Benha Veterinary Medical Journal 48 (2025) 36-40

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Variation of regional distribution of enteroendocrine cells in small and large intestine of camel: histological and immunohistochemical study

Tasnim Y. El keliny, Ehab M. El Zoghby, Hossam F. Attia, Mona N. Hussien

Histology and Cytology Department, Faculty of Veterinary Medicine,Benha University

ARTICLE INFO

Keywords

Intestine
Enteroendocrine cells
Camel
Immunohistochemistry

Received 27/05/2025
Accepted 24/06/2025
Available On-Line 01/07/2025

ABSTRACT

The enteroendocrine cells (EECs) are gut-associated endocrine cells. They secrete various kinds of locally and systemically acting amines and peptides. In this study, the EECs' distribution, shape, and staining properties were demonstrated in the intestine of the camel (*Camelus dromedarius*). hematoxylin and eosin, iron hematoxylin, Churukian and Schenk, and chromogranin A immunohistochemistry were used to identify EECs in various intestinal segments. Our results indicated that EECs have three different shapes: spindle, cone, and round. They were primarily located in the crypts of Lieberkühn. The number of positive immunostained EECs was higher in the small intestine than in the large intestine (P = 0.0030). The duodenum had the highest portion of EEC count in the small intestine, followed by the jejunum and the ileum. The colon had the highest portion in EECs count in the large intestine, followed by the rectum and then the caecum (P = 0.0030). In conclusion, our findings indicated the presence of three different kinds of EECs in the small and large intestines of camels.

1. INTRODUCTION

Camelids, particularly the one-humped camel (*Camelus dromedarius*), possess distinct physiological and anatomical traits, such as specialized gastrointestinal system features. Associated with their adaptation to desert conditions (Abdellatif, 2020).

The digestion, absorption, and hormonal regulation required to preserve homeostasis are greatly aided by the gastrointestinal system. EECs represent a specialized and diverse subset of the gastrointestinal mucosal cells that secrete hormones and are involved in controlling gut motility, secretion, food absorption, and metabolic processes. These cells link endocrine and digestive processes by releasing different peptides and amines that act locally or systemically in response to luminal contents (Furness et al., 2013; Gribble and Reimann, 2016). Despite increasing interest in camel biology detailed knowledge about the distribution and nature of EECs in camels' intestine remains limited, while previous studies have largely focused on ruminants and humans, investigations into non-ruminant herbivores like camels are comparatively scarce (Wang et al., 2022).

Understanding the regional variations in EEC distribution and architecture across intestinal segments is essential for exploring endocrine control of digestion in camels and conducting comparative gastroenterological studies. Therefore, this study aims to examine the histological and histochemical features of EECs in different regions of the camel intestine to provide insights into their structural characteristics

2. MATERIAL AND METHODS

2.1. Ethical approval

All procedures were conducted following the Guide for the Care and Use of Laboratory Animals. Ethical approval was granted by the Scientific Research Committee, Faculty of Veterinary Medicine, Benha University, Egypt (Approval No. BUFVT M 34-09-22).

2.2. Sample collection

The samples were collected from six healthy adult dromedary camels aged between 5 and 6 years, slaughtered at Toukh abattoir, Qalyubia Governorate, Egypt. Tissue samples were obtained from the small intestine (duodenum, jejunum, and ileum) and large intestine (cecum, colon, and rectum). The Samples were being gently rinsed with physiological saline to eliminate luminal contents. Then fixed in 10% neutral buffered formalin for one week.

2.3. Histological and Histochemical Technique

Tissue samples were dehydrated in ascending grades of alcohol, cleaned in xylene, and embedded in paraffin wax. Glass slides were used to mount serial slices with a thickness of 5 µm using a Leica RM2125 RTS microtome. Then sections were stained with general Hematoxylin and Eosin (H&E) stain following the protocol of (Bancroft and Gamble, 2008). Other sections were stained using iron hematoxylin and the Churukian and Schenk as silver stain to see argyrophilic granules in enteroendocrine cells, as described by Churukian (2002).

2.4. Immunohistochemistry

The immunohistochemical staining was performed according to (Hussein et al., 2021). Tissue sections were cleared by xylene, rehydrated by serial descending grades of alcohol, and washed triple with phosphate buffer saline (PBS). 3% hydrogen peroxide was used to inhibit the activity of endogenous peroxidase. Then, citrate buffer (pH 6.0) was used in a microwave oven to perform antigen retrieval. Then, 10% goat serum was added to tissue sections for 30 minutes at room temperature. A rabbit polyclonal anti-Chromogranin A (CgA) antibody (ab45179, Abcam, UK), a known marker for neuroendocrine cells, was used to perform immunohistochemical detection of enteroendocrine

Correspondence to: tasneem_yaser20@fvtm.bu.edu.eg

cells. The CgA primary antibody was diluted in goat serum 1/1000 and incubated with tissue sections for a whole night at 4°C. Then sections were washed with phosphate buffer saline (PBS) and incubated with Goat anti-rabbit IgG H&L (HRP) secondary antibody(ab6721) for one hour at room temperature. The positive reaction was visualized with diaminobenzidine (DAB). Then, tissue sections were counterstained with hematoxylin and observed by a Leica DM 3000 light microscope. The EECs were counted by Image J software in five captured images at x20 for each intestinal region.

2.5. Statistical analysis

The counted data for different intestinal regions were analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test for regional comparisons. The findings were presented as mean ± SEM. When $P < 0.05$, differences were deemed statistically significant. The GraphPad Prism (version 10.0.3) software was used to conduct the statistical analysis.

3. RESULTS

The present study showed the shape, localization, and number of EECs in different intestinal segments of the dromedary camel. The intestinal sections were stained with H&E, iron hematoxylin, and Churukian and Schenk stains were used for EECs identification. The Argyrophilic granules of ECCs appeared blue-black by iron hematoxylin and brown-black by Churukian and Schenck. The EECs were located in the villi and crypts of the intestinal gland regions in both the small (Fig. 1, fig 5) and large intestine (Fig. 2). The shape of EECs varied from spindle, round, to cone shape. The three morphologies of EECs were seen in the duodenum (Fig. 1 A, B, and C) and colon (Fig. 2 D, E, and F). while two types were seen in the other segment. In the jejunum, there were spindle and cone-shaped EECs (Fig. 1 D, E, and F). In the ileum (Fig. 1 G, H, and I), cecum (Fig. 2 A, B, and C), and rectum (Fig. 2 G, H, and I), there were spindle and round-shaped EECs.

Immunohistochemically, there was a noticeable variation in cellular localization, morphology, and number in the expression of chromogranin A among the different intestinal regions (Fig. 3).

Statistically, the number of enteroendocrine cells varied among different intestinal segments of the camel. The duodenum exhibited the highest mean count (19.0 ± 4.53), followed by the jejunum (17.4 ± 4.03), ileum (12.6 ± 3.44), colon (10.2 ± 1.64), rectum (9.6 ± 2.07), and the cecum showed the lowest count (7.0 ± 2.35). The statistical analysis revealed a significant difference in cell distribution between the segments ($P = 0.0030$).

Overall, the small intestine (duodenum, jejunum, and ileum) demonstrated a significantly higher density of enteroendocrine cells compared to the large intestine (cecum, colon, and rectum), highlighting a regional distribution that decreases distally along the intestinal tract (fig. 4)

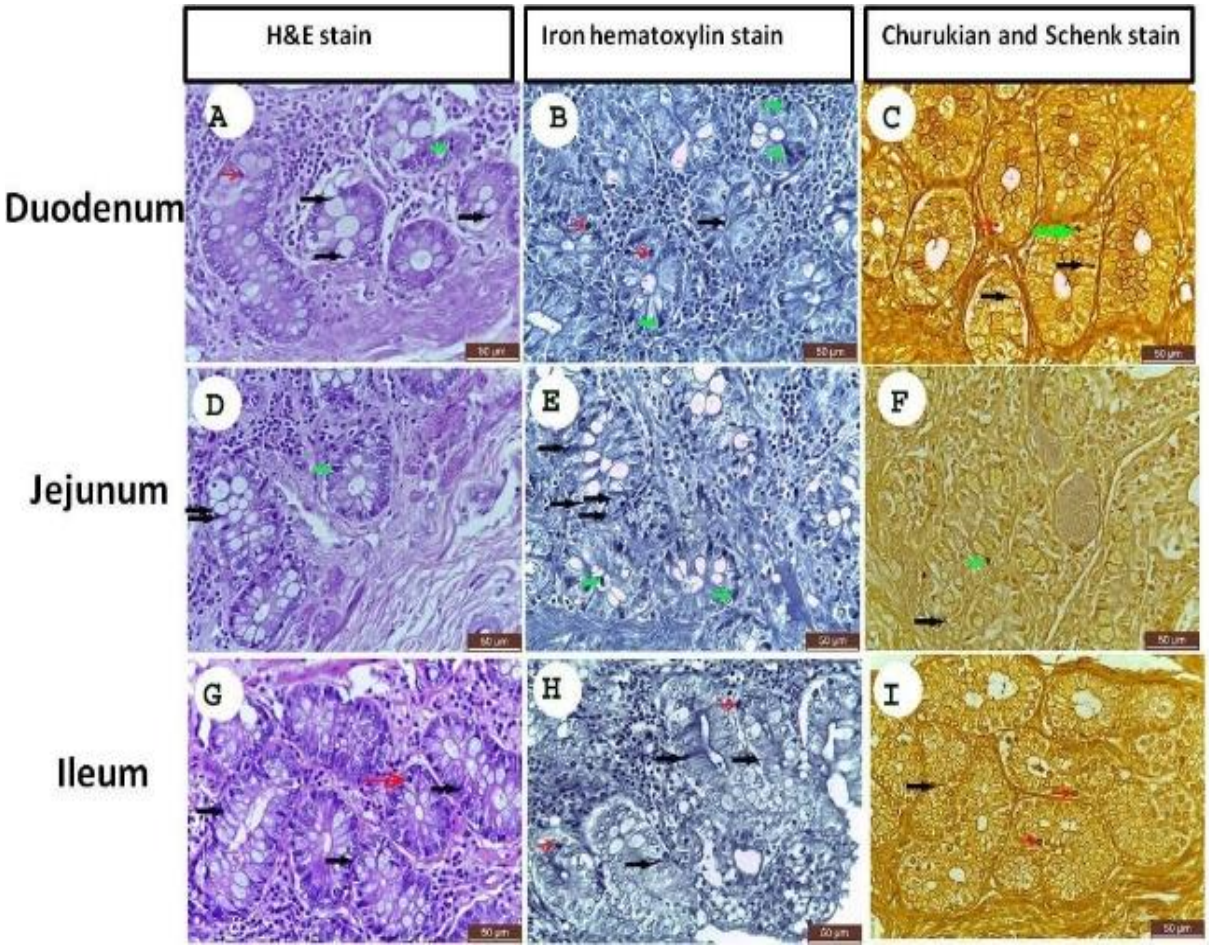


Fig. (1). distribution and morphology of enteroendocrine cells (EECs) in the small intestine of the camel (*Camelus dromedarius*). The black arrow referred to spindle-shaped EECs, the red arrow referred to round-shaped EECs, and the green arrow referred to cone-shaped EECs. The duodenum has three kinds of EECs (spindle, cone, and round shape). The jejunum has only two kinds of EECs (spindle and cone). The ileum has two kinds of EECs (spindle and round). Scale bar indicated 50µm.

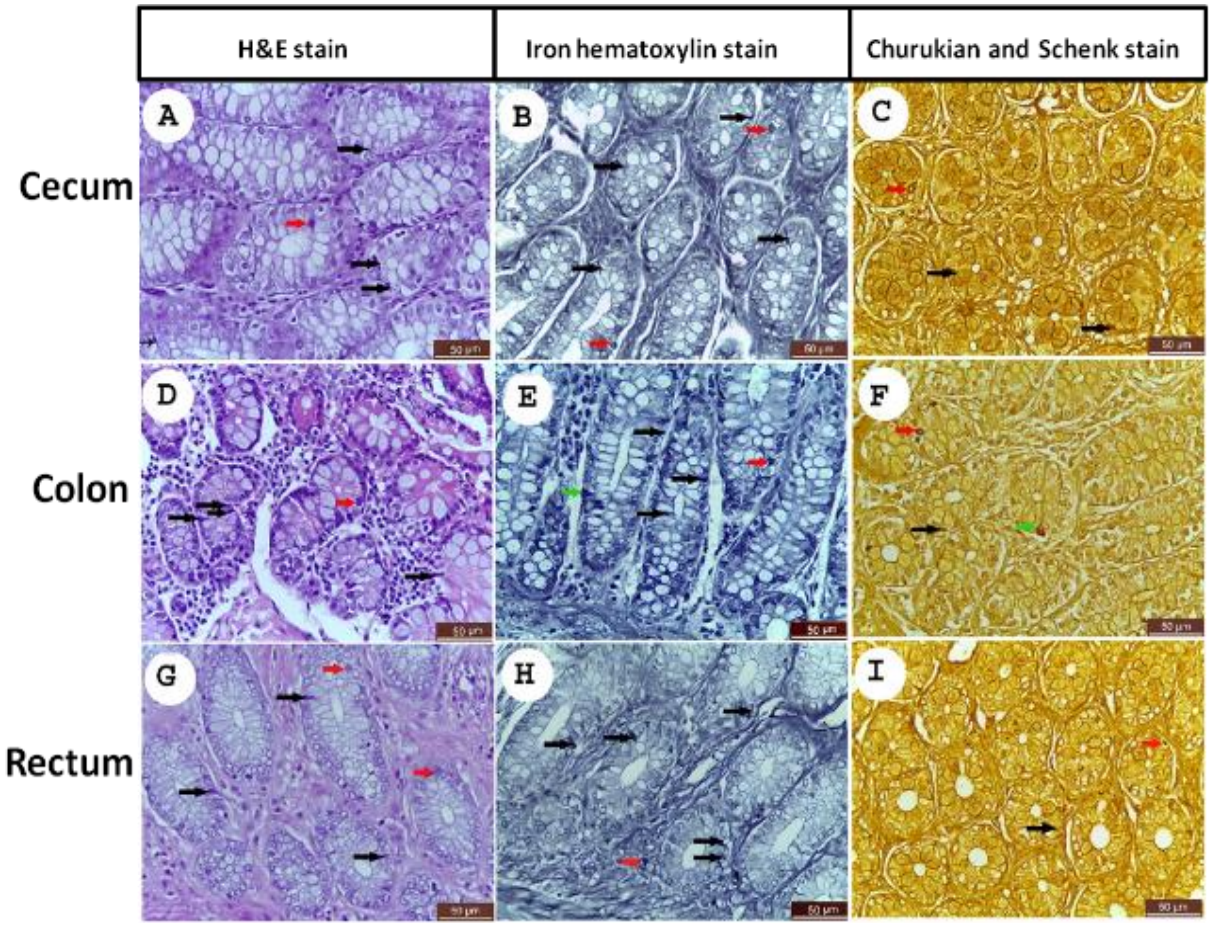


Fig. (2). The distribution and morphology of enteroendocrine cells in the large intestine of the camel. The black arrow referred to spindle-shaped EECs, the red arrow referred to round-shaped EECs, and the green arrow referred to cone cone-shaped of EECs. The EECs are spindle and round in cecum, spindle, cone, and round in the colon, and spindle and round in rectum. Scale bar indicated 50µm.

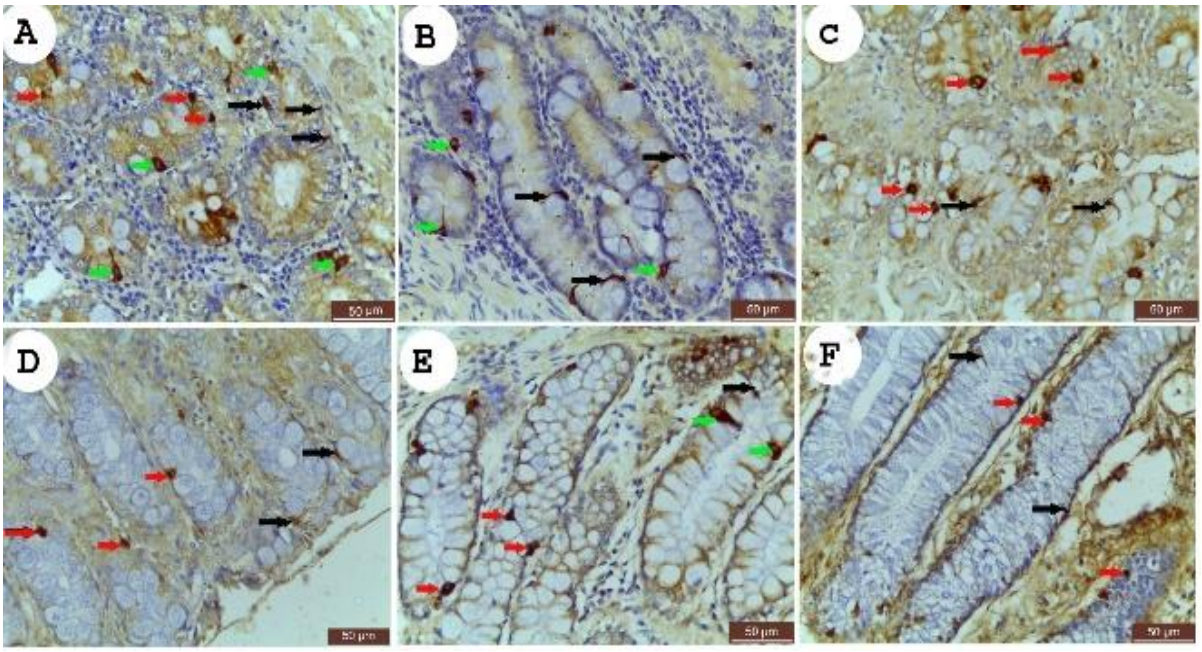


Fig. (3). Chromogranin A immunohistochemistry in the small and large intestine of the camel. The arrows show the EECs in different intestinal segments: duodenum (A), jejunum (B), ileum (C), cecum (D), colon (E), and rectum (F). The black arrow referred to spindle-shaped EECs, the red arrow referred to round-shaped EECs, and the green arrow referred to cone-shaped of EECs. Scale bar indicated 50µm.

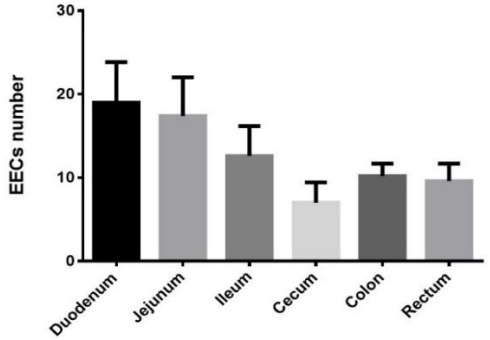
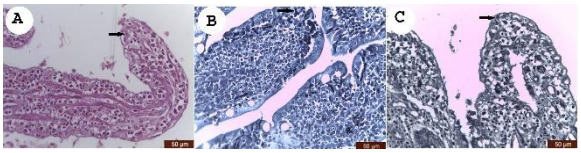


Fig. (4). Chart showing the number of EECs which exist in different intestinal segments of the dromedary camel (n=6). The EECs were counted following chromogranin A immunohistochemistry staining. The **P value is = 0.0030.



Figure(5) showed EECs cells in the villi of small intestine of camel ,(A) duodenum with H&E stain , (B) jejunum with iron hematoxylin stain ,(c) ilium with iron hematoxylin stain. EECs referred with black arrow. Scale bar indicated 50µm

4. DISCUSSION

This study confirmed a regional variation in enteroendocrine cells' distribution, shape, and staining properties throughout the camel intestine. The location, number, and morphology of EECs along the gastrointestinal tract suggest functional specialization based on intestinal segments.

EECs were regularly seen in the crypts of Lieberkühn in the small and large intestines, and they exhibited in a variety of shapes, such as spindle, round, and cone-like forms. These morphological changes are consistent with earlier findings in other mammalian species, indicating that EECs modify their morphology to fulfill secretory activities particular to a given location (Korkmaz et al., 2016; Salan et al., 2023).

All three EECs shapes—spindle, round, and cone—were found in the duodenum, suggesting a high level of functional activity and perhaps a role in starting the hormonal regulation of digestion, as the duodenum is the first part of the small intestine secreting hormones for digestion. On the other hand, the ileum and jejunum displayed more limited morphologies; the ileum had spindle and round types of cells, and the jejunum mostly contained spindle and cone-shaped cells. As the ingesta moves distally through the small intestine, needing less hormonal digestion, this decrease in cellular variety in the jejunum and ilium might indicate a change in EEC function.

In the large intestine, the colon showed three types spindle, cone, and round, whereas the cecum and rectum showed only spindle and round cells. This supports the hypothesis that the EECs exhibit regional specialization even inside the large intestine, especially in the colon, which it is function is mucosal immunological regulation and fluid reabsorption (Dockray, 2014). Cone-shaped cells in the colon might indicate that more varied hormonal responses are required to control these processes and perform this function.

Histochemically, EECs showed abundant secretory granules typical of peptide-secreting cells; these granules are argyrophilic granule stained using iron hematoxylin and Churukian and Schenk methods. The positive staining in every intestinal region supports that these techniques are used in comparative gastrointestinal research and validates their accuracy in identifying EECs (Gershon and Tack, 2007; Hussein, 2024).

The expression of chromogranin A (CgA) varied significantly by area across the digestive tract, according to immunohistochemical studies. There were more CgA-positive cells in the small intestine than in the large intestine. The small intestine of camels may have more active endocrine cells, perhaps as a result of its role in digestion by secreting hormones like secretin, motilin, and cholecystokinin and nutrient absorption. The density and dispersion of enteroendocrine cells can be evaluated using chromogranin A, a well-established marker for EECs (Rehfeld, 2004; Pucci and Batterham, 2020).

Conversely, the large intestine's comparatively reduced number of positive cells might be related to its microbial fermentation and absorption functions, which are less reliant on endocrine signaling.

The high density of EECs in the small intestine, particularly in the duodenum and jejunum, is indicative of their function in controlling the early stages of digestion using hormones such as secretin, cholecystokinin, and motilin. In the early stages of digestion, these hormones are crucial for promoting gastrointestinal motility, gallbladder contraction, and pancreatic secretions. EECs are more prevalent in these proximal areas, which is in line with earlier research on both ruminants and non-ruminants (Dockray, 2014; Wong and Chiu, 2025).

The presence and distribution of EECs in the small intestine of camels, confirming their morphological variety and crypt localization, was reported by Salan et al. (2023). These results are in line with the current work. EEC was also found in the camels' duodenum and jejunum, according to Habib and Al-Mayahi (2024). The present results support these findings but provide further information, such as comparisons between all intestinal segments (duodenum to rectum) and chromogranin A, an immunohistochemical indicator of EECs.

This study thus adds to the body of knowledge by offering a more thorough and spatially extensive investigation of EECs in the camel gut, in addition to validating earlier findings. Particularly in herbivores suited to the desert, the combined histology and immunohistochemical findings provide important information for the subject of comparative gastrointestinal endocrinology.

CONCLUSIONS

This study showed that the distribution, shape, and staining properties of enteroendocrine cells (EECs) in the camel's (*Camelus dromedarius*) gut varied significantly by location. EECs were more abundant in the small intestine than in the large intestine, especially in the duodenum and jejunum. These cells were regularly found within the crypts of Lieberkühn, and segments of the intestine showed a variety of morphologies, suggesting a functional adaptation particular to the segment. The endocrine nature of these cells was successfully confirmed by the application of histological and histochemical techniques, such as chromogranin A immunostaining. Overall, the findings highlight how important the small intestine is in camels' gastrointestinal endocrine regulation.

FUTURE PERSPECTIVES

The hormone types released by enteroendocrine cells in various camel's intestinal areas should be determined by additional research. Further information about species-specific adaptations of the gastrointestinal endocrine system may also be obtained through comparative studies, including other domestic or wild herbivores. Furthermore, assessing

alterations in EEC distribution under various physiological or pathological circumstances, such as dehydration, fasting, or gastrointestinal disorders, may improve our comprehension of their regulatory functions in camelid digestion and well-being.

4. REFERENCES

1. Abdellatif, A.M., 2020. Assessment of the endocrine cells and neural structures in the abomasum and pancreas of dromedary camel based on their synaptophysin immunoreactivity. Journal of Advanced Veterinary Research. 10,4, 226–232.
2. Bancroft, J.D. and Gamble, M., 2008. Theory and practice of histological techniques, 6th ed. London: Elsevier Health Sciences.
3. Churukian, C.J., 2002. Pigments and minerals. In: Bancroft, J.D. & Gamble, M. ,eds. Theory and practice of histological techniques, 5th ed. Edinburgh: Churchill Livingstone, pp.243–267
4. Dockray, G.J., 2014. Gastrointestinal hormones and the dialogue between gut and brain, The Journal of Physiology. 592,14, 2927–2941.
5. Furness, J.B., Rivera, L.R., Cho, H.J., Bravo, D.M., Callaghan, B.J., 2013. The gut as a sensory organ, Nature Reviews Gastroenterology & Hepatology. 10,12, 729–740.
6. Gershon, M.D. and Tack, J.J., 2007. The serotonin signaling system: from basic understanding to drug development for functional GI disorders, Gastroenterology. 132,1, 397–414.
7. Gribble, F.M. and Reimann, F.J., 2016. Enteroendocrine cells: chemosensors in the intestinal epithelium. Annual Review of Physiology. 78, 277–299.
8. Habib, K. and Al-Mayahi, M.J., 2024. Histomorphological and histochemical study of ileum and jejunum of the camel ,Camelus dromedarius. Asian Journal of Life Sciences 12, 10–14.
9. Hussein, M.N., 2024. Labeling of the serotonergic neuronal circuits emerging from the raphe nuclei via some retrograde tracers. Microscopy Research and Technique. 87,12, 2894–2914.
10. Hussein, M.N., Cao, X., El Asely, A.M., 2021. Characterization of peroxisome proliferator-activated receptor at different stages of development of intestine, liver, spleen, pancreas and posterior kidney of the loach ,Misgurnus anguillicaudatus. Egyptian Journal of Aquatic Biology and Fisheries, 25,4, 87–104
11. Korkmaz, D. and Kum, S.J., 2016. A histological and histochemical study of the small intestine of the dromedary camel ,Camelus dromedarius. Journal of Clinical Pathology and Research. 23,1, 111–116.
12. Pucci, A. and Batterham, R.L., 2020. Endocrinology of the gut and the regulation of body weight and metabolism. In: Feingold, K.R., Anawalt, B., Boyce, A., et al. ,eds. Endotext [Internet]. South Dartmouth ,MA: MDText.com, Inc.
13. Rehfeld, J.F., 2004. A centenary of gastrointestinal endocrinology. Hormone and Metabolic Research. 36,11/12, 735–741.
14. Salan, Z., Ali, S., Sadoon, A., 2023. Anatomical, histological, and immunohistochemical description of the abomasum in one-humped adult camel ,Camelus dromedarius in the south of Iraq. Archives of Razi Institute, 78,2, 549–560.
15. Wang, X., Zhaxi, Y., Li, P., Wang, W.J., 2022. The distribution of SIgA and IgG antibody-secreting cells in the large intestine of Bactrian camels ,Camelus bactrianus. Frontiers in Microbiology 81,4, 963–970.
16. Wong, C. and Chiu, I.M., 2025. Neurotransmitter and neuropeptide regulation of gut immunity. Current Opinion in Neurobiology, 92, 103036.