



Histological and Histochemical Studies on the Stomach of Guinea Pig (*Cavia porcellus*)

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ABSTRACT

The present work was conducted on the stomach of guinea pigs from neonate to adults (0-2 wks, 2-8 wks, 8-16 wks and 16-32 wks) to study the histological changes in the three regions of stomach. Present study revealed that tunica mucosa of all the three regions of the stomach was lined by the simple columnar epithelium. Simple tubular cardiac glands were lined mainly by mucous cells along with few parietal cells, enterochromaffin and undifferentiated cells. In fundic glands mucous cells and parietal cells were predominantly in both upper and lower half of the glands where as chief cells and enteroendocrine cells were predominantly occupied in the lower half of the fundic glands. Pyloric glands were simple branched tubular glands were lined by mucous acinar cells. Tunica muscularis of cardiac region comprised of skeletal and smooth muscle fibres whereas other two regions were comprised of smooth muscle fibres. The mean thickness of tunica mucosa was the maximum in fundic region and the minimum in pyloric region. The mean thickness of tunica muscularis external was the maximum in pyloric and the minimum in fundic region.

HIGHLIGHTS

- Simple tubular cardiac glands, fundic glands were lined mainly by mucous cells along with few parietal cells.
- Pyloric glands were simple branched tubular glands were lined by mucous acinar cells.

Keywords: Guinea pig, Histology, Histochemistry, Stomach

Guinea pigs (*Cavia porcellus*) are a descendant of the wild cavy (*Cavia aperea*) which is considered one of the common rodents lived in South America. The usage of rodents as experimental model was well known, guinea pigs being chosen as subjects in many studies (Stan, 2015). They are now widely distributed because of its popularity as a pet and a food source. This species is commonly used in biomedical research, for example in studies of the human immune system, since immunological genes of guinea pig are more similar to human than those from mouse so that considered one of the gold standards for modelling human disease (Guo *et al.*, 2012) beside others lab animals such as the rabbit (Al-Haak and Al-Saffer, 2017). There are wide variation in mammalian stomachs, which are greatly

influenced by adaptations, nature of the feed, frequency of food intake, duration, need for food storage, body size and shape Igbokwe and Obinna (2016). However, literature on detailed study of histological features of stomach of guinea pig is very much limited. Hence, the present study was conducted with an aim to explore the histological features of stomach in guinea pigs.

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MATERIALS AND METHODS

The histology and Histochemistry of stomach of guinea pig from postnatal age groups (Table 1) was conducted at the Department of Veterinary Anatomy, Madras Veterinary College, Chennai, Tamilnadu, India. Guinea pigs were procured from the Department of Laboratory Animal Medicine, Madhavaram Milk Colony, TANUVAS, Chennai-51 as per ethical committee approval (Lr. No. 1467/DFAB/IAEC/2018 dated 13.07.2018). After collection of the guinea pigs, they were euthanized as per the standard operating procedure by using the carbon dioxide asphyxiations as per CPCSEA norms and they were subjected for the dissection. After careful dissection of the animals, abdominal cavity was opened and stomach was removed out. Three areas of stomach (Cardiac, fundic and pyloric) was cut into small pieces. They were washed in the normal saline and fixed in 10% neutral buffered formalin and Bouin's fluid for general and special histological studies. Then the tissues were dehydrated in the ascending grades of the alcohol cleared in xylene and embedded in paraffin (58-60°C). Sections of 4-5 µm thickness were cut and used for the routine and special histological and histochemical staining techniques. The following histological and histochemical techniques were applied for the study (Table 2). Micrometrical measurements namely mean thickness of tunica mucosa, submucosa and muscularis of all the postnatal age groups

were recorded. Arithmetic mean and the standard error for the micrometrical data were calculated as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Histologically the stomach of guinea pig showed the four tunics mucosa, submucosa, muscularis and serosa in all the three regions of stomach namely cardiac, fundic and pylorus. Histologically the cardiac region of stomach showed small folded area surrounded the oesophageal entrance. Tunica mucosa of the cardiac region lined by the simple columnar epithelium (Fig. 1). The cardiac glands were lined simple tubular glands composed of predominate mucous secreting cells these observations were agreed with the findings of (Goshal and Bal, 1989) in rat, mice and guinea pig, (Chandana *et al.*, 2013) in albino rats, (Ranjan and Das, 2018) in rabbits (Al-rhman, 2016) in guinea pig. Mucous acinar cells found on the basal surfaces, few parietal cells, enteroendocrine cells and undifferentiated cells. The mucous secreting cells had cuboidal or low columnar shape with basally located nucleus and foamy cytoplasm, its apical portion filled with mucous (Fig. 2). Gastic pits were shallow in the cardiac region similar observations were given by (Goshal and Bal, 1989) in rat, mice and guinea pig, (Ranjan and Das, 2018) in rabbits and (Al-rhman, 2016) in guinea pig. The lamina propria made up of loose connective tissue and

Table 1: Details of postnatal age groups of guinea pigs used for research work

Age groups	Prewaning	Weaning	Young	Adult	Total
	0-2 weeks	2-8 weeks	8-16 weeks	16-32 weeks	
No. of animals	6	6	6	6	24

Table 2: Details of histological and histochemical techniques applied to the stomach of postnatal age group of guinea pigs

Sl. No.	Histological and histochemical staining technique	Purpose	Reference
1	Standard haemotoxylin and eosin method for paraffin sections	Routine histological observations	Bancroft and Stevens, 1996
2	Masson's trichrome staining	Demonstration of collagen and smooth muscle fibres	Luna, 1968
3	Picosirius red method	Demonstration of Collagen fibres	Bancroft and Stevens, 1996
4	Weigert's method	Demonstration of elastic fibres	Humason, 1979
5	Gomori's silver method	Demonstration of reticulum	Luna, 1968
6	Periodic acid – Schiff (PAS)	Demonstration of glycoproteins (Neutral mucosubstances)	Luna, 1968

was occupied by cardiac glands, collagen and reticular fibres, dense network of blood vessels and nerves (Fig. 3). The muscularis mucosa was thin and consists of circular arranged smooth muscle fibers. Elastic fibres predominant fibres were observed around the blood vessels and around the lamina propria. The tunica submucosa was thin (Fig. 4) consisted of loose connective tissue, adipocytes, fibrocytes, blood vessels and lymphocytes. The tunica muscularis was thick consisted of outer longitudinal and inner circular layer in between the two layers of the muscularis myenteric plexus was observed these findings were agreed with the finding of (Khalel and Ghafi, 2012) in rabbits and (Al-rhman, 2016) in guinea pig. The tunica serosa form the outer most layer of the stomach, it consists of areolar connective tissue, adipose connective tissue, blood vessels and nerves, this tunica was extended and intermingles with the former tunic in some areas. Histochemically, the cardiac region of stomach showed mild to moderate reaction towards the PAS reaction (arrows) apical surface of the lining epithelium and around the cardiac glands and lamina propria (Fig. 5) which means mucous which contains neutral mucus lining the epithelium was more observed in the guinea pigs in all the age groups studied. The intensity towards the PAS reaction was more around the glands than the apical surface these observations were similar with the findings of (Chande *et al.*, 2020) in guinea pigs. The level of intensity towards PAS increased as the age advanced from 0-2 week to 16-32 week-old guinea pigs studied.

The fundic region was the maximum, occupied the most entire area of the stomach between the cardiac region and pyloric region. It was more thick and less folded than cardiac region. Tunica mucosa of the fundic stomach was lined by the simple columnar epithelium with fundic glands and short gastric pits covers the tunica mucosa (Fig.06). The apical surface was covered by a thick layer of mucus these observations were concurred with the findings of (Goshal and Bal, 1989) in rat, mice and guinea pig, (Khalel and Ghafi, 2012), in rabbits (Chandana *et al.*, 2013) in albino rats, (Berghes *et al.*, 2011) and (Al-rhman, 2016) in guinea pig. The fundic glands were long, straight and simple tubular glands composed of mucous neck cells, parietal cells, chief cells and enteroendocrine cells (Fig. 7). The mucous neck cell was short columnar with large oval nucleus, it occupied a large proportion of the cytoplasm. The parietal cell were predominantly present in the upper

and lower half of the fundic glands, it was the largest cell types, had oval shape broad at the base, pink cytoplasm and central nucleus (Fig. 8), there was a clear whitish region present in the cytoplasm of some of these cells but in rabbits the parietal cells are polymorphic centrally located nucleus with eosinophilic cytoplasm these findings were similar with the observations of (Ranjan and Das, 2018) in rabbits (Al-rhman, 2016) in guinea pig and contrary to this in Rats, parietal cells are pyramidal and largest cells among the fundic region (Chandana *et al.*, 2013). The chief cells are mostly located at the lower half of the fundic stomach squeezed between the parietal cells. These are pyramidal cells with spherical shape nuclei with darkly stained nucleus basophilic in nature. The enteroendocrine cells found in the lower third of each fundic gland, it was small and had weakly stained cytoplasm. The lamina propria was occupied by heavily irregular tubes of fundic glands, blood vessels and nerves. The muscularis mucosa was thin and consists of circular arranged smooth muscle fibers. The tunica submucosa consists of loose connective tissue, collagen and reticular fibers, large blood vessels, lymphocytes (Fig. 9). Elastic fibres were noticed around the blood vessels in the submucosa and some places of the tunica muscularis layer. The tunica muscularis externa was thick consists of internal spiral and circular muscle fibers and outer thin longitudinal muscle fibers. The tunica serosa form the outer most layer of the fundic region it consists of areolar connective tissue, adipose connective tissue, blood vessels and nerves, this tunica was extended and intermingles with the former tunic. Histochemically the fundic region of stomach showed moderate reaction towards the PAS reaction (arrows) on the apical surface of the lining epithelium around the fundic glands and lamina propria (Fig. 10). This reaction was more in the lower third of the fundic glands than upper third in all the age groups studied. The intensity towards the PAS reaction was more in the fundic region than cardiac region as more chief and parietal cells will more in the fundic region secrete more mucous than other regions of the stomach these findings were similar with the findings of (Chande *et al.*, 2020) in guinea pigs. The level of intensity towards PAS increased as the age advanced from 0-2 week to 16-32 week-old guinea pigs studied.

Tunica mucosa of pylorus was lined by the simple columnar epithelium showed pyloric glands opened into pyloric pits. Gastric pits were longer than other two regions

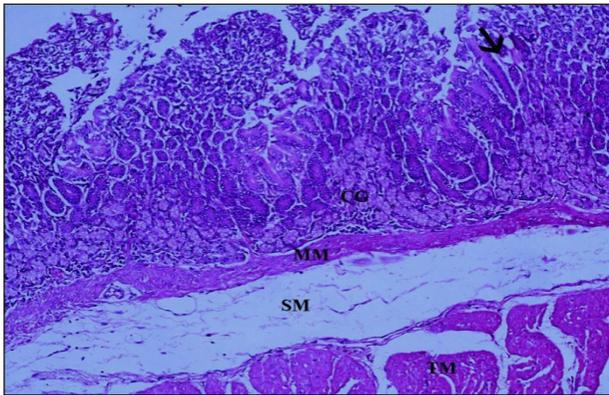


Fig. 1: Photomicrograph showing the different tunics of cardiac region of the stomach in eight week-old guinea pig. Arrow-Gastric pit, CG-Cardiac glands. MM-Muscularis mucosa, SM-Tunica submucosa, TM-Tunica muscularis H&E × 40

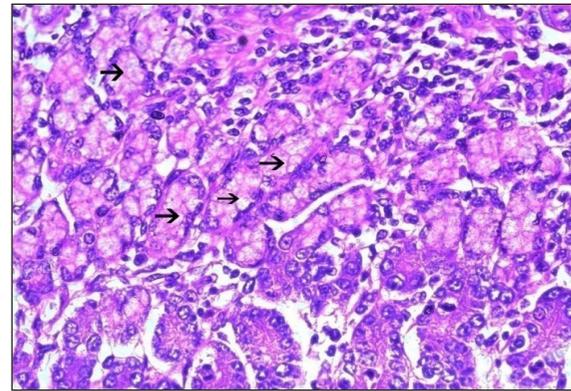


Fig. 2: Photomicrograph showing the mucous cells (Arrows) in the cardiac region of the stomach in twenty four week-old guinea pig. H&E × 400

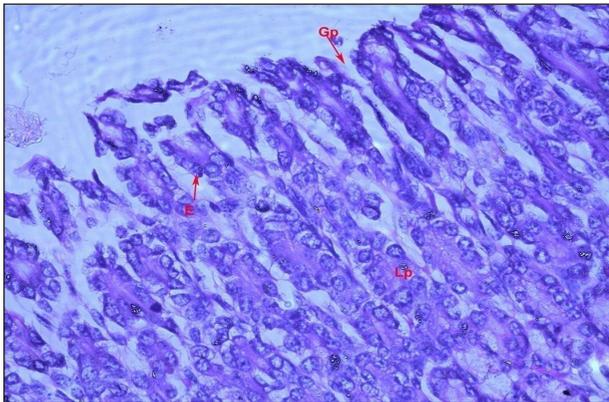


Fig. 3: Photomicrograph showing the epithelium and lamina propria in cardiac region of the stomach in eighteen week-old guinea pig. GP-Gastric pit, E-Epithelium, LP-Lamina propria. H&E × 100

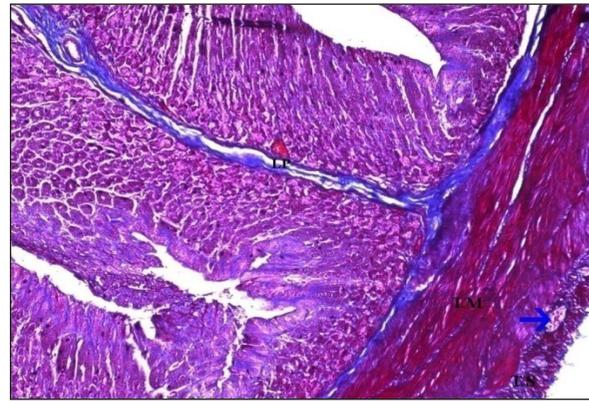


Fig. 4: Photomicrograph showing the different tunics of cardiac region of the stomach in twenty four week-old guinea pig. Arrow-Myenteric plexus. LP-Lamina propria, TM-Tunica muscularis, Masson's trichrome stain × 40

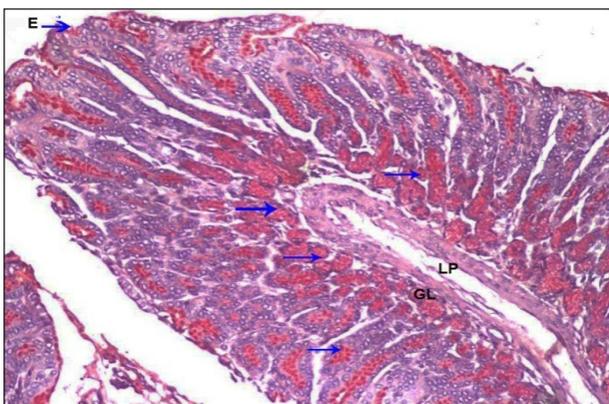


Fig. 5: Photomicrograph showing the mild to moderate reaction to periodic Schiff reaction in the epithelium (E), cardiac glands and lamina propria in cardiac region of the stomach in twenty four week-old guinea pig. Arrows-PAS reaction, LP-Lamina propria, GL-Cardiac glands, Periodic Schiff reaction × 100

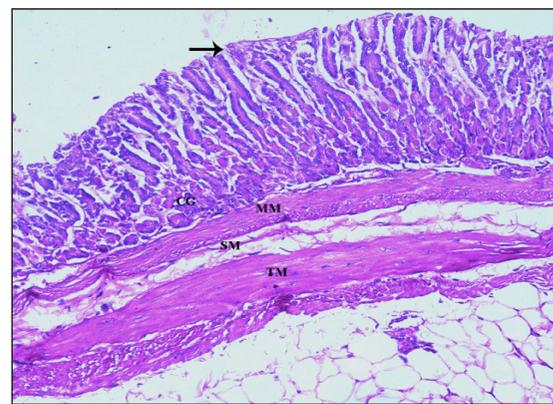


Fig. 6: Photomicrograph showing the different tunics of fundic region of the stomach in eight week-old guinea pig. Arrow-Gastric pit, CG-Cardiac glands. MM-Muscularis mucosa, SM-Tunica submucosa, TM-Tunica muscularis H&E × 40

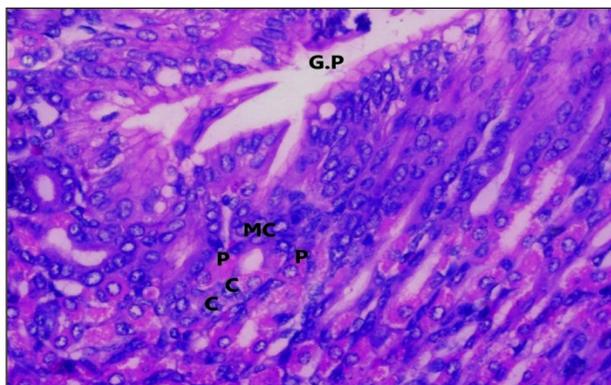


Fig. 7: Photomicrograph showing the different cells in the fundic region of the stomach in eighteen week-old guinea pig. GP-Gastric pit, MC-Mucous neck cells, P-Parietal cells, C-Chief cells. H&E × 400

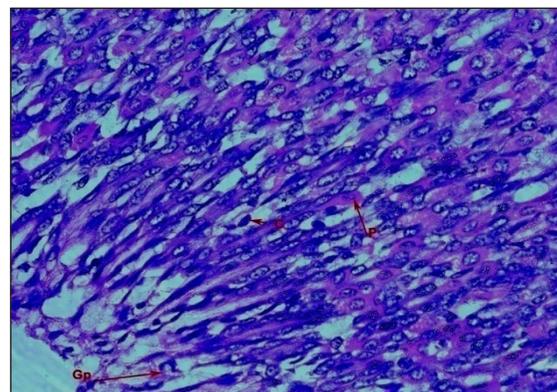


Fig. 8: Photomicrograph showing the different cells in the fundic region of the stomach in eight week-old guinea pig. GP-Gastric pit, P-Parietal cells, C-Chief cells. H&E × 100

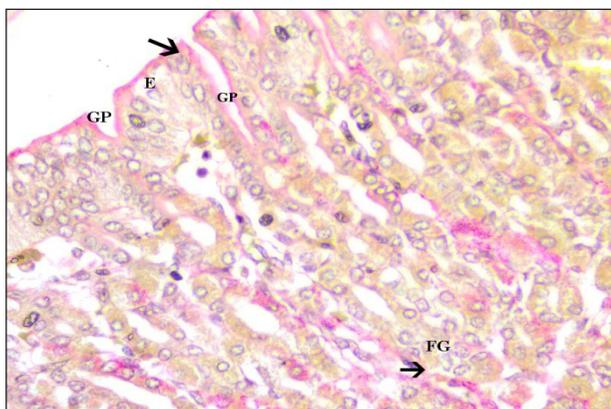


Fig. 9: Photomicrograph showing collagen fibres around the lamina propria and fundic glands (arrow) twenty four week-old guinea pig. Surface epithelium shows mucus. E-Epithelium, GP-Gastric pit, Picrosirius Red for collagen fibres × 100

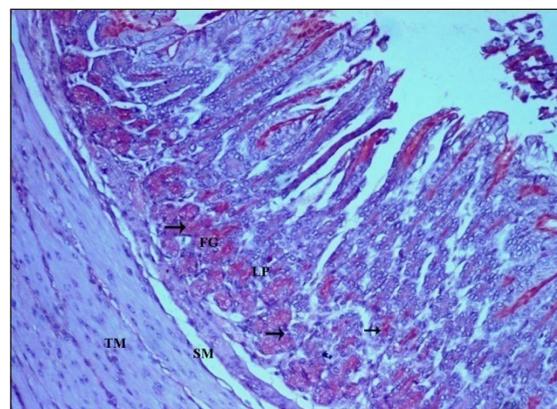


Fig. 10: Photomicrograph moderate reaction towards the PAS reaction (arrows) around the apical surface, fundic glands and lamina propria in twenty four week-old guinea pig. FG-Fundic glands, LP-Lamina propria, SM-Tunica submucosa, TM-Tunica muscularis. Periodic schiff's reaction × 40

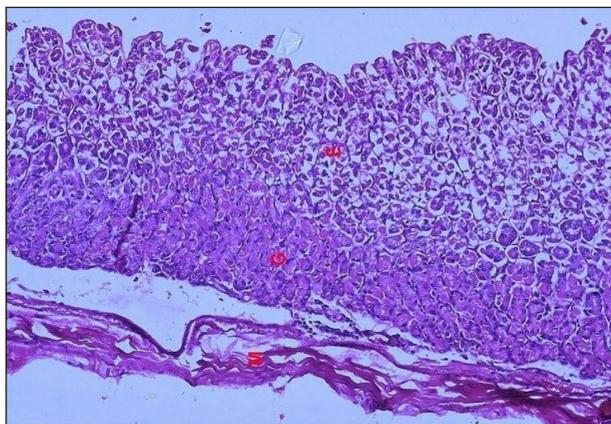


Fig. 11: Photomicrograph showing the different tunics of the pyloric region of the stomach in twenty four week-old guinea pig. E-Epithelium, G-Pyloric glands, M-Tunica muscularis. H & E × 40

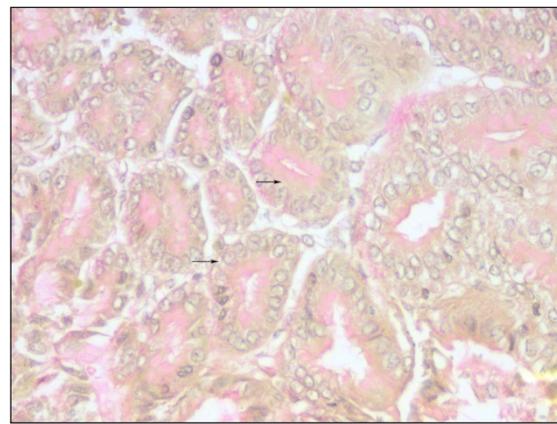


Fig. 12: Photomicrograph showing the pyloric glands in the stomach (arrows) in twenty four week-old guinea pig. Picro Sirius red for collagen fibres × 100

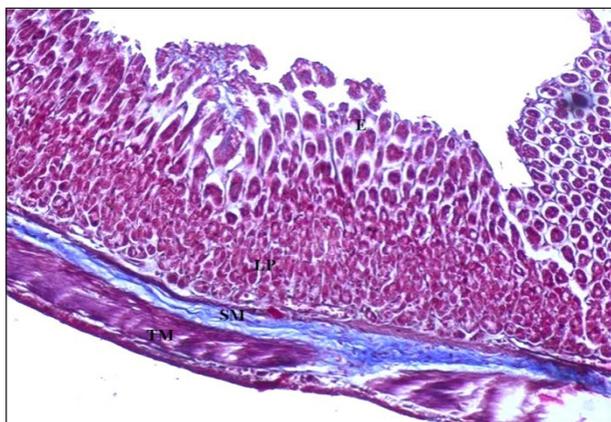


Fig. 13: Photomicrograph showing the presence collagen fibres in the submucosa and around the lamina propria in the pyloric region of the stomach in twenty four week-old guinea pig. E-Epithelium, LP-Lamina propria, SM-Tunica submucosa, TM-Tunica muscularis. Masson's trichrome $\times 40$

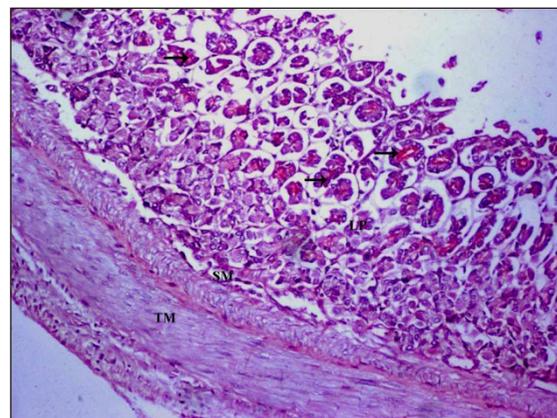


Fig. 14: Photomicrograph mild reaction towards the PAS reaction (arrows) in the pyloric glands and lamina propria in twenty four week-old guinea pig. LP-Lamina propria, SM-Tunica submucosa, TM-Tunica muscularis. Periodic schiff's reaction $\times 40$

of the stomach (Fig. 11). The apical surface was covered a prominent layer of mucus. The pyloric glands were long simple branched tubular glands coiled at the base with deep pits these findings were concurred with the findings of (Chandana *et al.*, 2013) in albino rats, (Berghes *et al.*, 2011) and (Al-rhman, 2016) in guinea pig. The pyloric glands composed of predominantly mucous acinar cells along the length of the front and basal surfaces and randomly parietal cells, few chief cells and enteroendocrine cells (Fig. 12). Mucous cells were Predominant with flat basally located nucleus and basophilic cytoplasm these observations were concurred with the observations of the (Eurell and Frappier, 2006) in domestic animals, (Ranjan and Das, 2016) and (Khalel and Ghafi, 2012) in rabbits. The tunica submucosa was thick extends and intermingles with the tunica muscularis. It is composed of loose connective tissue, collagen fibers (Fig. 13), blood vessels and lymphocytes. The tunica muscularis externa was consists of thick internal spiral circular muscle fibers and thin outer longitudinal muscle fibers. The tunica serosa form the outer most layer of the fundic region , it consists of areolar connective tissue, adipose connective tissue, blood vessels and nerves , this tunica was extended and intermingles with the former tunic. Histochemically the mild PAS reaction was observed on the apical surface of lining epithelium and around the pyloric glands and lamina propria (Fig. 14) in all the age groups studied. The intensity towards PAS reaction was less when compared

with cardiac and fundic region which indicate less amount of neutral mucopolysaccharides were observed in pyloric region similar observations were given by Chande *et al.*, 2020) in guinea pigs.

Micrometrical observations

The mean thickness of the tunica mucosa of cardiac, fundic and pylorus region of the stomach was (105.9 ± 1.03 to $335.4 \pm 1.14 \mu\text{m}$), (209.9 ± 1.13 to $491.8 \pm 1.07 \mu\text{m}$) and (64.8 ± 4.56 to $416.7 \pm 3.14 \mu\text{m}$) in 0-2 weeks to 16-32 week old guinea pigs indicated that the tunica mucosa thickness was more in the fundic region than other two regions of the stomach in all the age groups studied. The mean thickness of the tunica sub mucosa of cardiac, fundic and pylorus of the stomach was (24.5 ± 1.1 to $72.3 \pm 6.54 \mu\text{m}$), (35.1 ± 1.03 to $68.3 \pm 3.03 \mu\text{m}$) and (29.8 ± 1.11 to $64.8 \pm 3.14 \mu\text{m}$) in 0-2 weeks to 16-32 week old guinea pigs these findings were similar with the findings of the (Khalel and Ghafi, 2012) and (Ranjan and Das, 2018) in rabbits The mean thickness of the tunica muscularis of cardiac, fundic and pylorus region of the stomach was (138.8 ± 1.03 to $215.8 \pm 1.38 \mu\text{m}$), (98.9 ± 1.03 to $178.8 \pm 4.03 \mu\text{m}$) and (245.1 ± 1.5 to $485.8 \pm 1.18 \mu\text{m}$) in 0-2 weeks to 16-32 week old guinea pigs. The tunica muscularis of cardiac gland region also increased significantly than that of the fundic region but was less thick as compared to the pyloric region. This might be the

because of the need of higher muscular contraction at the pyloric region for propulsion of food particles towards the intestine. Similarly, the thickness was minimum at the fundic region where the food had to be retained for longer time for digestion. Likewise the thickness was more in the cardiac region than that of the fundic region to receive the food from the esophageal end. These observations suggested that the tunica mucosa, submucosa and muscularis was more in the fundic region of the stomach than the other regions and it was increased as the age advances in the all the age groups of guinea pigs studied.

CONCLUSION

Present study revealed that tunica mucosa of all the three regions of the stomach was lined by the simple columnar epithelium. Simple tubular cardiac glands, fundic glands were lined mainly by mucous cells along with few parietal cells. Fundic glands mostly occupied in the lower half of the stomach. Pyloric glands were simple branched tubular glands were lined by mucous acinar cells. The mean thickness of tunica mucosa was the maximum in fundic region and the minimum in pyloric region The mean thickness of tunica muscularis external was the maximum in pyloric and the minimum in fundic region.

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