

## Histological and Histochemical Study of Stomach of Local Adult Male And Female Cape Hare *Lepus Capensis*

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### ABSTRACT

The current study's to describe the stomach's histological and histochemical properties in male and female *Lepus capensis*, samples of cardiac, fundic, and pyloric regions were taken from 14 hare, exposed to histological techniques and stained with H&E, PAS, AB, Masson Trichrome and Mallory Trichrome. The stomach was glandular type and mucosa divided into cardiac, fundic, and pyloric portions. Mucosa, submucosa, muscularis, and serosa were the layers that make up stomach wall, the thickness of every layers in the fundic part was greater than that in the cardiac and pyloric parts, simple columnar epithelium lining the mucosa, with gastric pits that open into tubular or alveolar gastric glands. The mucosa's glands were shown as short, branched, coiled, tubular glands in the cardiac and pyloric regions, and they primarily include mucous-secreting cells with a very small number of parietal cells, while the fundic glands were simple branched tubular that were long, straight, and parallel to one another. They were coil-shaped at the base and had the chief cells and parietal cells, As connective tissue, the submucosa included numerous blood and lymphatic vessels, Inner and exterior smooth muscle fiber layers make up the muscularis externa, the mesothelium-based serosa. The gastric pits' surface lining cells in the cardiac glands take positive reaction with PAS and negative with AB; while in fundic glands and pyloric glands no response to each of PAS and AB. Male and female stomach had the same number of gastric glands in each of stomach segments.

## INTRODUCTION

The Cape hare is a nocturnal herbivore that is found all over the world and is native to Africa, Arabia, and parts of India. It is also known as the desert hare or the wild rabbit (1).

Because they are valued for their meat and fur, are kept as pets, are regarded as economically significant animals (1). The digestive system is made up of organs involved in the intake and digestion of food, as well as the movement of the unabsorbed materials through the body and the explosion of the digested food (2,3). The rats' stomachs are separated into glandular and nonglandular sections, with the glandular section having columnar secreting epithelium lining the glandular region (4). The walls of the rabbit stomach are thin and not sectioned, but consider as only glandular stomach (5). The glandular stomach found in dogs, pigs, and monkeys, and contains the mucosa of the cardiac, fundic, and pyloric regions (6), the pig's gastric mucosa covers a bigger percentage of the stomach. Chief and parietal cells can be found in the mucosa covering the gastric and pyloric regions, however the cells covering the cardiac part generate mucus (7,8). (9) discovered that within the first several days following birth, weight of the stomach of rabbit is grows dramatically. The weight of the stomach has risen to 2.5 times its original weight by 3 days after birth. Similarly, (10) reported that over the first five days after birth, weight of stomach of suckled rabbits rose steadily, and by postnatal day 7, the stomach weight had more than doubled. Prior to weaning, which took place four weeks after birth, the stomach weight of the pig significantly rose during postnatal development. Simple columnar epithelium lines the stomach mucosa of Grass cutter, which is punctuated by gastric pits that open into the alveolar or tubular gastric glands. Goblet cells are incredibly uncommon in this epithelium, and the columnar epithelial cells exude the mucus (10,11). The mucosa, submucosa, muscularis externa, and serosa are the four distinct tunicae that make up the gastric wall, In addition to the intrinsic innervations that represented by the submucosal plexuses in submucosa and myenteric plexuses between muscular layers, the sphincter separates the stomach from the intestine, and the transition from esophageal to gastric mucosa is abrupt. The stratified epithelium of the esophagus is replaced by the secretory columnar epithelium (12-15), This effort was done to research the structure of the cape hare's stomach due to the rarity of the references regarding them that were available in Iraq and their availability as fundamental data for other sciences.

## Materials and method

The study was performed using fourteen of the healthy local adult of cape hare *Lepus capensis*, at age about 11-12 months, seven male and seven female, during May and November 2022. They were given an overdose of ketamine to induce anesthesia (administered intramuscularly). The ventral abdominal wall of all animals was cut away, the stomach was quickly cut open along its larger curve, and all of its contents were evacuated and saline-washed. The tissue samples were then divided into cardiac, fundic, and pyloric sections from the stomach, samples were collected from various parts of the (cardiac, fundic, and pyloric), Five samples were obtained from each area and fixed in 10% formalin for 24 hours before being processed using standard histology procedures, the stains that used; Harris hematoxylin and eosin (H&E) stain for highlighting the tissue's general histological components; Periodic acid Schiff (PAS) stain for show the mucopolysaccharides, basement membrane, mucoprotein, and glycoprotein, Alician blue (pH2.5): For acid mucopolysaccharides and Masson Trichrome Mallory trichrome stains; to goblet cells, collagen and smooth muscles (16). Mean ( $\bar{x}$ )  $\pm$  standard error (S E) were calculated for each the measurement, Ten slides of the each part of the stomach (cardiac, fundic, and pyloric) of each sex (17).

## Results

The cape stomach was J-shaped, with thin walled and located in the left side of the abdominal cavity, it was simple monogastric and divided into three parts according to the sorts of glands they contain, cardiac, fundic and pyloric glands parts in both male and female, The cardiac part was a small region at the gastroesophageal junction, close to the fourth and fifth ribs, and lay along a horizontal line that went through the coxal tuberosity. The fundic portion was relatively large. The pyloric portion was close to the dorsal portion of the rib wall, orientated dorso-laterally, and cranially extended to the plane via the 7th intercostal area, The mucosa, submucosa, muscularis, and serosa were the four tunics that make up the stomach wall in all the cardiac, fundic, and pylorus portions (Fig.1-3), The thickness of tunics in fundic part was higher than that in the both cardiac and pyloric glands part in both sexes (Fig.1,2,3). When the stomach is contracted, the mucosa and submucosa are folded into longitudinal folds that are abundant in the fundic and pyloric gland regions, however when the stomach is swollen, these folds flatten out (Fig. 1,2).

The mucosa was made up of columnar, mucus-secreting surface lined cells which extend into relatively long gastric pits. According to the parts of the stomach forming the gastric pits, which were line with the same epithelium also where the glands opened in the base of it, the surface of the cape stomach was lined by a simple columnar epithelium with a lightly cytoplasm that formed the surface mucous lining cells that invaginate into varying depth into the lamina propria (Fig.3,4). Simple columnar epithelium made up the epithelial cells also lined the gastric pits in all stomach parts (Fig.4), As the fundic glands separate, the gastric mucosa is thrown into conspicuous longitudinal folds that protrude into the lumen of the contracted stomach. The submucosa forms the folds' center (Fig.5). The chief and parietal cells that make up the fundic glands are simple branched tubular structures that are long and straight for the most of their length in a parallel orientation (Fig.4), the glands in the fundic part were coiled, branching, tubular glands and shorter than that in the cardiac part, and primarily mucous secreting cells (Fig.4), whereas the pyloric glands are short tubular branched and coiled and open at the base of lengthy pits (Fig.3). The thickness of mucosa of the cardiac, fundic and pyloric parts in male stomach was ( $503.4 \pm 2.1$ ;  $761.3 \pm 0.3$ ;  $547.9 \pm 2.4 \mu\text{m}$ ) respectively, while in female stomach was ( $483.2 \pm 0.1$ ;  $725.8 \pm 2.3$ ;  $506.1 \pm 3.2 \mu\text{m}$ ) respectively, and thickness of epithelium of the cardiac, fundic and pyloric parts in male stomach was ( $23.1 \pm 1.4$ ;  $28.2 \pm 1.3$ ;  $25.1 \pm 0.2 \mu\text{m}$ ) respectively, while in female stomach was ( $21.2 \pm 2.1$ ;  $25.1 \pm 0.1$ ;  $23.7 \pm 0.1 \mu\text{m}$ ) respectively, (Table 1).

The lamina propria appears as connective tissue; has numerous simple branched tubular glands, which were once coil-like, extend through the lamina propria and into the muscularis mucosa where they are visible as a very long straight over the majority of their length, the cardiac glands were located in the lamina propria as a small, branching, tubular, coiled gland that opened into the bottom of pits that were relatively lengthy (Fig.5,7,8). The muscularis mucosa, which separates the mucosa from the submucosa, is the other part of the mucosa, and they were orientated both longitudinally and circularly (Fig.7-10). The thickness of lamina propria and muscularis mucosa of the cardiac, fundic and pyloric parts in male stomach was ( $424.5 \pm 2.4$ ;  $52.1 \pm 0.3$ ;  $683.1 \pm 0.3$ ;  $50.2 \pm 0.1$ ;  $462.6 \pm 3.1$ ;  $54.1 \pm 0.2 \mu\text{m}$ ) respectively, while in female stomach was ( $411.2 \pm 2.1$ ;  $47.1 \pm 0.1$ ;  $645.5 \pm 0.2$ ;  $45.1 \pm 0.1$ ;  $432.1 \pm 2.3$ ;  $52.1 \pm 0.3 \mu\text{m}$ ) respectively (Table 1).

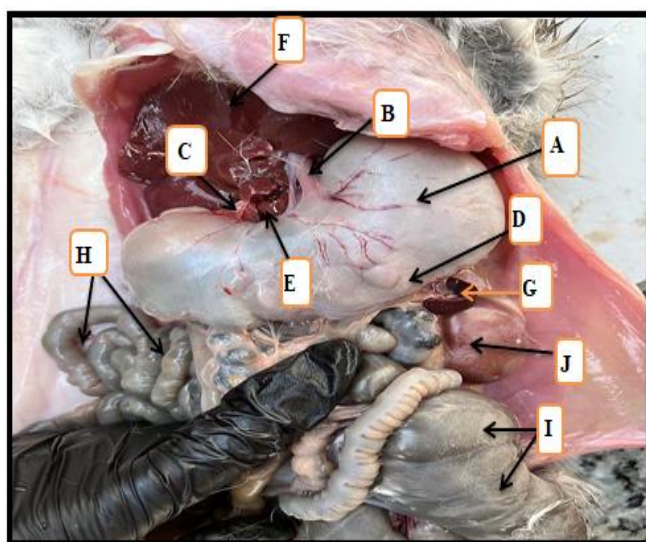
The submucosa as a connective tissue including blood and lymphatic vessels in addition to fat cells (Fig.1,7) The thickness of submucosa of the cardiac, fundic and pyloric parts in male

stomach was ( $132.1 \pm 1.2$ ;  $251 \pm 1.4$ ;  $150.2 \pm 1.6 \mu\text{m}$ ) respectively, while in female stomach was ( $121.3 \pm 2.3$ ;  $233.2 \pm 0.5$ ;  $141.1 \pm 1.2 \mu\text{m}$ ) respectively, Two layers of the smooth muscle fibers make up the muscularis externa, the inner of which is circular and the outer of which is longitudinal (Fig.8). The thickness of muscularis of the cardiac, fundic and pyloric parts in male stomach was ( $376.5 \pm 2.3$ ;  $513.3 \pm 1.2$ ;  $325.8 \pm 1.5 \mu\text{m}$ ) respectively, while in female stomach was ( $332.2 \pm 1.1$ ;  $507.2 \pm 1.4$ ;  $312.1 \pm 2.5 \mu\text{m}$ ) respectively. And the thickness of inner and outer layers of muscularis of the cardiac, fundic and pyloric parts in male stomach was ( $287.6 \pm 1.5$ ;  $90.9 \pm 3.2$ ;  $392.3 \pm 1.2$ ;  $117.3 \pm 1.2$ ,  $241.2 \pm 1.4$ ;  $88.5 \pm 1.1 \mu\text{m}$ ) respectively, while in female stomach was ( $262.9 \pm 4.2$ ;  $85.3 \pm 1.2$ ;  $385.7 \pm 2.1$ ;  $112.1 \pm 3.2$ ;  $221.5 \pm 1.3$ ;  $82.4 \pm 1.2 \mu\text{m}$ ) respectively, The thickness of serosa of the cardiac, fundic and pyloric parts in male stomach was ( $27.1 \pm 0.4$ ;  $31.2 \pm 0.3$ ;  $26.1 \pm 1.3 \mu\text{m}$ ) respectively, while in female stomach was ( $24.2 \pm 0.1$ ;  $28.1 \pm 0.2$ ;  $25.1 \pm 0.2 \mu\text{m}$ ) respectively (Table 1). Due to the mucous secreting cells in the cardiac and pyloric glands, the lining cells of the gastric pits in these organs provided a positive reaction to PAS and appeared pink, but the fundic glands in both males and females gave a negative response (Fig.3,4).

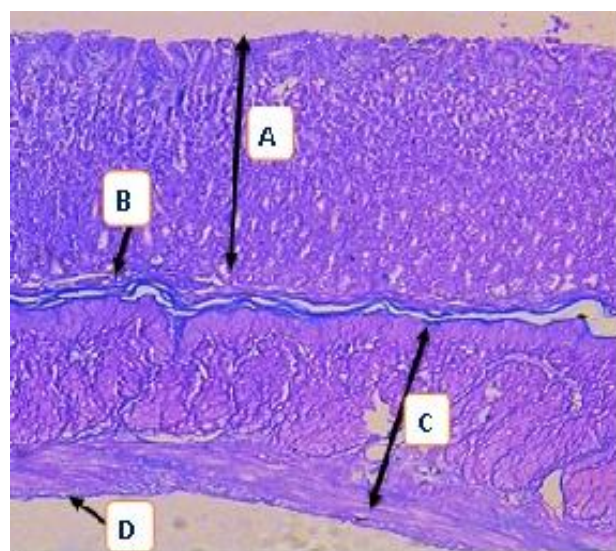
Table (1): Measurement of thickness of the wall layers of stomach parts of cape hare  $\mu\text{m}$  ( $\bar{X} \pm \text{S.E}$ )

Tunica Part	Mucosa				Submucosa	Muscularis externa			Serosa
	Total	Epithelium	Lamina propria	Muscularis mucosa		Total	Circular	Longitudinal	
Cardiac									
Male	$503.4 \pm 2.1$	$23.1 \pm 1.4$	$424.5 \pm 2.4$	$52.1 \pm 0.3$	$132.1 \pm 1.2$	$376.5 \pm 2.3$	$287.6 \pm 1.5$	$90.9 \pm 3.2$	$27.1 \pm 0.4$
female	$483.2 \pm 0.1$	$21.2 \pm 2.1$	$411.2 \pm 2.1$	$47.1 \pm 0.1$	$121.3 \pm 2.3$	$332.2 \pm 1.1$	$262.9 \pm 4.2$	$85.3 \pm 1.2$	$24.2 \pm 0.1$
Fundic									
Male	$761.3 \pm 0.3$	$28.2 \pm 1.3$	$683.1 \pm 0.3$	$50.2 \pm 0.1$	$251.1 \pm 1.4$	$513.3 \pm 1.2$	$392.3 \pm 1.2$	$117.3 \pm 1.2$	$31.2 \pm 0.3$
female	$725.8 \pm 2.3$	$25.1 \pm 0.1$	$645.5 \pm 0.2$	$45.1 \pm 0.1$	$233.2 \pm 0.5$	$507.2 \pm 1.4$	$385.7 \pm 2.1$	$112.1 \pm 3.2$	$28.1 \pm 0.2$
pyloric									
Male	$547.9 \pm 2.4$	$25.1 \pm 0.2$	$462.6 \pm 3.1$	$54.1 \pm 0.2$	$150.2 \pm 1.6$	$325.8 \pm 1.5$	$241.2 \pm 1.4$	$88.5 \pm 1.1$	$26.1 \pm 1.3$
female	$506.1 \pm 3.2$	$23.7 \pm 0.1$	$432.1 \pm 2.3$	$52.1 \pm 0.3$	$141.1 \pm 1.2$	$312.1 \pm 2.5$	$221.5 \pm 1.3$	$82.4 \pm 1.2$	$25.1 \pm 0.2$

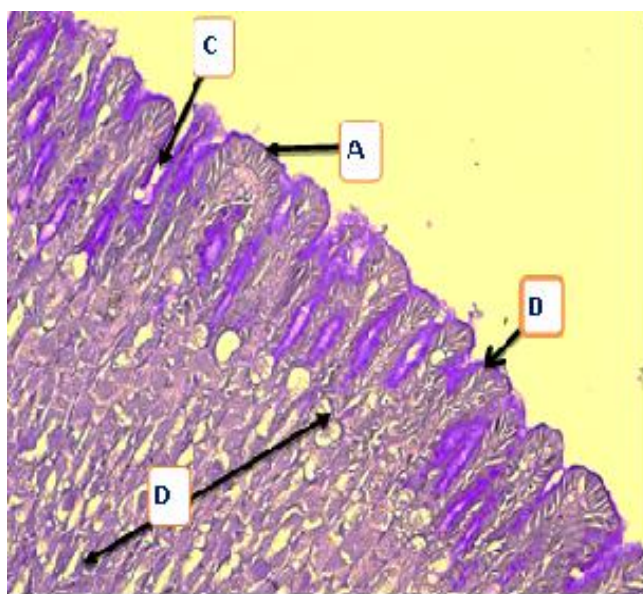




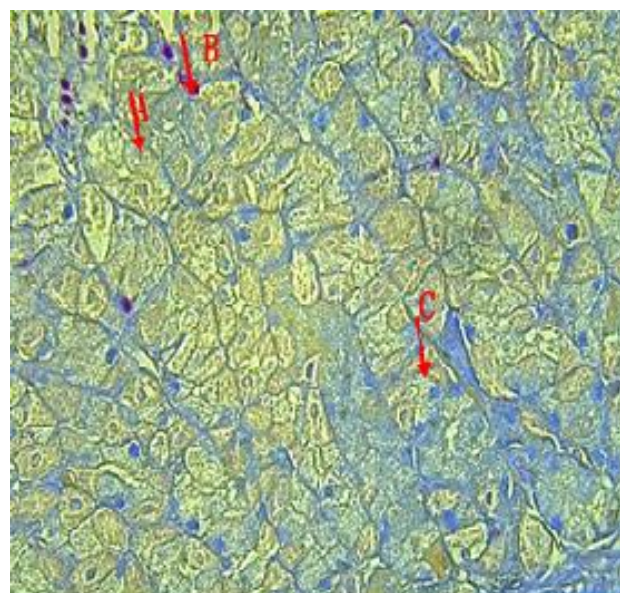
**Fig.1:** Gross appearance of internal viscera in rabbit ; A. stomach as J\_ shape, B. cardiac orifice, C. pyloric orifice, D. greater curvature, E. lesser curvature, F. liver, G. spleen, H. small intestine, I .large intestine, J. left kidney .



**Fig.2:** Microscopic section of stomach, in rabbit showed the : A. Mucosa, B. Sub mucosa, C. Muscularis externa, D. Serosa, **Masson Trichrome X 40.**



**Fig.3:** Microscopic section of mucosal layer stomach, in ten day: A. Epithelium, B. mucous cell, C. Gastric pits, D. Lamina propria , **PAS X 200.**



**Fig.4: Cross section of the cardiac glands part of male stomach; mucous cell (A), blood vessels (B), parietal cells (C), **Mallory 400X.****



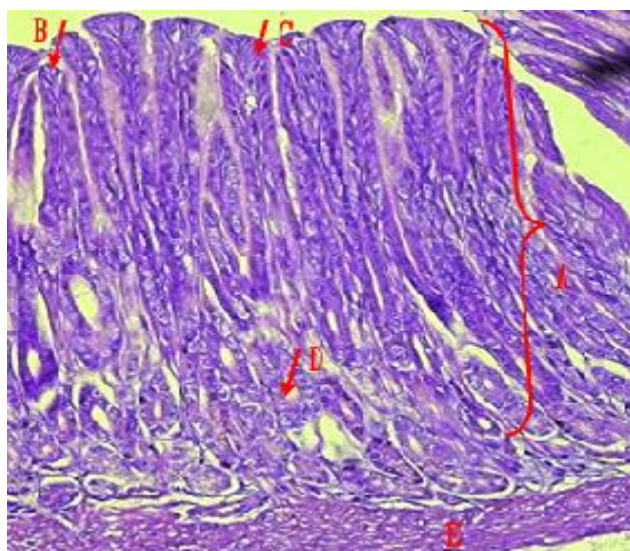


Fig.(5): **Cross section of the cardiac part of female stomach;** mucosa (A), pits (B), epithelium (C), cardiac glands (D), Muscularis mucosa (E), AB 200X.

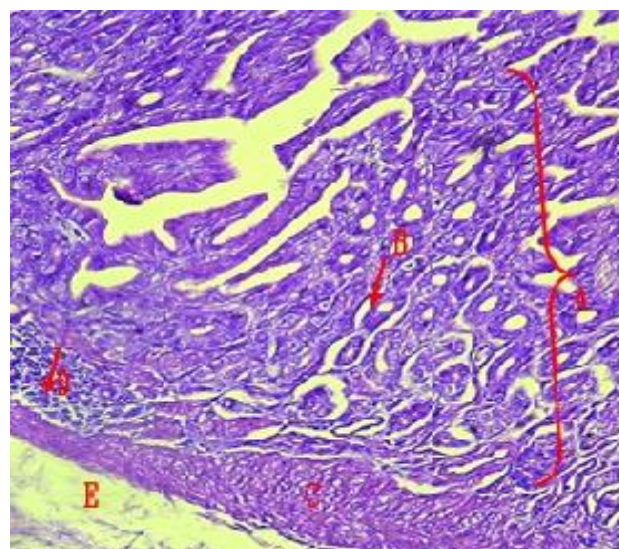


Fig.(6): **Cross section of the pyloric part of female stomach;** mucosa (A), pyloric glands (B), Muscularis mucosa (C), blood vessels (D), submucosa (E), PAS100X.

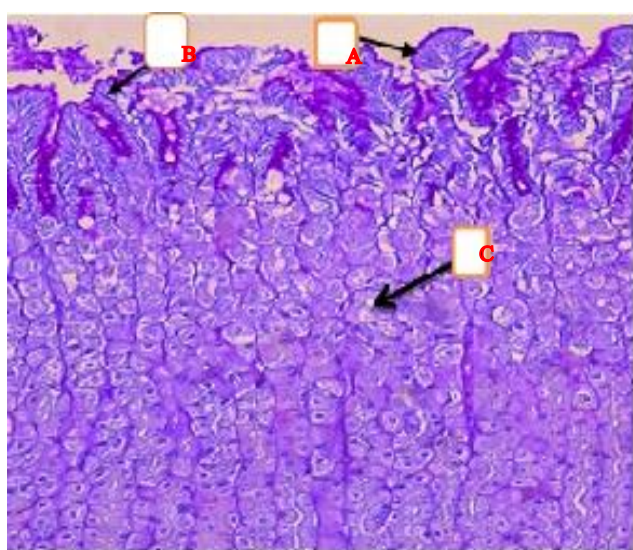


Fig.(7): Microscopic section of stomach, in fifteen day age showed : A. epithelium , B. mucous cell , C. gland, PAS X 200

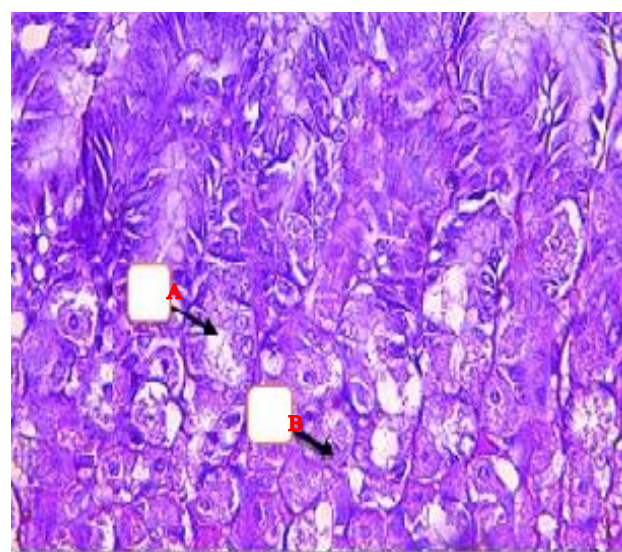


Fig.(8): Microscopic section of stomach, in ninety day age showed the : A. Gastric glands, B. Parietal cells, H&E X 400.



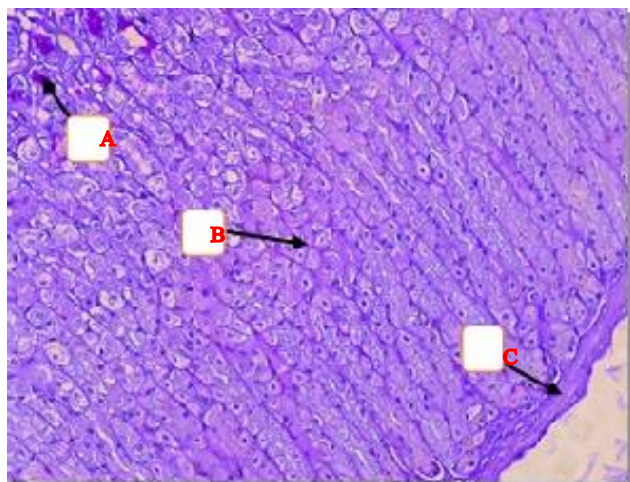


Fig.(7): Microscopic section of mucosa of stomach, in forty day age showed the : **A.** epithelium., **B.** Gastric glands, **C.** Muscularis externa , **.PAS X 200.**

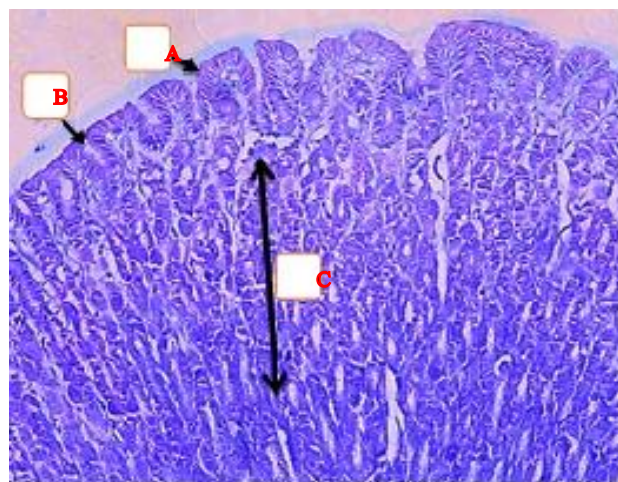


Fig.(10): Microscopic section of stomach, in fifteen day age showed : **A.** Epithelium, **B.** Gastric pits., **C.** Lamina propria with gastric glands, **Mallory Trichrome X 200.**

## Discussion

The cardiac, fundic, and pyloric gland regions make up the three portions of the cape stomach, which is glandular in each male and female. (18) mention the distinction between the glandular stomach and fore stomach in the rat. The basic wall structure of the cape stomach resembled that of monogastric mammals previously described by (6,19). Nonetheless, they differ from ruminants stomachs is feature by permanent spiral folds (20), that expand the mucous membrane's secreting surface area and take in a lot of food, the presence of gastric glands in cape stomach that resemble those seen in the stomach of most mammals (9). There is no difference between the histological structure of the glands in cape stomach from that in simple stomach in domestic mammals described by (5). And no differ in number of gland between male and female. (20) said the number of mucosal glands in ruminants differed from that in the three gland zones in the stomach of males and females, possibly due to a difference in the sex.

The majority of the mucous-secreting cells in the cardiac glands outnumber the parietal cells by a small margin, but there are not chief cells to be found, this agree with (5). The muscularis mucosa, lamina propria, and epithelium make up the mucosa of the gastric portions, the mucosal surface of the stomach has shallow gastric pits that are bordered by simple columnar epithelium, there are numerous depressions along the mucosal layer and into the lamina propria in the only fundic region, which together with the underlying tubular glands comprise the gastric pits, the gastric pits in the fundic part of the stomach are deeper than those in the cardiac part, and the lamina propria has tubular gastric glands that are open at their bases, Mucosa and submucosa are

separated by the muscularis mucosa, the submucosa was a dense connective tissue, the muscularis, consists of inner layer and outer layer, the serosa composed of loose connective tissue, this similar to (5) in mammals.

The lamina propria of fundic part had numerous collagenic bundles and blood arteries, and it was thicker than the lamina propria of the cardiac portion (6). The fundic glands are positioned perpendicular to the surface mucosa, straight parallel, and less coiled (12). The fundus, which contains the majority of the stomach's secretory cells, secretes pepsinogen, the precursor to pepsin, along with intrinsic factor and hydrochloric acid from parietal cells and hydrochloric acid from peptic cells (7). The abrupt removal of gastric glands, as well as large, deep gastric pits, make it easy to identify the pyloric portion, and its line by a simple columnar epithelium, with microvilli. When compared to the layers of the cardiac and fundic sections, the submucosa is the thinnest. The muscularis, the thickest part of the gastrointestinal sphincter, is made up of three layers of smooth muscles: thin inner, outer longitudinal, and thick middle circular layers, this similar to (6). The muscle wall in the pyloric area is significantly thicker to prevent the vomiting by an incredibly well-developed sphincter (12). Pyloric part lined by a same epithelium, but not has gastric glands, this agree to (8). (7) Mention the pyloric portion has a lot of smooth muscle. At the intersection of the stomach and intestine, more mucous cells, and thick muscularis allows food particles to enter into the intestine (12).

The mucous and parietal cells of the cape stomach are similar to those of the simple stomach as described by (13), and the composition of the fundic glands has been verified by (7) in monogastric animals, and parietal cells exhibit the same histological traits in monogastric mammals and ruminants. Because parietal cells have many packed mitochondria, which indicates a high metabolic activity used in the secretion of components of gastric fluids, their cytoplasm is eosinophilic and granulated (12). The chief cells are dispersed and crammed in among a parietal cells, as described histologically by (6) in monogastric animals that contend the ribosome's large contact with the rough endoplasmic reticulum is the cause of the highly basophilic granular cytoplasm. The description of pyloric glands' appearance is identical to (13). This result concurs with the hypothesis that the pyloric glands primarily consist of mucous with few parietal cells (8,20). Additionally, they noted that the transition zone between the fundic and pyloric glands contains parietal cells in addition to mucous cells, and they defined the pyloric glands region as being close to the pylorus and only having mucous cells. This does not concur with (7), who demonstrated that only mucous-secreting cells make up the pyloric glands, that are separated from the fundic glands by the sudden disappearance of chief cells.

Due to their mucous-secreting cells, the exterior lining cells in the stomach pits, the cardiac, the fundus, and the pylorus showed a positive reacted to the stain PAS and a negative response to the stain AB. The polysaccharide in the mucin is positive to PAS because the mucus cells shield the epithelium from self-digestive processes (9). The mucopolysaccharide act as a barrier against acidic substance and may be crucial for mucosal lubrication (4). The present study is consistent with (3). Additionally, the histological analysis of the chief and parietal cells showed a positive reaction with PAS. Some glands are completely made up of mucous-secreting cells, while others contain very few parietal cells as clear cells. The main cells of the fundic glands, parietal and chief cells, gave a negative reaction to PAS because serve in the production of hydrochloric acid and proenzyme, respectively (6), the finding of this study agrees with (20) in laboratory animal. The glands in pyloric part gave a positive reaction with PAS in the area the



pyloric glands. Since mucous secreting cells predominate in the pyloric glands, some glands have a very small amount of parietal cells. This result is agree with (19). The different ways that the gastric glands respond to PAS may be because of the various functions of these glands perform in the stomach.

**Conclusion:** There was no sex difference in the stomach of the cape hare's histological characteristics, but there were variations in the thickness of histological layers, with each layer being thicker in the male than the female.

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