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Postnatal Microanatomy of the Gall bladder in Guinea pigs (*Cavia porcellus*)

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ABSTRACT

The objective of the present study was to document the postnatal microanatomical features of the gall bladder in four postnatal age groups of guinea pigs. Six guinea pigs in each age group irrespective of sex were collected as per ethical committee approval and used for the study. After collection, the animals were dissected, gall bladders were removed, cut into small sections and subjected to routine tissue processing methods. The paraffin blocks were cut at 3-4 µm thickness and stained with routine and special staining techniques. The wall of the gall bladder consisted of tunica mucosa, submucosa, muscularis and serosa. The tunica mucosa of gall bladder consisted of irregular folds with primary, secondary and tertiary folds. The tunica mucosa consisted of lining epithelium and lamina propria in all postnatal ages. The lining epithelium was simple, tall columnar epithelium with irregular microvilli at the luminal border. Goblet cells and muscularis mucosa were absent. Lamina propria – submucosa consisted of loose connective tissue with collagen, reticular fibres, fibroblasts, lymphocytes and blood vessels. Tunica muscularis was a fibromuscular coat that consisted of a loose arrangement of smooth muscular layers in longitudinal and circular fashion. Tunica serosa consisted of a loose connective tissue layer which was covered by a single layer of mesothelium.

Key words: Microanatomy, Postnatal study, Gall bladder, Guinea pig .

INTRODUCTION

The gall bladder is a cyst-like muscular sac lined by simple columnar epithelium. It receives and stores bile from the liver and conveys it via cystic duct to the duodenum. The gall bladder is stimulated to expel the bile into the duodenum by the hormone cholecystokinin produced by the endocrine cells of duodenum. Enzyme production is stimulated by the presence of fat in the duodenal mucosa. The simple columnar epithelial cells had microvilli in human gall bladder. Below the epithelium is the lamina propria. Gall bladder does not have a muscularis mucosa and submucosa and contains a muscularis and serosa layer (Ozkan, 2023). Gall bladder is located on the visceral surface of mammalian liver in the gall fossa. Gall bladder is absent in horses, elephants, rats, pigeon and parrots. Animals without gall bladder had the oddi sphincter which controls the secretion of bile in the liver and bile ducts as a gall bladder in vertebrates (Oldham-Ott and Gilloteauz, 1997). The microscopic study of gall bladder with developmental nature is scanty in lab animals like guinea pigs. So, the present study was aimed to explore the microanatomical features of the gall bladder in the guinea pigs of various postnatal ages.

MATERIALS AND METHODS

The microanatomy of the gall bladder of guinea pig from postnatal age groups (Table 1) was conducted at the Department of Veterinary Anatomy, Madras Veterinary College, Chennai-7. Guinea pigs were procured from the Department of Laboratory Animal Medicine, Madhavaram Milk Colony, TANUVAS, Chennai-51, as per the 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 Carbon dioxide asphyxiation as per CPCSEA norms and they were subjected to dissection.

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Table1. Details of age groups of guinea pig used for the research work

	Postnatal Age Groups				Total
	0-2 weeks	2-8 weeks	8-16 weeks	16-32 weeks	
No. of Guinea pigs	6	6	6	6	24

The gall bladder of guinea pigs of postnatal age groups (Table 1) was collected. They were washed in normal saline and fixed either in 10% neutral buffered formalin, Zenker's fluid, or Bouin's fluid for histological studies. Then the tissues were dehydrated in the ascending grades of alcohol cleared in xylene and embedded in paraffin (58-60ºC). Sections of 4-5µm thickness were cut and used for the routine and special histological staining techniques. The following histological techniques were applied for the study. 1) Standard Haemotoxylin and Eosin method for paraffin sections (Bancroft and Stevens, 1996) for routine histological observations.2) Van Gieson's technique for collagen fibres, Masson's Trichrome for connective tissue fibres, Gomori's silver method for reticulum (Luna, 1968)

RESULTS AND DISCUSSION

The gall bladder of male and female guinea pigs had almost the same structure with no significant differences. The wall of the gall bladder consisted of tunica mucosa, submucosa, muscularis and serosa in all postnatal ages (Fig. 1) as reported by Mobini (2012) in avian gall bladder, but they did not discuss the tunica submucosa.

Tunica mucosa

The tunica mucosa of gall bladder consisted of irregular folds with primary, secondary and tertiary folds (Fig. 2). All the folds were visible in the gall bladder of postnatal ages, with the folding increased as age increased and the maximum fold was observed from 16-32 weeks of age (Fig. 3). Preweaning animals showed only primary folds of less height. The tunica mucosa consisted of lining epithelium and lamina propria in all postnatal ages as stated by Yamada (1959) in hamster gall bladder, Hatami-Monazah and Abdallah (1978) in goat gall bladder, Mobini (2012) in avian gall bladder. Similar to the present observation Yamada (1959) in hamster gall bladder also did not find goblet cells in the lining epithelium whereas, Bamaniya *et al.* (2016) in Marwari goat gall bladder and Mobini (2013) in quail found goblet cells in the lining epithelium. Mucularis mucosa was not observed in all ages. So, lamina propria and submucosa formed a continuous layer as recorded by Mobini (2012) in avian gall bladder. The lining epithelium was simple tall columnar epithelium with irregular microvilli at the luminal border (Fig. 4). Reticular fibres were found in the basement membrane of the lining epithelium as stated by Mobini (2012) in avian gall bladder (Fig. 5). The lining epithelium of gall bladder had dark and light cells and some basal cells towards the basement membrane (Fig. 4). Goblet cells were absent in the lining epithelium in the present study. Similar results were also recorded in Japanese quail (Mobini, 2013). Lamina propria – submucosa consisted of loose connective tissue with collagen, reticular fibres, fibroblasts, lymphocytes and blood vessels as stated by Mobini (2012) in avian gall bladder. Cask-like cells, crayon-like cells and glands in the lamina propria were not observed in the present study but were seen in the hamster gall bladder (Yamada, 1959). Mucous glands were seen in subepithelial layer of goat gall bladder (Hatami-Monazah and Abdallah, 1978) which was not observed in the present study. This may be due to species differences.

Tunica muscularis

Tunica muscularis was a fibromuscular coat, consisting of a loose arrangement of smooth muscular layers in longitudinal and circular fashion. Similar results were also reported by

Hatami-Monazah and Abdallah (1978) in goat gall bladder, Cai and Gabella (1984) in guinea pig gall bladder and Maya *et al.*(2012) and Mobini(2012) in avian gall bladder. Dense connective tissue separated the muscular bundles (Fig. 6) with the occurrence of blood vessels.

Tunica serosa

Tunica serosa covered the external surface of the gall bladder in all ages and consisted of a loose connective layer which was covered by a single layer of mesothelium (Fig. 6) as recorded by Cai and Gabella (1984) in guinea pig. The serosa was thicker in some areas and thin in others. The loose connective tissue of the tunica serosa had many blood vessels as reported by Yamada (1959) in hamster gall bladder and Maya *et al.* (2012) in avian gall bladder. Elastic fibres were observed in the wall of the blood vessels. Glands in serosa were absent in the avian species (Maya *et al*., 2012), similar to the present observation. The gall bladder of 16-32 weeks of age showed the presence of large blood vessels.

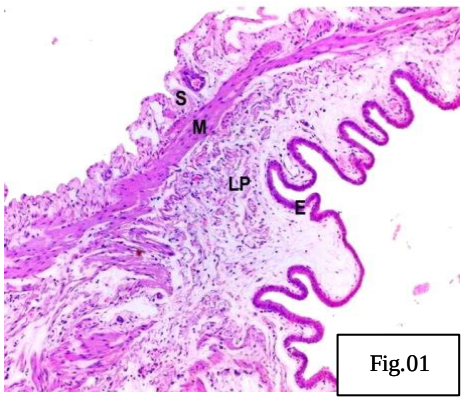
Cystic duct

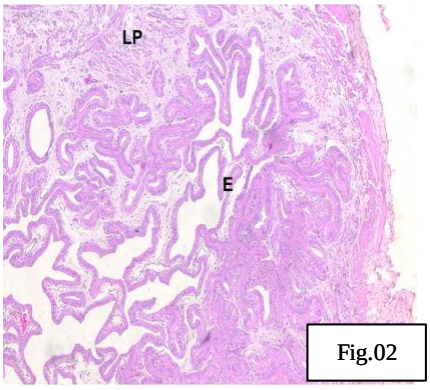
The extrahepatic bile duct had tunica mucosa, submucosa, muscularis and serosa in all the postnatal groups. The mucosa was thrown into longitudinal folds and lined by simple, high-columnar cells with brush borders at the apical or luminal surface. The columnar cells had a large basally located elongated or oval-shaped nucleus. The columnar cells rested on the basement membrane. The lamina propria contained collagen, reticular fibres, blood vessels and smooth muscle fibres in various directions. The muscularis mucosa was absent. The lamina propria and submucosa formed a continuous layer. The tunica muscularis layer was thick with smooth muscle fibres with a circular arrangement. The thin serosa of connective nature with blood vessels covered the bile ducts and was mesothelium. Similar findings were reported by Cai and Gabella (1984) in guinea pig, but recorded well-developed serosa in the cystic duct, hepatic duct and common bile duct in contrast to the present observation.

Duodenal papilla

The bile duct was observed to open into the duodenum and formed a dome-shaped appearance with irregularly folded lining epithelium. Cai and Gabella (1984) stated the dome-shaped appearance of bile duct in the present study as ampulla and the bile duct communicated to the intestinal lumen and opened at the duodenal papilla. Cai and Gabella (1984) found in guinea pigs that the pancreatic duct did not join the common bile duct at the ampulla, as in the present observation. The angle formed between the bile duct and duodenum was 240-300^0 in the guinea pig of all postnatal ages, whereas Cai and Gabella (1984) recorded the angle as 300^0 in the adult guinea pig.

The lining epithelium was simple columnar with basally located, elongated to oval nuclei and many crypts. This lining epithelium was surrounded by a thick loose connective tissue and thin discontinuous layer of smooth muscle fibres which were the continuation of the lamina propria and muscularis mucosa of the duodenum (Fig. 7). The muscularis layer was thicker at the duodenal papilla where the bile duct opened. Opposite to the opening of the bile duct at the duodenal papilla, there was another papilla within the duodenal lumen for the opening of the pancreatic duct. The tunica muscularis layer of the duodenal papilla consisted of a basal layer of circular arrangement of muscle fibres, above that muscle fibres were arranged in longitudinal and oblique fashion (plate 59). Similar findings were observed by Cai and Gabella (1984) in guinea pig gall bladder. Similar observations regarding the muscle fibres of the duodenal papilla were reported by Grace *et al.* (1988) in prairie dogs but they stated the muscle fibres arrangement as sphincter choledochus or sphincter of Oddi.





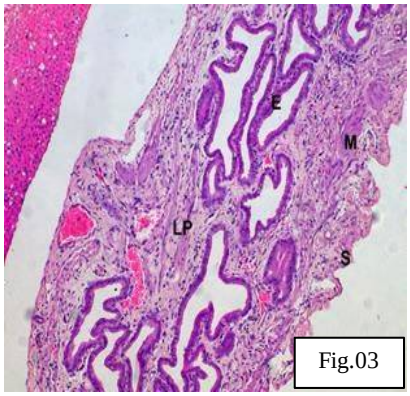
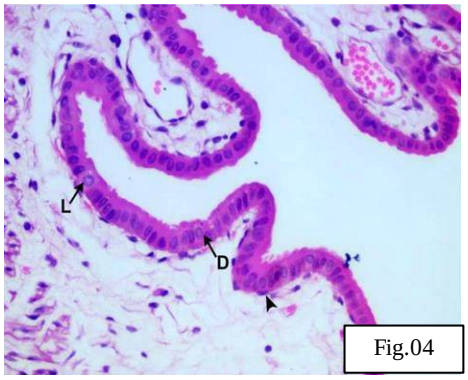
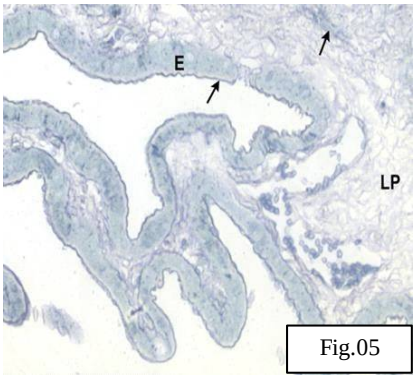


Fig 1: Photomicrograph of 2-week-old guinea pig gall bladder showing Epithelium (E), lamina propria (LP), muscularsis (M) and Serosa (S) H & E x 400 **Fig 2:** Photomicrograph of 20 week-old guinea pig gall bladder showing Epithelium (E) and lamina propria (LP) H & E x 100 **Fig 3:** Photomicrograph of 10 day-old guinea pig gall bladder showing primary epithelial folds. Epithelium (E), lamina propria (LP), muscularsis (M) and Serosa (S) H & E x 100





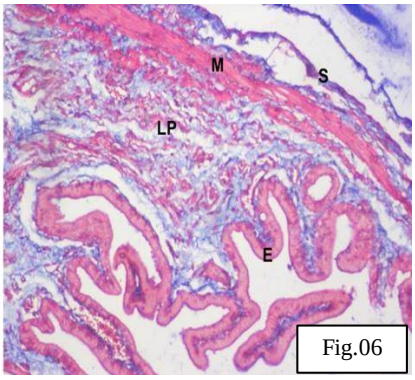


Fig 4: Photomicrograph of 30 week-old guinea pig gall bladder showing dark (D) and light cells (L) and some basal cells (arrow head) towards the basement membrane H & E x 400, **Fig 5**: Photomicrograph of 2 week-old guinea pig gall bladder showing reticular fibres (arrow) in the apical part of epithelium (E), lamina propria (LP), muscularsis (M) and Serosa (S) Gomori's method x 400, **Fig 6:** Photomicrograph of 10 day-old guinea pig gall bladder showing blue collagen fibres in the lamina propria (LP) and Serosa (S) and red colourled muscle fibres in the muscularis (M) layer. E - epithelium Masson's Trichrome x 100.

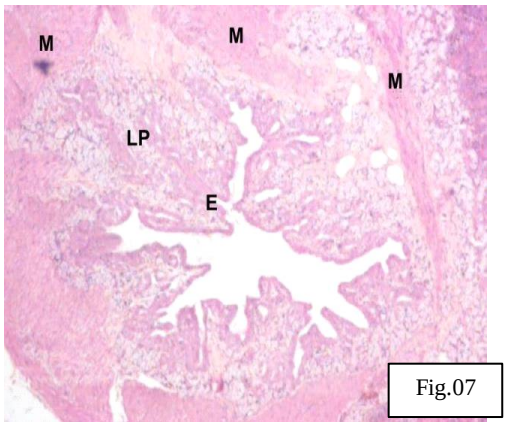


Fig 7: Photomicrograph of 30 week-old guinea pig duodenum showing duodenal papilla. E - epithelium lamina propria (LP) and muscularsis (M) H & E x 100

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