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Morphological, Histochemical and Ultrastructural studies on the Gallbladder of Tilapia Nilotica (Oreochromus Nilotica).

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ABSTRACT

This study was undertaken to study the anatomical features and morphologic peculiarities of the tilapia nilotica gallbladder, with special focus on its epithelium. Ten Specimens of sexually mature tilapia nilotica were collected and manually dissected. The gallbladders were anatomically examined and then removed, carefully separated. Sections were stained with different stains for histological, histochemical examination. The gross anatomical results revealed that the tilapia gallbladder was large sac and formed of narrow neck carried the two openings of cystic and bile ducts. The bile duct, opened in the ventral curvature of the duodenum. Histologically, the epithelium of tilapia gallbladder was simple columnar and formed of two types of light and dark cells. Ultrastructurally, both light and dark cells, nucleus spherical with prominent nucleolus with translucent face in light cells and abundant heterochromatin in dark cells, small Golgi apparatus stalks present juxtanuclear and extensive rough endoplasmic reticulum (rER) around nucleus and in basal part. The apical border showed irregular microvilli, the basal part showed irregularity in the basal lamina. Histochemically, the reaction to PAS; Alcian blue and best's carmin showing strong reaction with PAS stain at supranuclear part while the secretory granules in the apical part reacted strongly with Alcian blue and with Best's carmine stain, the most apical part of the cells gives strong positive reaction and become weaker in the supranuclear part.

Keywords: Gallbladder anatomy, Gallbladder epithelium, Tilapia Nilotica

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INTRODUCTION

In those animals which possess a gallbladder, the function is essentially the same in all species: to function as an accessory organ of the digestive tract in storing bile, concentrating the bile by removing water and exchanging electrolytes, actively secreting, modifying, and circulating the bile through the enterohepatic system, and affecting the digestive uptake of lipid soluble compounds. Thus, the gallbladder functions as a reservoir and mechanical pump **[1].**

In general, variability in gallbladder morphology is dependent mainly upon diet. Carnivores (except whales) invariably possess a gallbladder. The gallbladder may or may not be present in omnivores and herbivores **[2 and 3]**. In animals which eat only periodically, as well as in humans, the bile received from the liver becomes more concentrated and is stored for longer time periods than is the bile in vertebrates that eat frequently, such as guinea pigs **[4].** Some animals, such as pigeons, rats, and deer, which eat almost continuously, have no gallbladder; the constant flow of bile from the liver to the intestine is adequate for their digestive needs **[3 and 5].** Diet is not the only factor involved in the differences seen in gallbladder morphology.

The abundant literature on gallbladder epithelia strikingly shows that the early electron microscopical investigation by **[6]** on the carp (Cyprinus carpio, L.) is still the only one available on teleost gallbladder epithelial structure. Histochemical data are not known at all.

In this paper no particular information about the feeding conditions of the fishes and the season when the experiments were carried out are given. Recently it has been reported that fasting **[7 and 8]** or special diets **[7, 9 and 10]** induced distinct micro-morphological alterations in mouse gallbladder epithelial cells. With regard to possible seasonal variations of gallbladder epithelial cells, which might be expected especially in hibernating animals or animals with a hibernation-like winter rest, no information is given in the literature.

This study was undertaken to study the anatomical features and morphologic peculiarities of the tilapia nilotica gallbladder, with special focus on its epithelium.

MATERIAL AND METHODS

Specimens of tilpia nilotica (*Oreochromis niloticus*) were obtained from River Nile at Giza. A total of 10 male and female sexually mature Nile tilapia fish were collected over the period in August 2017. Fish were transported alive to the central lab of Cytology and Histology department, faculty of Veterinary Medicine Cairo University. Fish were physically examined to ensure that they were free from any pathological changes. Each Nile tilapia was weighted (500-750 gms) to ensure that all fish sexually mature as mentioned by **[11]**. Fish were opened from the right side for gross anatomical study of the gallbladder. After anatomical examination, the gallbladders were removed immediately after decapitation and sample were carefully separated of 1 cubic cm. Sections were taken quickly fixed in neutral buffered formalin for about 24hs., also Bouin's fluid, Susa and Zenker's formol were used for 12hs. The use of different methods of fixations was experienced in order to reach the proper fixative of choice. After proper time of fixation for each fixative the samples were dehydrated, embedded in paraffin and following that sections were cut at 5-6 µm.

Section stained with Harris haematoxylin and eosin for general histological examination**;** Crossmon's method for demonstration of collagen fibers**,** Periodic acid Schiff - Alcian blue (PH 2.5) combination for identification and differentiation of both neutral and acid mucopolysaccharides and Best's Carmin for identification of glycogen **[12].**

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RESULTS

Gross anatomical findings

The liver and the gallbladder (Plate 1)

The liver of tilapia was large located just behind the transverse septum in the anterior part of the peritoneal cavity. The liver was reddish light brown in color and divided by faint incomplete fissure into 2 lobes; The right and the left lobes **(plate 1/B&C)**.

The right lobe was smaller than the left one and was vertically situated with 1.4 cm width and 2.9 cm height. The left lobe was longitudinally situated with 6.8 cm length, its cranial end was wide with 2.3 cm width and its caudal end became narrower and cylindrical with o.8 cm width **(plate 1/A).**

Tilapia gallbladder was oval hollow sac with film like thin wall distended with bile that gave it characteristic greenish coloration. The gallbladder body width was 1.6 cm while its entire height was 2.45 cm. The gallbladder cranial border was at the level of the origin of pectoral fin **(plate 1/A)**.

The gallbladder situated between the visceral surface of the left lobe of liver ventrally, the visceral surface of the right lobe of liver cranially, the duodenum dorsally and its right side and the stomach caudally **(plate 1/A, B&C)**.

The gallbladder formed of narrow neck carried the two openings of cystic and bile ducts. The gall bladder dilated distally to form the wide body and rest on the fundus which is the most cranial part of the gall bladder.

Accessory ducts of the tilapia liver and gallbladder showed the hepatic biliary ducts in all examined specimens, a right biliary duct arose from the right lobe of liver. Eight examined fishes showed 2 left hepatic biliary ducts derived from the left lobe of liver while the other 2 had single left hepatic biliary duct. The right and left hepatic biliary ducts united to form a common hepatic biliary duct on the craniodorsal border of the gallbladder that passed caudally to open in cystic duct on the dorsal surface of the gallbladder neck. The bile duct, arose from the dorsal surface of the bladder near its neck and opened in the medial surface of the ventral curvature of the duodenum where the duodenum appeared wider in diameter than other intestinal loop **(plate 1/D&E)**.

Histological, Histochemical and Ultrastructural findings

In tilapia, light microscopy investigation of gall bladder in different state of empty and extended shows the surface epithelium in Semithin section examination of distended tilapia gallbladder appear wide large diameter lumen, surface epithelium low columnar cells, with vesicular spherical to oval nucleus has prominent nucleolus **(fig 1).** Propria submucosa is highly cellular connective tissues. Meanwhile, examination of empty gallbladder semithin section reveals large diameter lumen with small bulging folds covered with high columnar epithelium with clear brush border **(fig 2).** Propria submucosa layer appeared to occupy significant width of the wall of the gallbladder and was made of loose connective tissue contained slender fibrocyts and smooth muscle fibers. External muscular layer is made of intertwined smooth muscle bundles arranged from circular to oblique. The outermost subserosal layer is mainly composed of flat mesothelium **(fig 3).**

In further observation of the epithelial surface of distended status the simple columnar epithelium appears heavily contrasted with Toluidine blue than other adjacent tissue of its wall. The apical part of the cells appears darkly contrasted by presence of heterogenous, densely stained granules in the supra nuclear regions **(fig 1).**

The apical part of the columnar epithelium of the gallbladder either in distended or empty status has long pointed fuzzy surface extensions **(fig 1& 2).** Most of the supranuclear regions appears either empty or filled with small pale green granules that gave poorly stained apical parts of the cells **(fig. 2).**

The columnar epithelial lining of Tilapia gallbladder represented two types of epithelial light and dark cells **(fig 4).** Frequently, a third cells of migrating blood cells lymphocytes, monocytes and macrophage seen in the base or in between the columnar cells **(fig 7)**.

The columnar cells were tightly adhered with continuity of the apical part in light microscopic examination with apparent dilated space between their basolateral region **(fig 5).**

In some sections, the epithelia of tilapia gallbladder showed a balloon like cells with faint stain and vacuolated parts with basally-located nucleus, these cells found mainly between principle columnar lining epithelia **(fig 6).**
Ultrastructural, the light and dark cells are differing in the density of cytoplasm but not in other fine structural details. The apical border showed irregular microvilli, the basal part showed irregularity in the basal lamina that extended between cells **(fig 8).** The subepithelial fibrous connective tissue with fibrocytes formed the inner fibrous layer and externally appear endothelium of blood vessels **(fig 9).**

TME of light cells shows translucent nuclei with apical part packed with dark granules protruding markedly the free surface, also it has cytoplasmic protrusions in the lumen bounded by plasma membrane and contain cytoplasm and others protrusion appeared with no content **(fig 10).**

General aspects of epithelium of gallbladder of tilapia, in both light and dark cells, nucleus spherical with prominent nucleolus with translucent face in light cells and abundant heterochromatin in dark cells, small Golgi apparatus stalks present juxtanuclear and extensive rough endoplasmic reticulum (rER) around nucleus and in basal part **(fig 11).** Few Smooth endoplasmic reticulum and glycogen granules noticed in apical part.

The epithelial cell of gallbladder of Tilapia connected apically by desmosomes and frequently seen in lateral sides of cells and more distal position an extensive lateral surface interdigitation is well recognized **(fig 10 & 11).**

Cytoplasm of epithelial cells of gallbladder of Tilapia is filled with numerous populations of mucus rich vesicles, that differentiated by their either tightly granular or finely fibrillar content, appearing somewhat dense than adjacent cytoplasm **(fig 11).** Remarkable vacuole of different size was present in the apical part. Amid the granules rare but small, elongated to spherical mitochondria can be seen.

In the TEM, few dense bodies are also observed with heterogenous electron dense matrix, those present in the basal part of the cells exhibit electron dense core and translucent periphery resembled multivesicular bodies **(fig 11).**

Histochemically, epithelium of gallbladder of Tilapia reacted strongly with PAS stain in the supranuclear part **(fig 12).** Meanwhile, the balloon-like vacuolated cells which appear between epithelial cells of gallbladder are reacting faintly to PAS (**fig 13).** The secretory granules in the apical part reacted strongly with Alcian blue and the degree of reaction was more condensed in the superficial layer of cells **(fig 14).** With Best's carmine stain, the most apical part of the cells gives strong positive reaction and become weaker in the supranuclear part **(fig 15).**

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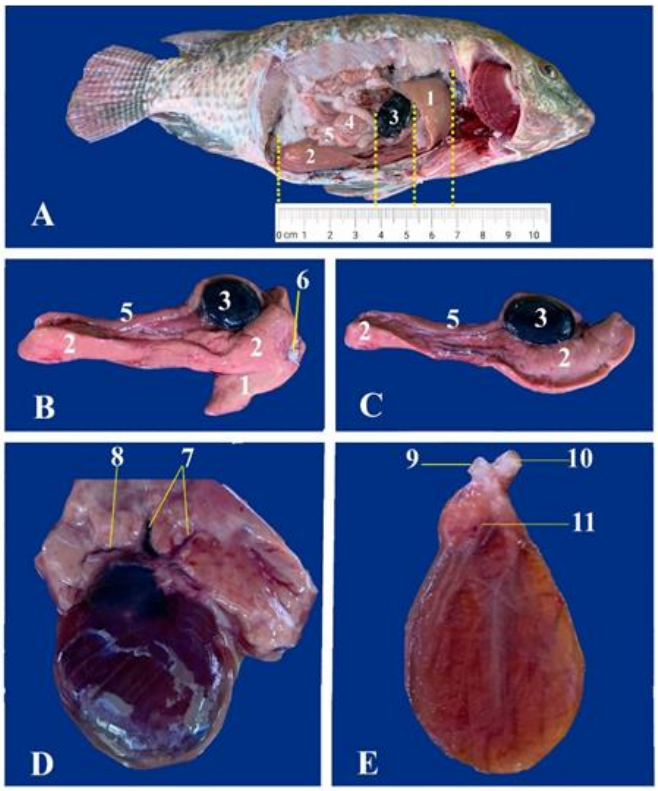


Plate 1: A, photograph showing the liver and the gall bladder dimensions in Tilapia, right side **B,** photograph showing the parietal surface of the liver and the gall bladder **C,** photograph showing the visceral surface of the liver and the gall bladder **D,** photograph showing the hepatic biliary ducts and the common hepatic duct and **E,** photograph showing the cystic duct and the bile duct. **1,** right lobe of liver **2,** left lobe of liver **3,** gall bladder **4,** stomach **5,** duodenum **6,** transverse septum **7,** hepatic ducts **8,** common hepatic duct **9,** cystic duct **10,** bile duct **11,** neck of the gall bladder.

List of Figure

ISSN: 0975-8585

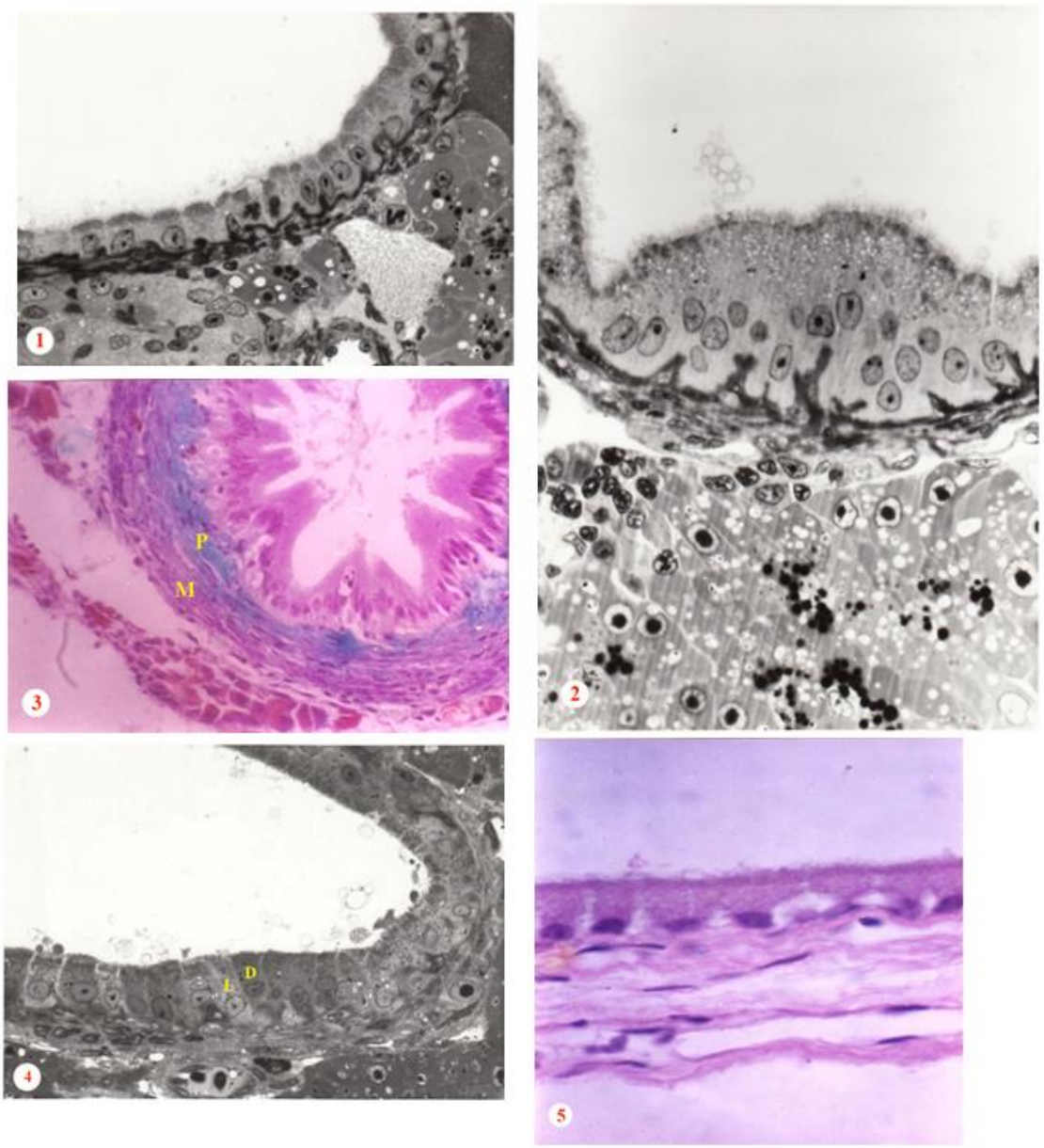
Fig (11): TEM of tilapia gallbladder showing cells connected apically by zonula occludents, (arrow head) desmosomes frequently seen laterally & the lateral surface extensively interdigited, (arrow) notice the rough endoplasmic reticulum (r) few stalks of Golgi complex (G) isolated cisternae of rER, large vacuoles (V) & few dense bodies. (Lead acetate- urnyl citrate 8000X).

Fig. (12): Photomicrograph of tilapia gall bladder showing supranuclear strong PAS positive materials that appeared to be pinched off from the apical surface. (PAS – 800X).

Fig (13): Tilapia gallbladder showing the balloon vacuolated cells (B) with faint PAS reaction (PAS – 1024X).

Fig. (14): Tilapia gallbladder epithelium showing strong supranuclear alcian blue +ve reaction which become more condensed in the superficial layer. (AB 1024X).

Fig. (15): Tilapia gallbladder epithelium showing deep Best\s carmin reaction in the most apical part of the cells and weak reaction at the supranuclear position. (best's carmin 1024X).



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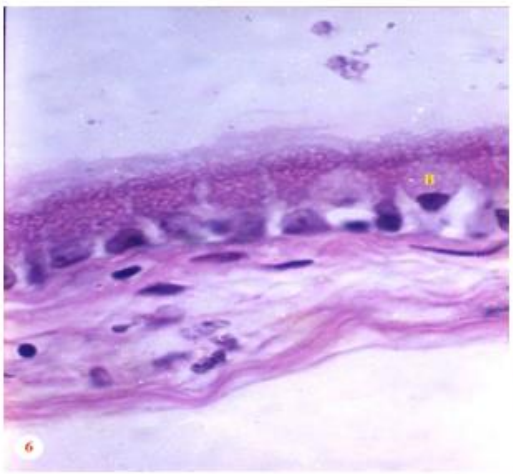

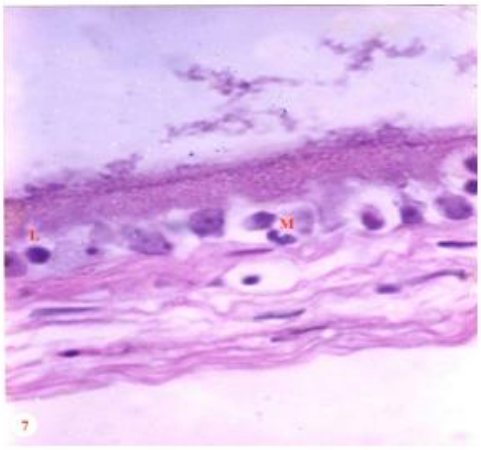

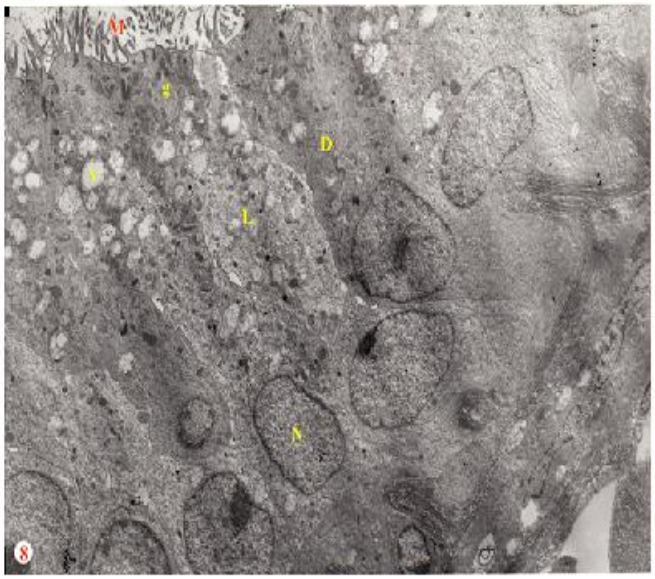

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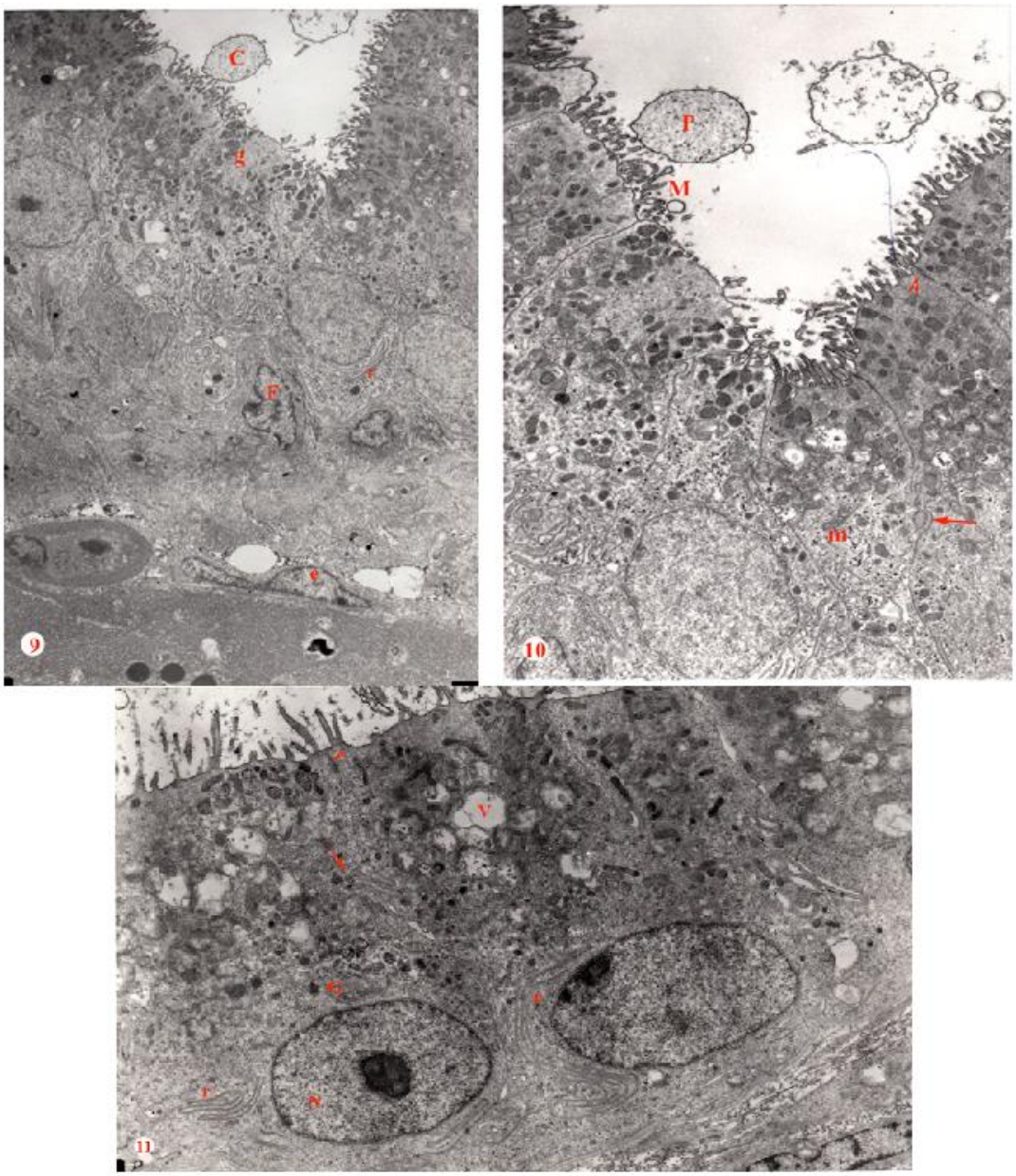
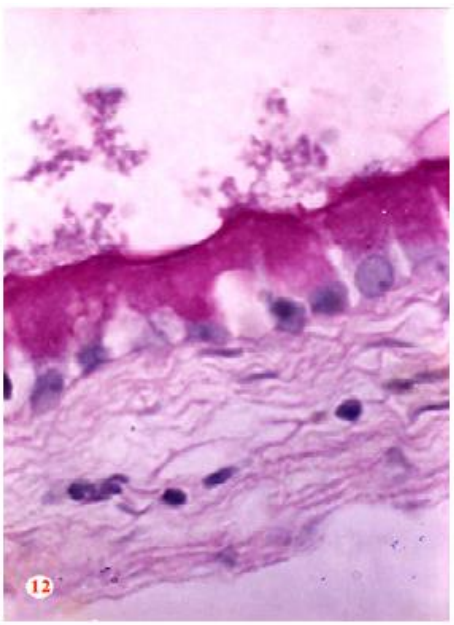
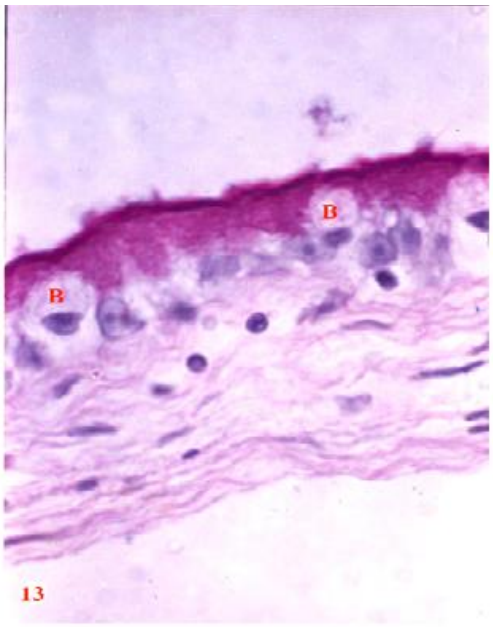
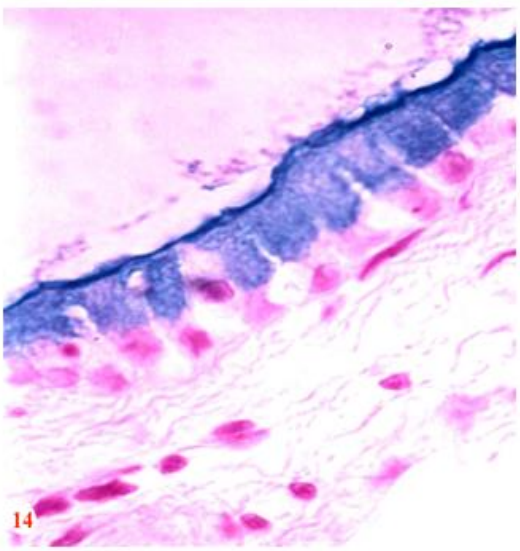
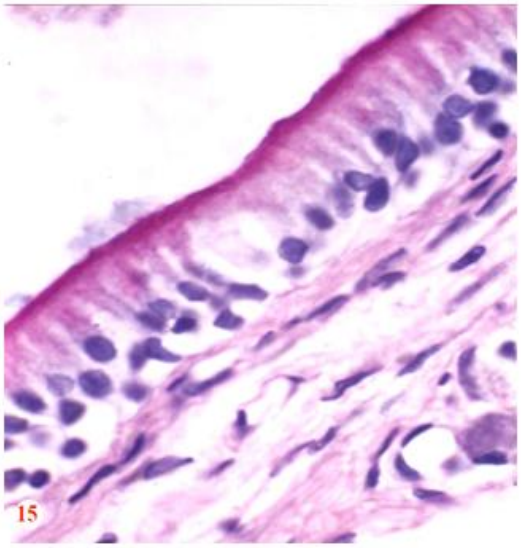


ISSN: 0975-8585









DISCUSSION

The present study showed that the liver of tilapia was reddish brown in color and longitudinally located in the cranial part of the abdominal cavity as found in catfish. The color of the liver differentiated according to the type of fish, reddish in carnivorous fish and light brown in herbivories fish depending on the amount of glycogen and fat content **[13]** while **[14]** in tilapia nilotica reported that the liver was dark brown in color.

Our results revealed that the liver of tilapia was divided by faint fissure into right small lobe and left large lobe similar to the findings appeared with **[14]** in Nile perch and **[15]** in the carnivorous fish; the brown spotted groups. On the other hand, **[16]** mentioned that the right lobe was larger and bigger than the left lobe in the *Rippon Barbell Barbus* fish while **[13]** added the presence of two accessory lobes as a subdivision of each hepatic lobes in catfish.

Carol, [17] in Nile tilapia, stated that the gallbladder was oval film like well distended with bile as mentioned by **[15]** in carnivorous fish **[14]** added that the gallbladder was elongated oval and contained a blue-green bile in catfish dissimilar to **[13]** who declared that the gall bladder was pear-shaped in catfish.

Our results revealed that in tilapia, the two hepatic ducts were united to form common hepatic duct where the bile was transmitted from the liver to the gallbladder via cystic duct. The above mentioned ducts were described and showed the same pattern by **[13, 19 and 20]** in the catfish but they didn't mention the common hepatic duct. While **[21]** in common carp used the MRI, fat-suppressed pulse sequence technique to differentiate between the fluid filled structures (gall bladder and ducts; hepatic, common hepatic, cystic and bile ducts) and the surroundings (liver and spleen).

The bile duct opened at the medial surface of the ventral curvature of the duodenum in the present study, similar to our findings in carnivorous fish, brown-spotted grouper, **[15]**, in catfish, **[22, 13]** and in zebra fish, **[23]**. While **[14]** in Nile perch added that the bile duct was attached to the liver and **[24]** in A. nigrofasciata asserted that the bile and pancreatic ducts opened together in the intestinal loop.

In the present study the tilapia gallbladder lined by Columnar epithelium, this was in accordance with most of studies investigated gallbladder epithelium **[25, 26, 27, 28 and 29]. Gilloteaux [29]** described that in some view falsely appeared pseudostratified as results or presences of some basal cells above the basal lamina. In some textbooks, the epithelium was described as composed of "transitional" epithelium, and this description was generally true and widely used to description of gallbladder epithelium of fish. While there may be some species differences, it seemed more likely that the miss description of the epithelium as "transitional" come from viewing oblique section of empty gallbladder at the mucosal fold. This might give columnar epithelium the appearance of being "transitional".

A light and dark gall bladder cell, as seen in the tilapia has been described since the beginning of the 19th century **[30, 31, 32, 33, 34 and 26].** Electron microscopically they had been detected in carp and Frog **[6],** rabbit **[35 and 36],** mouse **[35 and 37],** guinea pig **[35 and 38],** cat **[35],** sheep **[39],** electric Ray **[29]** as earlier described by **[32 and 34]** this was a transition stage, there should be speculated that the light and dark cells were in different physiological states. **Aschoff**, **[32],** considered the dark cells as degenerated cells. Furthermore, **[34]** surmised they could be exhausted cells, it might be at the beginning of a new secretion, because he never saw them dropped off into the gallbladder lumen. Many years later, both theories were combined by **[40],** he completely agreed with **[34]** but he also noted, that few dark cells could be degenerated epithelial cells. After many years **[26],** described that within the dark cells two types must be distinguished: normal less hydrated epithelial cells and degenerating cells. The degenerated cells be eliminated from the epithelium by monocytes and macrophages, which possibly be consistent with the basal regenerative cells of earlier investigations. As they were mainly present near the basement membrane, a hypothesis was put onward that they did not entered the gallbladder lumen, but after phagocytosis migrate back into the subepithelial connective tissue. Light cells were specialized for absorption of water and dissolved bile salts and for secretion of mucous.

Many electron microscopical investigations became with antithetical results. So, the dark cells had been explicated as cells with reduced **[41]** or enhanced **[37 and 46]** metabolic activity, as a less differentiated cell-type occupy intermediate way from an undifferentiated basal cell to an ordinary light epithelial cell **[6, 35 and 42]**, as a less hydrated cell **[36]**, or even as an artifact induced by aldehyde fixation **[43].** In addition, in the guinea pig the endoplasmic reticulum was said to be better developed in the dark cells than in the light cells **[38].**

Both in rainbow trout and in tenches two types of dark cells can be distinguished. dark cells differ only in the density of their cytoplasm and their karyoplasms from the light cells. Moreover, dark cells with degenerative alterations are also seen **[27].**

Cytoplasmic protrusions of gallbladder epithelial cells had been described in various animals. **Togari, [44]** described in teleosts cytoplasmic protrusion as "secretory droplets" accumulating in the apical portion of gallbladder epithelial cells, protruding markedly the free surface and finally being liberate into the lumen together with the prominent portion of the cell. **Viehberger, [26]** in trout and tench described that the protrusions extending from the apical region contains no secretory material. Therefore, the term "secretion" should be avoided for the process. In the present study the cytoplasmic protrusions appeared as bulging from apical surface bounded by plasma membrane contain cytoplasm and no organelles this come in accordance with

what was described by **[26]** in trout, who further added in the tench that the protrusion were contain glycogen granules. Meanwhile, **[29]** in Electric Ray described that hemispherical-shaped bulging apices detached and rich of mucinous content which appeared poorly contrast with toluidine blue. Viehberger's conclusion was that cells release degenerated cytoplasm into the lumen of the organ as part of the regeneration process; perhaps this loss was counterbalance by synthesis of replacement material.

Our results showed that the free surface of the epithelial cells in the gallbladder in tilapia was characterized by well-developed microvilli irregularly arranged. In contrary, in the tench they were regularly arranged, but form a true brush consisting of regularly aligned microvilli in the rainbow trout **[26]**. A brush border consisted of irregularly aligned microvilli as seen in the tilapia had not been reported in literature. The brush border of the principle gallbladder epithelial cells of the Tilapia may be related to the feeding habits of the animal. However, the abundant literature on gallbladder epithelia of various mammalian species did not allowed a correlation in the respect. Meanwhile, **[45]** described that the apical surfaces of the cells of biliary epithelia bear numerous microvilli, which were reflective of their absorptive nature, refereed by their structural features, it was apparent that the cells of the bile ducts of the larval lamprey were probably active in fluid transport **[46]**. The tilapia apical surface was heavily populated with microvilli, signifying a high rate of uptake, and intercellular spaces were wide, indicating that flow rates were high. the functional length of the intercellular channel was increased, to a moderate degree, by the presence of lateral interdigitation. These intercellular spaces may be more than simply sites of cell attachments and ionic flux.

Bader, [6] described that a regenerative process in the carp gallbladder where undifferentiated cells at the base of the epithelium differentiate into epithelia cells proper. As the differentiation proceeded, the cells passed from small and dark to dark, normal-sized forms; then to light epithelial cells. **Viehberger, [27]** did not note this progression in the tench or trout gallbladder. He also concluded that the "basal" cells described by **[6]** were phagocytic cells that functioned to remove degenerated epithelium, our results support this hypothesis for presence of macrophage in between or basal to the epithelial cell. **Viehberger, [27]** emphasized that in Rainbow trout as well as tench, numerous lymphocytes, monocytes and macrophages occurred at the ridge of the mucosal folds, where degenerative alterations were seen. So, it looked that degenerated epithelial cells were removed by phagocyting monocytes and macrophages. Possibly, these cells leave epithelium not into the lumen but the basement membrane into the connective tissue, because they were mainly founded on the basement membrane.

In the present study there was a lateral space between epithelial cells that was might exhibit transport of fluid as earlier described that in fish, rabbit and man, gallbladder cells were water transporting epithelia that modified the concentration of bile by active removal of sodium and three anions: chloride, bromide, and bicarbonate, along with water **[8].** Fluids and electrolytes might then be transported into intercellular spaces which act as cell compartments for making of an upright osmotic gradient **[47].** These lateral intercellular spaces were of variable width depending upon the rate of fluid movement **[41, 36 and 47]** and the lateral plasma membranes form complex interdigitations with adjacent cells **[47].**

Bile was highly alkaline, and the strict environment in the lumen of the bladder might require a high turnover of the lining epithelial cells, and if that was the case, the separation might be considered normal. However, **[27]** stated that rainbow trout and tench (as well as human and laboratory rodents) had a slow degradation and turnover of the gallbladder epithelium, so if rapid turnover of the epithelium occurs in the mollie, it differed somewhat from other species in this respect. The investigation in this study did not show large numbers of mitotic figures, which would be predicted if turnover were high, as in intestinal epithelium, that was not support or proved the high turnover.

Previously it was reported the lateral and the basal separation of intestinal epithelium in mollie **[48]**, and lateral separation of gallbladder epithelium could be observed in micrographs of the channel catfish gallbladder in **[25]** atlas, although the authors didn't comment specifically on it. **[6]** had described slight separation in the carp gall bladder cells, with detached lateral interdigitating projecting into the intercellular space as finger – like structures.

Sidon, [45] mentioned that the intercellular spaces containing prominent brush border in lampery. Vacuole-like intracytoplasmic cisternae formed a peripheral network within the cells and were confluent with intercellular spaces at the site of numerous pores in the lateral plasma membranes.

We observed a slight separation of gall bladder epithelial cells in tilapia in this study. That epithelial separation has been observed in several species by several independent investigators makes it unlikely that it was an artifact, but it's difficult to see what function might serve. Basolateral separation of cells in columnar epithelial sheets in both the intestine **[48]** and the gall bladder in the mollie digestive tract may indicated some sort of species-specific variation of the phenomenon.

The present work revealed some large balloon -like cell with well-defined borders present between the gall bladder epithelial cells. These cells contained dark stained nuclei that occupy one pole of the cell. These cells may be resembled to the rodlet cell that described by **[49]** in angel fish and **[51]** in freshwater bream, who found it in the collecting tubule only. These cells were faint reacted to PAS. This result appeared in agreement to that described by **[49],** who emphasized that the rodlet cell produce secretory material of proteinceous nature. The same suggestion offered by **[51]** in bluegill fish. Finally, we did not conclude much about the task of these strange cells and in our opinion a comprehensive study is needed to clarify the exact nature and function of such cells.

The strong histochemical reactivity with bests Carmin indicate glycogen bodies in the apical part of gall bladder epithelia of Tilapia and it have never been reported before in any gallbladder epithelium. Possibly, the ergastoplasmic Nebenkern-formations in carp gallbladder epithelial cells **[6]** correspond with the glycogen bodies in the tilapia. Nevertheless, differently from the findings in the tenth, the smooth membranes in the carp were described without any reference to glycogen. Glycogen bodies are known from several other tissues, but their function is still not clear. A role in glycogen metabolism is presumed **[52, 53 and 54]**. These morphological findings, however, indicate differences of the gallbladder epithelia regarding cell biological processes (e.g. absorptive rate, glycoprotein and glycogen metabolism). The final question of a possible relation of these morphological distinctions to the various feeding habits of the omnivorous tilapia cannot be answered at the present, but it should be taken into account in further investigations on teleost gallbladder epithelia.

CONCLUSION

In conclusion, this morphological findings of tilapia gallbladder described peculiarities epithelium with regards to the cell biological processes; absorption and secretion. The important question, is there was any possible correlation between epithelium traffic and feeding habit in tilapia? The answer of this question should be considered in further investigations to analyze the different secretory materials and fluid movement across the epithelium.

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