Histological and histochemical study of large intestine in Camel *(Camelus Dromedarius)*

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Abstract

Current study was performed to describe the Histohistological and histochemical structures of the large intestine in Iraqi one humped camel (*Camelus Dromedarius*). Five healthy male camels Approximately 3 to 4 years old were chosen and their specimens from cecum, colon and rectum. The specimens were directly transferred after the animal's slaughtering to the histology lab at College of Veterinary Medicine for subsequent histological techniques. Tissue specimens from various parts of the large intestine (cecum, colon and rectum) were collected and fixed with 10% neutral buffered formalin then subjected to routine tissue processing. Sections of 6 micrometers thickness were prepared and stained with hematoxylin and eosin (H&E) for general histology and Periodic Acid Schiff combine with Alcian blue (AB–PAS), Masson's trichrome as well as Verhoeff stains for histochemical description. Microscopic findings revealed four tunicae constructing the wall of cecum, colon and rectum mucosa, that were from the inner most mucosa, submucosa, muscularis and the outer most either serosa or adventitia. The tunica mucosa characterized by simple columnar epithelium rested on basement membrane and the presence of muscularis mucosa which separated mucosa from the underneath submucosa. Crypts of Lieberkühn and goblet cells were abundant in the colonic epithelium. The submucosa was made up of irregular connective tissue rich with adipose tissue and was supplied well with many blood vessels. Tunica muscularis in all over the three parts of large intestine was made up of inner circular smooth muscle fibers and outer longitudinal smooth muscle fibers. The tunica serosa, and in parts replaced by adventitia, was made up of loose connective tissues.

Keywords: Camel, cecum, colon, rectum, histochemistry, histomorphometric

Introduction

The Arabian camel (*Camelus dromedarius*) is an important source of meat, wool, skin, milk and for race and transportation. The camels are well adapted to life of the desert due to their unique metabolic pathways, more efficient fermentation in their stomach and high intestinal absorption. These features allow these animals to survive without food and water for a few days in many parts of the world, especially in the developing countries in Africa and Asia (1) (2) (3) (4) (5)

Dromedary and Bactrian camels were known as early ruminants, and their digestive anatomy were significantly different from those true ruminants because camelids have a maxillary dental pad and mandibular incisors. In general camelids tend to move around and selectively eat,

selecting food close to the ground, in contrast to cattle that consume as much as they can in one area. The upper lip of camelids is divided into two independently manipulated labia, allowing them to select certain foods and avoid foreign objects. This trait and ability was absent in members of cattle. Camelids do not use their tongue to manipulate or grab food. The main histomorphological difference is the presence of extensive glandular mucosal arrangements of camel stomach that have no counterpart in the stomach of ruminants. Moreover, camels do not have gall bladder and the hindgut is consisted of a large cecum and extremely long, spiral colon so that can produces a relatively dry, ball shaped feces (6) (7) (8) (9) (10)

The new world camels were substantially more efficient at digesting dry materials and their energy-protein requirements for maintaining live, body weight that were lesser than the recommended allowance for ruminants (11) (12) (13) (14). This study amid to investigate the histological and histochemical features of large intestine.

Materials and methods

Five Iraqi local male camels (dromedarius camel) were collected singly when they were slaughtered at the local abattoir present in hay Albasatin (near almuthana bridge) at Baghdad dated between October 2021 to February 2022. The studied specimens (large intestine) were excised and directly transferred to Department of Veterinary Anatomy Laboratory University of Baghdad to conduct such study. The chosen camels were clinically healthy of both sexes, aged approximately 3.5 to 5 years according to dental formula and their weights were ranged between 350 to 450 kg. Specimens excised from the mid part of each segment of the large intestine, collected and subsequently fixed in 10% neutral buffer formalin for 48-72 hrs. Tissue specimens were then after dehydrated using a series of ascending graded ethanol, then cleared with xylene, and then embedded in paraffin wax. Paraffin blocks of the tissues were sectioned by rotary microtome to prepare tissue section of 6 μm thicknesses. To stain these tissue sections, they were dewaxed in xylene and hydrated using descending series of graded ethanol. These specimens were stained by H&E stain, PAS stain Combined with Alcian blue (AB) pH 2.5 (15), Masson trichrome stain, and Verhoeff stain, Then the slides were examined by light microscope and the microphotographs were taken to the sections by digital camera.

Results & Discussion

The present study showed that the large intestine of Dromedary camel is divided into three segments: the short cecum, the colon (ascending, transverse, descending colon), and the rectum. These segments subdivisions were already mentioned by previous researchers such as (16) (17) (18) (19) in camel, ruminant, horse and human, respectively. They showed similar general structures with some regional variations in the large intestine of the studied subjects.

Cecum

Microscopic findings of the current study showed that the cecum was composed of four tunicae mucosa, submucosa, muscularis, and serosa (Fig. 1 A & C). The tunica mucosa was made up of three layers, i.e. epithelial mucosa, lamina propria and muscularis mucosa. The epithelial mucosa was buildup by simple columnar epithelial cells which covered the apical rim of the cecal glands. The lamina propria was composed of loose connective tissue which occupied by simple tubular cecal glands which in turn highly supplied with goblet cells (Fig. 1C). The muscularis mucosa was made up of irregular layer of circular smooth muscle fibers (Fig. 1, D). Approximately, similar description was recorded in the cecum of buffalo **(**20) and cattle **(**21) (22), who described similar subdivisions of the cecal mucosa. However, in buffalo, the researchers observed muscularis mucosa only in the apex and body of the cecum. In cattle and buffalo, as same as to current findings, simple columnar epithelium was described in addition to the presence of tubular cecal glands. Whereas, it has been stated that muscularis mucosa is made up of an inner circular layer and an outer longitudinal layer of smooth muscle fibers and absence of the glands (18).

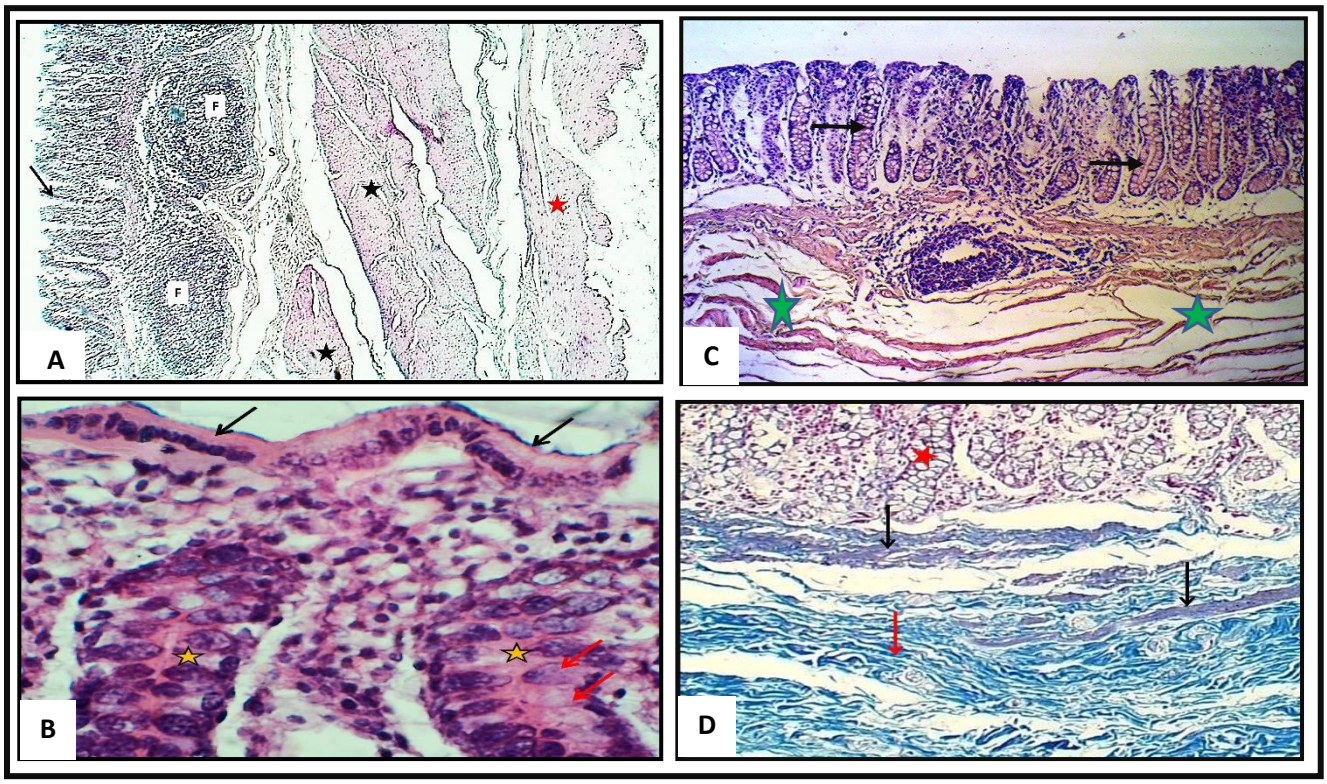

Figure 1 : Representative histological (A-B) and histochemical (C-D) sections of the cecum of Doromadery camel shows: (A) Mucosal simple tubular gland (black arrow), lymphoid follicles (F), submucosa (S), inner circular smooth muscle fibers (Black star) & outer longitudinal smooth muscles of muscularis (Red). H&E stain. 40x. (B) Low columnar epithelial cells (Black arrows), mucus secreting cells (Goblet cells) (Red arrow). H&E stain.400x. (C) Muscularis mucosa

(green star), Goblet cell (Black arrows). (D) Dense irregular collagenous connective tissue (Red arrows). Masson's trichrom stain 100x.

However, the tunica submucosa in camel's cecum of the current study was made up mainly of dense irregular collagen fibers in which invested numerous solitary lymphoid follicles (Fig.2 A&B). Recently, (16) and (23) recorded similar observations in cecal wall of the large intestine of camel and cattle, respectively. They described dense irregular connective tissue composed of large blood vessels positioned between the muscularis mucosa and tunica muscularis. Currently, the tunica muscularis in the camel's cecum was very thick layer which was build up of thick inner circular layer of smooth muscle fibers and an outer longitudinal layer of smooth muscle fibers. Autonomic nervous plexuses were existed between such two sublayers of smooth muscles (Fig. 2,B). Differently to the current studied camels, (18) described tunica muscularis of the cecum in horses and pigs which was arranged in such away to be called taeniae coli in which the outer layer, is organized in bands. Actually, elastic fibers are replacing the muscle fibers in horses. The tunica serosa was made up of a single layer of mesothelium characterized by single layer of flattened cells that was continued with peritoneum. This layer was supported by a layer of loose connective tissue. Similar features were described in camel and ruminant by previous investigations (18) (23) respectively.

The histochemical findings showed post staining with combined PAS-AB (pH 2.5) cecal simple tubular glands in the studied camels that were predominantly produces neutral mucopolysaccharides and for lesser extent of acidic mucopolysaccharides secretions (Fig. 3, A & B).

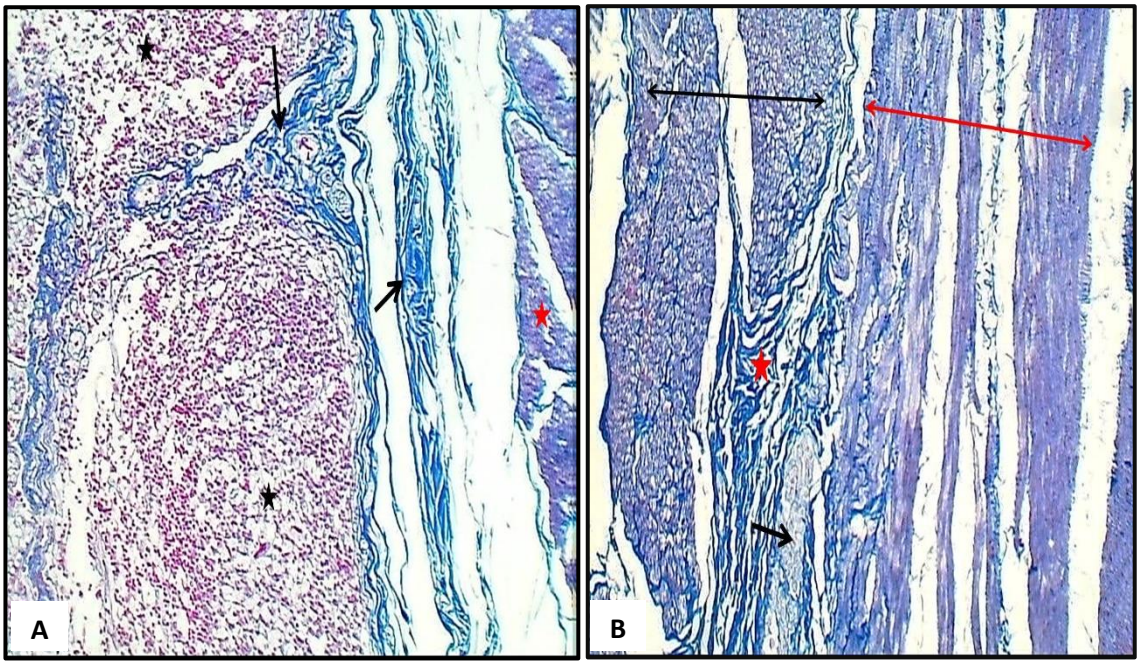

Figure 2: Representative Histochemical section of cecum of dromedary camel shows:A) collagen bundles (Black arrows,), inner circular muscle smooth muscle fibers (Red star) & solitary lymphoid (Black star). Masson trichrom stain.100x. B) Collagen bundles (Asterisk,), inner circular muscle smooth muscle fibers (Red double arrow), outer longitudinal layer of tunica muscularis (Black double arrow), nervous plexuses (Black arrow). Masson trichrom stain.100x.

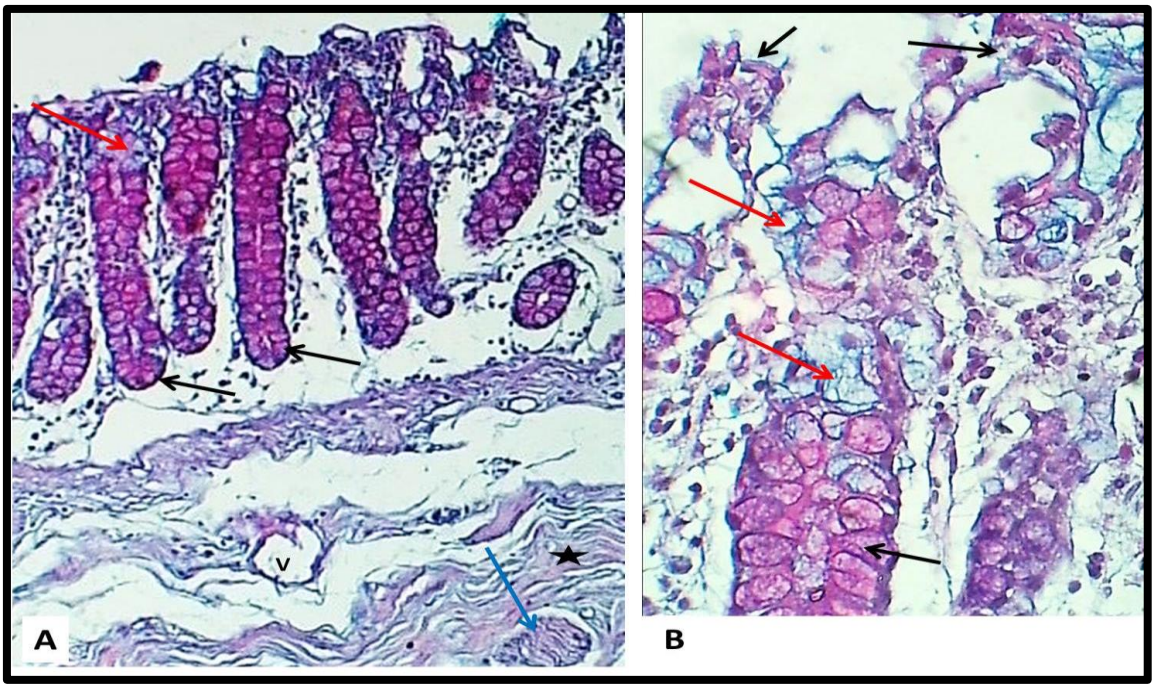

Fig 3: Representative histochemical section of cecal mucosa of dromedary camel shows: mostly neutral mucoploysacharrid (Black arrows), little of acidic mucopolysacharids secretion (Red arrows), veinule (V), collagen bundle (Asterisk) & nerve (Blue arrow). Combine (2.5pH)-PAS (A) 100x & (B)400x.

Colon

Histologically, the present findings of the colon showed that both ascending and transverse colon have the same wall structure. Their walls were very thick with unique feature because of the presence of numerous plica circularis (Fig. 4). The tunica mucosa was lined with simple columnar epithelium with abundant goblet cells. It is invaginated toward the lamina propria to form long simple tubular glands may name intestinal glands that were opened into surface to face intestinal lumen. Numerous solitary lymphoid follicles were distributed around such glands. Both the glands and follicles were invested in loose connective tissue with abundant fibroblast, lymphocytes, plasma cells and blood vessels. The related muscularis mucosa was well developed showing thick layer of inner circular and outer longitudinal sublayers of smooth muscles fibers (Fig. 5, A, B& E). These characteristic features were in agreement with recent findings of (18) in the colon of camels.

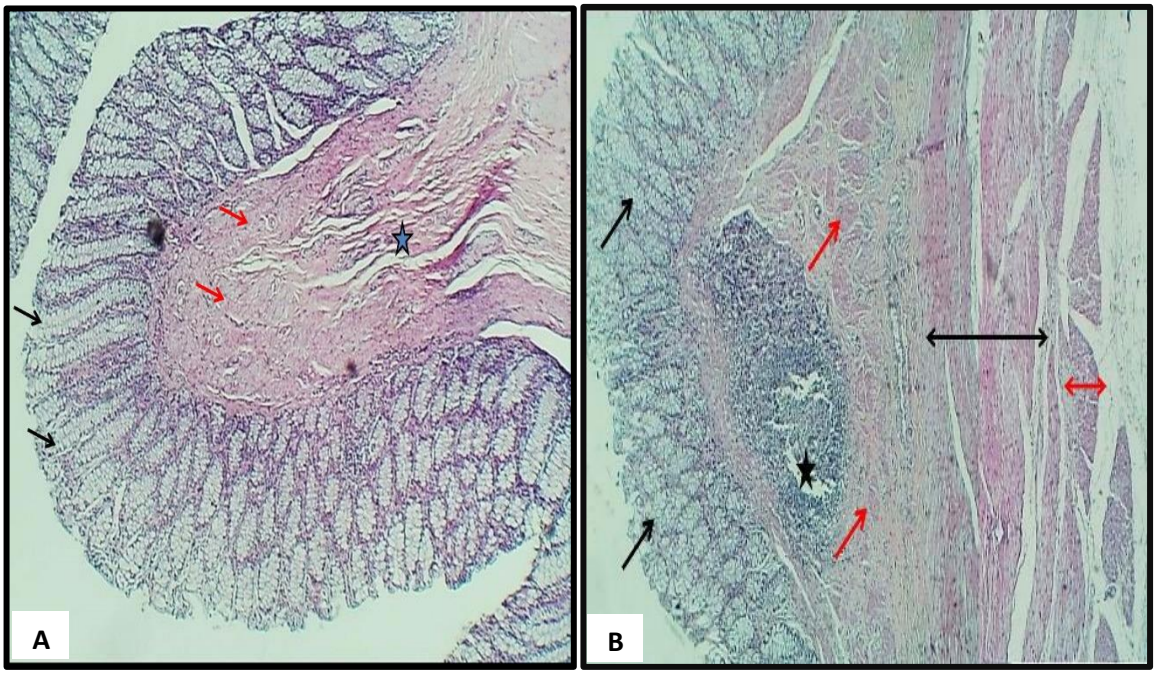

Figure 4 : Representative Histological section plica circularis of transverse colon in camel shows:A) very long mucosal simple tubular gland (Black arrows), muscularis mucosa (Red arrow),& thick fibrous tissue of submucoaa (asterisk). H&E stain.40x. B) Simple tubular glands of ascending colon shows: epithelium (E), mucus secreting cells (Goblet cells) (black arrow) & gland (black star). H&E stain.400x

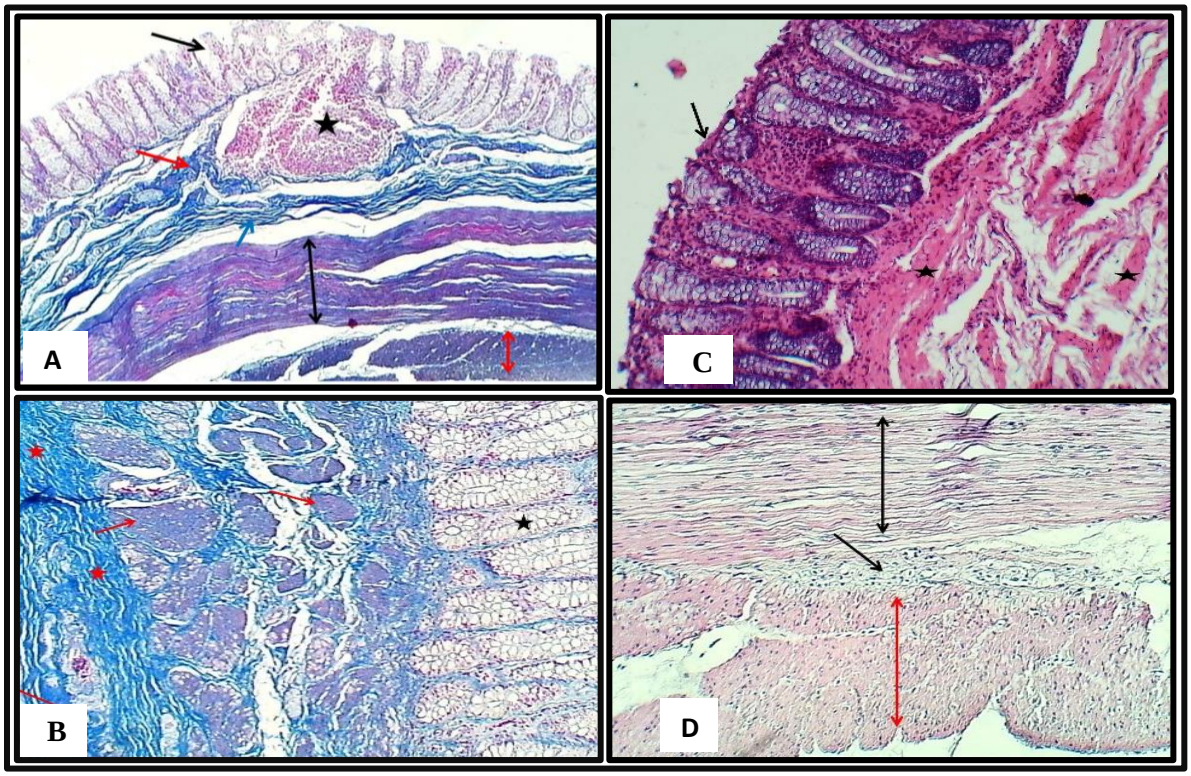

Figure 5: Representative Histological and histochemical section of colon in camel shows: A) mucosal simple tubular gland (Black arrows), mucosal lymphoid follicle (Asterisk), muscularis mucosa (Red arrow), inner circular smooth muscle fibers (Black double arrow) & outer longitudinal smooth muscles of tunica muscularis (Red double arrow) & (Black arrow). H&E stain.40x. B) Fibrous connective tissue of submucosa (Red star). Masson trichrom stain.100x. C) mucosal simple tubular gland (Arrows), inner and outer muscularis mucosa (Black asterisk) . H&E stain.100x.). (D): inner circular muscle smooth muscle fibers (Black double arrow), outer muscularis layer (Red double arrow) & nerve plexuses (Arrow). H&E stain.100x.

In camel the epithelium associated with few goblet cells and the underlining lamina propria possessed limited lymphocytes and plasma cells. However, the parallel arrangement of intestinal glands was similar in both camel and dog. Present findings were in a good agreement with those in camel recorded by (24). They recorded epithelium of colon that was densely packed with goblet cells. Goblet cells were globular shaped unicellular mucous glands scattered throughout the columnar cells of the lining intestinal epithelium.

The underlying submucosa of the camel's colon was built up of dense irregular connective tissue mainly of collagenous type (Fig. 5, D). These observations of the submucosa were comparable to those described recently by (25) and previously by (26) in other ruminants. As same as in current findings, (27) described a network of dense connective tissue, blood arteries and lymphatic nodules in the tunica submucosa of the camel's colon.

the tunica muscularis in colon found very thick because it composed of thicker inner circular layer of smooth muscle fibers and thinner outer longitudinal layer of smooth muscle fibers. Obvious connective tissue existed between these two layers of the muscularis tunica in which an autonomicnervous plexuses were detected (Fig. 5). In fact, these were myenteric plexus or called Auerbach's nerve plexus. These findings were comparable with previous records in camel's colon (24) (16). The latter researchers found tunica muscularis made of inner (circular) and outer (longitudinal) layers; the outer layer was divided into three distinct bands known as taeniae coli. Tunica serosa is made up of a single layer of flattened mesothelium cells that cover the loose connective tissues.

The histochemical techniques showed that simple tubular glands of ascending, transverse and descending colons were produced predominantly neutral mucopolysaccharides and for lesser degrees of acidic mucopolysaccharides secretions. The acidic secretory cells were detected at the bottom of the tubular glands (Fig. 6, A&B).

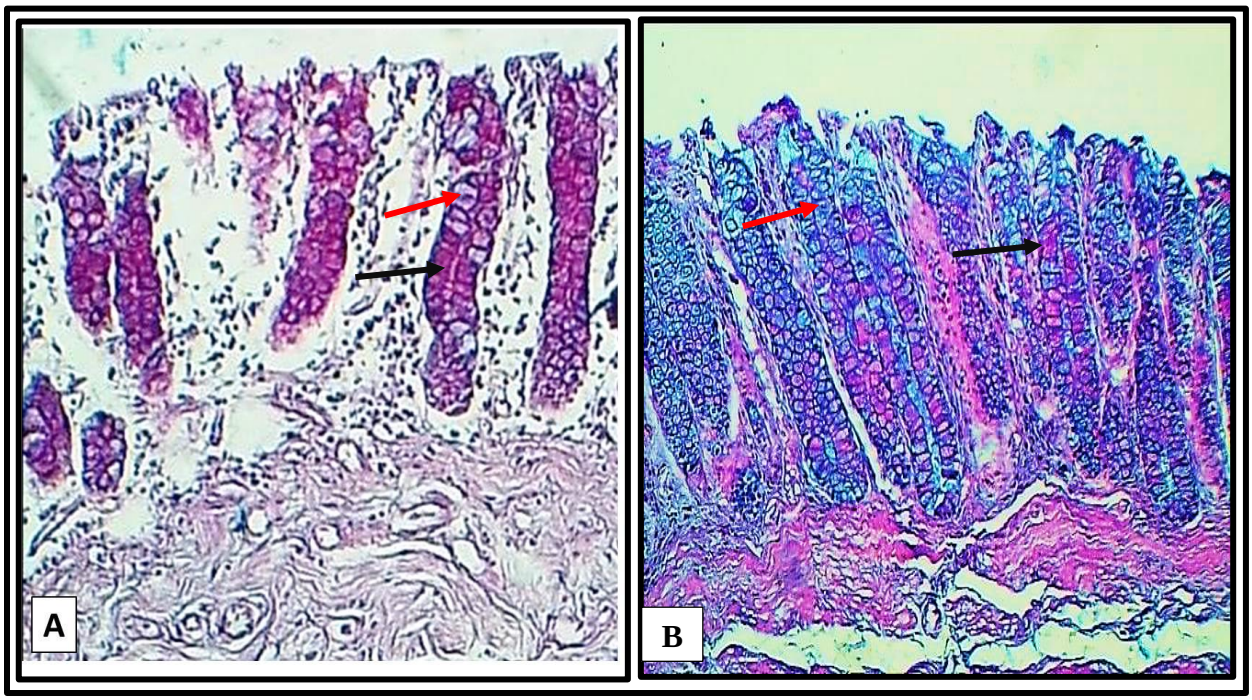

Figure 6: A Representative histochemical section of mucosa of descending colon in camel shows: mostly neutral mucoploysacharrid (Black arrows), little of acidic mucopolysacharids secretion (Red arrows), Combine (2.5pH)-PAS (A) 100x, B) Representative section of mucosa of transverse colon in camel shows: little neutral mucoploysacharrid (Black arrows), mostly of acidic mucopolysacharids secretion (Red arrows), Combine (2.5pH)-PAS (A) 100x .

Rectum

In the current study, histological examination revealed a similarity of the rectum with that of cecum and colon which made up of mucosa with numerous simple tubular mucus secreting glands associated with abundant goblet cells, but more than those found in the colon. It also had similar submucosa as in the other parts of large intestine (Fig.7). The tunica muscularis of the wall of rectum was typically more developed than that found in colon and it was made of an internal circular smooth muscle bundles and outer longitudinal smooth muscle bundles (Fig 8). The rectum was composed of serosa or adventitia (in its final section when united to the anus). The mucosa was made up of simple columnar epithelium with numerous goblet cells interspersed among the tall columnar cells, lamina propria with intestinal glands and muscularis mucosa. The lamina propria was made up of connective tissue fibers as well as lymphatic cells. The intestinal glands were tubular and branched which was lined by simple columnar epithelium and predominantly situated at the mucosal basal region, with lesser number of goblet cells than those present in the surface epithelium. The rectum's muscularis mucosa was distinguished by its inner (circular) layer and outer (longitudinal) layer of smooth muscle bundles. The submucosa is a layer of connective tissues that includes elastic fibers, collagen, blood vessels, and submucosal nerve plexuses. The tunica muscularis was made up of two layers of smooth muscle bundles: an inner (circular) layer and an outer (longitudinal) layer. The tunica serosa of rectum was the outermost layer, which was made up of loose connective tissues and blood arteries. At the anus-tunica junction, this layer is replaced by tunica adventitia. These histological features were similar to the corresponding rectum in cattle, sheep and goat (23). The thickest wall of rectum in camel appeared similar to same organ in horses and cattle (17). The latter reference postulated that in carnivores the outer longitudinal layer of tunica muscularis is thick compared to the other parts the author also stated that elastic fibers are most visible in the rectums of horses and cattle and least visible in the rectums of sheep and goats. In addition to that, the outer longitudinal layer of tunica muscularis includes more elastic fibers than the inner circular layer. In the lamina propria of the rectum, all domestic mammals have a large venous plexus and in dogs, the rectum is characterized by roughly 100 isolated lymphatic nodules.

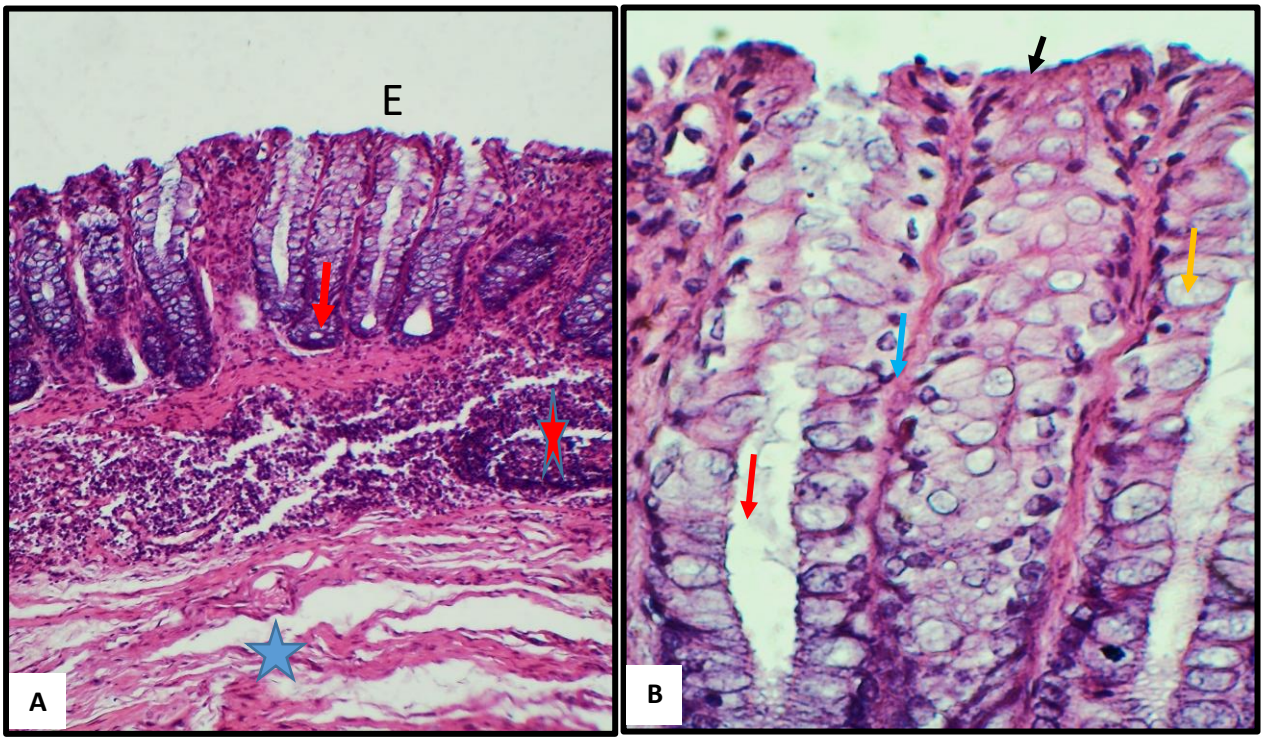

Figure 7: Representative Histological section of mucosal fold rectum of camel shows:A) simple tubular gland (Red Arrows), solitary lymphoid nodules(Red star), muscularis mucosa (Black asterisk) & collagen bundles (Blue star) H&E stain.40x. (B). The simple tubula glands with large number of Goblet cells H&E x400.

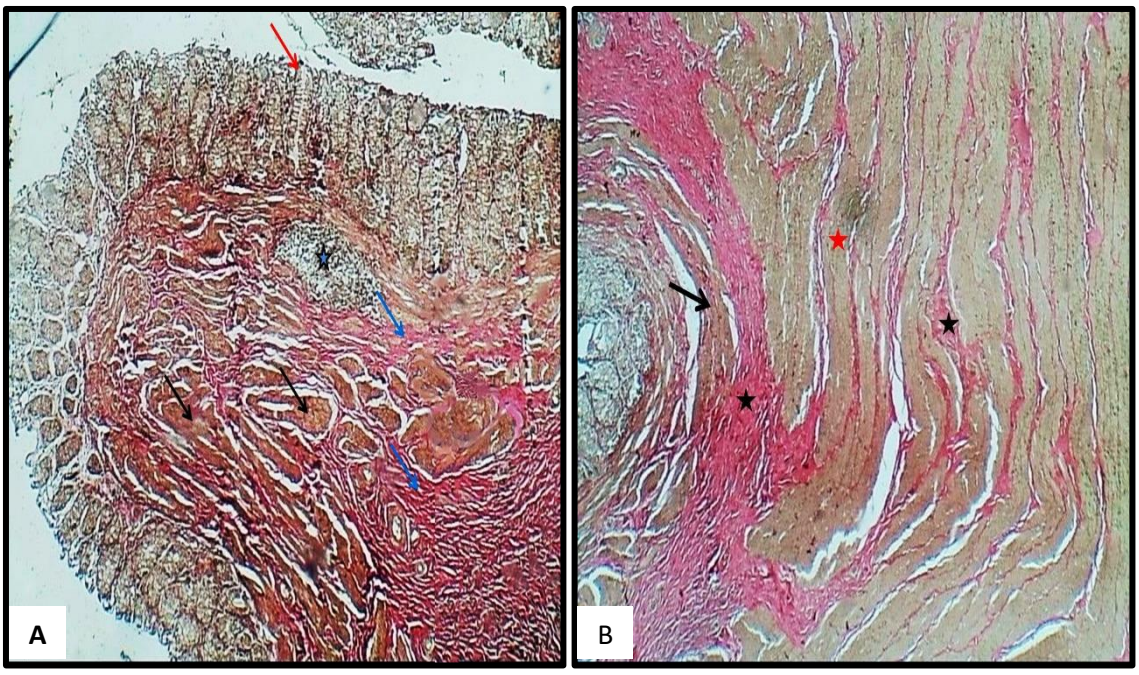

Figure 8: Representative Histochemical section of mucosal fold of rectum in camel shows :A) simple tubular glands (Red arrow) solitary lymphiod nodules (blue star) ,muscularis mucosa (black arrow) and collagen bundles (blue arrow) Verhoff stain x40. B) smooth muscle of muscularis mucosa (Black arrows), collagen bundles of submucosa (Black asterisk), inner circular layer of tunica muscularis (Red asterisk). Verhoff stain.100x.

Conclusion

This study of the large intestine of one humped camels (*Camelus Dromedarius*) demonstrated that the large intestine has the same layers (mucosa, submucosa, muscularis, and serosa) as the majority of other mammalians. The lining epithelium is composed of simple columnar tall cells and a large number of goblet cells, as well as the presence of intestinal glands (crypts of Lieberkühn), but with numerous differences in size and number of cells. While the muscularis mucosa was distinct and composed of two sub-layers of smooth muscle fibers which separate the mucosa from the submucosa and dense irregular connective tissues. Interestingly, the large intestine's columnar cells showed mild reaction to PAS-AB, whereas the goblet cells were significantly positive with the presence of sulfated acidic mucin contents in the tissue.

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