

## Histological and Histochemical Study on Stomach of *Salamandra infraimmaculata* (Amphibia: Urodela)

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### Abstract

In the current study, we aimed to investigate the histological and histochemical characteristics of stomach of *Salamandra infraimmaculata*. The stomach of *S. infraimmaculata* was composed of four distinct layers; mucosa, submucosa, muscularis externa and serosa. The inner surface of gastric mucosa was lined by surface mucous cells which were simple columnar epithelium. Gastric glands were observed in mucosa. Mucous neck cells were located at the upper portion of glands, besides oxynticopeptic cells which were predominant at the gland body. The secretory components of mucous cells were neutral and acidic glycoproteins. The mucosa was separated from muscularis externa through submucosa which was formed by loose connective tissue. Muscularis externa consisted of a thick layer of smooth muscle. The muscularis externa was surrounded by the serosa which was the outermost layer of digestive tract.

**Keywords** — Amphibian, glycoproteins, mucous neck cell, oxynticopeptic cell, stomach, surface mucous cell

### 1. Introduction

Digestive tract is one of the most active and essential system due to digestion of food, absorption and waste removal [1,2]. The entire of gastrointestinal epithelium is coated with mucus, which protects surface of it. Mucus covers most surface of body such as digestive and respiratory systems, genital tract, the skin of amphibians and fish [3-6]. Mucus is an effective semipermeable barrier which provides the exchange of nutrients, water, gases, hormones and gametes while being impermeant to many pathogens [4]. The viscoelasticity of mucus is correlated with concentration of mucins. Mucins are high molecular weight glycoproteins [7]. Glycoproteins are proteins which carry covalently-bonded sugar units [8].

Amphibians are study model for many biological processes. Therefore research on them encompasses many fields such as morphology, histology, embryology, ecology, endocrinology, genetic and public health [9-14].

The genus *Salamandra* is composed of 6 species and shows a wide geographical distribution. *Salamandra infraimmaculata* is a thick bodied, robust salamander with maximum total length of about 25–32 cm. The females are usually larger than males and cloacal region is swollen in males. *S. infraimmaculata* is black salamander with big

yellow dots over the whole body, except the belly. This is the largest fire salamander species which are active at twilight, spending most of the day under snags and stones. During rainy weather, salamanders leave their hiding places. The adults feed on insects, earthworms, slugs and snails [15,16].

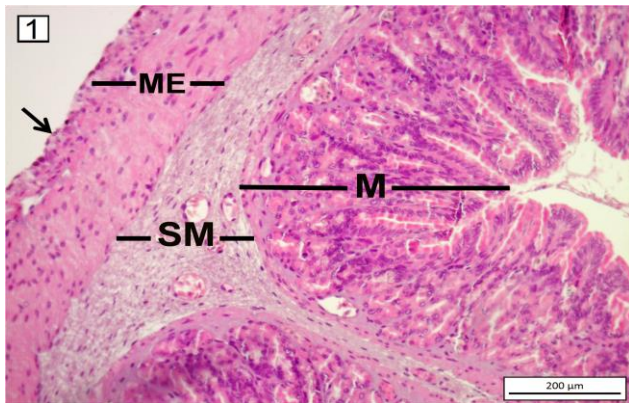
In the current study, we aimed to investigate the histological characterization and composition of the mucins in the gastric mucosa and discuss probable function of mucins in the stomach of *S. infraimmaculata*.

### 2. Materials and Methods

Two adult males/females of *S. infraimmaculata* were utilized in the current study. Salamanders were obtained from their natural habitat in Hatay/Turkey (36.492516 N, 36.203558 E), anaesthetized with ether, and euthanized by decapitation. Stomach samples were fixed in Bouin's fluid for 48 h. Then tissue samples were dehydrated in ethanol, cleared in xylol and embedded in paraffin. Sections (5 µm) were stained with Gill's hematoxylin-eosin (HE), Periodic acid-Schiff (PAS) and Alcian blue (AB). Sections were examined by Zeiss AxioScope A1 microscope attached to a digital camera (AxioCam Erc 5s).

### 3. Results and Discussion

The present study has been focused on the histological and histochemical characterization of the stomach in *S. infraimmaculata*. The stomach was composed of four distinct layers as described for digestive tract of other vertebrates [17-19]; mucosa, submucosa, muscularis externa and serosa (Figure 1).

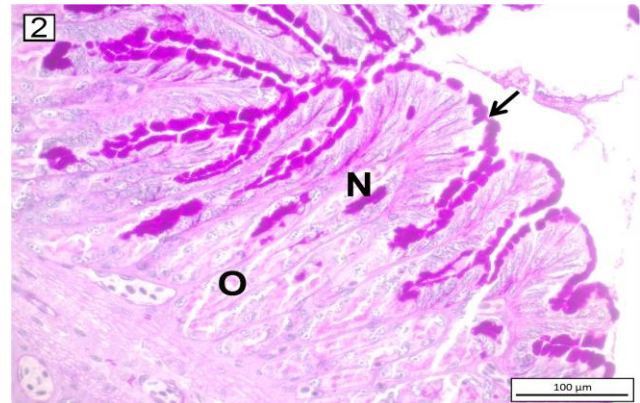


**Figure 1.** Light microscopic view of the stomach of *S. infraimmaculata*. The layers of stomach; mucosa (M), submucosa (SM), muscularis externa (ME), serosa (arrow). HE staining.

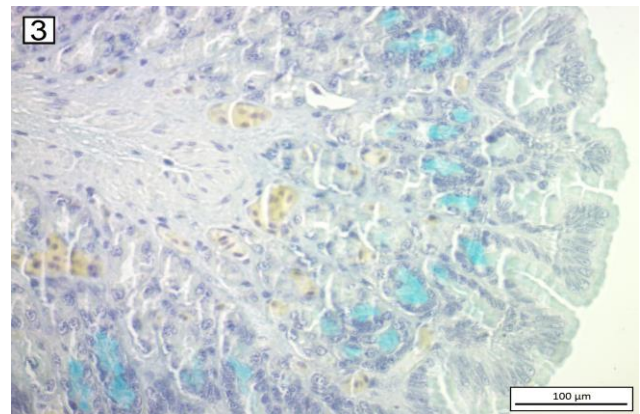
The mucosa was the innermost layer of the stomach. It was made up of three layers; epithelium, lamina propria and muscularis mucosa. The gastric mucosa showed numerous folds. It was made of the lining epithelium supported by loose connective tissue which was lamina propria. The inner surface of gastric mucosa was lined by surface mucous cells which were simple columnar epithelium as in other vertebrates [17,20,21]. Interestingly, for other anurans, the lining epithelium was constituted by ciliated cells as in the larval stage of *Xenopus laevis* [20] and in *Rana temporaria* [22].

Apical portion of the surface mucous cells was weak alcianophilic and exhibited strong staining after PAS (Figures 2, 3). These histochemical results indicated that the surface mucous cells were rich in neutral glycoproteins. Ferri et al. [23], who described epithelial cells of stomach of *Rana aurora aurora* produced neutral and acidic mucins. However, mucous cells of the stomach of *Triturus carnifex* [24], *Rhinella icterica* [25] mainly produced neutral glycoproteins. The mucus layer has important roles including the protection of underlying epithelium from mechanical and chemical stress, electrolyte absorption, increasing digestive efficiency and lubrication of tract [26-28]. Neutral glycoproteins have protective function against mechanical injuries, pathogens and pepsin [23].

Furthermore, neutral glycoproteins are poor in anions. This may prevent hydrogen ions and water molecules in the gastric juice being drawn into the protective mucus layer [29]. Alcian blue staining method indicated acidic glycoproteins that could be responsible for an increasing viscosity of the secretions [30]. Acidic glycoproteins were also reported to protect the intestinal epithelium against the degradative actions of glycosidases [27].



**Figure 2.** Gastric glands composed of mucous neck cells (N) at the upper portion of glands and oxynticopeptic cells (O) at the lower portion. Neutral glycoproteins were identified at the apical domain of surface mucous cells (arrow) and mucous neck cells (N), PAS method



**Figure 3.** Gastric mucosa of *S. infraimmaculata*. Neck cells were stained with AB method.

Mucous neck cells were located at the upper portion of glands, besides oxynticopeptic cells which were predominant at the gland body (Figure 2). Scillitani et al. [21] reported that in the base region of the fundic glands of *Natrix natrix*, there were mainly oxynticopeptic cells. According to Suganuma et al. [31], amphibians were the first vertebrates to develop true mucous neck cells. The mucous neck cells were stained with AB and PAS. These histochemical results demonstrated that their contents included in both neutral and acidic glycoproteins.

Oxynticopeptic cells were not stained with PAS and AB (Figures 2, 3). It was reported that oxynticopeptic cells produced and released both hydrochloric acid and pepsinogen in *Bombina variegata* [32], *Bufo marinus* [33], *Triturus carnifex* [34].

The mucosa was separated from submucosa through muscularis mucosa, which was a thin layer of smooth muscle that supports the mucosa and provides mucosa with the ability to move and fold. Crespo et al. [17] reported the absence of muscularis mucosa between the lamina propria of the mucosa and the submucosa in *Dentex dentex*. The submucosa was composed of loose connective tissue. Muscularis externa consisted of a thick layer of smooth muscle. The serosa was the outermost layer of digestive tract. It consisted of two layers, a thin layer of loose connective tissue and mesothelium. The connective tissue was surrounded by mesothelium which was simple squamous epithelium.

It can be concluded that the gastric wall of *S. inframaculata* was composed of four histologically distinct layers; mucosa, submucosa, muscularis externa and serosa as described for digestive tract of other vertebrates. The secretory components of mucous cells were neutral and acidic glycoproteins. Compared with the studies published to date, our results added information for characterization of stomach of *S. inframaculata*.

#### 4. Acknowledgements

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