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"Immunohistological study of localization of Enteroendocrine cells in the small intestine in the Iraqi buffalo"

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Abstract

a small number of parts of the intestinal tract, which slot in recreation a vital and main role in the secretion of persuaded hormones that control significant organs of the body. The aim of this study is to look at the site of endocrine cells in small intestine in Iraqi buffalo . The regions anywhere endocrine cells in the intestine layer of the five Iraqi buffalo examined via using immunohistochemistry process material. Specimens were taken start five animals from the ages ranging from 3-5 years. It was used immunohistochemistry fabric technology and (ChromograinnA), which is a special detector Endocrine intestinal cells, four types of hormones. Immune chemical assessment in the mucous layer duodenum and the presence of hormones, 'glucose insulin tropic polypeptide(GIP) results showed (GIP) and cholecystokinin (CCK) glucagon-like peptide2 (GLP-2)' in epithelial cells collection along the internal axis of the villi, chemical immunological consequences for many of the sections shown note chromogranin A detector chiefly for intestinal endocrine cells, the cells that have hormones (GIP) , glucose insulin tropic polypeptide cholecystokinin(CCK) and glucagon peptide GLP-1 \ 2)) containing this reagent representative confirm the site of these cells, a gastric endocrine cells . The attendance of these hormones in the intestine layer decreases as we move away from the duodenum.

Keywords: Enteroendocrine cells, small intestine, buffalo, Hormones.

الخلاصة

للخلايا الصماوية المعوية في بعض اجزاء القناة المعوية دور مهم ورئيسي في افراز بعض الهرمونات التي تنظم اجهزة الجسم المهمة من خلال تواجد الخلايا الصماوية في الطبقة المخاطية للأمعاء الدقيقة في الجاموس العراقي حيث تم الكشف عنها باستخدام التقنية الكيميائية المناعية النسيجية في المختبر المركزي لكلية الطب البيطري في جامعة القادسية . حيث تم اخذ خمسة عينات من امعاء الجاموس العراقي تتراوح اعمارها ما بين (1-2) سنة واجريت عليها التقنية باستعمال اربعة انواع من الهرمونات وهي كروموكرانين أ والكلوكوز بيببتيدو الكلوكاجون بيببتيدو الكوليستوستوكينين . ان الهدف من هذه الدراسة هو فحص مواقع الخلايا الصماوية في الطبقة المخاطية في الامعاء الدقيقة للجاموس العراقي . بحيث اظهرت نتائج الفحص وجود الهرمونات الاربع المذكورة اعلا على طول المحور الداخلي للطبقة المخاطية في الاثني عشري والصائم واللفائفي خصوصا في الزغابات وهذا يدل على احتواء كل من هذه الهرمونات على الكاشف كروموكرانين أ مما يدل على تأكيد مواقع هذه الخلايا في الطبقة المخاطية للثلاثي عشري وتبدأ بالانخفاض كلما ابتعدنا عن الاثني عشري.

Introduction:

In adult buffalos the regional distribution of endocrine cells is similar to that of other adult ruminants. During postnatal development, these cell types showed the following changes in their frequency and distribution: (1) 5-HT, neurotensin and gastrin/CCK immunoreactive

cells (i.c.) showed a decrease in frequency with age; (2) somatostatini.c. frequency remained stable with age; (3) motilin, GIP, secretin and CCK i.c. showed a slight increase in frequency with age; (4) GLU/GLI and PYY i.c. decreased in frequency with age in the small intestine, caecum and proximal colon

and an increase in frequency in the rectum. It was hypothesised that the endocrine cell types, whose presence and localisation is substantially stable in all examined ages, probably contain substances that are strictly necessary for intestinal function. In contrast the hormones contained in the cell populations that decreased with age, are probably involved in physiological needs during the milk and weaning diet or play a role in intestinal growth (1). Goat turn out to be an important component of animal manufacture in Mideast . At the financial level of individual families ,small ruminants serve as asset and indemnity due to their high fruitfulness, short generation gap, ability to produce under incomplete feed resources and their flexibility to harsh environment (2). Small Intestine: As partly digested feed enters the duodenum, the first piece of the small intestine, the enzymes produced and secreted by the pancreas and the Brunner's glands of the duodenum additional break down feed nutrients into straightforward compounds. These compounds are engrossed into the bloodstream or lymph by an active process carried on mainly in the jejunum and ileum second and third part of the small intestine, respectively. The small intestinal partition is lined with many small fingerlike projections called villi, which increase the amalgamation area of the small intestine. The ability of the small intestine of goats is approximately 2-1/2 gallons. Large Intestine: Undigested nourish and unabsorbed nutrients send-off the small intestine pass into this compartment. The functions of the large intestine comprise water absorption and additional digestion of feed materials by microorganisms. The large intestine is comprised of the colon and rectum. Fecal pellets are shaped in the end portion of the spiral colon. The capacity of the large intestine of goats ranges from 1-1/4 to 1-1/2 gallons(2,3). In domestic animals the small intestine is alienated into three parts: duodenum, jejunum and ileum. The three regions of the small intestine split a common histological pattern with some particular characteristics of each part. Their wall from outside to inside have the serosa, the muscularis, the submucosa and the mucosa(4) . The gastrointestinal endocrine system has

been on a huge scale deliberate by means of "immunocytochemistry". Different gut hormones have a major role on the digestive functions of the stomach and intestine [4]. The distribution of endocrine cells in the gastrointestinal tract has been extensively investigated in the gastrointestinal tract of many domestic [5,6] . Enteroendocrine cells (EEC) play manifold roles in the gastrointestinal response to food, coordinating secretory and motor events to maximize the efficient digestion and absorption of food. They also play a key function in maintaining epithelial integrity, and contribute to the mucosal innate immune system."They make up <1% of the whole gut epithelial inhabitants, but together are the largest endocrine organ of the body (7). Gastrointestinal endocrine cells discrete in the mucosa of gut synthesize different types of gastrointestinal hormones which played an considerable role in the physiology purpose of alimentary tract such as nutrient absorption , the secretion of intestinal and associated glands, gut motility and augmented intestinal blood pour (8). in addition the regional distributions and family affiliate frequencies of these endocrine cells differ according to the animal species and feeding behavior (9). huge interest is the function of these endocrine cells and the enteric nervous system contribution as neurotransmitters in the directive of muscular movement, oozing of intestinal glands and control of vascular permeability of gut" (10).

Material and methods

Five sampling were obtained start small intestine of Iraqi buffalo of both male and female , aged 3-5 year; weight 350-800kg), were collected from Al-Qadisiyah abattoir. Sections were wash in ice cold "0.9% (w/v) NaCl pH 7.4" and permanent in 10% formaldehyde, dehydrated through an ethanol-xylene sequence then that entrenched in paraffin for histological examinations and immunofluercent studies . Sections are incise at 5mm in thickness. Slides, containing wax embedded camel pancreas tissue, be dewaxed in 100% xylene for 3 x 10 minutes each. The tissue was located twice in 100% ethanol for 2 x 10 minutes and detached, allowable to air dry for 10 minutes and were circled with

ImmEdge Hydrophobic Pen and allowable to dry for 10 minutes. then, they were positioned 2 x 5 minutes in 70% ethanol. Slides were then rehydrated two times in distilled H₂O for 5 minutes each. Slides were engrossed in antigen repositioning buffer (10mM Tris/HCl pH 10.0) and autoclaved (Series A1200086, LMS CONS. Ltd, Germany) 2 x 15 minutes at 121°C and 15 psi. next, slides were allowed to cool in antigen rescue buffer for 30-60 minutes at room temperature and washed for 3 x 5 minutes in phosphate buffer saline (PBS). distracted antibody compulsory sites were infertile by incubating the tissue sections for 1 hour in the blocking solution 10% (v/v) donkey serum in a humidified assembly at room temperature. Sections were incubated

during the night at 4°C with primary antibodies (Table 1). Every slide was then washed in PBS for 5 x 5 minutes. FITC-conjugated IgG / IgY (Table 1) (Stretch, technical imperfect, Suffolk, UK) were second-hand at a intensity of 1:500 for 1 hour incubation at room temperature. Finally, slides were wash with PBS for 5 x 5 minutes and mount in Vectashield firm Set rising Media with DAPI "Vector Laboratories Ltd, Peterborough, UK". Sections were visualized by an "epifluorescence microscope" (MEIJI TECHNO, Model MT4300, Japan) and imagery were captured with a "Canon digital camera" (DS126371, Canon INC, Japan). Omission of primary antibody was routinely used as negative control.

Table(1): Primary and Secondary Antibody.

"Primary antibody Host Dilution clonality and starting place"			
<u>Polyclonal, GIP(Y-20):sc-23554, Santa Cruz Biotechnology, INC., Santa Cruz, CA,USA.</u>	1-100	buffalo	Anti-GIP
<u>Polyclonal, GLP-1(C-17):sc-7782, Santa Cruz Biotechnology, INC., Santa Cruz, CA,USA.</u>	1-100	buffalo	Anti-GLP-1
<u>Polyclonal, GLP-2(C-20):sc-7781, Santa Cruz Biotechnology, INC., Santa Cruz, CA,USA.</u>	1-100	buffalo	Anti-GLP-2

Secondary antibody Label Dilution Source

<u>Fluorescein-conjugated IgG (705-095-147), Stratech Scientific Limited, Suffolk, UK.</u>	<u>1:500</u>	<u>FITC</u>	<u>Donkey anti-buffalo IgG</u>
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The relative frequency of "IR" cells was located into 1of 5 categories, not detect (-) , uncommon (±; mean standards were underneath 2/one field) , few (+; mean standards were below 5/one filed); reasonable (++; mean values were below 10/one filed) and elevated (+++; mean values were up to 20/one filed) , according to their experiential mean numbers as seen under one filed of "epifluorescence microscope" (200X) and the surveillance of each region of small intestine was conducted as triplet by three histologists.

Results

In the 'small intestine' of the buffalo, the results were indicated clearly by using immunohistochemistry that the GIP, CCK, GLP-1 and GLP-2 were spoken in a separation of cells along the villus (Fig. 1.B).Gut hormones show no labeling ; at what time the primary antibodies was absent

from the control section (fig.1.A). The results showed the relative frequency and

sharing of endocrine cells in the small intestine of goat were examined by using four type of 'antisera . The "immunoreactive" cells were recognized in the small intestine, the majority of the gut hormone were situated in the basal portion of glands. The "IR" cells

were triangular or slim in shape .They appear as close type cells as they did not have lamina get in touch with their apical cytoplasmic

processes. Observed some open-type cells with apical cytoplasm process that arrive at the intestinal lumen .

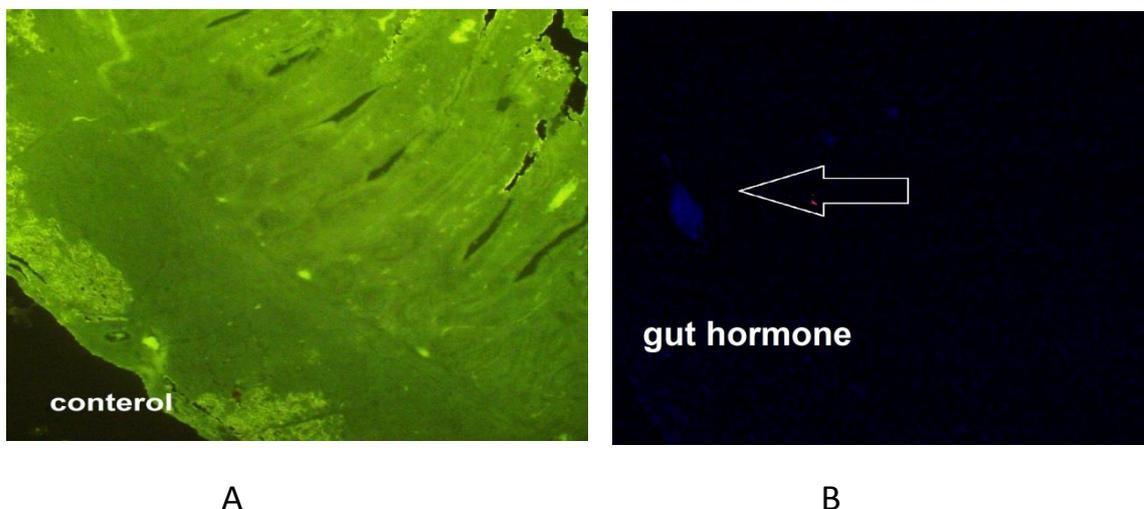


Figure 2, A, B: "Wax embedded intestinal tissue sections from goat were probed with the antibodies to gut hormone (GIP, GLP-1, GLP-2 and CCK). Typical image showing that gut hormone (blue) is expressed in a subset of intestinal cells. When the primary antibody was lost from the control section there is refusal labeling for gut hormones show in control image. Images are 200 X magnified. Nuclie are stained blue with 4 6'- di a m i d i n o - 2 - p h e n y l i n d o l e (D A P I) ' ' .

The relative frequency and sharing of enteroendocrine cells in the duodenum on the intestinal villi , in the crypts (intestinal glands) and in Brunner's glands (duodenal glands). These results show in (Table 1). Generally, the endocrine cells were experiential in high frequently in the villi of duodenum, less frequently on the intestinal crypts and rarely in Brunner's glands. In the jejunum GIP IR-cells were experiential in rare frequency on the intestinal villi. In the ileum GIP IR-cells were not detect. The duodenum contains the most variety of endocrine cell types in the gut. the L-cells which containing GLP-1 and GLP-2 the relative frequency is an increase caudally along of the buffalo small intestine compare with duodenum and jejunum (Table.1).In the duodenum and jejunumGLP-1\2 IR- cells were not observed.

Table (1) Regional distribution and Relative Frequencies of the Enteroendocrine Cells in the Intestinal Tract of goat''.

Hormone	Duodenum	Jejunum	Ileum
GIP	+++	±	-
GLP-1	-	-	++
GLP-2	-	-	++

Relative frequencies: +++ : high, ++ : moderate, + : few , ± : rare, : not detected.

By using double immunostaining, cell type expressing gut hormones was employed with an antibody to chromogranin A (ChA), a classical marker for endocrine cells. With double immunostaining technique, primary antibodies to each protein were raised in different species. This

allows labelling with two secondary antibodies anti-goat IgG labelling with one fluorochrome and anti-rabbit IgG labelled with another. When viewed singly, staining was red for one protein and green for another. When the images were merged, areas of co-expression showed as orange / yellow. Sections of small intestine from buffalo were incubated with antibodies to alimentary canal hormones (GIP, CCK, GLP-1 and GLP-2) and ChA (Fig.2). The results of immunostaining techniques showed that enteroendocrine cells containing gut hormones and ChA were co-expressed in the same cells in goat small intestine. The immunoreactive cells were identified in the small intestine, most of the gut hormone were located in the basal portion of glands. The IR cells were triangular or cerephical in shape (Fig.3).

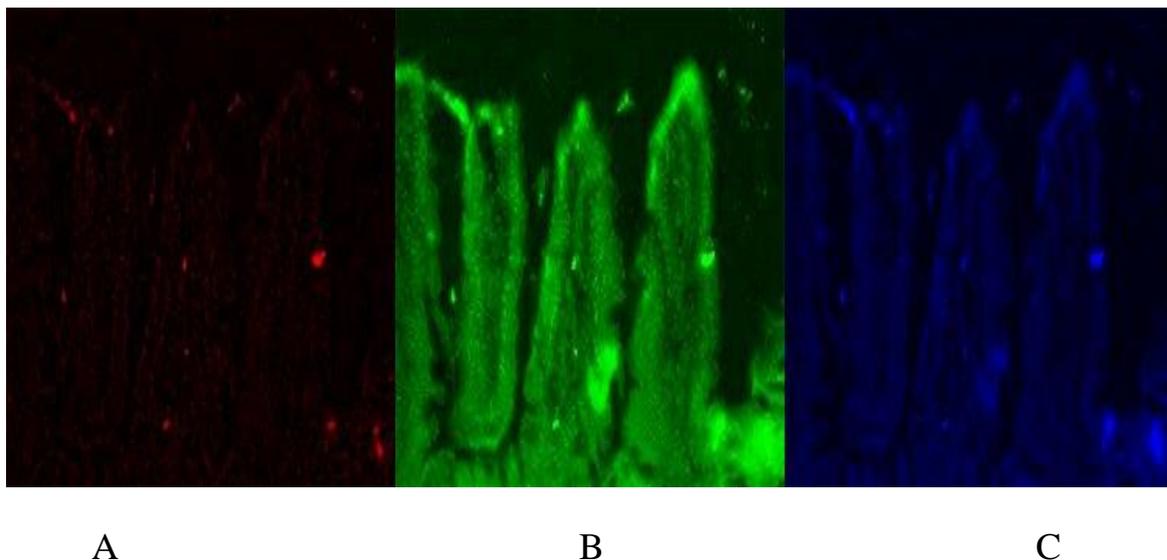


Figure.2 A.B.C: Typical images showing gut hormones (GLP-1 and GLP-2 in duodenum) green and red in a subset of buffalo duodenum. Image C is (200 X) magnified A and B are (400 X) magnified. Nuclei are stained blue with 4', 6-diamidino-2-phenylindole (DAPI).

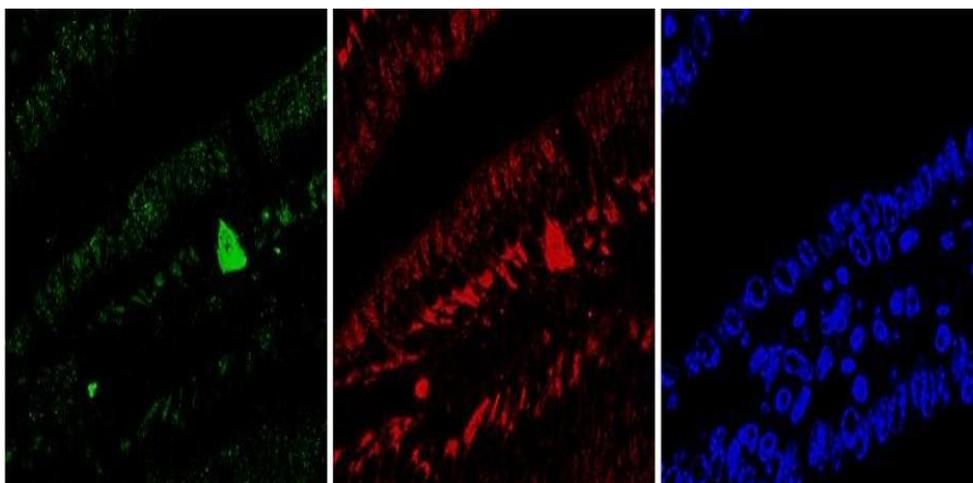


Fig. 3: typical immunofluorescence images showing co-localisation of gut hormones (GIP) (green) with ChA (red) in duodenum.

Discussion

In this study, the structure of small intestine is similar to other mammalian species especially to the duodenum of the porcine support by (Ross and Pawlina, 2011).

In the "duodenum, jejunum and ileum" of the buffalo, the results were indicated clearly by using immunohistochemistry that the GIP, CCK, GLP-1 and GLP-2 were spoken in a separation of cells along the villus. Gut

hormones show no labelling; when the primary antibodies was absent from the control section .

The results showed the relative frequency and sharing of endocrine cells in the small intestine were examined by using four type of antisera. The immunoreactive cells were recognized in the small intestine, most of the gut hormone were situated in the basal portion of glands. The IR cells were triangular or slender in form .They appeared as close-type cells as they did not own lamina contact with their apical cytoplasm process. experiential some open-type cells with apical cytoplasmic processes that reach the intestinal lumen more frequency in duodenum area.

The relative frequency and sharing of IR endocrine cells in the duodenum on the intestinal villi, in the crypts (intestinal glands) and in Brunner's glands (duodenal glands). These results show in .Generally, the endocrine cells were observed in high frequently in the villi of duodenum, less frequently on the intestinal crypts and rarely in Brunner's glands. In the jejunum GIP IR-cells were observed in rare frequency on the intestinal villi. In the ileum GIP IR-cells were not detected. The duodenum contains the maximum diversity of endocrine cell types in the digestive tract. This finding supported by (Solcia *et al.* 1987).

CCK IR- cells were observed in high frequently in the villi and the intestinal crypts of the duodenum. In the jejunum CCK IR-cells were observed in few frequency on the intestinal villi. In the ileum CCK IR- cells not detected .It is well established that the duodenal mucosa plays a very important role in digestion and influences pancreatic secretion and gall bladder emptying in higher mammals via gastrointestinal hormones released from the proximal small intestine. The proximal duodenum is thought to be protected, at least in part, from acid-pepsin entering from the stomach by secretions from Brunner's glands. Similar findings concluded that CCK have been established on dromedary camel (Ali *et al.*, 2007), (Krause, 1985), (Al-Rahabi and Al-Rammahi, 2014).

In the small intestine K-cells which contain GIP and I-cells containing CCK, were detected . The relative frequency is a caudally

decrease along of the buffalo intestine (Table 4.1), the I-cells and K cells that expressed the CCK and GIP hormone in duodenum and subsequently ,was decreased in jejunum and ileum. This finding was supported by Rindi *et al.* , (2004), Ali *et al.*, (2007) and Filed *et al.*, (2010).

References

- 1- Lucini C¹, De Girolamo P, Coppola L, Paino G, Castaldo L. (1999) . Postnatal development of intestinal endocrine cell populations in the water buffalo. J Anat. Oct;195 (Pt 3):439-46.
- 2- Robert N, (2004). Anatomy and physiology of goat www.agric.nsw.gov.au
- 3- Church, D. C. (1993). The ruminant animal: Digestive physiology and nutrition. Prospect Heights, IL Waveland Press, Inc
- 4- Junqueira ,L.C. and Carneiro , J. (2005) : Basic Histology text and atlas 11th .ed. McGraw – Hill, Pp : 281 - 311.
- 5- Capella C., Solcia E. (1972). The endocrine cells of the pig gastrointestinal mucosa and pancreas. Arch. Histol. Jpn., 35 : 1-29.
- 6- Ceccarelli P., Gargiulo A.M., Pedini V. (1990). Endocrine cells of the GEP (gastro-entero-pancreatic) system in horse intestine (in Italian). Atti Societa Italiana Scienze veterinarie , LIV, 311-313.
- 7 - Sternini, C., Anselmi, L. & Rozengurt, E. (2008): Enteroendocrine cells : asite of taste'ingastro intestinal chemosensing. Curr. Opin. Endocrinol. Diabetes Obes. 15(1):73-78.
- 8- Huang, X.G. and Wu, X.B. (2005): Immunohistochemical study on gastrointestinal endocrine cells of four reptiles. World J. Gastroenterol., 11: 5498-5505.
- 9- Ku, S.K., Lee, H.S., and Lee, J.H. (2004): An immunohistochemical study of the gastrointestinal endocrine cells in ddY mice . J. Vet. Sci. 5(2): 87-95.
- 10- Moran, A.W., Al-Rammahi, M., Zhang C., Bravo, D., Calsamiglia, S., Shirazi-Beechey, S.P. (2014): Sweet taste receptor expression in ruminant intestine and its activation by artificial sweeteners to regulate glucose absorption. J Dairy Sci.; 97(8):4955-72.

- 11- Rehfeld, J.F., Friis-Hansen, L., Goetze, J.P., and Hansen, T.V. (2007): The biology of cholecystokinin and gastrin peptides. *Curr Top Med Chem.* 7:1154–65.
- 12- Rindi, G., Leiter, A.B., Kopin, A.S., Bordi, C., and Solcia, E. (2004) : The “normal” endocrine cell of the gut: changing concepts and new evidences. *Ann NY Acad Sci*; 1014:1–12.
- 13- Druker D.j. (2007) the role of gut hormones in glycohomeostasis .J. Clinic Invited 117-(1)24-32
- 14- AL Rakabi F,S and Al rammahi M (2014). Distribution of enterendocrine cells in the small intestine of camels
- 15- Ganong, W.F. (2003): Review of medical physiology. 21st ed. New York: *Lange Medical Books/McGraw-Hill Companies*. P: 137-151.
- 16- Hansen, C. F., Vrang, N., Sangild, P. T., and Jelsing, J. (2013): Novel insight into the distribution of L-cells in the rat intestinal tract . *Am J Transl Res*; 5(3):347-358.
- 17- Mortensen, K., Christensen, L.L., Holset, J.J., Orskov, K. (2003): GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine .*Regul. pept.* 114, 189-196.
- 18- Theodorakis, M.J., Carlson, O., Michopoulos, S., Doyle, M.E., Juhaszova, M., Petraki, K. & Egan, J.M. (2006) : Human duodenal enteroendocrine cells: source of both incretin peptides, GLP-1 and GIP. *Am. J Physiol. Endocrinol. Metab.* 290(3):E550-E559.
- 19- Ahlman, H. and Nilsson, O. (2001): The gut as the largest endocrine organ in the body. *Ann. Oncol.*, 12:S63-S68.
- 20- Rehfeld, J.F. (2004): A centenary of gastrointestinal endocrinology. *Horm. Metab. Res.* 36: 735– 741.
- 21- Al-Rahabi, F., and Al-Rammahi, M. (2014): Immunofluorescence study of the Enteroendocrine cells in the Small Intestine of One Humped Camel (*Camelus Dromedarius*). Master Thesis; University of AL-Qadissya, College of Veterinary Medicine.
- 22- Kuru, N., Cinar, K., Senol, N., Demirag, E., and Diler, D. (2010): Endocrine cells in the Gastrointestinal Tract of Garrarufa . *Kafkas Univ. Vet. Fak Derg.* 16(Suppl-B): S235-S241.
- 23- Ross, B.M.H. and Pawlina, W. (2011): *Histology: A Text and Atlas*. Wolters Kluwer. Lippincott Williams & Wilkens. Two Commerce Square, 2001 Market Street, Philadelphia, PA 19103.

