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Histological and Histochemical Aspects of the Digestive Tract of *Lyciasalamandra billae arikani* Göçmen & Akman, 2012 (Urodela: Salamandridae)

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Abstract: The present study describes the histological and histochemical features of the digestive tract of *Lyciasalamandra billae arikani* Göçmen & Akman, 2012. The wall of the oesophagus, stomach, small intestine and large intestine are formed by four layers from inside to outside: mucosa, submucosa, muscularis externa and adventitia or serosa. The oesophageal epithelium of *L. billae arikani* consists of ciliated columnar epithelial cells with many goblet cells. The inner surface of the stomach mucosa is lined by columnar mucus-secreting cells. The small intestine has many deep folds lined by cylindrical cells and goblet cells. The mucosa of the large intestine had small folds, which are lined by columnar epithelial cells. An increased number of goblet cells is observed from the small intestine to the large intestine. According to the histochemical results, the secretion of mucous cells has distinct properties (neutral, acidic and sulphated glycoproteins) characteristic for the different parts of the digestive system. The secretion of mucous cells participates in maintaining tissue moisture, has protective functions against chemicals and pathogens as well as contributes to the reduction of mechanical friction by lubricating.

Key words: Histology, digestive system, glycoproteins, amphibian, *Lyciasalamandra*

Introduction

The fundamental functions of the digestive system are the digestion of food, the absorption of nutrients and the removal of waste. Food is ingested, macerated, mixed with digestive enzymes, broken down into smaller particles and pushed through the digestive system. The mucosa of the digestive tube is covered by a mucus layer having important roles such as protection of the epithelium against mechanical friction and chemicals, absorption of water and electrolytes and maintenance of optimal pH required for digestion. The mucus contains mainly water and mucins, which are glycoproteins with extremely high molecular weight (Díaz 2003, Stevens & Hume 2004, Bansil & Turner 2006, Kim & Ho 2010).

Amphibians are a suitable model to study physiology and many biological processes (Feder 1992). However, there are few studies related to the amphibian alimentary tract. Several studies have reported data on the histochemistry of glycoproteins in the digestive system of some anurans (Liquori et al. 2002, Pelli-Martins et al. 2012, Akat et al. 2014, Machado-Santos 2014). Although many salamander species have been described in the world, there are far fewer studies related to the histomorphology of the digestive system of salamander species and there are no reports on the histology of the digestive tract of the genus *Lyciasalamandra*. In the present study, we aim to assess the glycoproteins produced by epithelial cells of *Lyciasalamandra billae arikani* in order to identify possible correlations between the different composition of the glycoproteins and cell types in the digestive tract.

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Materials and Methods

The current study was approved by the Animal Ethical Committee of the Ege University, Faculty of Medicine (permission number: 2011-091) and the Ministry of Forestry and Water Affairs of the Republic of Turkey (date: 11 April 2011, number: 42694). Four individuals (two adult males and females) of *Lyciasalamandra billae arikani* were obtained from Antalya, Turkey (Göçmen & Akman 2012). Salamanders were anaesthetised with ether and their alimentary canals were quickly removed and then fixed in Bouin's fluid for 48 h. Thereafter, the tissue samples were washed with ethanol 70% for 24 h before embedding them in paraffin. Five-micrometer-thick sections were deparaffinised, hydrated and stained with Gill's haematoxylin-eosin (HE). Histochemical techniques were performed for the identification of glycoproteins (GPs) as detailed in Table 1. Counterstaining was carried out with haematoxylin, after the stain with PAS, AB and AF. Sections were evaluated and photographed by Leica DM3000 microscope (Leica Microsystems) connected to a Leica digital camera (DFC290).

Table 1. Histochemical features of the mucosa of alimentary canal in *Lyciasalamandra billae arikani* (PAS: periodic acid/Schiff; AB: Alcian blue; AF: Aldehyde fuchsin; GPs: glycoproteins).

No.	Procedures	Interpretation of staining reactions
1	PAS	GPs with oxidizable vicinal diols and/or glycogen
2	AB pH 2.5	Acid rich GPs
3	AF	GPs with sulphate

Results

The digestive tract of *L. billae arikani* was composed of four layers from outside to inside: adventitia or serosa, muscularis externa, submucosa and mucosa (Figs. 1C, F). The mucosa of the oesophagus in *L. billae arikani* had longitudinal folds composed of the columnar ciliated epithelium with numerous goblet cells overlaying the lamina propria (a loose connective tissue). The secretion of goblet cells gave positive reaction with AB (Fig. 1A) and PAS, demonstrating their secretion contained neutral and acidic glycoproteins. Secretory products of goblet cells also gave positive reaction with AF staining (Fig. 1B) due to the presence of sulphated glycoproteins.

The stomach of *L. billae arikani* was subdivided into a wide corpus or fundus and short pyloric regions. The stomach was covered by serosa which was lined with a simple flat epithelium. The muscularis externa was composed of a thick layer of smooth muscle fibres. The submucosa was formed of loose connective tissue rich in blood vessels. The epithelial layer of mucosa in the stomach was lined by mucous secreting columnar epithelial cells. The epithelial layer folded into the lamina propria, forming many gastric pits. Gastric glands were observed in the mucosa. Mucous neck cells were located in the upper portion of the glands, whereas oxynticopeptic cells were predominant at the gland body. The secretion of mucous neck cells consisted of mainly neutral glycoproteins (Fig. 1C). The apical portion of the surface mucous cells was weakly alcianophilic and exhibited strong staining after PAS (Fig. 1D) and moderate after AB. However, they did not give reaction with AF.

The small intestine of *L. billae arikani* was lined by columnar absorptive cells (enterocytes) with brush border and scattered goblet cells. The mucus secretion of goblet cells showed PAS positive reaction and the glycocalyx of brush border also reacted with PAS (Fig. 1E). The intestinal glands and glandular crypts were not examined in the mucosa of small intestine.

The surface of the large intestine had many irregular wavy folds. The mucosal surface of the large intestine consisted of columnar cells and abundant goblet cells (Fig. 1F). The mucus secreted by goblet cells in both small and large intestine was stained with AB and AF. In both small and large intestine, the submucosa was formed of loose connective tissue containing a number of blood vessels. The next layer was the muscularis externa layer, composed of layers of smooth muscle. The outermost layer was the serosal layer, composed of a thin layer of epithelial tissue.

Discussion

The present histochemical study on the digestive system of *L. billae arikani* has enabled us to identify glycoproteins secreted by distinct mucous cells. Histochemical staining methods demonstrated that the secretion of mucous cells contained neutral, acidic and sulphated glycoproteins.

The oesophagus epithelium of *L. billae arikani* consists of the columnar ciliated type with widespread goblet cells. Similarly, Liquori et al. (2007) have reported that oesophageal mucosa in *Triturus carnifex* is lined by columnar ciliated epithelium with many goblet cells. Domeneghini et al. (2005) have determined that many mucous cells with flat-

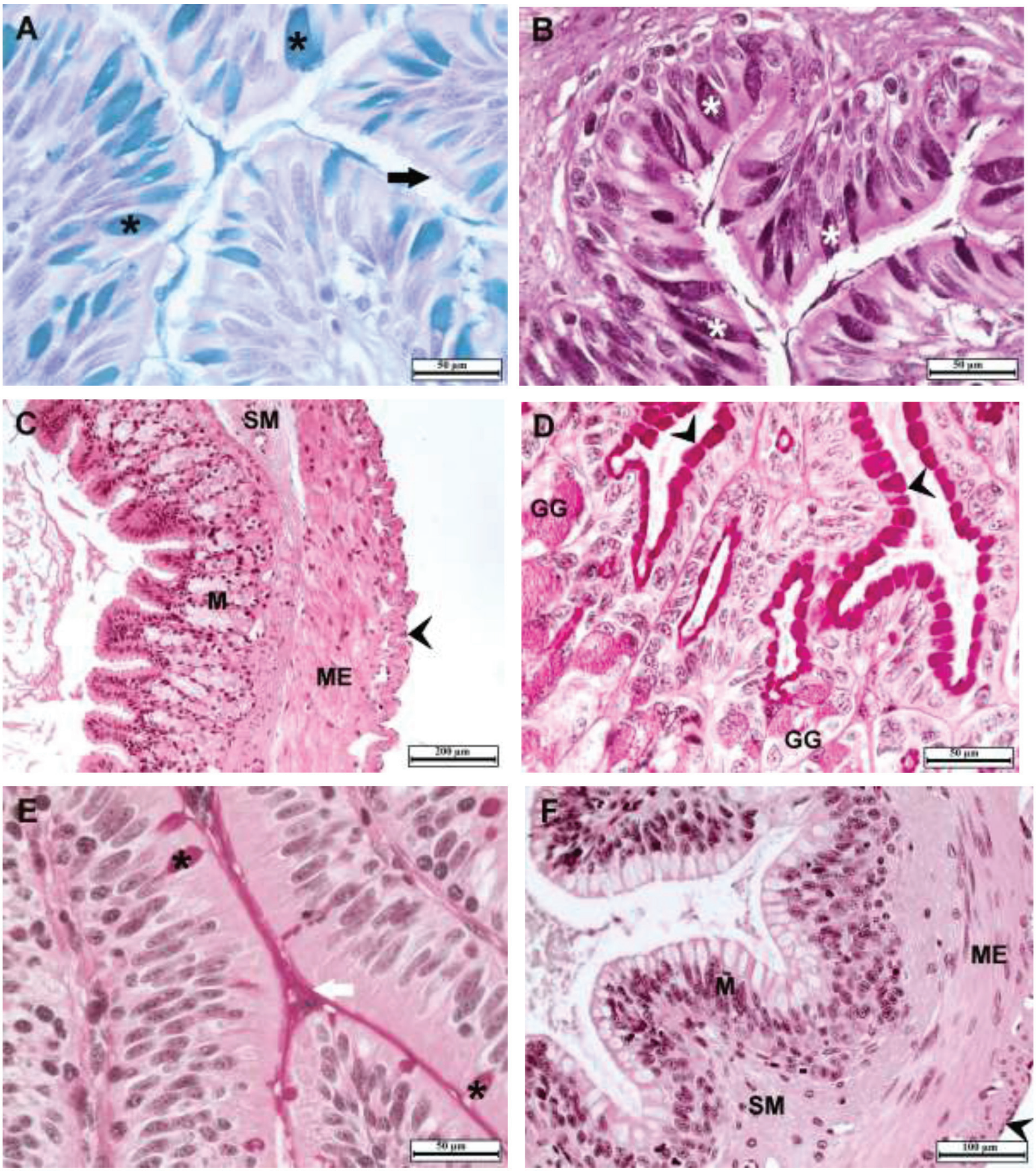

Fig. 1. Histology and histochemistry of the digestive tract in *Lyciasalamandra billae arikani*. A. The oesophageal epithelium of *L. billae arikani* was of the columnar ciliated type (black arrow) with goblet cells (asterisk). Goblet cells secretory product showed affinity for AB. B. Sulphated glycoproteins (GPs) in goblet cells (asterisk), AF. C. The layers of the stomach, HE. Mucosa (M), submucosa (SM), muscularis externa (ME), serosa (arrow head). D. Surface cells (arrow head) of gastric mucosa and mucous neck cells in gastric glands (GG) stained with the PAS reaction. E. Goblet cells (asterisk) and the glycoproteins in the brush border membrane (white arrow) reacted with PAS, revealing the presence of neutral glycoproteins. F. The mucosal surface of large intestine consisted of simple columnar cells and abundant goblet cells, HE. Mucosa (M), submucosa (SM), muscularis externa (ME), serosa (arrow head).

tened basal nuclei are located in oesophageal mucosa of *Anguilla anguilla*. Scillitani et al. (2012) examined mucin-secreting cells in oesophagus of *Natrix natrix*. The oesophagus of adult mammals is lined by a stratified squamous epithelium without goblet cells in individuals feeding on soft food or with a keratinised stratified squamous epithelium in individuals feeding on coarse food (Parakkal 1967, Raymond 1991). It is conceivable that the secretion of oesophageal goblet cells could perform a similar function of mammalian saliva in preserving the mucosa of the first part of the digestive tract due to absence of salivary glands in amphibians. The structure of the oesophageal epithelium of *L. billae arikani* of columnar ciliated epithelial cells and goblet cells is probably related to the absence of salivary glands in amphibians. The secretion of the oesophageal epithelium may serve as a protection against the mechanical effects during the passage of food through the oesophagus.

The gastric mucin exhibits a positive reaction with PAS in the apical portion of surface mucous cells and mucous neck cells because of the presence of neutral GPs as it has been reported for *Bufo viridis* (Liquori et al. 2002) and some reptiles (Ferri & Liquori 1992, Çakici & Akat 2013). Apical portion of the surface mucous cells is weakly alcianophilic after AB. However, gastric mucin does not react with AF. Neutral GPs protect the stomach from mechanical friction, pathogens and aggressive factors such as pepsin and hydrochloric acid (Ferri et al. 2001). Leknes (2011) suggests that neutral GPs are poor in anions. Based on these data, we can suppose that neutral GPs may inhibit motion of hydrogen ions and water molecules from the stomach extract to the preservative mucus layer.

The epithelium in the small intestine of *L. billae arikani* is lined by simple columnar epithelial cells with scattered goblet cells. An increased number of goblet cells is observed from the small intestine to the large intestine. The mucins of goblet cells of both small and large intestine were stained with all histochemical methods (PAS, AB, AF). No differences were observed among the different parts of the intestine in terms of glycoprotein secretion. The glycocalyx of the brush border gave positive reaction with PAS due to the existence of neutral glycoproteins, which indicate an emulsification task of food into chyme and absorptive functions (Murray et al. 1996).

The mucin of goblet cells in the oesophagus, small and large intestine reacted positively with all used histochemical methods (PAS, AB, AF). The AB staining method indicated acidic GPs which could provide a high viscosity of the secretions (Díaz et al. 2008). Furthermore, acidic GPs have been reported to protect the intestinal epithelium from the degenerative activity of glycosidases (Carrasson et al. 2006). AF staining technique indicated that the goblet cells of *L. billae arikani* included sulphated glycoproteins. The presence of numerous microorganisms is generally linked with the secretion of sulphated glycoproteins (Liquori et al. 2002). High level of secretion of sulphated glycoproteins by goblet cells of intestinal tracts are recorded where GP-mediated trapping of pathogens and protein digestion occur and probably have a function in stimulating immunity (Murray et al. 1996, Domeneghini et al. 1998).

In conclusion, the secretion of mucous cells in the oesophagus probably acts similarly to the mammalian saliva. The secretion of mucous cells in the stomach was made by mainly neutral GPs which may inhibit motion of hydrogen ions and water molecules. No differences have been observed among the different parts of the intestine in terms of glycoproteins secretion. The secretion of goblet cells in the intestine consisted of neutral, acidic and sulphated GPs, each with a prominent role such as maintaining tissue moisture, lubrication with decreasing mechanical stress and preserving from chemicals and microorganisms.

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