological aspect and concerning diseases of these organs in rabbit and other species.

1. 2. Aim of the study

Accordingly to the above reasons the current study will be conducted to first investigate the regional distribution, volume density, and relative frequency in the islets of cells secreting insulin in pancreas of Iraqi catfish (silurus triostegus) using antisera against mammalian counterparts to provide better understanding data for the immunohistochemical aspect of this organ.

Chapter two: Literature reviews

2. Literature reviews

2. 1. Gross reviews

2. 1. 1. Gross reviews of pancreas

The pancreas of the adult grass carp (*Ctenopharyngodon Idella*) appeared as groups of yellowish masses that distributed in mesenteries around the intestine, spleen, and intestinal bulb. It also was diffusely spread within the hepatic tissue to form hepato-pancreas, but the pancreas could not be seen macroscopically within the liver (Mokhtar, 2015).

Previously, The pancreas of teleosts fish was classified into disseminated, compact, and intrahepatic types. The disseminated type occurs around gall bladder and in mesentery; the compact pancreas is located in the splenic mesentery, and acts as a discrete organ, while the intrahepatic pancreas is located inside the liver (Rizkalla,1967).

2. 2. Histological Reviews

2. 2. 1. Histological reviews of pancreas

On the same animal species, recent study on the pancreas, the grass carp was a highly lobulated gland, each lobule was composed of closely packed secretory pancreatic acini separated by thin connective tissue septa. It was divided into exocrine and endocrine portions. Exocrine pancreatic tissues of the grass carp were observed in two forms: the first one was disseminated in mesenteries around the intestinal bulb and the anterior portion of the intestine, as well as embedded in splenic tissue, the second form was intrahepatic, around the branches of the portal vein separated from the hepatic parenchyma by a thin layer of connective tissue mainly of collagenous fibers. Abundant amounts of reticular fibers were determined in the pancreatic tissue that appeared as a black colored meshwork encircling the pancreatic acinar cells and concentrated around the blood vessels. Fine and few elastic fibers were also demonstrated in the pancreatic tissues (Mokhtar, 2015).

In Actinopterygian (ray-finned) fish the exocrine pancreas commonly diffuses in the mesentery that extends between the bile ducts, blood vessels in the abdomen, intestine, stomach, liver, and gallbladder, and exocrine tissue commonly presents in intrahepatic sites among the teleosts. Islet tissue may be

observed in any of those regions, with the exception of intra-hepatic sites (Youson and Al-Mahrouki, 2006)

Exocrine pancreatic tissue consisted of scattered serous acini. Two alveolar cell types were present in the acini; centroacinar and typical acinar cells. The centroacinar cells were polyhedral cells attached by their basal ends toward the central lumen, and represent the terminal end of the pancreatic duct system. The typical acinar cells were polyhedral in shape, arranged in groups characterized by rounded to oval shape and intracytoplasmic granules or surrounded by a thick capsule. around the lumina of the pancreatic acini (Wang *et al.*, 1986).

The exocrine pancreas composed of large principle islets and small secondary islets. Principal islets diffusely existed in the exocrine pancreas and were irregular, oval, or round in shape, and in small quantities. They were observed to be located mainly in the exocrine pancreas tissue, which was surrounded by adipose tissue. The small secondary islets were oval or round in shape and scattered throughout the exocrine pancreas, and were more in point of numbers than the principal islets. in turn, the exocrine pancreas was also observed around the bile ducts entering the liver; however, the endocrine cells

or islets could not be detected in those portions of the exocrine pancreas (Burak, 2019).

The endocrine pancreas of the channel catfish Ictalurus punctuates is localized into a single primary islet (or Brockmann body) and numerous secondary islets. The primary islet, a fusiform structure approximately 4 mm in diameter, is found at the inferior border of the gall bladder. The secondary islets are more spherical, usually less than 2 mm in diameter, and are found lying within the mesentery of the common bile duct. It is unknown if these size and spatial differences between islets in the catfish reflect differences in the embryological development of the respective islet tissue (Brinn, 1971).

In most vertebrate species studied,4 endocrine cell types have been described in the endocrine pancreas. Immunohistochemical, these cell types have been shown to contain glucagon, insulin, somatostatin, and pancreatic polypeptide, A, B, D, and pancreatic polypeptide-containing cells, respectively. The topographic location of these cell types varies from species to species (Erlansen *et al*., 1976).

Three endocrine cell types were demonstrated with immunohistochemistry using antibodies against mammalian insulin, glucagon, and somatostatin in the pancreatic islets, ducts, and exocrine regions. The volume density of each immunoreactive endocrine cell within the principle and secondary islets were 54.03±2.50%, 28.70±0.94% and 11.37±0.98% whereas, 52.08±2.05%, 27.31±3.15% and 13. 61±1.47% respectively. the similar measurements of insulin-, glucagon- and somatostatin immunoreactive cells were counted at about54%, 28%, and 11%, in the principle islets, while they were counted at about 52%, 27%, and 13% in the secondary islets respectively. Significant differences were not found between any of the IR cell types in the principle and secondary islets (Burak, 2019).

The distribution of insulin-immunoreactive cells well corresponded to observations from the aldehyde fuchsine staining and those cells were determined to be localized throughout the islets. Insulin-immunoreactive cells were commonly observed in the central region and a small number in the peripheral areas were intermingled with other endocrine cells in the principle islets. A similar trend in the distribution of those cells was also observed in the secondary islets. Insulin-immunoreactive cells were also found in the exocrine parenchyma, either individually or with two or three grouped together. Those cells were found around the exocrine ducts, either individually or with two or

three grouped together, and were rarely noted in the epithelium or sub-epithelial connective tissue of the exocrine ducts (Yang *et al.,* 1999).

2. 2. 2. The duct system of exocrine pancreatic tissue

The duct system of the pancreas consisted of intralobular, interlobular pancreatic ducts, and main duct. The intralobular pancreatic ducts were small (80-100 μm in diameter), present in the pancreatic lobules and consisted of a simple cuboidal epithelium surrounded by layers of collagenous and elastic fibers. The interlobular pancreatic ducts were larger (500-750 μm in diameter), present between the pancreatic lobules and the mucosa was thrown into mucosal folds. The wall of the interlobular duct consisted of simple columnar epithelium with goblet-like cells surrounded by layers of collagenous fibers. The main duct was the larger duct (1100-1400 μm in diameter), opened in the middle portion of the intestinal bulb, and surrounded by collagenous fibers. The main pancreatic duct was composed of a simple columnar epithelium with a PAS-positive apical portion and some silver- positive argyrophilic cells (Mokhtar, 2015).

Chapter 3: Conclusions and Recommendations

3. 1. Conclusions

1. Gross findings showed the presence of only one minor pancreatic duct in the pancreas of Iraqi catfish (*silurus triostegus*) and such result was significantly different than most rodents by having major pancreatic duct and that in rabbit have two ducts i.e. the minor as well as an accessory ducts.

2. Most branches of the duct system were lined by columnar epithelium with goblet cells.

3. Percentage of ß cells in the islets of the pancreas was higher than α, and δ. Such observations were parallel positively with hormones analysis of insulin glucagon and somatostatin which were a corresponding products to these cells.

3. 2. Recommendations

1. Comparative histological and immunohistochemical study of the pancreas of Iraqi catfish (silurus triostegus) with other local lab animals such as rabbits.

2. Light and EM comparative histological and immunohistochemical study of the pancreas of Iraqi catfish (silurus triostegus) with golden hamster.

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الخلاصـــــــة

الغرض من هذه الدراسة هو وصف التوزيع الإقليمي ، وكثافة الحجم ، والتكرار النسبي لبعض خلايا غدد البنكرياس الصماء في سمك السلور العراقي *(silurus triostegus)* الذي تم بحثها عن طريق الكيمياء المناعية باستخدام أجسام محددة مضادة للثدييات. ينقسم البنكرياس إلى أجزاء من الغدد الداخلية والخارجية. تتكون أنسجة البنكرياس الخارجية من اسناخ مبعثرة ، وتلاحظ في شكلين: 1) منتشرة في أنسجة الطحال ، في المساريق حول الأمعاء والمصباح المعوي ، و 2) داخل الكبد ، حول فروع الوريد البابي.

يكون الجزء ذات الافراز الخارجي للبنكرياس معظم تركيب العضو مكونا الفصيصات مختلفة الاحجام والتي تتكون من وحدات افرازيه بشكل مكثف. التركيب الغدي مجهز بنظام قنوي ممتاز وفيه تظهر معظم القنوات بين الفصيصيات مبطنه بظهاره عموديه مع خلايا كأسية.

تم فحص خلايا الغدد الصماء للبنكرياس ، بما في ذلك الجزر والغدد ذات الافراز الخارجي وقنوات البنكرياس. ووفقًا للكيمياء المناعية ، لوحظ أن خلايا بيتا التي تفرز الأنسولين تتمركز في جميع أنحاء وبدرجه اقل على خلايا الفا بينما نادرا ما توجد او تظهر خلايا دالتا.

وزارة التعليم العالي والبحث العلمي
جامعة ديالى/كلية العلوم
قسم التقانة الاحيائية
الدراسة الصباحية

التوزيع الكيميائي المناعي للأنسولين في بنكرياس اسماك السلور العراقي (silurus triostegus)

بحث تخرج مقدم الى

مجلس قسم التقانة الاحيائية/كلية العلوم/جامعة ديالى

وهو جزء من متطلبات نيل درجة البكالوريوس في التقانة الاحيائية

من قبل

رند محسن حسين
اسامه توفيق قدوري
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سجاد مجيد مهدي

باشراف

م. د. رياض حميد نصيف

2020م – 1441هـ