Frequency and distribution of the enteroendocrine cells in small and large intestine of one humped camel (*Camelus dromedarius*)

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Abstract

Enteroendocrine cells in some portions of intestinal tract plays as endocrine portion by secreting some hormones that play key roles in the regulation of certain important organs. The aim of this study is to examine in detail the relative frequency and regional distribution of enteroendocrine cells in some portions of intestinal tract of the camels. The regional localization of the endocrine cells in the some portion intestinal tract of the camels (*Camelus dromedarius*), are inspected using immunohistochemistry techniques. Specimens from eight dromedarian camels *(Camelus dromedarius)* of both sexes with age ranging from 2 - 6 years are investigated. The immunohistochemistry was performed using chromogranin A (ChA) and four types of hormones. Immune detection findings demonstrated that in the camel small intestine, glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2) and cholecystokinin (CCK) are expressed in a subset of epithelial cells along the crypt villus axis. While, in large intestine (colon) of camels, glucagon-like peptide-1(GLP-1) and glucagon-like peptide-2 (GLP-2) expressed in colonic gland. The cells are contained gut hormones appeared to be either triangular or flask-like in shape as indicate that they are enteroendocrine cells. Immunohistochemistry of serial sections showed that (ChA), which is a specific marker for enteroendocrine cells, is indeed expressed with glucose dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1(GLP-1), glucagon-like peptide-2(GLP-2) and cholecystokinin (CCK) confirms the site of expression to be in enteroendocrine cells. There is caudal decrease in K-cells and I-cells along of the camel small intestine, while increase in L-cells in the colon.

Key words: Endocrine cells, intestinal tract, immunohistochemistry, hormones, camel.

التكرار و انتشار الخلايا الصماوية المعوية في الأمعاء الدقيقة والغليظة للجمال وحيدة السنام (*Camelus dromedarius*)

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الخلاصة

الخلايا الصماوية المعوية في بعض أجزاء القناة المعوية تلعب دور مهم ورئيسي في إفراز بعض الهرمونات التي تنظم أجهزة الجسم المهمة. إن الهدف من هذه الدراسة هو فحص تفاصيل التكرار النسبي ومناطق انتشار الخلايا الصماوية في بعض أجزاء القناة المعوية في الجمال وحيدة السنام. أن مناطق تواجد الخلايا الصماوية في بعض أجزاء الأمعاء للجمال وحيدة السنام (*Camelus dromedaries*) تم فحصها بواسطة استخدام تقنية الكيمياء المناعية النسيجية. كانت العينات مأخوذة من ثمانية جمال عربية لكلا الجنسين بأعمار تتراوح ما بين 2-6 سنوات. وتم استخدام تقنية الكيمياء المناعية النسيجية وكروموكرانين (Chromograinn A) الذي يعتبر كاشف خاص للخلايا الصماوية المعوية ، وأربعة أنواع من الهرمونات. أظهرت نتائج الفحص الكيميائي المناعي في الأمعاء الدقيقة للجمال وحيدة السنام وجود هرمونات ، الجلوكوز إينسولينوتروبيك الببتيد (GIP) و كولسيتوسكينين (CCK) الجلوكاجون ببيتيد (GLP-1\2) في مجموعة الخلايا الظهارية على طول محور الداخلي للزغابات ، بينما في الأمعاء الغليظة (القولون) وجود هرمون الجلوكاجون ببيتيد (GLP-1\2) فقط في الغدد المعوية. ويبدو أن شكل الخلايا الصماوية المعوية التي تحتوي على الهرمونات يكون ما بين مثلث الى دورقي وهذا الشكل يدل على كونها خلايا صماوية معوية. أظهرت النتائج الكيميائية المناعية للعديد من المقاطع ملاحظة

الكروموكرانين وهو كاشف خاص للخلايا المعوية الصماوية ، بأن الخلايا التي تحتوي على الهرمونات (GIP) إينسولينوتروبيك الببتيد ، كولسيتوسكينين (CCK) و الجلوكاجون ببيتيد (GLP-1\2) تحتوي على هذا الكاشف مما يدل على تأكيد موقع هذه الخلايا وهي خلايا معوية صماوية. أن وجود هذه الهرمونات في الأمعاء الدقيقة والغليظة يختلف على طول الأمعاء حيث أظهرت النتائج بأن خلايا (K,I) تنخفض كلما ابتعدنا عن الاثني عشري للأمعاء الدقيقة. على العكس من خلايا (L) حيث أنها تزداد باتجاه الارتباط اللفائفي القولوني.

الكلمات المفتاحية: الخلايا الغدية ، القناة المعوية ،المناعة النسجية الكيميائية ، الهرمونات ، الجمل.

Introduction

The dromedary camels (*Camelus dromedaries*) are mostly present in the desert areas (Africa and Asia) and known as the ship of the desert in Arabian countries. It is used as a main source of milk, meat, wool and lashing for people especially who were living in the desert. The adult camels can produce 9.1-14.1 Kg of milk per day (1). The camels is ruminants, but its stomach differs morphological from that of other ruminants, they are pseudo-ruminants that possess a three chambered stomach, lacking the omasum that is part of the four-chambered stomach of the order Ruminantia (2). The mammalian gastrointestinal tract is an important endocrine organ because it contains an array of endocrine cells, which produce a range of regulatory peptide such as, somatostatin, neurotensin, gastrin, serotonin, gastric inhibitory polypeptide (GIP), glucagon like peptide-1(GLP-1), glucagon like peptide-2 (GLP-2) and cholecystokinin (CCK) (3). Many immuno-histochemistry studies have carried on the distribution of endocrine cells of human, cattle, pigs, lesser mouse deer, sheep, horse, water buffalo, babirusa and camel (4, 5, 6, 7). Studies on the expression, relative frequency and reginal distribution of enteroendocrine cells in some portions of intestinal tract of the one humped camel (*Camelus dromedaries*) virtually lacking. The present paper were undertaken to study the relative frequency and regional distribution of enteroendocrine cells in some portions of intestinal tract of the camel using immunohistochemistry techniques.

Materials and methods

Samples were obtained from small and large intestine of eight dromedarian camels (*Camelus dromedarius*; four females and four males, aged 2-6 years; 250-400kg). Tissue samples were collected from small intestine approximately 50 cm in length were removed from the proximal (distal to the pylorus), mid (half way along the small intestine), and distal (proximal to the ileocaecal junction) intestine. Two or three pieces of fresh large intestinal tissues each measuring 1-2 cm were collected from colon. Sections were opened longitudinally, rinsed in ice-cold 0.9% (w/v) NaCl pH 7.4, and blotted with paper towels to remove excess mucous. Samples of the tissue were fixed in 10% formaldehyde for 48 hours, dehydrated through an ethanol-xylene series, and embedded in paraffin for histological examinations and immunohistochemical studies. Sections were cut at 5mm in thickness. Slides, containing wax embedded camel small and large intestinal tissue, were dewaxed in 100% xylene for 3 x 10 minutes each. The tissue was placed twice in 100% ethanol for 2 x 10 minutes. Sections were removed, allowed to air dry for 10 minutes and were circled with ImmEdge Hydrophobic Pen and allowed to dry for 10 minutes. Subsequently, they were placed 2 x 5 minutes in 70% ethanol. Slides were then rehydrated twice in distilled H2O for 5 minutes each. Slides were immersed in antigen retrieval buffer (10 mM Tris/HCl pH 10.0) and autoclaved, 2 x 15 minutes at 121°C and 15 psi. Subsequently, slides were allowed to cool in antigen retrieval buffer for 30-60 minutes at room temperature and washed for 3 x 5 minutes in phosphate buffer saline. Non specific antibody binding sites were blocked by incubating the tissue sections for 1 hour in the blocking solution 10% (v/v) donkey serum in a humidified chamber at room temperature. Sections were incubated overnight at 4°C with primary antibodies (Table 1). For double immuno-staining, primary antibodies raised in different species were mixed with one another without changing the final required

concentration and were incubated at 4°C overnight. Each slide was then washed in PBS for 5 x 5 minutes. FITC-conjugated IgG/IgY and Cy3-conjugated IgG/IgY (Table 1) Scientific were used at a dilution of 1:500 for 1 hour incubation at room temperature. Finally slides were washed with PBS for 5 x 5 minutes and mounted in Vectashield Hard Set Mounting Media with DAPI. Sections were visualised using an epifluorescence microscope and images were captured with a Canon digital camera images from serial sections were merged using imaging products laboratory imaging software (Bio-Vision Technologies, Exton, PA, USA). Omission of primary antibody was routinely used as a control.

The relative frequency of immuno-reactive cells was placed into one of five categorise, not detected (-), rare (+; mean values were below 2/filed), a few (±; mean values were below 5/ filed); moderate (++; mean values were below 10/filed) and numerous (+++; mean numbers as seen under one filed of epifluorescence microscope (X200) and the observation of each region of GI tract was conducted as triplet by 3 histologists.

Table (1): Primary and secondary antibodies were used

Primary antibody	Host	Dilution	Clonality and source
Anti-chromogranin A	Rabbit	1:100	Polyclonal, chromogranin A (H-300): sc-13090 Santa Cruz Biotechnology, INC., CA, USA.
Anti-chromogranin A	Goat	1:100	Polyclonal, chromogranin A (C-20): sc-1488, Santa Cruz Bio-technology, INC., CA, USA.
Anti-GIP	Goat	1:100	Polyclonal, GIP (Y-20): sc-23554, Santa Cruz Biotechnology, INC., Santa Cruz, CA, USA.
Anti-GLP-1	Goat	1:100	Polyclonal, GLP-1 (C-17): sc-7782, Santa Cruz Biotechnolo-gy, INC., Santa Cruz, CA, USA.
Anti-GLP-2	Goat	1:100	Polyclonal, GLP-2 (C-20): sc-7781, Santa Cruz Biotechnolo-gy, INC., Santa Cruz, CA, USA.
Anti-CCK-8	Rabbit	1:200	Polyclonal, CCK (C2581): Sigma, Saint Louis, Missouri, USA.

Secondary antibody	Label	Dilution	Source
Donkey anti-rabbit IgG	Cy3	1:500	Cyanine-conjugated IgG (711-165-152), Stratech Scientific Limited, Suffolk, UK.
Donkey anti-rabbit IgG	FITC	1:500	Fluorescein-conjugated IgG (711-095-152), Stratech Scienti-fic Limited, Suffolk, UK.
Donkey anti-goat IgG	Cy3	1:500	Cyanine-conjugated IgG (705-166-147), Stratech Scientific Limited, Suffolk, UK.
Donkey anti-goat IgG	FITC	1:500	Fluorescein-conjugated IgG (705-095-147), Stratech Scienti-fic

Results

Integrity of small and large intestine tissues

The villi of small intestine were seen intact, and the epithelial cells attached indicate the integrity of the tissue of small intestine (Fig. 1). The crypt of colon were seen intact with the cells attached indicate the integrity of the tissue of colon (Fig. 2).

Expression of gut hormones in the small intestine of mice

Intestinal tissues of mice were first used as a positive control to investigate the expression of GIP, GLP-1/GLP-2 and CCK by using immunohistochemistry. Results in mice intestinal tissues, presented that GIP, GLP-1/GLP-2 and CCK were expressed. There was no staining when the primary antibody was omitted from the control section. Typical image meaning that gut hormone (GIP, GLP-1/GLP-2 and CCK) were expressed in duodenum, jejunum and ileum (Fig. 3).

Expression of gut hormones in the intestine of camel

Results of using immunohistochemistry in duodenum, jejunum and ileum of the one-humped camel indicate clearly expression of the GIP, CCK, GLP-1 and GLP-2 in a subset

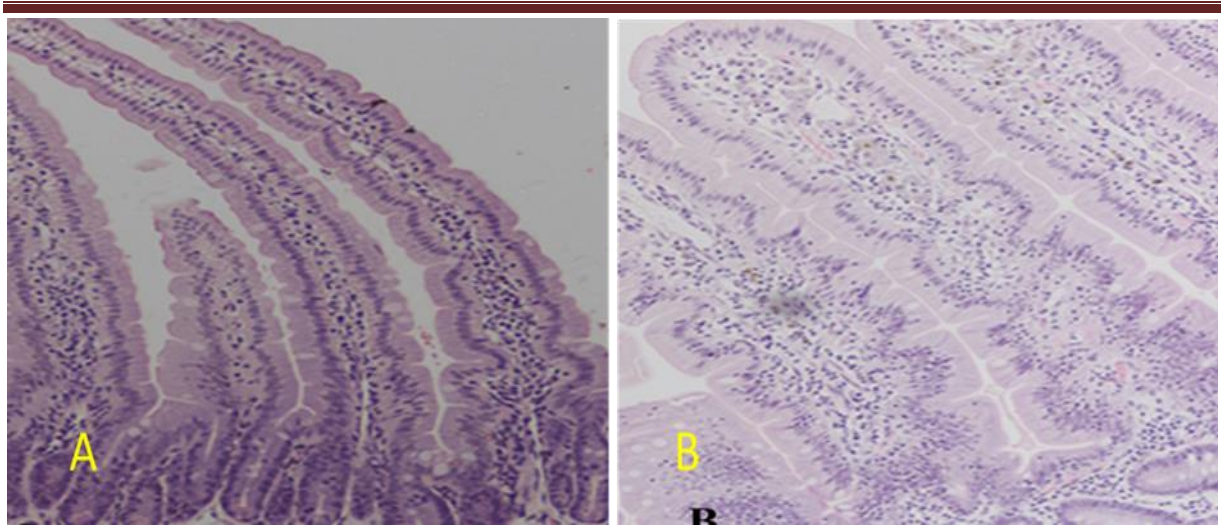

Fig. (1): Cross sections of small intestine of mice (A) and camel (B). The villi are intact, and the cells are attached indicated the integrity of the tissue. (H&E stain (A) X200 and (B) X400).

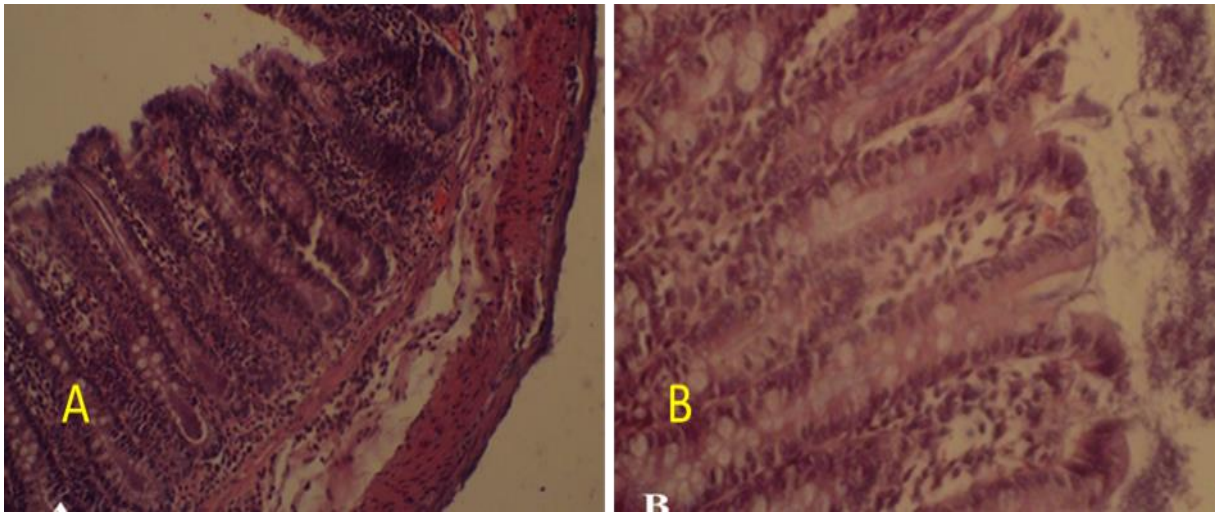

Fig. (2): Cross section of colon of mice (A) and camel (B). The crypts of colon are intact with the cells attached indicated the integrity of the tissue. (H&E stain (A) X200, and (B) X400).

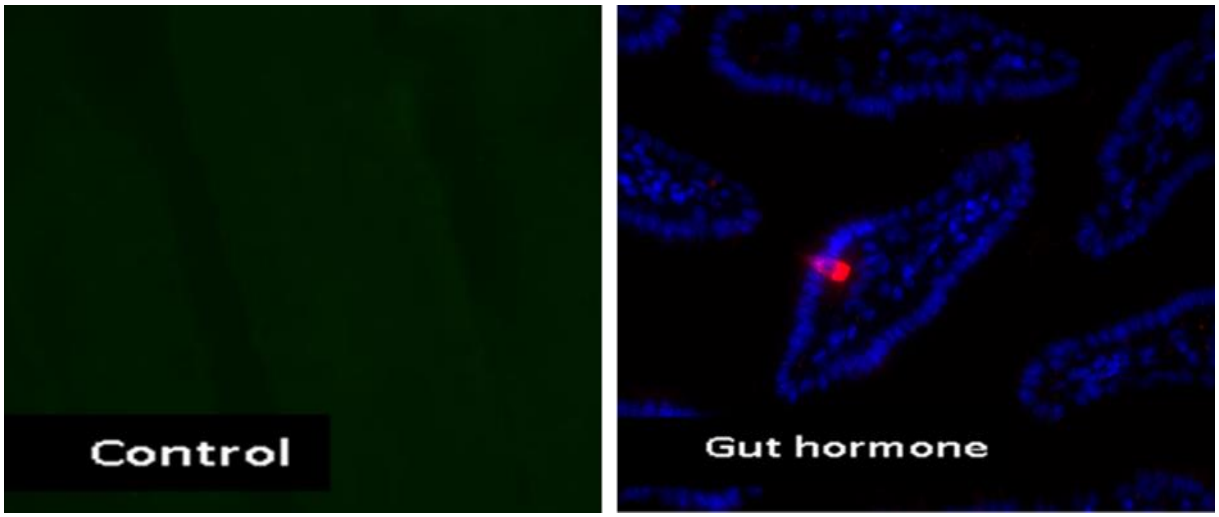

Fig. (3): Intestinal section of mice probed with antibodies gut hormones (GIP, GLP-1, GLP-2 and CCK), show the gut hormone (red) expressed in a subset of cells. There is no labelling for gut hormones in control section. Blue and 46-diamidino-2-phenylindole stain (DAPI) X200.

of cells along the villus. The K-cells containing GIP, and I-cells containing CCK. The gut hormones labelling were specific; when the primary antibodies were omitted it was not observed in control section (Fig. 4). Results were displayed the relative frequency and distribution of endocrine cells in the small intestine of one humped camels examined by four types of antisera. The immuneoreactive cells were identified in the small intestine, and most of the gut hormones were located in the basal portion of glands. The IR cells were triangular or slender in shape. They appeared as close-type cells as they did not possess lamina contact with their apical cytoplasmic processes, some open-type cells with apical cytoplasmic processes observed reach the intestinal lumen more frequency in the colon region. The relative frequency and distribution of IR endocrine cells in the duodenum on the intestinal villi, in the crypts (intestinal glands) and in Brunner's glands (duodenal glands)(Table 1). 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 the GIP IR-cells were observed in rare frequency on the intestinal villi, and were not detected in the ileum. The duodenum contains the greatest variety of endocrine cell types in the digestive tract. CCK IR-cells were observed in high frequently in the villi and the intestinal crypts of the duodenum. In the jejunum the CCK IR- cells were observed in few frequencies on the intestinal villi, and not detected in the ileum. It was 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 was thought to be protected, at least in part, from acid-pepsin entering from the stomach by secretions from Brunner's glands. In the small intestine K-cells which containing GIP and I-cells containing CCK, were detected (Fig. 4). The relative frequency is a caudally decrease along of the camel intestine (Table 1), the I-cells and K cells that expressed the CCK and GIP hormone in duodenum and subsequently was decreased in jejunum and ileum. However, the L-cells which containing GLP-1 and GLP-2 the relative frequency was increase caudally along of the camel small intestine compare with duodenum and jejunum (Table 1). In the duodenum and jejunum the GLP-1\2IR- cells not observed.

Co-localisation of gut hormones with ChA

By using double immunostaining, cell type expressing gut hormones was employed with an antibody to (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 images were merged, areas of co-expression showed as orange / yellow. Sections of small intestine from camel were incubated with antibodies to gut hormones (GIP, CCK, GLP-1 and GLP-2) and ChA. Results of immuneostaining showed that enteroendocrine cells containing gut hormones and ChA were co-expressed in the same cells in camel small intestine (Fig. 5).

Expression of gut hormones in the large intestine of camel

The immunohistochemistry show the gut hormones were expressed solely in a subpopulation of cells along the crypt in the colon of the one-humped camel (Fig. 6). There was a caudally increase in L-cells containing GLP-1/2, but not observed immuneodetection for K-cells containing GIP and I-cells containing CCK (Table 2). The enteroendocrine cells that expressed gut hormones were the open type which the apex of the cells found in the epithelial lining of the lumen. The relative frequency and distribution of IR endocrine cells in the large intestine K-cells containing GIP and I-cells containing CCK were not detected (Fig. 6). Only the GLP-1\2 IR cells were observed in high frequently in the colon, while both the CCK IR- cells, and GIP IR-cells were not detected in colon. The GLP-1\2 cells were not detected in duodenum and jejunum, while it was moderate frequently in ileum (Table 1).

Fig. (4): Intestinal section of camel probed with antibodies gut hormones (GIP, GLP-1, GLP-2 and CCK); show the gut hormone (red) expressed in a subpopulation cells. There is no labeling for gut hormones in control section. Blue and 46-diamidino-2-phenylindole stain (DAPI) X200.

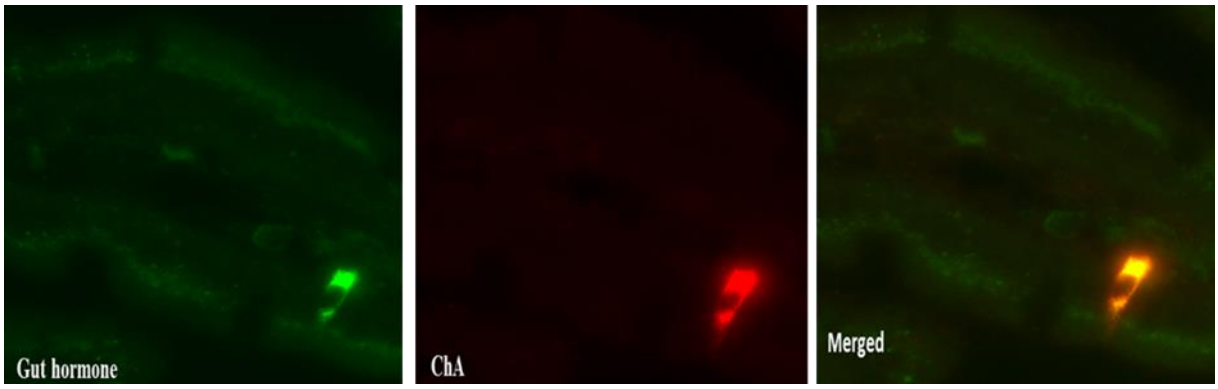

Fig. (5): Immunofluorescence sections of camel small intestine incubated with antibodies of gut hormones (GIP, CCK, GLP-1 and GLP-2) and ChA; showing co-localisation of gut hormones (GIP, CCK, GLP-1 and GLP-2) (green) with ChA (red). When the sections were overlaid, gut hormones and ChA were shown to be co-expressed in a same cell (yellow) (X400).

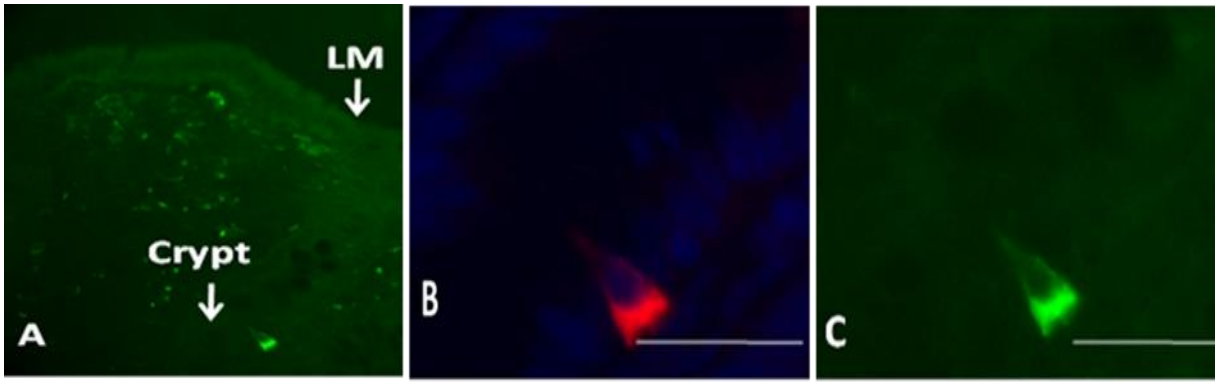

Fig. (6): Immunofluorescence sections of camel colon showing gut hormones (GLP-1 and GLP-2) green and red in a subset of camel colon cells. A (X200), B and C (X400). Nuclei are stained blue with 4', 6-diamidino-2-phenylindole (DAPI).

Table (2): Regional distributions and relative frequencies of the gastrointestinal endocrine cells in the GIT of one humped camels (*Camelus dromedaries*).

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

Relative frequencies; (high: +++, moderate: ++, few: +, rare: ±, not detected: -).

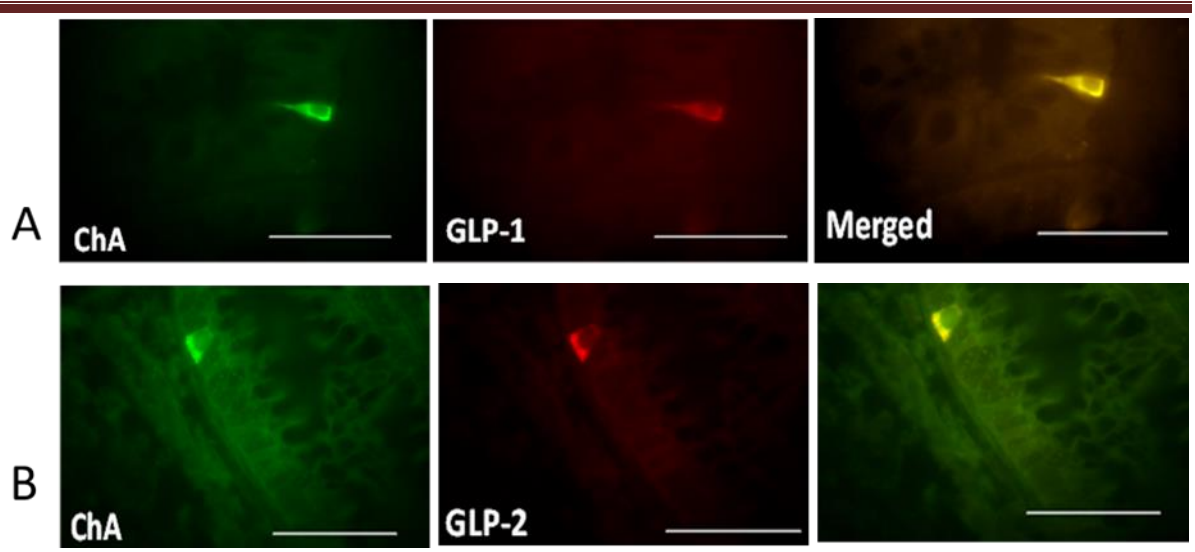

Fig. (7): Immunofluorescence sections of camel colon were labelled with the primary antibodies to GLP-1 (A), GLP-2 (B) and ChA, showing co-localisation of ChA (green) with GLP-1 and GLP-2 (red). When the sections were overlaid, GLP-1/2 and ChA were shown to be co-expressed in a same cell (yellow) (X400).

Co-localisation of GLP-1/2 with ChA

The presence of the gut hormones have demonstrated in large intestine of camel. The cells that contain gut hormones appear to be either triangular or flask-like in shape and located mainly in crypt (Fig. 6). The shape of these cells indicated that they enteroendocrine cells. Consequently to investigate the cell type expressing gut hormones, double immunohistochemistry was employed with an antibody to chromogranin A (ChA), a classical marker for endocrine cells. Large intestine sections from camel were incubated with antibodies to GLP-1/2 and ChA. Immunostaining showed that enteroendocrine L-cells containing GLP-1/2 and ChA were co-expressed in the same cells in camel large intestine (Fig. 7; A and B).

Discussion

Enteroendocrine cells secrete hormones and peptides and the effect of these hormones and peptides on food intake and appetite, the regulation of glucose homeostasis, gut motility and various other physiological functions (8). The endocrine cells in each part of the gastrointestinal tract differ remarkably between animal species in term of regional distribution, relative frequency and cell type (5). L-enteroendocrine cells secrete GLP-1and GLP-2 in response to dietary carbohydrates, amino acids and lipids (9,10) This pattern of distribution of the GLP-1 has also been shown in other mammals including sheep and cow (11,12), rabbit (13), buffalo (14), human (15), pig (16), horse (10) and camel (6). Enteroendocrine cells constitute 1% of the cells lining the intestinal epithelium, and there are twenty or more subtypes of enteroendocrine cells based on the major products they secrete (16). In each part of the gastrointestinal tract, the endocrine cells in the small and large intestine expresses hormones for GIP, GLP-1, GLP-2 and CCK (16,17,18) and differ remarkably between animal species in term of regional distribution, relative frequency and cell type (5). In response to dietary carbohydrates, amino acids and lipids K-enteroendocrine cells secrete GIP; I-enteroendocrine cells secrete -CCK, while L-enteroendocrine cells secrete GLP-1and GLP-2 (9, 10). Enteroendocrine cells secrete hormones and peptides play a vital role in the function of the digestive system with enteric nervous system and the effect of these hormones and peptides on food intake and appetite, the regulation of glucose homeostasis, gut motility and various other physiological functions (3, 6). Our results support the important digestive role of endocrine cells in the gut of camel. The present study, demonstrated the expression, distribution and

relative frequency of three types of entero-endocrine cells in the duodenum, jejunum, ileum and colon that secrete, at least, GIP, GLP-1, GLP-2 and CCK. The results shown are support by (4), who reported that GLP-1/2 CCK and GIP were expressed in the two-humped camel. The localised, relatively stable presence of GIP, GLP1/2 and CCK in the small and large intestine may be related to the role of these hormones in the stimulation of intestinal and gallbladder smooth muscle and pancreatic secretion (19).

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