

Comparative Histology and Histochemical Analysis of the duodenum between Persian Squirrel (*Sciurus anomalus*) and Hamster (*Mesocricetus auratus*)

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Abstract—The purpose of this study was to describe, comparison the histological structures and histochemical of the histological layers in the different parts of the duodenum, with observation of the reactions of cells and tissues using histochemical stains (PAS, AB-PAS and toluidine blue). The study was carried out on twelve samples of duodenum of each of squirrel and hamster. The duodenum divided into proximal part, middle part and distal parts. six specimens were taking from squirrel and six specimens from hamster. The duodenum is squirrel and hamster fixed in 10% formalin for 24 hours and treating by routine histological technique. The duodenum consists of four main tunicae that is mucosa, submucosa, muscularis, and serosa. The mucosa layer is consists of three secondary layers the first layer is epithelial lining layer, lamina propria, and end layer is muscularis mucosa. The epithelial covering comprises simple columnar epithelial tissue composed of the columnar cells and goblet cells. The lamina propria under epithelial layer that contains simple straight tubular glands called the intestinal glands or the crypt of Lieberkühn. The muscularis mucosa that end layer is composed of the a very thin, single layer of the smooth muscle fibers arranged circularly. The submucosa layer consists of the dense connective tissue, while the tunica muscularis appears composed from two layers of smooth muscle fibers. The tunica serosa consists from a loose connective tissue that is covered by a mesothelium.

Keywords — histological, histochemical, comparative duodenum

INTRODUCTION

The Persian squirrels are Mammals classified to the Rodentia. The family Sciuridae is estimated to include about 50 genera and 273 species (1). The Caucasian squirrel (*Sciurus anomalus*) is the only species from the found *Sciurus* in the middle East. Its distribution large area in Greece and even Azerbaijan and Iran passage through (2). Lebanon and Syria. The Caucasian squirrel inhabits temperate mixed of forests, which are

distributed fluctuating climate characterized by evergreen with deciduous tree, this environment provides suitable conditions for both food and shelter (2). The hamster (*Mesocricetus auratus*) is rodent and belonging to the cricetinae, which are called also gold hamster or Syria hamster. The hamsters are most common type of hamsters and is largest type of hamster, there are main diet is vegetables, fruits, seed and sometime of small insect such as worms and grasshoppers (3). The hamster gold or Syria maturity is about 4-5 week unlike the squirrel, maturity about 6-8 months (4) The duodenum, the first anterior section of the small intestine, consists of three segments: the ascending, descending, and transverse parts. The pancreas is present between the ascending and descending parts. The pancreas secretes enzymes through the pancreatic duct into the duodenum to complete the stage of digestion and break down lipids, creating an immense surface area with which to digest enzymes (5) (6). The first part of small intestine that follow the stomach. the consist of four layers arranged from inside to outside an organized and methodical manner (mucosa, submucosa, muscularis, serosa). The tunica mucosa is inner layer of duodenum lining of columnar cell epithelial. Cells epithelial buds are structure to form cluster surround of protrusion villi and to found in the surface of epithelial (7)

Tunica mucosa is consisting of three layers (lumen, epithelium, lamina propria), these parts are arranged one part another part is monolithic between them. Epithelium a group of cells arranged vertically and enveloped the inner layer and extended to the lumen and top of this cell is microvilli (7). Gland intestine is the simple tubular gland present between base villi structure is also called crypts of Lieberkühn beginning is form lower surface of villi to the muscular area of mucosa (8)

MATERIALS AND METHODS

Samples collection:

The twelve male adult Persian squirrels (*Sciurus anomalus*) and hamster (*Mesocricetus auratus*) The animals used have

been verified and are in good health. They were euthanized with an overdose of ketamine and xylazine administered intramuscularly. After dissected of the squirrel and hamster the organs immediately removed from the abdominal cavity and washed with saline solution to remove blood and any other adhering debris, six of the specimens (duodenum) from each Squirrel and, six of the specimens (duodenum) from hamster were collected and fixed in 10% neutral buffered formalin. The specimen passed through of series of ethyl alcohol ascending (70, 80, 90 and 100%) embedded in the paraffin wax and cut blocks of 5 micrometers thickness, and stained with H&E, PAS, AB-PAS and Toluidine blue. Then, that done take of the photographed for sections the using a compound light microscope with the camera (9,10)

Statistical Analysis

The Statistical Analysis System The data were using the SPSS program (version 16). One-way ANOVA was applied to determine significant differences, followed by the Least Significant Difference (LSD) test to identify the minimum significant difference. The independent t-test was used. Results were expressed as Mean \pm Standard Error (SE), and differences were considered statistically significant at $P \leq 0.05$.

Results and Discussion

The results observed during the microscopic examination of the male persain squirrel and hamster Syria showed that it consists of four tissue layers, starting from the innermost layer directed toward the lumen is the mucosa, followed by the submucosa, then the muscularis, and finally the outer layer is the serosa in all such as (10). (Fig.1)

Mucosa

Microscopic examination of the duodenum in both squirrels and hamsters showed no difference in the layers comprising the mucosa, which consists of three layers: epithelium, lamina propria, and muscularis mucosa. The epithelial layer, which is the first layer of the mucosa, faces the lumen and contains villi. Microscopic examination of the duodenum in both squirrels and hamsters revealed a similarity in the general structure of the mucosal layer, which consisted of the three main layers: the epithelium, the lamina propria, and the muscularis mucosae. This conserved histological organization between the two species reflects the importance of these layers in performing the fundamental functions of the small intestine, namely protection, absorption, and secretion. This finding is consistent with the description provided by (11) in a previous study on rodents (12,13) (Fig.1).

These villi consist of a group of columnar cells called absorptive cells, which contain a round or oval nucleus and clear cytoplasm. Their apical surface is covered with microvilli (forming brush border). Goblet cells are interspersed between the absorptive cells they have a typical goblet-shaped appearance with their theca (containing mucinogen granules) and an oval, basally located nucleus finding in (13) (Fig 3).

The apical surface of the absorptive cells was lined with a distinct brush border, representing dense microvilli that significantly increase the absorptive surface area this his study is consistent with (13), who pointed out present at surface villi

brush border. This structural adaptation was observed in both species, indicating a high absorptive efficiency regardless of the difference in villus shape. As for the goblet cells, they were regularly distributed among the absorptive cells, characterized by basally located nuclei and mucinogen granules in the apical cytoplasm. This typical cellular arrangement allows for efficient synthesis, storage, and release of mucus) this his study is consistent with (14). The high frequency of these cells in the duodenum reflects the essential role of mucus in protecting the intestinal lining from the acidic chyme entering from the stomach (Fig.3)

A strong positive reaction was observed in squirrels when PAS dye was used on Goblet cells, resulting in a dark purple color. This indicates that the Goblet cell reserves in squirrels contained large amounts of neutral mucins. However, the situation was slightly different in hamsters, where only a pink color appeared. The reason for this is that the reserves of contained a small amount of acid mucins in hamsters and consistent with (14) (Fig.5).

Use AB-PAS stain the appearance of purple color in the Goblet cells in the duodenum is evidence that the mucus is neutral and that the reaction is positive because this dye reacts with the sugar compounds of neutral mucus, giving it this color. This statement is consistent with (15) (Fig.6)

It was observed that when toluidine blue stain was used, granular cells-stained blue appeared in the duodenum of the squirrel, whereas appear blue color all of crypts of Lieberkühn the same stain was applied in the hamster (Fig.7) because Toluidine blue staining appeared clearly defined metachromatic granules within the Paneth cells of the squirrel, comparable to the findings described in rabbits by (16). But in the hamster samples showed little to no metachromatic response, suggesting interspecies differences in the chemical nature of the secretory granules of Paneth cells this study is consistent with (17).

Moreover, the statistically significant increase in both the tunica muscularis externa and the submucosa in the hamster (60 μm) compared with the squirrel (35 μm) likely represents an integrated mechanical propulsion system. This enhancement may compensate for lower nutrient density by increasing mixing efficiency and propulsive force. Collectively, these morphometric differences should not be regarded as incidental anatomical variations; rather, they represent an evolutionary signature reflecting the balance between the energetic cost of tissue development and the nutritional return obtained from the environment.

The statistical results for both the Caucasian squirrel and the Syrian hamster showed of the average mucosa layer of the Caucasian squirrel was 1900 ± 133.4 lesser than that recorded by in Syrian hamster 2050 ± 24.4 .

submucosal

The results microscopic examination that the submucosal layer, located beneath the mucosa, contains collagen fibers, dense connective tissue, with a dense presence of Brunner's glands in the proximal duodenum, gradually decreasing toward the distal duodenum. Brunner gland is compound tubular gland present in submucosa od duodenum show in Hematoxylin and Eosin stain light pink color in squirrel and pink dark in hamster

(18) (19). when used Masson's Trichrome show submucosa blue in squirrel and red and blue in hamster (Fig 2) (Fig 4)

The statistical results for both the Caucasian squirrel and the Syrian hamster showed that the average tunica submucosal of the Caucasian squirrel was 60 ± 6.12 larger than that recorded by in Syrian hamster 40 ± 6.12

muscular

The results microscopic examination that the muscular layer in both the squirrel and the hamster consists of two layers: an inner circular layer and an outer longitudinal layer. In muscularis layer is between outer longitudinal and line inner circular muscular and myenteric nerve plexus (Auerbach's plexus) was observed between the inner circular and outer longitudinal muscle layers in both species (20). (Fig 4) (Fig 8). Morphometric analysis revealed a slight difference in the thickness of the muscular layer between the two species, with the squirrel exhibiting a greater thickness than the hamster. However, this difference was not statistically significant. The thickness and organization of the muscular layer are proportional to the contractile activity required for peristalsis and the functional demands of the proximal duodenum, which may be influenced by the secretory activity of Brunner's glands consistent with (21). The statistical results for both the Caucasian squirrel and the Syrian hamster showed that the average tunica muscularis of the Caucasian squirrel was 105 ± 9.35 lesser than that recorded by in Syrian hamster 95 ± 14.5

serosa

appeared as a thin layer of simple squamous epithelium composed of flattened cells with a single flat nucleus. Beneath this epithelial layer, loose connective tissue containing blood vessels and collagen fibers was observed. No significant difference in the thickness of the serosal layer was detected between the Caucasian squirrel and the Syrian hamster (Fig. 1) (Fig 4) the finding in (22). The statistical results for both the Caucasian squirrel and the Syrian hamster showed that the average tunica serosa of the Caucasian squirrel was 40 ± 6.12 larger than that recorded by in Syrian hamster was 30 ± 5 .

Table 1. The histometric characteristics of the duodenum of the squirrel and hamster

animal	Mucosa	Submucosa	muscularis	Serosa
squirrel	1900 ± 133.4	60 ± 6.12	105 ± 9.35	40 ± 6.12
hamster	2050 ± 24.4	40 ± 6.12	95 ± 14.5	30 ± 5
T value	1.11	2.30	0.577	1.265
P value	0.299	0.049*	0.580	0.242

Results were expressed as Mean \pm Standard Error (SE), and differences were considered statistically significant at $P \leq 0.05^*$.

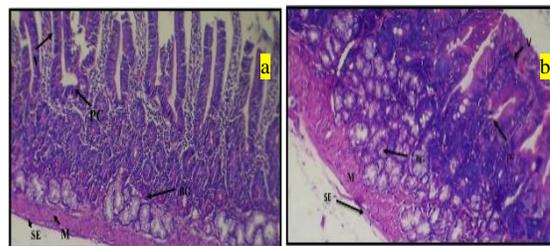


Figure 1. Cross section of proximal duodenum for the squirrel(a) and hamster (b) show: villi (V), Plicae circulares (PC), Brunner gland (BG), Muscularis(M) and Serosa (SE). H&E 10x

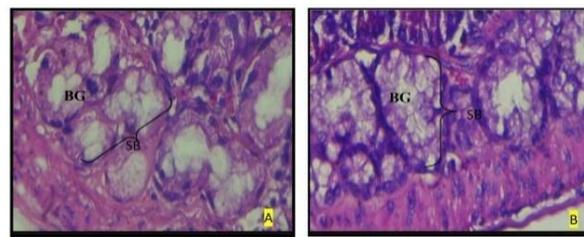


Figure 2. Cross section of duodenum for squirrel (a) and hamster show submucosa (SB) and Brunner gland (BG). 40x H&E

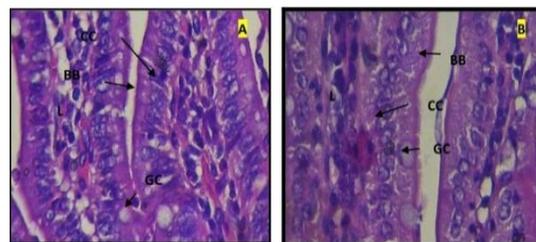


Figure 3. Cross longitudinal of villi for squirrel (a) and hamster(b) show: Columnar cell (CC), brush border (BB), Goblet cell (GC) and lacteal (L). 40x H&E

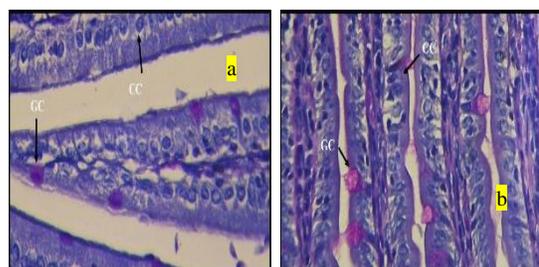


Figure 4. Cross section of the proximal duodenum for the squirrel (a) and hamster(b) show: Brunner's gland (BG), Submucosa (SM), Outer longitudinal muscular (LO), Inner circular muscular (IC) and Serosa (SE). Masson's Trichrome 10X

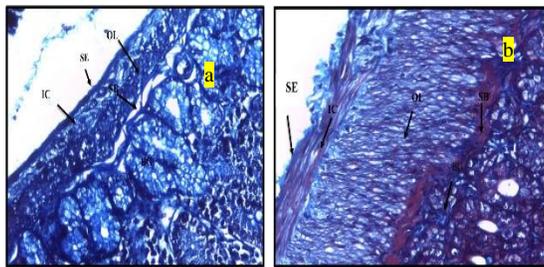


Figure 5. Cross section of the villi for squirrel (a) and hamster (b) show: Goblet cell (GC) and columnar cell (CC). PAS 40X

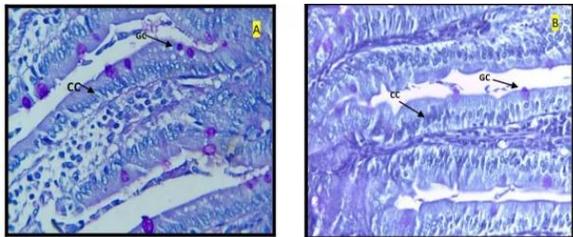


Figure 6. cross longitudinal of the villi for squirrel(a) and hamster (b) show: goblet cell (gc), columnar cell (cc) and lacteal (l). pas-ab 40x

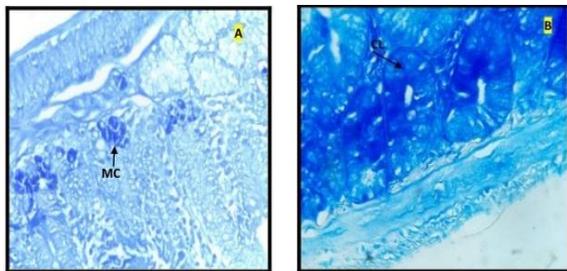


Figure 7. Cross longitudinal of duodenum for squirrel (a) and hamster (b) show: Crypts of Lieberkühn (CL). Toluidine blue 40x

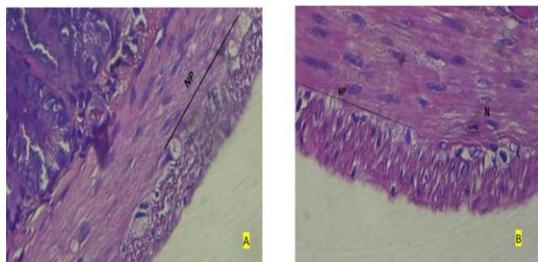


Figure 8. Cross section of jejunum for squirrel (a) and hamster (b) show: inner circular muscular (IC), outer longitudinal muscular (OL), nerve (N), nerve plexus (NP) and serosa (SE). H&E 10X

CONCLUSION

To sum up, our study shows that the duodenum in squirrels differs significantly from that in hamsters, both anatomically and functionally. These differences are mainly due to their different diets and lifestyles. Squirrels are better adapted for nuts and fibers, whereas hamsters have their own digestive adaptations. These results provide a better understanding of

how rodent digestive systems change based on their environment.

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