Short Communication

Histomorphological and Histochemical Study of Esophagus and Stomach in the Neonate Guinea Pigs (*Cavia porcellus*)

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Abstract | This study aimed to investigate the histomorphological changes that occur in the esophagus and stomach during the postnatal periods of guinea pigs' lives. For this purpose, pregnant guinea pigs in good health condition were obtained from public markets and housed in the animal facility until delivery to obtain neonate guinea pigs. Ten pups were separated from their mothers on the second day of their lives and euthanized using an overdose of ketamine HCl (85-95 mg/kg BW). The topographic position of organs was observed in situ, and samples were collected in 10% neutral buffered formalin for routine histological procedures. The results showed that in neonate guinea pigs, the esophagus was a narrow muscular tube approximately 1.47±0.02 cm in length and 0.044±0.02 gm in weight, divided according to its position into three portions. The stomach was a transverse, transparent, whitish-pink sac-like structure of the digestive system with a weight of 1.13±0.05 gm, a length of 2.76±0.04 cm, and a capacity of 2.57±0.08 ml. The stomach lacks an outward demarcation-limiting ridge between its divisions and appears relatively lobulated. This age was characterized by longitudinal and translational folds along the entire inner surface. Histologically, both organs were composed of the same four basic layers. Due to some of its distinct anatomical and physiological characteristics, this animal species is valuable as a model in numerous scientific investigations. Therefore, this study sheds light on the histomorphological and histochemical properties of these organs, supporting further studies on vital digestive tract functions such as food processing and absorption.

Keywords | Histology, Guinea Pig, esophagus, stomach, Suckling Period.

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INTRODUCTION

The guinea pig (*Cavia porcellus*) ancestor is one of the most prevalent rodents in South America. Due to their appeal as a pet and food source, they are widely spread, belong to the order *Rodentia*, suborder *Hystricomorpha*, and the Family *Caviinae* (Shomer et al., 2015; Al-Saffar and Nasif, 2020). Since animals consume most feed materials, they are primarily complex and insoluble. Therefore, the goal of the digestion process is to gradually transform the feed materials into soluble, simple forms that are suitable for consumption and absorption (Al-Aaraji and Addi Ali, 2022). The gastrointestinal tract (GIT), has a very diverse morphological shape and function as a result of adaptation through long-term natural selection processes (Jiang et al., 2015; Al-Aaraji and Al-Kafagy, 2016; Cai et al., 2022). Rodent GITs have highly specific morphological, histological, and functional traits that have developed over time to accommodate these environmental variations. That includes the esophagus and stomach, among its most important vital parts (Borghesi et al., 2015; Zhang et al., 2022). The esophagus is a narrow muscular folded tube connected the pharynx to the stomach's cardio in a direct line, and it travels through the cervical region and the thoracic cavity before crossing the diaphragm and entering the stomach at the cardiac sphincter. The lower esophageal sphincter is the endpoint of the esophagus, which starts at the inferior edge of the cricopharyngeal muscle. Therefore, a macroscopic feature of the esophagus has previously been described as having three regions: the cervical, thoracic, and abdominal (Erman et al., 1998; Abidu-Figueiredo et al., 2008; Dyce et al., 2010; Kadhim, 2019). The largest part of the gastrointestinal tract is the stomach which stores, breaks down, and churns food into a semi-liquid called a chyme before transporting it to the duodenum. The stomach is a vertical organ that lies between the small intestine and the esophagus in the abdominal cavity (Vdoviaková et al., 2016). It is mostly located in the left half of the abdomen, behind the diaphragm, and under the ribs for the majority of the left side of the median plane. As far as concerned with the anatomical features, stomach are greater curvature (major curvatura ventriculi), convex posterior surface; less curvature (minor curvature ventriculi), and concave anterior surface (Erman et al., 1998; Dyce et al., 2010). Simple stomachs are composed of four parts: the cardia, fundus, body, and pylorus. Here, the esophagus enters the fundus part of the stomach through the tiniest region, called the cardiac part (Singh and Singh, 2011; Aspinall and Cappello, 2015). Histologically the esophagus is lined with a papillated thick stratified squamous epithelium. The epithelium keratinization also depended on species variation and diet, while the stomach was simple glandular and lined with simple columnar epithelium (Erman et al., 1998; Igbokwe and Obinna, 2016; de Sousa Cavalcante et al., 2021). Due to the importance of these two organs (esophagus and stomach) of the digestive system, which plays a major role in converting food into energy for all parts of the body to operate; and considering the significance of guinea pig as an essential laboratory animal in biomedical research, current research was designed to provide a clear understanding of the qualitative changes in the esophagus and stomach of newborn guinea pigs.

MATERIALS AND METHODS

Ten neonate guinea pigs, weight 64.7±0.59gm and 13.0300±0.17cm in length, were obtained at the second day of birth from their mothers, who had gathered from the public spinning market and housed in Animal House cages at the University of Baghdad's College of Veterinary Medicine until they were delivered. They had outwardly normal appearances after the classic clinical examination and no evidence of systemic illness was visible in them (Enas and Hadaf, 2020). The pups at first anesthetized by intramuscular injection of 85-95 mg/kg BW ketamine HCl, and then sacrificed (Amin and Atiyah, 2014; Bonhomme et al., 2019; Harith and Suhaib, 2020). For the morphological investigations, the classic dissection method was used, in such a way as to allow the highlighting of the best conditions of the esophagus and stomach in situ to determine how each of them relates to the other structures (E'atelaf, 2015; Das and Ranjan, 2016; Abd Elkader et al., 2020). The esophagus and stomach were dissected gently and removed from the body cavity examined and measured for length, width, and weight as soon as they were isolated from the abdominal cavity. After emptying the stomach of all contents and repeatedly washing it with water, a gradient syringe was used to fill the stomach with a precise volume of water. For histological and histochemical investigations fragments from the esophagus and stomach were collected and fixed with 10% neutral buffered formalin for two days, with daily change of the fixative solution. The samples passed successfully by classical histological sectioning with a thickness of 5 micrometers, on which histological and histochemical staining was performed (Al-Saffar and Al-Samawy, 2016; Al-Aaraji and AL-Kafagy, 2016; Al-Aaraji and Ali, 2022). The specimens were stained with Hematoxylin Eosin, and Masson's trichrome stains for a general histological feature and micro morphometric measurements. While the histochemical stains Periodic acid Schiff (PAS) and Alcian blue were used to stain and detect the glands and cells of the esophagus and stomach (Enas and Mohammed, 2020; Salih and Hamza, 2022). Ethical approval was granted through the local committee of animal care and use at the Veterinary Medicine within University of Baghdad (Number 500 P.G 2024/24/02) before starting this study.

RESULTS AND DISCUSSION

Esophagus

Morphologically the esophagus is a hollow body with a laminated musculomembranous structure white to pink in color and shiny appearance that connects the pharynx to the stomach's cardio in a direct line, it can be systematized into three portions: cervical, thoracic, and abdominal (fig 1A). The cervical section runs the entire neck length; it begins at the pharynx esophagus's caudal end and terminates where the right thoracic inlet meets the first set of ribs. Its length approximately was 1.47±0.02 cm and 0.044±0.02 gm in weight. This part was surrounded by the left and right common carotid artery ventrolaterally, the cervical vertebra dorsally, omohyoid and sternohyoid muscles laterally, and the trachea ventrally. These results agree with the earlier studies (Davies and Davies, 2003; Kadhim, 2019). The thoracic portion of the esophagus extended from the thoracic inlet dorsally connected to the caudal vena cava and served as an entrance into the diaphragm, pauses via the mediastinum of the lung lobes dorsally to the heart; before entering the esophageal hiatus the length was 1.67±0.01 cm and 0.03±0.001 gm in weight. The diaphragm's esophageal hiatus is where the vagus trunk gathers ventrolaterally with the thoracic portion of the esophagus (Fig 1A). This observation is similar to (Abd Elkader et al., 2020) who stated that the esophagus of the cates was located dorsally to the trachea and ventrally to the ventral cervical muscles except for measuring information. From the esophageal hiatus of the diaphragm until the gastroesophageal at the stomach cardiac orifice, there was the short abdominal part of the esophagus 1.31±0.02 cm long, 0.019±0.001 gm in weight, and situated on the posterior surface of the left lobe of the liver's esophageal groove (fig 1A). There is a thickened part where there are muscle fibers that surround the base of the esophagus and make up a circular muscle called the esophageal sphincter. These results agreed with (Dyce et al., 2010; Cai et al., 2022) with differences in morphometrical measurements. The histological results showed that the esophagus in all regions (cervical, thoracic, and abdominal) was formed from four tunics: mucosa, submucosa, muscularis, and adventitia or serosa. The tunica mucosa was folded forming a wide, irregular lumen appear as star-like shape visibly (fig 2_1). This is comparable to the findings published by (Das and Ranjan 2016), who observed that the rabbit esophageal lumen looked like a star-shaped lumen in cross-section. The mucosa consists of epithelium, lamina propria, and muscularis mucosae which measured thickness 119.6±14.9, 108.9±3.65, 239.7±19.9 um subsequently in cranial, middle, and caudal esophageal regions. The epithelia were keratinized stratified squamous epithelium along its length and consisted of the following layers: stratum corneum, stratum granulosum, stratum spinosum, and keratin (fig 2_3). In the cervical part, the height of epithelia was 93.8±6.4 um, the thoracic part 79.7±3.2 um, and the abdominal part 118.2±8.2 um. The basal lamina served as a barrier between the epithelium and the lamina propria. Relatively dense connective tissue, sporadic lymphocytes, and vascular structure that make up the lamina propria were interdigitated with epithelium is poorly developed in the cervical region while well developed in the other two regions (fig 2_4,6). This finding is agreed with (Igbokwe and Obinna, 2016) who mentioned that the esophagus lining of the camel was keratinized stratified squamous epithelium along its length and the lamina propria appeared interdigitated with the epithelium. With a longitudinal arrangement of thin smooth muscle cell layers, the muscularis mucosae were made up of a well-developed structure and became thicker in the middle and caudal region of the esophagus except for the cranial part, which consists of scattered thin smooth muscle fibers (fig 2_5,6). However, these results disagree with (Kadhim, 2019) who states that the lamina muscularis in grey mongooses was thin and consisted of scattered interrupted skeletal muscle bundles in the cervical part. The submucosa is composed of loose connective tissue that is rich in collagen fibers and contains a small number of elastic fibers that allow mobility between the mucosa and the muscularis externa and also enter the axis of each longitudinal fold, and loose glands (fig 2_4,5,6). The thickness of this layer was 23.8±2.3,

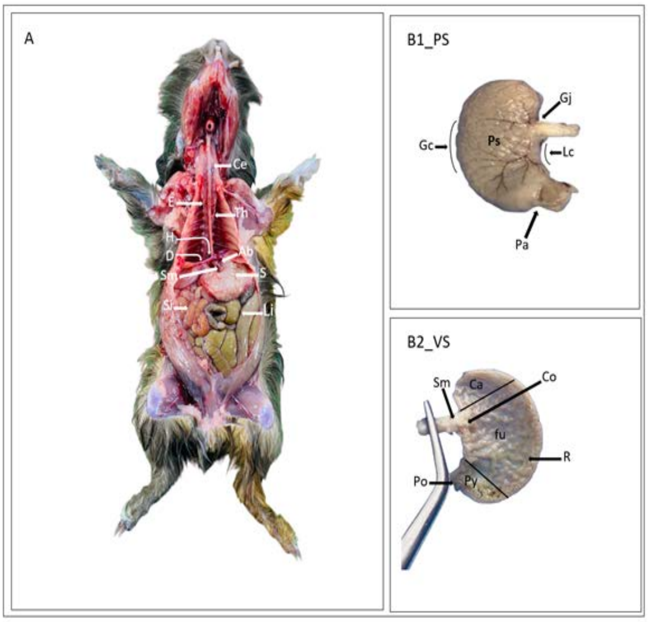

Figure 1: Topographic photograph in newborn guinea pig shows (**A**): The esophagus(E), stomach(S), small Intestine(Si),large Intestine(Li), esophagus cervical section (Ce), esophagus thoracic section (Th), esophagus abdominal section (Ab), esophageal hiatus (H), diaphragm (D), esophageal sphincter muscle (Sm). (**B1_PS**): stomach parietal surface: gastroesophageal junction (Gj), pyloric antrum (Pa), lesser curvature (Lc), greater curvature (Gc). (**B2_VS**): stomach visceral surface: stomach cardiac region (Ca), stomach fundic region (Fu), stomach pyloric region (Py), esophagus sphincter muscle (Es), cardiac opening (Co), pyloric opening (Po), rugae (R).

21.7±2.7, and 47.3±4.08 subsequently in the three parts. This observation was consistent with the findings published by (Cai et al., 2022), and consisted of some elastic and collagen fibers without any visible submucosal glands. The tunica muscularis in the cranial region of the esophagus consisted of striated muscle fibers, which progressively changed into mixed fibers in the intermediate region and smooth fibers in the caudal region, formed by three layers from outside to inside: The external thinner layer that formed by longitudinal oriented muscle fibers, the middle thicker layer its fibers having a circular disposition, and the internal layer with a longitudinal arrangement of muscle fibers that are present in less than half of the esophageal circumference is not on the entire circumference which agreed with study of (Das and Ranjan, 2016). However, some areas have just two layers (middle and external). The most exterior layer of the esophagus is called the adventitia, and it is made up of loose connective tissue that is deeply innervated and highly vascularized in the pre-diaphragmatic segment and serosa in the post-diaphragmatic segment which is simple squamous epithelium similar to the results that have been observed by (Calamar et al., 2014; Das and Ranjan, 2016; Kadhim, 2019).

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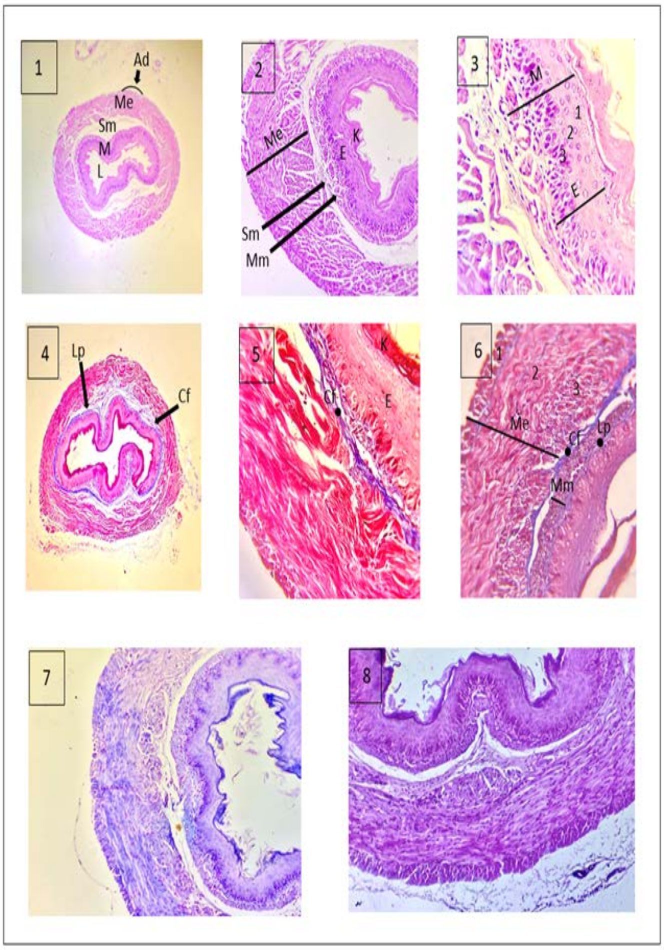

Figure 2: Histological cross section of the esophagus in neonate guinea pig shows **1**. Lumen (L), mucosa (M), submucosa (Sm), muscularis mucosa (me), and adventitia (Ad). X4, H&E stain. **2**. Keratin layer (K), Stratified squamous epithelium (E), muscularis externa (Me), Submucosa (Sm), and muscularis mucosa (Mm). X10, H&E stain. **3.** Mucosa (M), epithelium (E), stratum corneum(1), stratum granulosum(2), stratum spinosum (3). X40, H&E stain. **4.** Lamina propria (Lp), collagen fibers)Cf). X4, Masson's trichrome stain. **5&6.** Keratin layer (K), Stratified squamous epithelium (E), collagen fibers) Cf), Lamina propria (Lp), muscularis mucosa (Mm), and muscularis externa (Me) three layers1,2,3. X40, Masson's trichrome stain. **7&8.** No esophageal glands. X10, Alcian blue and PAS stains.

Stomach

Grossly the stomach in the current study was positioned vertically in the abdominal cavity, between the small intestine and the esophagus; retro-diaphragm, and retro-hepatic situated to the median plane farther to the left than the right. It is a sac-like structure relatively simple shaped uniform and pink in color, with two openings (cardiac and pyloric), two surfaces (parietal and visceral), two curvatures (lesser and greater), and two extremities (fig 1_B1, B2). This observation is similar to (Vdoviaková et al., 2016) who stated that the stomach of rat is a huge vertical organ lying in the abdominal cavity while is J shaped sac in rats, bulky sac-like with distinct ridges in *Laonastes aenigmamus*, in African rodents it was U-shaped, and in laboratory mice with oval features (Davies and Davies, 2003; Calamar et al., 2014; Scopin et al., 2015). The neonate guinea pig's stomach was simple (unilocular), appeared lobulated, and missing any exterior boundaries (*margo plicatus*) between its divisions. Lesser curvature was very short and connected to the small intestine by the omentum, whereas greater curvature was very wide and related to the spleen, which extends somewhat dorsally (fig 1_A, B1, B2). In comparison with those of other rodents: grey mongoose, rabbits, and southern African rodents it was a compound unilocular stomach, with an unspecialized unilocular hemiglandular stomach; a bilocular discoglandular stomach with a visible limiting ridge between non-glandular areas and glandular area of the stomach; or a multichambered stomach (Calamar et al., 2014; Scopin et al., 2015; Kadhim, 2019). A shallow angle created by the small lesser curvature formed the angular notch, also known as the incisura angularis located at the esophageal entry. Ascending ventrocaudally, the greater curvature was seen. This finding was similar to that of the African rope squirrel while, deep situated esophagal entrance in African rodents, and medially situated esophagal entrance to the lesser curvature in *Chinchilla laniger* (Calamar et al., 2014; Vdoviaková et al., 2016; Igbokwe and Obinna, 2016). The stomach was composed of three main internal sites: the cardiac region, which is thicker and rougher than others surrounds and is connected to the esophagus at the stomach's superior opening, the fundic region, which includes the fundus and the body is made up a larger portion of the stomach, and the pyloric region, which has a funnel-shaped connection to the duodenum. In the pyloric region, there was a pyloric antrum as a depression connected with the stomach body and the pyloric canal which continues to the duodenum at the pyloric sphincter (fig. 1_B1, B2). Our result was unlike with study of (Kadhim, 2019) who states that the stomach of grey mongooses has narrowed in its middle part. Numerous longitudinal and transverse wrinkles (rugae) were present along the internal surface; these folds were higher and larger at this age period (fig 1_B2). These folds may appear when the stomach is empty. Additionally, the presence of these folds may allow the stomach to empty more efficiently and shortly after feeding a large amount of milk. However, because particularly wild animals require compact organs for digestion, the grey mongoose stomach possesses longitudinal folds to break up and digest food effectively. In contrast, other species' stomachs, such as those of hamsters and rabbits, are completely flat (Davies and Davies, 2003; Dyce et al., 2010). The mean stomach weight was 1.13±0.05 gm, the length was 2.76±0.04 cm and the capacity was 2.57±0.08 ml. The presence of morphomet

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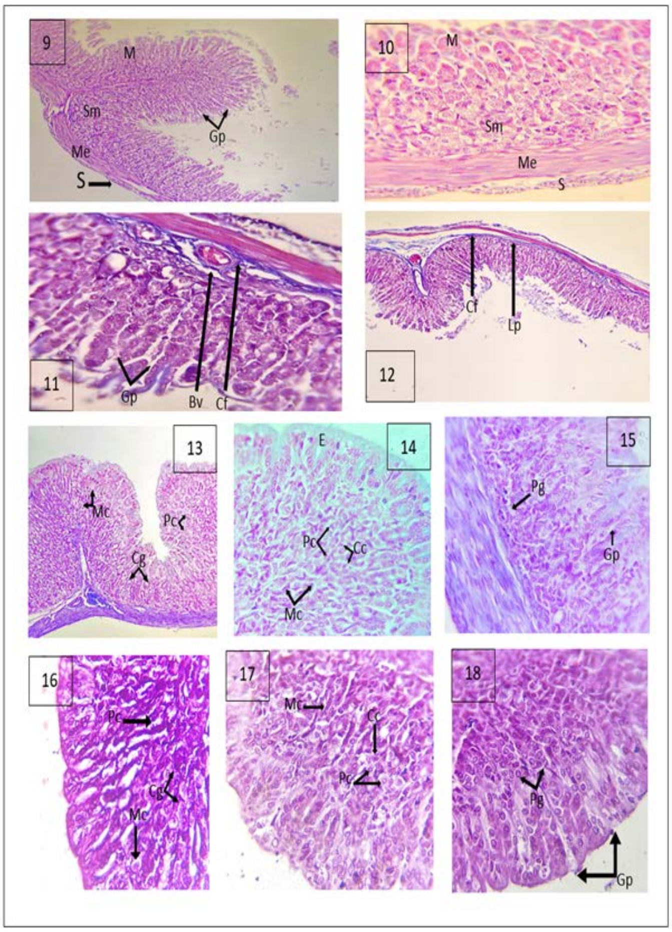

Figure 3: Histological cross section of the stomach in neonate guinea pig shows: **9,10.** Tunica mucosa(M), tunica submucosa(Sm), tunica muscularis (Me), Serosa(S), gastric pit(Gp). X10, X40 H&E stain. **11,12.** Lamina propria (Lp), collagen fibers)Cf), gastric pit(Gp), blood vessel(Bv). X10, X40 Masson's trichrome stain. **13,16.** The cardiac region: cardiac glands(Cg), mucous cell (Mc), and parietal cells (Pc). X10, X40 Alcian blue and PAS stain. **14,17.** The fundic region: chief cells(Cc), parietal cells(Pc), mucous neck cells(Mc). X40 Alcian blue and PAS stain. **15,18.** The pyloric region: gastric pit(Gp), pyloric glands(Pg). X40 Alcian blue and PAS stain.

ric results varies from results recorded in *Cavia Porcellus* pups and other rodents (Erman et al., 1998; Calamar et al., 2014; Kadhim, 2019). Histologically it was simple glandular and had three regions, cardiac, fundic, and pyloric. The wall of each region had four basic layers: mucosa, submucosa, muscularis, and serosa (figs 3_9, 10). As mentioned in the study of (Khalel and Ghafi, 2012) for rabbits, the cardiac area revealed a tiny, folded region encircling the esophageal opening. The simple columnar epithelium lines the cardiac region's tunica mucosa. The cardiac glands were simple tubular glands made up of mostly mucous-secreting cells that were arranged along the glands' frontal surfaces, mucous acinar cells that were located on the glands' basal surfaces cuboidal in shape with a foamy cytoplasm and a basally situated nucleus, a small number of parietal cells, enteroendocrine cells, and undifferenciated cells (fig 3_13,16). The lamina propria was home to a complex network of blood vessels, neurons, and cardiac gland tubes. Smooth muscle fibers arranged in a circle make up the thin muscularis mucosa. The thin tunica submucosa was made up of lymphocytes, blood vessels, and loose connective tissue (figs 3_11,12). The tunica muscularis was thick and made up of narrow longitudinal muscle fibers on the outside and spiral or circular muscle fibers inside. The outermost layer of the stomach is the tunica serosa consisting of blood vessels, nerves, and areolar adipose connective tissue, this tunica was stretched and in certain places, it blends in with the former tunic. The last three layers have the same histological structure along of stomach wall and similar to those reported by (Khalel and Ghafi, 2012; de Sousa Cavalcante et al., 2021). The fundic region was the largest and took up the whole space in the stomach between the pyloric and cardiac regions. The tunica mucosa has been covered in a simple columnar epithelium with fundic glands and relatively long pits. There was a thick layer of mucus covering the apical surface. Mucous neck cells, parietal cells, chief cells, and enteroendocrine cells make up the basic tubular fundic glands. The mucous neck cell took up a significant amount of the cytoplasm and was short and columnar with a huge oval nucleus. The largest cell type, the parietal cell was mostly found in the upper and lower part of the fundic glands region. It had an oval shape, was broad at the base, had pink cytoplasm, and a central nucleus (fig 3_14, 16). Some of these cells also had a distinct white area in their cytoplasm. The chief cells were spherical, with a pale granule-filled top area and a darkly pigmented basal nucleus. The tiny enteroendocrine cells with faintly stained cytoplasm were located in the lowest part of each fundic gland. Blood vessels, nerves, and very asymmetrical tubes of the pyloric glands were present in the lamina propria (fig 3_12). The same results were noted in African rope squirrels and *Chinchilla laniger* by (Calamar et al., 2014; Scopin et al., 2015). There was a thick layer of mucus covering the apical surface. The long, simple, branching, tubular pyloric glands have deep pits in them. The pyloric glands consist primarily of mucous acinar cells extending along the front and basal surfaces, sporadic parietal cells, a small number of chief cells, and enteroendocrine cells. The histochemical results showed a positive for both the PAS and Alcian blue reactions, which suggests they synthesize both neutral and acidic mucins. The superficial gastric glands appear magenta, while the deep glands positioned in the mucosa's base appear mixed purple-blue (fig 3_15, 18). Similar findings were noticed in the study of the stomach by (Calamar et al., 2014; Khudair and Mohamed, 2019; Salih and Hamza, 2022).

CONCLUSION

The current study explored the stomach and esophagus of day-old guinea pigs, an important model animal for in vivo research. The study sheds light on the histomorphological and histochemical properties of these organs and revealed that submucosal glands in the esophagus were absent. Furthermore, the distribution of glands in various parts of the stomach was directly proportional to its role in digestion. Further studies are warranted on vital digestive tract functions such as food processing and absorption.

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CONFLICT INTEREST

There is no conflict of interest.

NOVELTY STATEMENT

The current study revealed submucosal glands in the esophagus were absent, and the distribution of glands in the stomach depends on the parts and which one has the greatest role in the digestion process.

AUTHOR CONTRIBUTION

The work was completed by researcher Manar Neamah Al-Shreefy and the title was written by Suhaib A.H. Al-Taai.

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