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Histological and histochemical Study of Brunner's glands in Wild Brown Rat (*Rattus norvegicus*)

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Abstract

The goal of the current study is to describe the characteristics of Brunner's glands and their location throughout the wild rat duodenum. Six healthy brown wild rats were purchased from a local animal shop in Baghdad city. Xylazine and ketamine were used to euthanize the experimental animals. Dissection was done, and specimens were obtained from various sections of the duodenum and fixed with 10 % neutral buffered formalin. Routine histological processing procedures were used for samples, and 7 µm sections were prepared and stained with Hematoxylin, eosin, and Alcian blue (2.5 pH) stain to detect neutral mucin. Then, the samples were examined under a light microscope, and images were captured. Various glands were seen in wild brown rats, including mucous-based intestinal glands. The glands were made up of tightly connected acini within the submucosa. These glands were distributed as crowded acinar groups close to the pancreatic duct. However, the most obvious Brunner's glands were mucous type. Nonetheless, the mixed type was also seen close to the pancreatic duct. Sections also showed the extension of glands from mucosa to submucosa and individually drained by a single excretory duct. Additionally, the individual gland ducts progressively expand after penetrating the muscular smooth muscle layer, showing huge pyramids of cells that comprise the secretory units.

Keyword: Histology, Mucous, Brunner's glands , Wild brown Rat.

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Introduction

Numerous vital processes, including sustenance breakdown, nutrient absorption, immune system support, and waste removal, depend on the gastrointestinal tract (Greenwood-Van Meerveld B et al., 2017; Jahan-Mihan et al., 2011). A specific portion of the small intestine, the

duodenum, is chiefly responsible for the enzymatic breakdown of nutrients. In addition, hormones secreted by the pancreas trigger the opening of the pancreatic duct, leading to the easy flow of pancreatic juice and bile into the duodenum (Duvnjak M & Smirčić-Duvnjak L., 2018; Jurij Dolenšek et al., 2017; Leung, 2007) . The submucosal glands of the duodenum are critical for nutrient absorption across all mammals, as they produce mucus that enhances the efficacy of digestive enzymes. These glands are situated within the submucosa of the proximal duodenum (A. A. MOHAMMADPOUR, 2011; Moran, 2017; Yen, 2000). The duodenum is resistant to sour chyme due to the formation of the protective mucus compared to other areas of the intestinal tract. The chyme acts to neutralize the duodenum contents before entering the jejunum. This protecting mechanism prevents the harmful effect on the reminder parts of the small intestine (Han et al., 2023). Brunner's glands, characterized by their tubular-alveolar structure, resemble mucous acini. These glands' ducts usually reach the most profound areas of the Lieberkühn crypts, passing through the (lamina muscularis) to discharge their contents into the intestinal lumen. These glands are situated within the submucosa of the duodenum and are present in all mammals (Dina H. Sadiq & Luay Abdulwahid Shihab, 2023; Patrice Spitalnik, 2016). Throughout the stages of development from infancy to adulthood, the duodenal glands in various animals undergo growth and transformation in both size and structure. As they expand, these glands ultimately coalesce into a dense cluster. In the case of Mole-rats, the duodenal submucosa develops coiled tubules postnatally (MN et al., 1970). Both acid and neutral mucins are produced by the epithelial cells. The presence of sulfate characterizes sulfomucins, whereas sialomucins are distinguished by sialic acid. Acid mucins exist in two distinct forms. The morphogenetic process that leads to the formation of finger-like projections known as villi and crypts significantly enhances the gut's nutrient absorption capacity, thereby meeting daily nutritional needs(Gipson & Argüeso, 2003; Kemp et al., 2004) . The villi are made from special, important cell types that contribute to the digestion of important nutrients. They safeguard the intestine against harmful invasive organisms that usually colonize the adult gut. Analyzing the data supporting the idea that a complex web of correlated indicating conduits controls the overall differentiation of the gut mucosa is crucial (CORFIELD et al., 2000; Zhen et al., 2007). The rapid and constant renewal of the gastrointestinal mucosal layer is a characteristic that often leads to the production of immature cells. Rapid cell multiplication creates an initial amplifying barrier as these cells move along the axis that connects the crypt to the villus. Particular cells with epithelial properties, including enterocytes, goblet, entero-endocrine, and Paneth cells, are various (Gunawardene et al., 2011; Shaoul et al., 2005). Research on Brunner's gland secretions has shown that they are less alkaline than pancreatic secretions, have a thick consistency because of their glycoprotein content, and contain very little digesting enzyme. It has been proposed that the primary role of Brunner's glands is to release substances that are acidic and potentially abrasive from the stomach to shield the mucosal lining of the proximal duodenum (Jawad et al., 2019; Simawy et al., 2024). A literature review revealed scarce publications regarding the histology of Brunner's gland in Wild Brown Rats (*Rattus norvegicus*) in Iraq. Therefore, this study is designed to describe the histological features of Brunner's gland and its location throughout the wild rat duodenum.

Materials & Methods

Ethical statement : This study approved by by the Animal Ethical committee/ College of Veterinary Medicine / University of Kerbala (2024-AHD)

Six healthy wild rats were purchased from a local animal shop in Baghdad city and used in the present investigation. The rat's weight averaged about 350 grams. The animals were kept for one week in the animal house at Karbala University's College of Veterinary Medicine for acclimatized and fed with pellet and ad libitum water A sensitive digital balance was used for calculating the weight in grams (gm)to calculate the dose of ketamine and xylazine as 75–100 mg/kg and 5–10 mg/kg, respectivelly (Thurmon JC et al. 1996), which were used for sedation of the rats via Intramuscular injection. All animals were dissected via the duodenum, which was detached from the pyloric sphincter by slicing it immediately before it reached the second part of the small intestine. All duodenum samples were preserved in 10% neutral buffered formalin. Then, the samples were processed using routine histological methods, and multiple serial sections were cut at seven µm. Later, the tissue sections were stained with Hematoxylin and eosin, while an Alcian blue (2.5 pH) stain was used to detect neutral mucin. All stained sections were examined using light microscopy. Then, images were captured by a digital Camera (Canon's products 550D, 20, Japan) connected to the microscope. An optical micrometer matched the objective lens with the application (Hussein Bashar Mahmood et al., 2020).

Results & Discussions

Brunner's glands were found in the upper duodenum's submucosa of wild rats. It revealed several branched tubules, categorizing them as compound tubular organs. Although the glands vary in size and quantity, they were generally more prevalent in the duodenum's initial sections than in its later sections. Brunner's glands were situated in the (proximal) part of the submucosa of the duodenum, which was just after the stomach's pylorus. These glands are frequently seen together and can be easily identified since they are larger than other glands in the intestinal tract. They expand from the mucosa to the submucosa, and their ducts open at the base of the intestinal villi, allowing their secretions to mix with the contents of the intestinal tract. Brunner's glands are individually drained by just one excretory duct. Individual gland ducts progressively expand after penetrating the muscularis' smooth muscle layer. Massive cells in the shape of pyramids make up the secretory units. Except for horses and rabbits, every species seen so far has secretory units that produce mucous cells called Brunner's glands (Figure. 1,2).

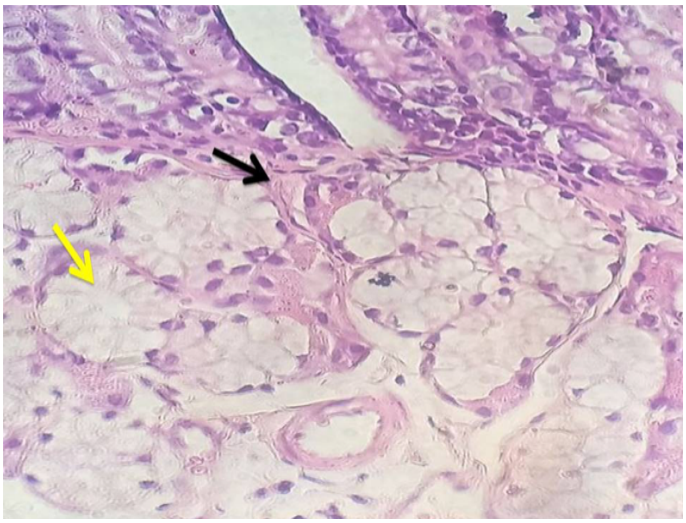

Figure. 1: The duodenum of wild rats shows the distribution of Brunner's glands in submucosa in groups (yellow arrow). These glands separated from mucosa by thin layers of muscular laminae (black arrow).H&E stain.400x

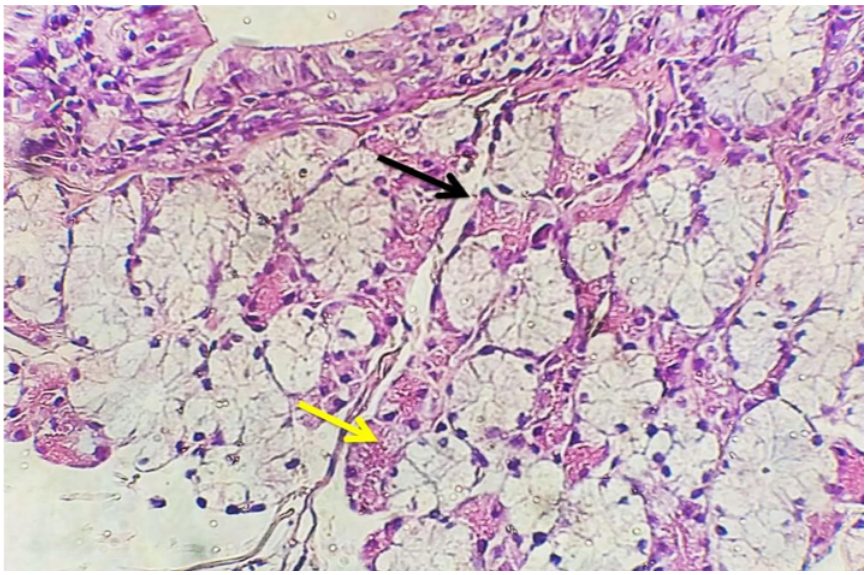

Figure. 2: Duodenum of wild rats shows the Mixed types of Brunner's glands (yellow arrow). These glands drained in longitudinal ducts lined by simple squamous epithelia (black arrow).H&E stain.400x

The current study's findings are similar to those reported previously (Sultan, Al-Haaik, and Alhasso, 2023), who claimed that Brunner's glands varied by species. The cell type may resemble a mucous cell referred to traditionally with a resembling nucleus located basally close to the basal cell membrane, and most of the supranuclear cytoplasm stuffed with mucous particles, or aspect cells may have a more serious appearance, with an oval or circular nucleus within the fundamental cytoplasm. Encircled by simple squamous epithelia, individual gland ducts penetrate the smooth muscle layer of the muscularis mucosae and often connect to the undersides of the covered intestinal glands. As the ductal cells of Brunner's gland approach the intestinal glands, they gradually grow taller (Figure. 3).

The present study mentions that the high numbers of myoepithelial cells are distinguished by their stellate-like form and numerous lengthy processes that connect the basal lamina beneath the epithelial cells. They usually live in the lamina propria, which envelops the glandular structures of intestinal glands (Figure. 4). This investigation agrees partially with (Moore, Kim, and Vanner 2000), who found that these cells were situated between the glands' epithelial cells and the basement membrane. Contributing to the general operation of Brunner's glands, they contract to help expel glandular secretions into the ducts.

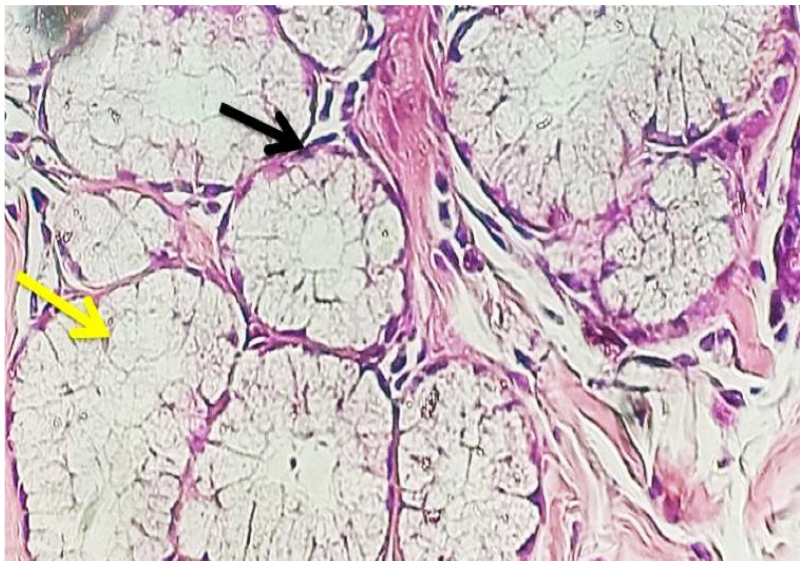

Figure. 3: The duodenum of wild rats shows that the mucous cells have the most of these cells (yellow arrow). Myoepithelial cells are distinguished by their stellate-like (black arrow). H&E stain.400x

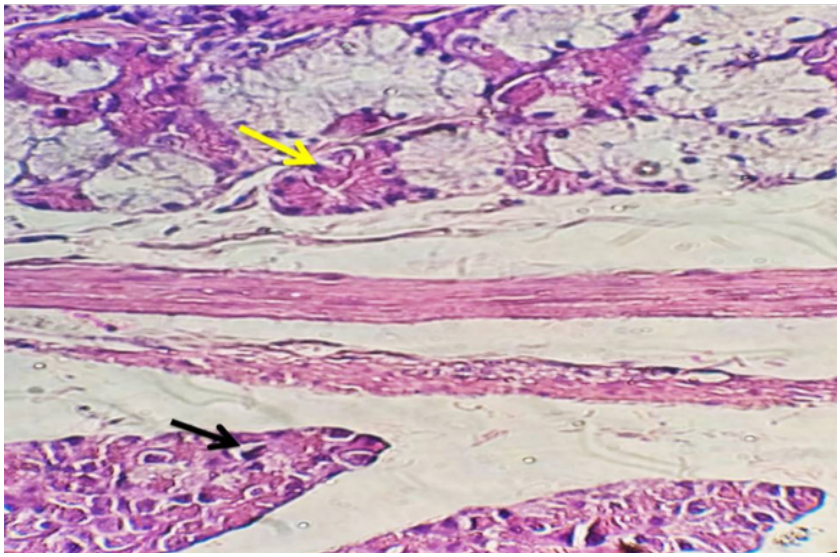

Figure. 4: Duodenum of wild rats shows the mixed cells near the pancreatic duct (yellow arrow)—Pancreas acini (black arrow).H&E stain 400x.

According to the current experimental study, the Brunner's glands in every wild rat exhibited a strong positive interaction with Alcian blue coloration, which revealed a mixture of neutral mucin and mixed acini (Figure. 5), giving the glands a magenta appearance. In addition, serous glands featured denser cells with centered nuclei and a narrower lumina than mucus glands, which featured pale appearance cells with a comparatively broader lumen. These findings concur with a previous study (Ergun et al., 2010), which showed that the pancreatic duct induces the discharge of digestive juice and bile due to hormones produced by the duodenum. Furthermore, because of

the mucus released by submucosal glands, the duodenum is significantly less susceptible to acidity than the rest of the small intestine (R Andleeb et al., 2017).

Figure. 5: The Brunner glands in the duodenum of wild rats have a strong dyeing response (yellow arrow). Scant amount of collagen fibers (black arrow).H&E stain.400x.

In conclusion, the current study documents the histological features of the Brunner's gland of wild brown rats (Rattus norvegicus). Comparing the results of the current study to those of previously published articles showed a development variation due to dietary habits, which leads to variations in gland number, distribution, and organization.

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Ethical statement

Routine care and experimental protocols used in this study has been done according to the international procedures for using the laboratory animals in scientific research.

Author's contribution

The author suggested the Conceptualization, Investigation, writing original draft, Methodology, Validation and Formal analysis of the current study. The data for this study are available on request.

Conflict of interest

The authors report no conflict of interest.

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