

## Histological and histochemical study on the large intestine of one-humped camel in Iraq

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### Abstract

The aim of the study was to investigate the histological aspects of the large intestine of adult one-humped camels (*Camelus dromedarius*) domiciled in Iraq. To conduct that, large intestines samples of twelve adult camels (six males and six females) obtained directly after animals slaughtering. Tissue specimens from different segments of the intestine (cecum, colon, and rectum) were collected, fixed, processed routinely, and subsequently stained with H&E, Lillies Allochrome, Periodic Acid Schiff, Combined AB-PAS, and Gomori Aldehyde-Fuchsin stains. The microscopic examination showed the composition of tunica mucosa, submucosa, muscularis, and serosa layers. Lining epithelium of all segments showed their consistency of simple columnar and goblet cells. Distinctly, the muscularis mucosa separates mucosa from submucosa layers. Tunica muscularis structured of two distinct layers (inner circular and outer longitudinal); the outer layer is organized by three separated bands, that is known as taeniae coli. The histochemically stained sections revealed scattered goblet cells of all segments that were strongly reacted to PAS. Staining with PAS-AB showed negative or faintly bluish stained epithelial cells due to the reaction with AB contents, whereas goblet cells showed toughly positive reaction due to the PAS contents. The gomori Aldehyde-Fuchsin stain showed goblet cells strongly reacted to the acidic, non-sulfated, mucopolysaccharides but columnar epithelium were showing poor reaction toward this stain. the unique structure of large intestine of one-humped camels has investigated and its composed layers have remarkably observed in this work.

**Keywords:** One humped camel, Large intestine, Colon, Cecum, Rectum, Mucin

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### Introduction

One-humped camel (*Camelus dromedarius*) or Arabian camel, is a herbivores mammalian inhabits

the geographic area of middle east and horn of Africa, which considers one of the essential sources of meat, wool, leather, milk, as well as, a mean of transport in the desert during past ages (Robert, 2006). The



average of its life expectancy ranged between 40-50 years, the fully grown adult camel can stand up to 1.85 meters at the shoulder, and 2.15 meters at the hump. The adult male camel is weighing 300 to 600 kg, whereas the adult female is 300-450 kg. This camel can run at 65 km/hr in short bursts, but generally can maintain a speed of 40 km/hr (Fayed, 2001). Arabian camels are adapted to live in the desert due to their very unique metabolic pathways, especially the efficient fermentation occur within their stomach, and the highly intestinal absorption occur within their intestine, these characteristic features enable the camel to survive for few days without food or water (Robert, 2006; Ouajd and Kamel, 2009). One-humped camels are known for their high efficiency in the digestion of dried materials and requirements to energy of proteins for maintaining their body weight, which are lesser than the recommended allowance for other ruminants (Iqbal and Khan, 2001). However, the main anatomical difference between this camel and rest of ruminant is the presence of large cecum and extremely long spiral colon, in addition, the extensive glandular mucosal arrangements in camel's stomach, that are not encountered in the ruminants. In addition, the camel does not have gall bladder (Abdel-Magied et al., 1994; Farid, 1995; Larson, 2010).

## Material and Methods

Large intestine samples were collection from May 2016 to May 2017. Specimens of twelve, clinically healthy dromedary camels of both sexes, aged 2-8 years (estimated according to the dental equation of the camel), collected at Al-Simawa abattoir. Samples from the mid part of each segment of large intestine collected and fixed in 10% neutral formalin buffer (for 48-72 hrs), dehydrated using series of ascending grades of ethanol, then after embedded in paraffin wax. Tissue sections were prepared at 6µm thicknesses by a rotary microtome, dewaxed in xylene, and hydrated using series of descending grades of ethanol. The sections then stained using conventional staining procedures, as follow:

- A. Hematoxylin and eosin (H&E); used for general description of general histological structures (Luna, 1968).
- B. Lillies Allochrome, used to differentiate the connective tissue (Reticular fibers) (Pearse, 1968).
- C. Periodic Acid Schiff (PAS); used to observe the

vicinal diol containing glycoconjugate (Pearse, 1968).

- D. Gomori Aldehyde Fuchsin stain; used to observe the non-sulfated mucin (Spicer et al., 1967).

Combined AB (pH 2.5)-PAS; used to demonstrate acidic and neutral glycoconjugates (Spicer et al., 1967).

## Results

### The morphologic findings

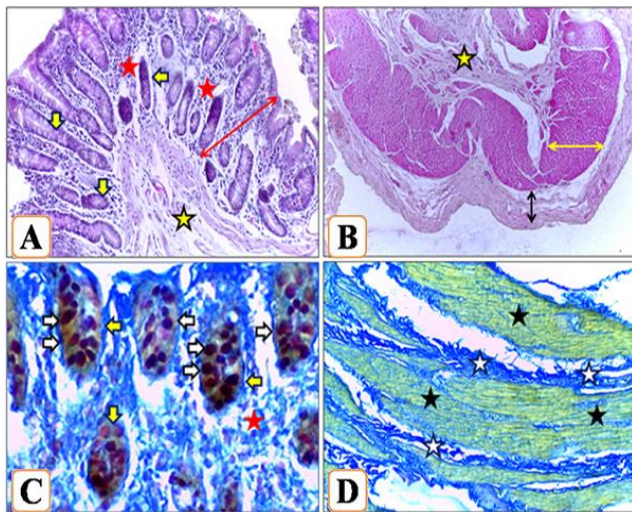
Gross examination of the large intestine of all camel samples did not show any pathological or histopathological changes. The microscopic examination showed colon distinct of three segments; cecum, colon, and rectum. The colon is differentiated into three main portions; the ascending, the transverse, and the descending. The cecum of one-humped camel found as a blind ending piece of the gut, which roses at the junction of the ileum with the first part of the colon. The ascending colon elongates from cecum to the surface right lobe of the liver. The transverse colon occupies the area across of the abdomen of umbilical region formed a wide U-shaped curve. This part was bended downward near the spleen forming left colic flexure then continues as descending colon. Latter extends to the pelvic below that occupied the left upper and lower quadrants.

### The histological findings

The microscopic examination of the outer wall of cecum revealed the presence of four tunicae, called tunica; including mucosa, submucosa, muscularis, and serosa. While mucosal membrane showed three regions, which were the lining epithelium, the lamina propria, and the lamina muscularis. The epithelium composed of simple columnar epithelial cells with a thin brush border of numerous goblet cells. Villi were absent in cecum and in all parts of large intestine in of the one-humped camel. The crypts of Lieberkühn glands were straight, unbranched, and lined distinctly with goblet cells. Lamina propria has made of loose connective tissue with infiltrated lymphocytes in different regions of the mucosa, while lamina muscularis mucosa arranged only in one longitudinal smooth muscles layer. The submucosa found to be formed of irregular dense connective tissue composed of large blood vessels situated between the muscularis mucosa and tunica muscularis. Underneath submucosa, the muscular coat consists of the smooth



muscles fibers arranged into inner circular and outer longitudinal layers. The serosa composed of single layer of mesothelium (flattened cells) covering the layer of loose connective tissue (Figure 1).

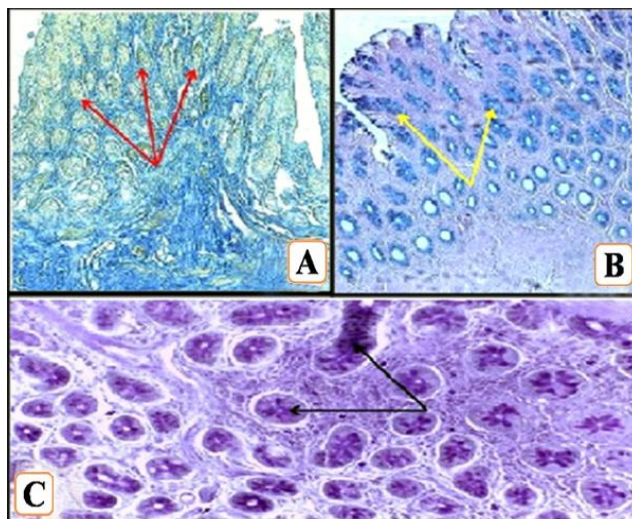


**Figure 1:** Cross section in the wall of cecum showed mucosa (red double head arrow), submucosa (yellow star), muscularis externa (yellow double head arrow), serosa (black double head arrow), intestinal glands (yellow arrows), goblet cells (white arrows), connective tissue (white stars) between smooth muscle bundles of muscularis externa (black stars) and Lymphocytes (red stars) infiltrated in the lamina propria. A & B: H&E, X200. C & D: Lille's Alcchrome stain, X200.

### The histochemical findings

The organs such as cecum, colon and rectum were well studied histochemically by applying three types of histochemical staining, that were PAS, combined PAS-AB (pH 2.5), and Gomori Aldehyde Fuchsin, these conducted to investigate the presence or absence of neutral mucin, acidic mucin, and non-sulfated mucin. The histochemically stained specimens of the cecal wall showed the mucosal layer and the circular folds of the mucosa, and submucosa were possessed of columnar and goblet cells. The columnar cells demonstrated negative reaction to PAS stain, while the goblet cells reacted strongly to it. The connective tissue in lamina propria, submucosa, and serosa, showed gentle reaction to PAS, whereas, the smooth muscle fibers (which were constructs the muscularis mucosa as well as tunica muscularis) showed raise fair reaction to PAS stain. By using the combined PAS-AB stain onto tissue sections of the cecum wall; the epithelial cells showed negative reaction while goblet

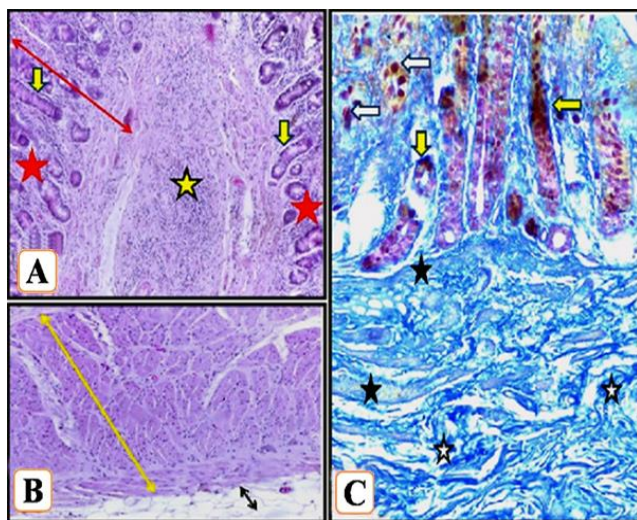
cells were gained tough positive reaction. The intense reactivity of this stain may be for the presence of acid mucopolysaccharides in the goblet cells. Moreover, red stained neutral mucous of the basal regions of goblet cells have been encountered. Both connective tissue and smooth muscle fibers, that were structured the wall of the cecum, were softly stained with this staining procedure on camel's colon tissue. Whereas, by using the Gomori Aldehyde Fuchsin stain, the goblet cells presented in the epithelium were showing a strong reaction for non sulfated mucopolysaccharides, but the columnar epithelium were showing poor reaction to this stain. In addition, the connective tissue of the submucosa showed a positive reaction for Aldehyde Fuchsin stain (dark blue), while smooth muscle fibers of tunica muscularis were showed moderate reaction to this stain (Figure 2).



**Figure 2:** Cross section in the wall of cecum showed intestinal glands non sulfated acidic mucin (red arrows), intestinal glands with neutral mucin (yellow arrows), crypts of Lieberkühn glands with acidic and neutral glycoconjugate (black arrows), A: Gomori Aldehyde Fuchsin stains, X100. B: AB (pH 2.5) – PAS, X100. C: PAS, X200.

The tunica mucosa of the colon formed of simple layer of columnar epithelial with goblet cells, contained in a sub-layer of intestinal glands. The lamina propria formed of loose connective tissues with numerous lymphocytes, collagen, lymphatic vessels, and plasma cells, and underneath lamina demarcated by presence of lamina muscularis mucosa. The tunica submucosa found to be composed of a network of dense connective tissue, blood vessels, and lymphatic nodules. The tunica muscularis was composed of inner

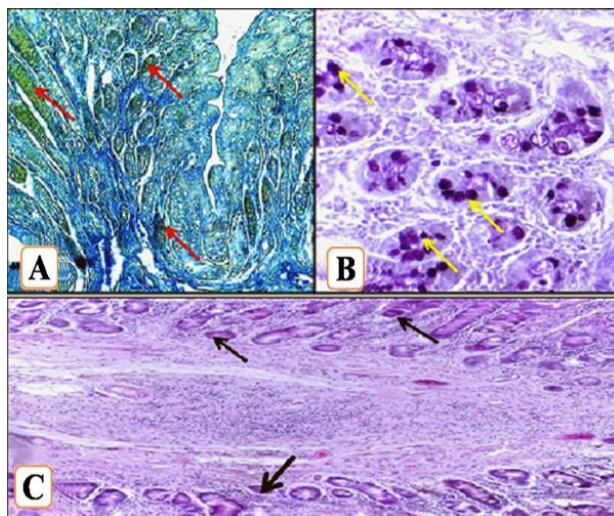
(circular) and outer (longitudinal) layers; the outer layer was organized into three separate bands, known as taeniae coli. The teniae coli were formed by flattened strands of the outer longitudinal muscles, the inner circular layer of muscles was formed the usual sheath surrounds the large intestinal tube. In addition, a thin layer of longitudinal muscles was surrounding the inner layer of circular muscles between the taeniae coli. Tunica serosa was formed of a single layer of flattened mesothelium cells covering the loose connective tissues. The myenteric plexus (Auerbach's nerve plexus) observed between the two sub-layers of muscularis (Figure 3).



**Figure 3:** Cross section in the wall of colon showed mucosa (red double head arrow), submucosa (yellow star), muscularis externa (yellow double head arrow), serosa (black double head arrow), intestinal glands (yellow arrows), goblet cells (white arrows), connective tissue (white stars) between smooth muscle bundles of muscularis externa (black stars) and Lymphocytes (red stars) infiltrated in the lamina propria. A & B: H&E, X100. C: Lille's Alchrome stain, X200.

Histochemical staining procedures showed the colon mucosa, mucous goblet cells, and striated border of columnar cells were exhibited positive reaction to AB and PAS-AB stains. Plica of camel's colon (which characterized by circular apical part and wide basal part), and goblet cells were strongly reacted to PAS. The connective tissues that structured of the propria, submucosa, serosa, and smooth muscle fibers in tunica muscularis, were negatively reacted to PAS (pink color), whereas, the mucosal goblet cells reacted

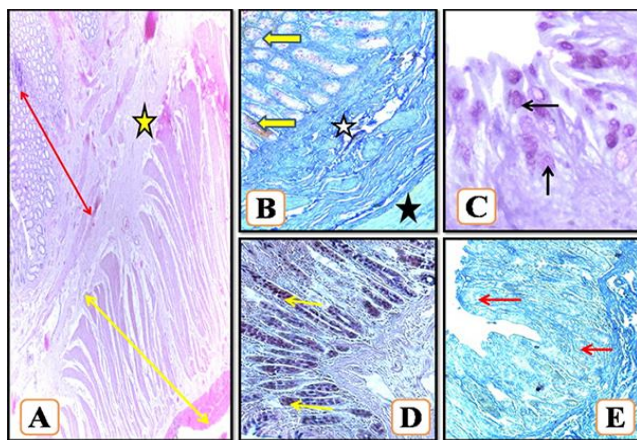
positively (dark blue) to the combined PAS-AB stain, although the rest of epithelial cells reacted negatively. The plica showed moderate to weak reaction to Alcian blue stain, while the basement membrane of the epithelium throughout the intestine and the connective tissue showed moderate reaction to Alcian blue in all sections of camels' samples. Additionally, the smooth muscle fibers in the tunica muscularis were negatively reacted with PAS stain. The sections stained with Gomori's Aldehyde Fuchsin showed positive reaction (yellow color) in the goblet cells and negative reaction (blue color) in the epithelial layer of the mucosa, indicating the presence of sulfated and non-sulfated mucopolysaccharides respectively, whereas connective tissue was faintly stained, and smooth muscle bundles were colored as pink (Figure 4).



**Figure 4:** Cross section in the wall of Colon showed intestinal glands non sulfated acidic mucin (red arrows), intestinal glands with neutral mucin (yellow arrows), crypts of Lieberkühn glands with acidic and neutral glycoconjugate (black arrows), A: Gomori Aldehyde Fuchsin stains X100. B: AB (pH 2.5) – PAS, X400. C: PAS, X200.

The rectum wall of camels' samples showed compositions of mucosa, submucosa, muscularis, and serosa or adventitia (in its last part when joined the anus). The mucosa consists of simple columnar epithelium with abundant goblet cells interspersed within the tall columnar cells, lamina propria with intestinal glands, and muscularis mucosa. The lamina propria consisted of connective tissue fibers with lymphatic cells. The intestinal glands were branched, tubular, and tortuous, lined by simple columnar cells,

and located mostly at the basal region of the mucosa with lesser number of goblet cells than seen in the epithelium. Characteristically, the muscularis mucosa of the rectum was comprised of inner (circular) layer and outer (longitudinal) layer of smooth muscle bundles. The submucosa formed of a layer of connective tissues, which contains elastic fibers, collagen, as well as, blood vessels, and submucosal nerve plexuses. The tunica muscularis showed composition of two layers of smooth muscle bundles; inner (circular) layer and outer (longitudinal) layer. Tunica serosa of the rectum was the outermost layer comprised of loose connective tissues and blood vessels. This layer replaced by tunica adventitia at junction with the anus.



**Figure 5:** Cross section in the wall of rectum showed mucosa (red double head arrow), submucosa (yellow star), muscularis externa (yellow double head arrow), intestinal glands (yellow arrows) connective tissue (white stars) between smooth muscle bundles of muscularis externa (black stars), crypts of Lieberkühn glands with acidic and neutral glycoconjugate (black arrows), intestinal glands with neutral mucin (yellow arrows) and intestinal glands non sulfated acidic mucin (red arrows). A: H&E, X100. B: Lille's Alchrome stain, X200. C: PAS X200. D: AB (pH 2.5) – PAS, X400. E: Gomori Aldehyde Fuchsin stains, X100.

The rectum mucosa revealed a strong red coloration in goblet cells of both the fold and the crypts of Lieberkühn when stained with PAS; however, the cytoplasm of the simple columnar cells were slightly stained, connective tissue and smooth muscle fibers showed gentle reactions to PAS. The positive reaction to PAS by goblet cells was indicating their importance in lubricating the tract and facilitating the movement

of ingesta. The histological sections of the rectal wall subjected to PAS-AB staining technique displayed an intense reactivity of acidic mucin substances present in the goblet cells, which was indicated by strong bluish coloration and the red-stained neutral mucous in their bases. The connective tissue present in the propria and submucosa showed negative reaction to PAS and part of PAS-AB stains, similarly, the tunica muscular showed negative reactions to these staining procedures as well. The sections stained with Gomori's Aldehyde Fuchsin showed positive reaction (dark yellowish color) in goblet cells of lining mucosal, indicating the secretion of non-sulfated and sulfated acidic mucopolysaccharides within this layer. The connective tissue of propria and submucosa reacted strongly to PAS staining, indicating that connective tissues of this layer were contained trace amount of both non-sulfated and sulfated mucosubstances. Smooth muscles showed mild reaction to Aldehyde Fuchsin part of the stain (Figure 5).

## Discussion

The description of the three gross portions of the one-humped camel colon was appeared as similar as the described colon of most of other mammals, e.g. Saleh et al. (2016; 2017) described similar three portions in rabbits colon, and Byanet et al. (2008) named same portions in the colon of cane rats. The cecum of camel's intestine is also as similar to other ruminants as the presence of a large blind pouch, that is situated the beginning part of the large intestine, which have approximately 3 feet long with two gallons capacity in matured cow (Parish, 2011). Unlike in the ruminant, the horse's cecum for example accounted nearly 15% of the digestive tract, because it is main vat where microbial fermentation occurs in this herbivores animal (Mackie et al., 1997). In rabbit and rodents, cecum accounted more than 40% of the digestive contents (O'Malley, 2005), while the cecum in domestic cat is obviously small and macroscopically relatively undifferentiated compared to most herbivores as it was considered that the carnivore cecum as rudimentary organ (Snipes, 1984). The findings of the cecum of the one-humped camels observed in this study were agreed with previous description of Kadam et al. (2011) to the cecum of cow, goat, and sheep. Dellmann and Brown (1987) described the characteristics features of large intestine, including the absence of villi, the longer, straighter, and more compact intestinal glands with

large number goblet cells. Whereas, the histological structure of the lamina propria was different from that observed in cow and other small ruminants (because of their formation of thin and discontinues layers of inner circular and outer longitudinal ones) (Kadam et al., 2011). Similarly, Nzalak (2010) has described the lamina propria in giant rat, which constitutes of loose connective tissues, numerous lymphatic vessels, lymphocytes, and collagen fibers, separated from the submucosa by the presence of lamina muscularis mucosa. The observations of the submucosa were similar to those described by Bacha and Bacha (2000) and Călămar et al. (2014) in other ruminants.

The mucosa is made of simple columnar epithelial layer and lamina propria (composed of intestinal glands, lymphatic nodules, and lamina muscularis). The lymphatic nodules were aggregated approximately near the latter structure. Numerous tall columnar cells and goblet cells were lined other intestinal glands, the goblet cells seen at their highest numbers in the rectum (Kadam et al., 2011). Pakowadee et al. (2008) indicated that the histochemical stained specimens of the cecal surface lining and crypts of Lieberkühn in goats were reacted as similar as seen in camel tissue.

The histochemical reactions were similarly as found in different mammalian species as well (Saleh et al., 2016; 2017), which are agreed to our findings in this study. The taeniae coli observed in camel's colon were likely as tunica muscularis of colon in both pigs and horses as documented previously by Perez (2008) and Eurell (2004). Similar findings recorded on ruminants' colon were in accordance to ours as well (Wedel, 1999; Wei et al., 2012; Bello and Umar, 2016). Studies on other mammalian species, such as human colon (Ganns et al., 2006; Kustermann et al., 2011), mice (Mazzuoli and Schemann, 2012; Salih et al., 2014), rats (Bridges et al., 1986; Hanan et al., 2014), equine (Freitag et al., 2008), carnivores; cats and dogs (Christensen et al., 1984), and rabbit colon (Alhaaik, 2017), indicated the histological structure of camel's rectum similar to the structure in these species, especially of ruminants' rectum, such as cattle, sheep and goat (Kadam et al., 2011).

## Conclusion

As a conclusion, the gross section of the one-humped camel large intestine showed its characterization of large, U-shaped, colon. The rectum, which is the last part of the tract, is too small in comparison other parts.

The large intestine is consist of the typical layers (mucosa, submucosa, muscularis, and serosa) as known in the majority of other mammalian species, where the lining epithelium composed of simple columnar tall cells and large numbers of goblet cells, with the presence of intestinal glands (crypt of Lieberkühn), but with numerous differences in size and number of cells along with these three different segments. The layer of muscularis mucosa is distinct and constructed of two sub-layers of smooth muscle fibers (that are separating mucosa from submucosa), formed of dense irregular connective tissues. The columnar cells of the large intestine have shown either faint or negative reactions to PAS, PAS-AB, and Gomori Aldehyde Fuchsin stains, whereas goblet cells have shown to be strongly positive to these stains (indicating the presence of sulfated acidic mucin contents in the tissue).

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## Contribution of Authors

AL-Samawy ERM: Conceived idea and collected data  
Jarad AS: Developed research design, collected data and wrote manuscript

Al-Saffar FJ: Conducted literature review and wrote manuscript

Kadhim DMH: Collected and analysed data

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