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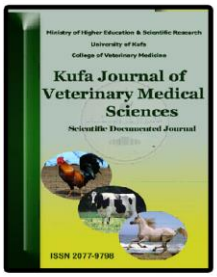

Relative Frequency and Regional Distribution of Enteroendocrine cells in small and large intestine of goat

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Abstract

Some parts of the intestinal tract, which involve in secretion of certain hormones that regulate significant organs of the body, the purpose of the research to study the site endocrine cell in small intestine & colon in goat, the present study included 5 specimens of intestine goat from the ages ranging from 1-5 years to determine the site endocrine cells in the parts of intestine examined via using immunohistochemistry procedure material.

It was used immunohistochemistry textile technology and (ChromograinnA), which is a special detector Endocrine intestinal cells, three 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 glucagon-like peptide2 (GLP-2)' in epithelial cells collection along the internal axis of the villi , the cells appeared triangular or slender shape indicate that they are enter ondocrine cell 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 and glucagon peptide GLP-1) containing this reagent representative confirm the site of these cells, a gastric endocrine cells ,the result attendance that presence the GIP was in the duodenal region higher than ilium & jejunum while the GLP-1/ GIP-2 was clear to a large extent in colon and obtrusive in ilium .

Key words : Immunohistochemistry , goat , villi , intestinal tract.

الخلاصة :

بعض اجزاء القناة المعوية تقوم بافراز بعض الهرمونات التي تنظم عمل اعضاء الجسم ان الهدف من هذه الدراسة هو البحث عن مواقع الخلايا الصماوية في طبقات الامعاء الدقيقة والقولون في الماعز لغرض ايجاد هذه الخلايا تم فحص عينات لخمس حيوانات بطريقة تقنية الكيمياء المناعية النسيجية ' تم اخذ العينات من حيوانات باعمار من 1-5 سنوات واستعملت تقنية الكيمياء المناعية الكروموكرانين أالذي هو كاشف خاص للخلايا الصماوية في الامعاء ، وثلاثة انواع من الهرمونات.

اظهرت نتائج الفحص المناعي الكيميائي لاجزاء الامعاء الدقيقة للماعز وهي الاثني عشري والصائم واللفائفي وجود GIP في منطقة الاثني عشري اعلى من اللفائفي والصائم وانعدامه في منطقة القولون وجد على طول المحور الداخلي للزغابات ، بينما في الامعاء الغليضة (القولون) وجود الهرمونين (GIP-2, GIP-1) ، ان شكل الخلايا التي تحتوي على الهرمونات يكون بين مثلث الى دورقي وهذا الشكل يدل على انها خلايا صماويه معويه .

من خلال تقنية الكيمياء المناعية وجد ان هرمون الكروموكرانين هو الكاشف الرئيسي للخلايا الصماوية التي تحتوي على الهرمونات المستخدمة في التقنية بحيث اعطت تفاعل ايجابي مع هرمون الكروموكرانين أ كما لوحظ انخفاض بمستوى التفاعل كلما زاد البعد عن الاثني عشري .

الكلمات المفتاحية : المناعة النسجية الكيميائية ، الماعز ، الزغابات ، القناة المعوية .

Introduction

Goats one of the ruminants they eat plants& grass, including pastures and many weeds, and trees , digestion is the process of breaking down this material in the stomach and intestines into components which can be absorbed and used by the goat , the enteroendocrine cells have been extensively investigated by secreting some hormones which & play important role in the regulation of gut functions in domestic animals out of maximize the absorption & digestion of the food, and maintain inteegrity of the epithelial , and last hase immune mechanism (1,2, 3, 4) .

The "neurroendocrine spread between the epithelial cells like paneth cells & goblet and enterocyte, and the several hormones manufactured by these cells including : GIP, GLP-1 , GLP-2 , (PYY), and there are many other hormones which performs many functions such as growing and mend of the elementary canal epithelium , pancreatic secretion , glycemia, motility of the gastrointestinal tract wall

, GLP-1and GIP secreted by the L- and K-cells, correspondingly play important role in homeostasis and regulation of blood glucose level (5,6, 7).

The "immunohistochemistry " was executed using Chromogranin A (ChA), and types of hormones, "GIP ,GLP-1 & GLP-2" were spoken in epithelial cells in the crypt villii , these cells containe hormones look as triangular & flask-like shape , "Immunohistochemistry" of sequential sections showed that ChA, which is a explicit marker for endocrine cells, was definitely spoken with GIP, GLP-1, and GLP-2 checks the site of expression to stay in endocrine cells (8 ,9 ,10) they are unicellular endocrine glands , among other types of the cells that lie in the villi and crypts of the mucosa , the enteroendocrin divided into tow type first exist in the apex of microvilli & contact found in the epithelial of lumen , the second type located in the cellular "found in the intestinal region " (11), entero endocrine secrete, depending on the type more than 20 gut hormones (12).

The villi increase surface area of absorption and probably were device that facilitates rapid absorption of water and nutritive materials, the recent investigation revealed the small and large intestine. In sheep who mentioned increased length and thickness of the villi in proximal part of small intestine more than that in distal part , indicated most of sucking of materials occur in proximal part of intestine, the circular folds in addition to enhancing surface area for assist facilitates easy absorption of water and nutritive material by increasing the time of contact with absorptive surfaces, they act as effective barrier against of the bacteria, viruses, toxins and different antigens, so that these folds were more frequency toward last parts , entero endocrine cells differ according to feeding behavior & the species of the animal (13).

The purpose of the research to study the site endocrine cell in small intestine and colon in goat by using " immune histochemistry "and also the studies about the goat are lacking.

Material and methods:

Five specimen were obtained from three parts of intestine & colon of goat of both male and female , aged 1-5 year; weight 100-150kg),were collected from al-qadisiyah abattoir. sections were wash in ice cold "0.9% (w/v) NaCl pH 7.4" & put in 10% formaldehyde, dried out by an ethanol-xylene sequence, to prepare for histological examinations and immunofluercent studies, pieces are incise at 5mm in thickness, containing wax embedded goat intestine 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 & leave to dry for 10 minutes, then, they were positioned 2 x 5 minutes in 70% ethanol, and then rehydrated two times in distilled water to 5 minutes , slips were engrossed in antigen repossession buffer (pH 10.0) and auttoclaved aerated conncrete (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 in the normal temperature after that washed for 3 x 5 minutes in

phosphate buffer saline (PBS), the tissue sections for 1 hour in the blocking solution 10% (v/v) donkey serum in a humidified chamber and incubated during the night at(4°C) with primary antibody (Table 1), each slide was then washed in PBS for 5 x 5 minutes. FITC-conjugated IgG / IgY (Table 1) were second-hand at a intensity of 1/500 for one hour cultivation in temperature of the room, finally, slides wash with PBS for 5 x 5 minutes and mount use "epifluorescence "for the purpose photography, Omission of primary antiibody was routinely used as negative control.
(14).

Table (1): "Primary and Secondary Antibodies" .

1-100	Goat	Anti-GIP
1-100	Goat	Anti-GLP-1
1-100	Goat	Anti-GLP-2
1-500	Goat	Fluorescein-conjugated IgG (705-095-147),

Results

Fixed sections and stained with haematoxylin & eosin to confirm the integrity of these organ were intact, with epithelial cells attached. (Fig. 1.A) show the integrity of the tissue as the villi are intact with the cells attached while the finding in the (Fig. 1.D) indicate integrity of the tissue as the crypt of colon are intact with the cells attached.

The present study revealed the three parts of intestine & colon' of the goat, consist of four tunicae from inside to outside : mucosa, submucosa, muscularis and serosa ,small intestine lined by simple columnar epithelium, they were transverse folds of the mucosa and submucosa of the goat intestine,increase in size and frequency toward fifth part of the duodenum , solitary lymph nodules are found between the crypts, the intestinal glands or crypts of Leiberkühn are simple straight tubular downgrowths extending from the surface to the muscularis mucosae they are lined by columnar cells, abundant goblet cells, stem cells, enteroendocrine cells .

The mucosal layers of colon are similar in arrangement and histology to the cecum.The mucosa of the colon is smooth and devoid of villi , epithelium is simple columnar with an increase number of goblet cells, the lamina propria is formed of loose lymphoreticular tissue entirely occupied by intestinal crypts,the submucosa is devoid of glands except in some parts of the colon, the tunica muscularis is formed of the usual muscular layers with myentric plexux , outer layers of colon not continuous, being concentrated into three bands, taeniae coli, serosa.(Fig1.D)

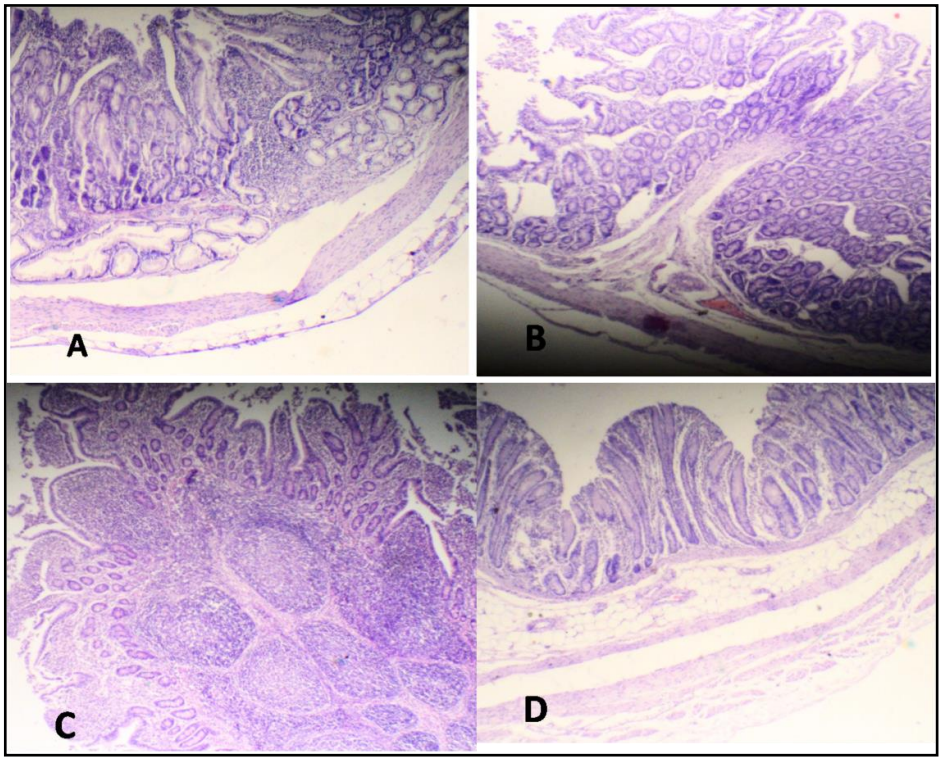
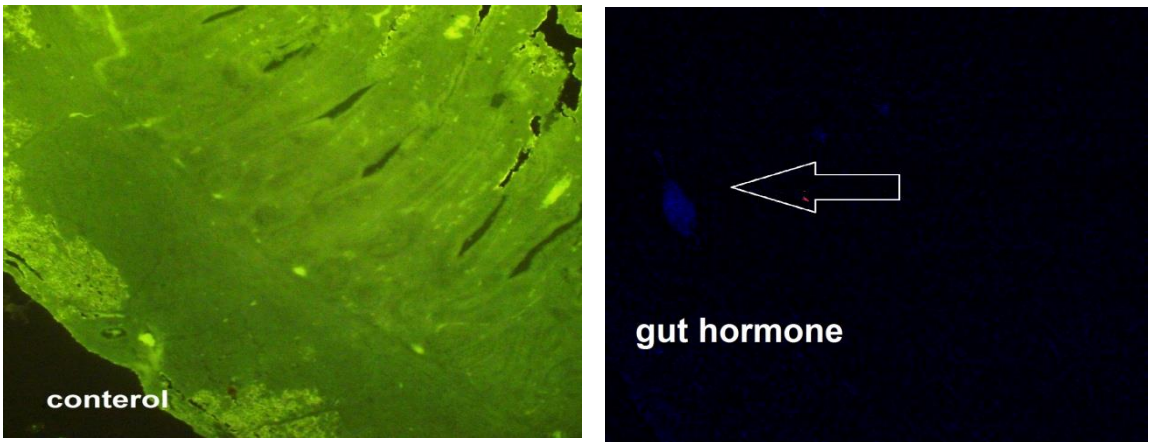


Fig.(1): Wax embedded intestinal tissue sections from the small intestine of Goat were stained with routine H&E. As the villi are intact with the cells attached A(duodenum),B(jejunum) , C (ileum). 100 X (A, B and C) magnified. and figure. D- sections from the colon were stained with routine histology stain (H&E). The findings indicated the integrity of the tissue as the crypts of colon Image are 100 X magnified.

Small &and large tissues of goat were first used as positive controle to investigate the expression of GIP , GLP-1/GLP-2 by using immunohistochemistry (Fig.2,3,4) , gut hormones show no labeling at what stage the priimary antibodies was missing foreign the negative control section (Fig.2.A), after examination by 'antisera" the result showed relative frequency and sharing of enddocrine cells in the alimentary canal of goat that appeared hormones situated in the lower basal portion of glands, the immunoreactive cells showed triangular , slender shape (Fig. 7).



A B

Fig.2 A,B gut hormone (blue) is expressed in a subset of intestinal cells. When the primary antibodies 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 46ʿ- diamidino -2- phenylindole (DAPI)".

The relative frequency and arrgement of GIP IR enteroendocrine cells in the duodenum on intestinal villi , crypts (intestinal glands) in Brunner's glands (duodenal glands), the result showed endocrine cells were experiential in high frequently in villi of duodenum, less frequently on the intestinal crypts and rarely in Brunner's glands, jejunum GIP IR-cells were experiential in rare frequency on villi, in ileum GIP IR-cells were not identify (Table 1).

L-cells which containing GLP-1 and GLP-2 the relative frequency is an increase caudally along the "ilium & colon " of the goat compare with the jejunum &duodenum GLP-1\2 IR- cells not observed (Table 1).

Table:(1):''Regional sharing and Rellative Frequency of the Enteroendocrine Cells in the part of small intestine and colon of goat''.

Hormone	Duodenum	Jejunum	Ileum	Colon
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 IgGlabelling with one fluorochrome and anti-rabbit IgGlabelled 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 goatl were incubated with antibodies to alimentary canal hormones "GIP, GLP-1 and GLP-2" & ChA.

The results of immunostaining technique showed that enteroendocrine cells containing gut hormones and ChA were co-expressed in the same cells in small intestine of goat, The immunoreactive cells were recognized in the three parts of small intestine , the gut hormone take the site in lower basal part of glands, the IR cells as triangular or selender. (Fig. 7)

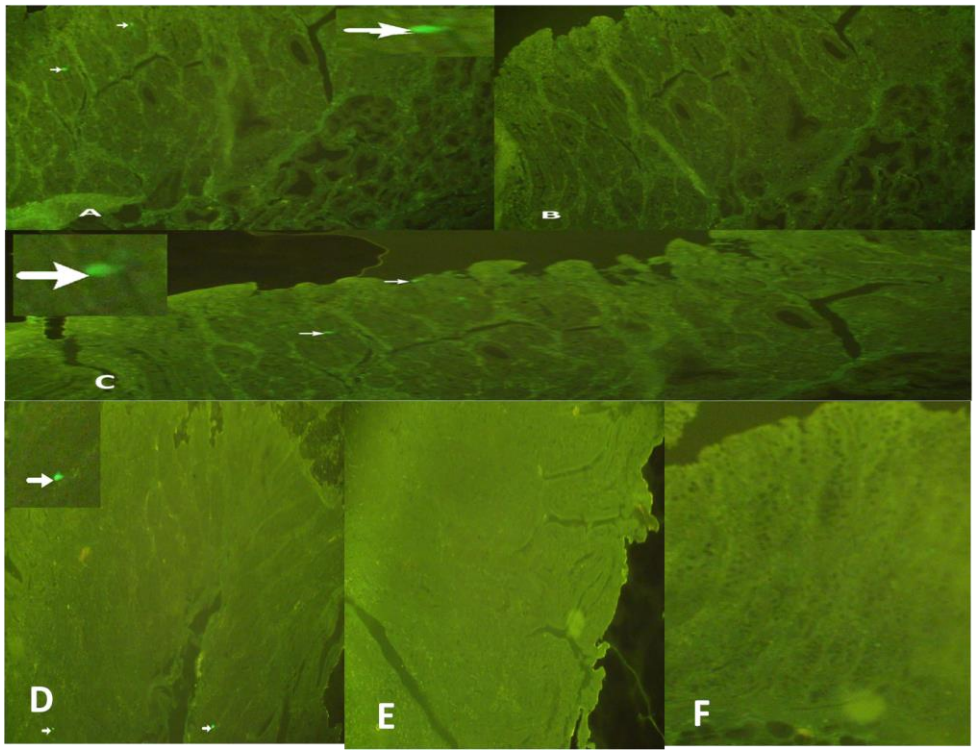


Fig-3 -A: In duodenum. GIP- immunoreactive cells were detected in the lower half of the glands. B- Control image show that there is no react for gut hormones. C- GIP- immunoreactive cells were detected in villi and lower half of the glands of duodenum. D- GIP-immunoreactive cells were detected in jejunum . no detected in ileum(E) and colon (F). Images are 200 X magnify

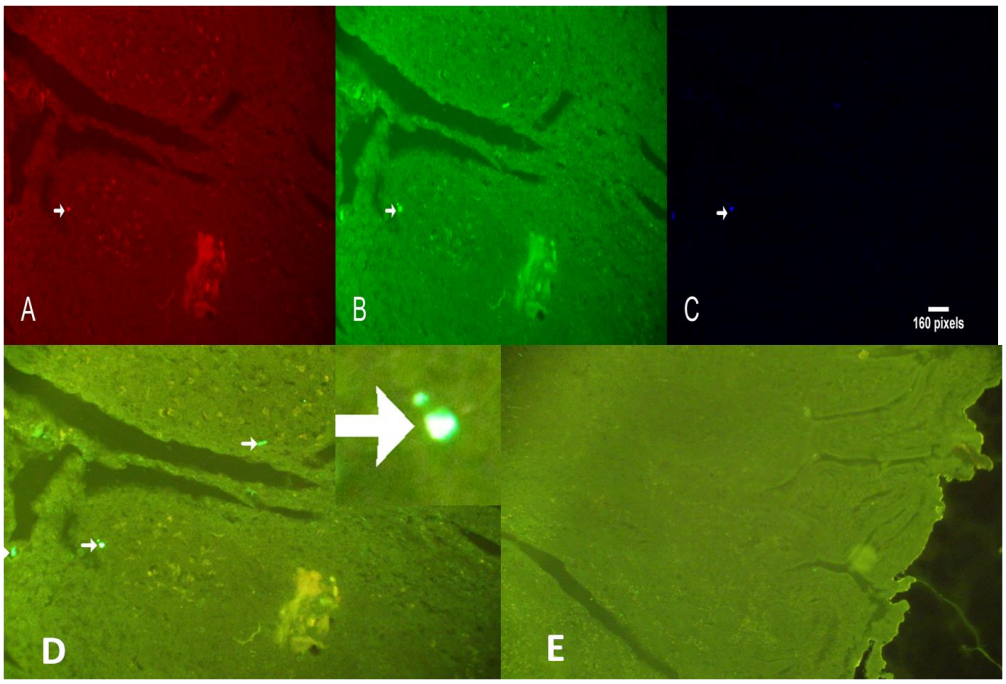


Fig-4: Typical images(A and B) showing gut hormones (GLP-1 and GLP-2 in ileum) green and red in a subset of goat ileum. Nuclei are stained blue with 4', 6-diamidino-2-phenylindole (DAPI) in fig.(C) . while fig. E. is negative control . Image A , B , C , D and E are(200 X) magnified .

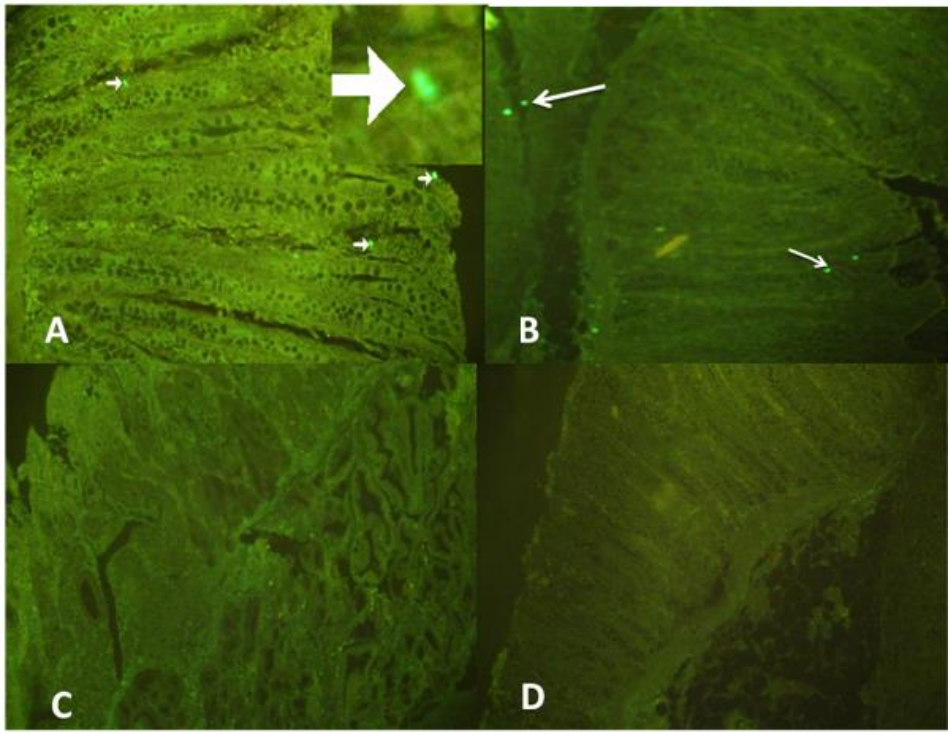
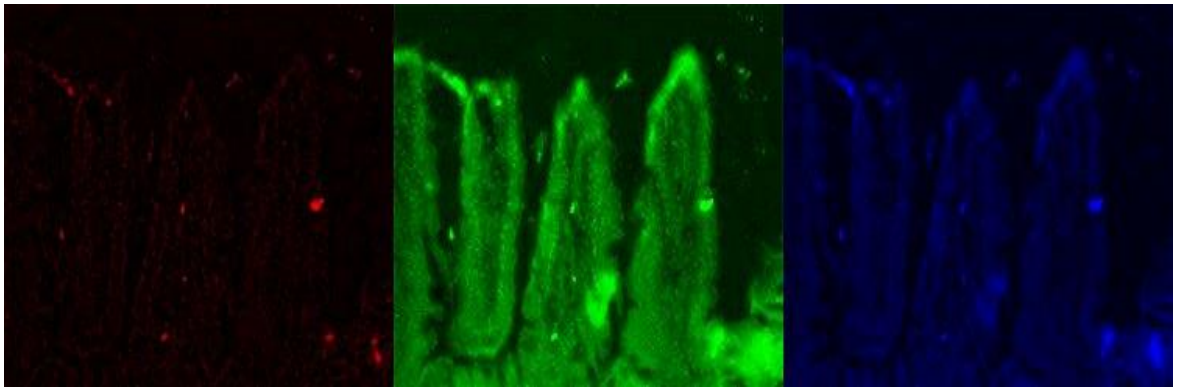


Fig.(5) : Typical images showing gut hormones (GLP-1(image A in colon) and GLP-2(image B in colon) in a subset of goat colon. Image A in colon , B in colon, C(duodenum) and D(jejunum) are not react . image A,B,C and D (200 X) magnified .



Fig(6): typical immunnoflurescence images showing (GLP-1&GLP-2)green and red in duodenum of goat image are 400X

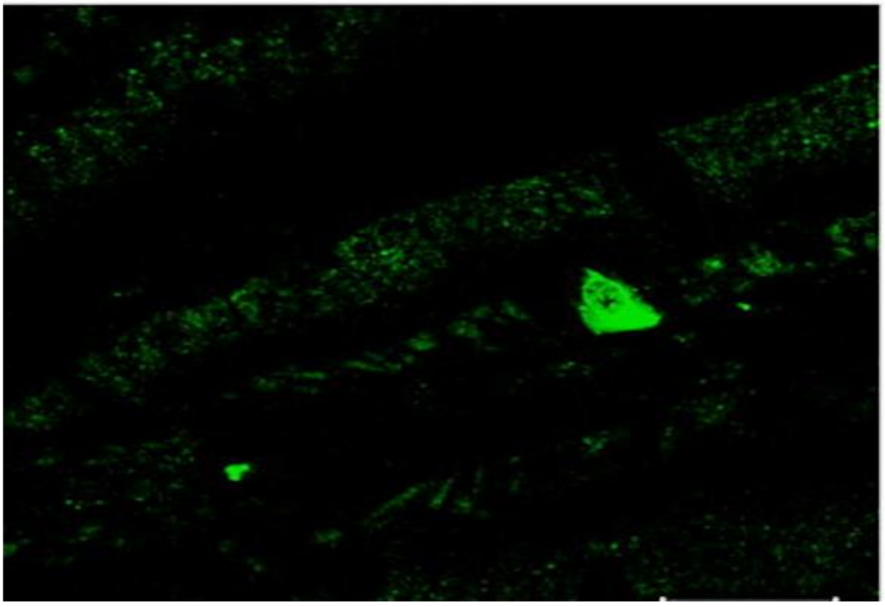


Fig.(7): A typical immunofluorescence images showing co-localization of gut hormones & the immuunoorective cells appear triangular &selender in shape **Images are 400 X magnified**

Discussion:

The present study revealed the three parts of intestine & colon of the goat, consist of four tunicae from inside to outside : mucosa, submucosa, muscularis and serosa, lined by simple columnar epithelium, same findings records by (15,16) in mammals, they were transverse folds of the mucosa and submucosa of the goat intestine, increase in size and frequency toward fifth part of the duodenum,this finding confirmed with (17) . In this study, the structure of colon in one goat is similar to other mamhgumalian species especially to the colon of the porcine support by (18).

In the three parts of small intestine of the goat, the results were indicated clearly by using immunohistochemistry that the GIP, GLP-1& GLP-2 were spoken in a separation of cells along the villuii , gut hormones show no labelling; once the primary antibodies was absent from the control section .

The relative frequency and sharing of IR entrerondocrine cells in the duodenum on the intestinal villi,in the crypts (intestinal glands) and in Brunner's glands (duodenal glands). and noticed in high frequently in the villi of duodenum, less frequently on the intestinal crypts and rarely in Brunner's glands,n the jejunum GIP IR-cells rare frequency on the intestinal villi, in the ileum GIP IR-cells were not detected ,the duodenum has the maximum diversity of endocrine cell , This finding supported by (19).

the duodenal mucosa plays a very important role indigestion 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. Correspond with (20) on adult opossum .

In the small intestine K-cells which contain GIP were detected , the relative frequency is a caudally decrease along of the goat intestine , that expressed the GIP hormone in duodenum and subsequently ,was decreased in jejunum and ileum , this finding was supported by(21,22).

L-enteroendocrine cells secrete GLP-1& GLP-2 in reply to dietetic carbohydrates, and lipids this pattern of distribution of the GLP-1 ensures also been shown in cow (23), buffalo (24). Entero-endocrine cells institute , in each

part immunohistochemistry was employed with an antibody to chromogranin A (ChA), a classical marker for endocrine cells. Large intestine sections from goat 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 goat. .

Result support the important digestive role of enterooendocrine cell in alimentary canal of goat in this research there are three types of endocrine cell secrete , GIP, GLP-1, GLP-2 were identified in three parts of intestine & colon the localized relatively stable presence of GIP in the duoduenum may be related to the role of these hormones in the stimulation of and panecreatic juice secretion.

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