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Original Article

Histomorphological, histochemical, and ultrastructural studies on the stomach of the adult African catfish *(Clarias gariepinus)*

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ABSTRACT

This study investigated the morphology, histochemistry, and ultrastructure of the adult African catfish *(Clarias gariepinus)* stomach in order to detect the functional aspects involved in gross and histological studies to get detailed information about the precise cellular structures of different cells lining the stomach. Forty fishes were used in this study. The stomach is a J-shaped sac divided into three regions: (1) cardiac; (2) fundic; and (3) pyloric. Histologically, its wall is composed of four tunics: (1) mucosa; (2) submucosa; (3) muscularis externa; and (4) serosa. The mucosa of the three portions showed thick longitudinal folds lined with simple high columnar cells containing oval basally located nuclei. These cells contained apically located mucosubstances that reacted positively with Periodic–acid Schiff and negatively with Alcian blue stains. Many gastric pits were formed by invaginations of the mucosal layer into the underlying lamina propria and continuous with the openings of the gastric glands. Only the cardiac and fundic regions contained mucosal glands. The fundic glands were lined with oxynticopeptic cells. Enteroendocrine cells were distributed in the gastric wall within the epithelial cells of the gastric mucosa and gland. The lamina propria composed of extensive collagen fibers, many blood vessels, and nerves. Strands of smooth muscle fibers situated between the lamina propria and the submucosa forming lamina muscularis mucosa. Loose connective tissue was the main component of the tunica submucosa. The pyloric portion had the thickest musclosa and the serosal coat of the stomach was formed of loose connective tissue containing blood vessels.

1. Introduction

African catfish (*Clarias gariepinus)* is widely distributed in Africa and parts of Asia (Syria and South Turkey). Its main habitats are calm lakes, rivers, and swamps in areas that flood on a seasonal basis [12].

The digestive system of fishes has remarkable diversity in its morphological structures and functions; this diversity may be related to taxonomy and feeding habits [1]. The feeding activities of fishes are classified into three categories according to the nature of the food consumed by all fish species: (1) herbivores fish that eat plant material; (2) carnivores that consume animal material; and (3) omnivores fish that consume both plant and animal materials [16]. In addition, Grau et al [22] stated that the main

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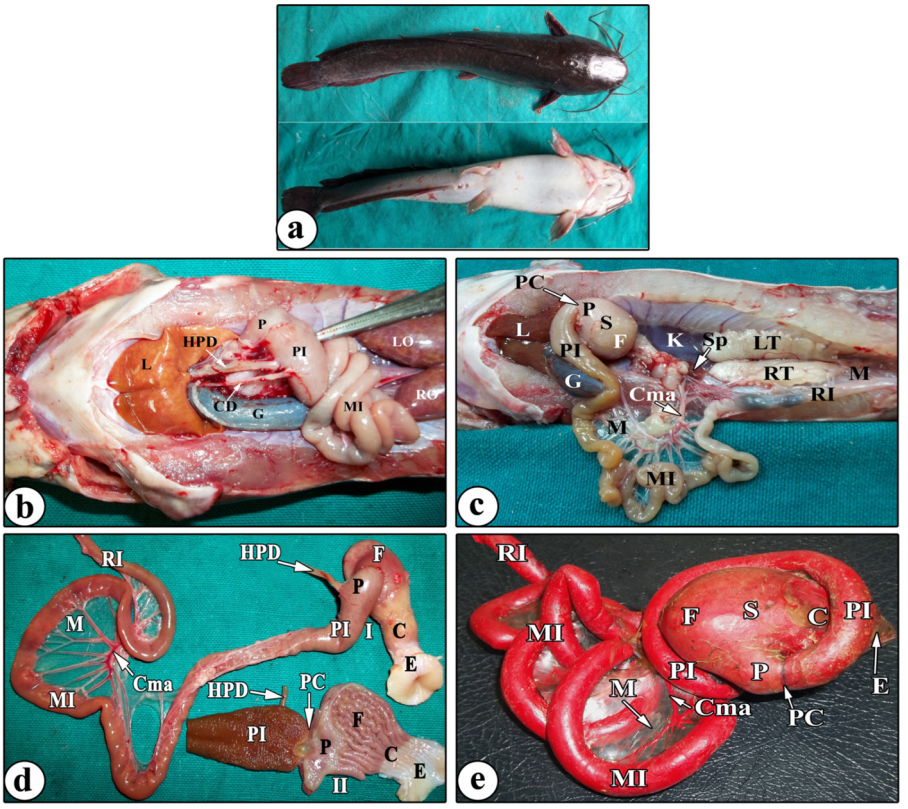

Fig. 1. Gross dissection of the African catfish (*Clarias gariepinus*): (a) dorsal and ventral views of the external features of the fish used in the present investigation; (b, c) contents of the body cavity and divisions of the digestive tract; (d) divisions of the digestive tract outside the body cavity showing the outer structures (I) and the internal structures (II) of the stomach; (e) epoxy corrosion casting of the ventral aspect of the gastrointestinal tract. Cardiac part of the stomach (C), cranial mesenteric artery (Cma), esophagus (E), fundic part of the stomach (F), gall bladder (G), hepato-pancreatic duct (HPD), kidney (K), liver (L), left ovary (LO), left testicle (LT), mesentery of the intestine (M), middle intestine (MI), pylorus (P), pyloric constriction (PC), pyloric intestine (PI), rectal intestine (RI), right ovary (RO), right testicle (RT), spleen (Sp), and stomach (S).

features of the digestive tract of carnivorous fish are the presence of a large stomach and a short intestine. The stomach of fishes is divided into many categories depending on their shape: (1) no stomach as in Cryprinids and Labrids; (2) J-shape in African and American catfish; (3) straight with enlarged lumen in pike; (4) channel catfish and halibut; (5) U-shaped in salmonide; and (6) Y-shaped in tilapia [45].

The approach to the histological structures of fish digestive tracts resides in the application of this knowledge in understanding the pathology of fish diseases, contributing to the development of fish farming, and a rational use of the natural resources [34]. Moreover, knowledge about the structure of the digestive system in various fish species is useful for nutritional development researches and preparation of diets [43].

Even though investigations of most teleosts have already been reported, there is a dearth of information about the histochemistry and ultrastructure of the stomach in African catfish from the Nile River. In this context, the aim of the present work was to illustrate the morphology, histology, histochemistry, and ultrastructure of the stomach of the African catfish. These observations will provide a basis for understanding the digestive physiology and help pathologists and nutritionists in future studies on diet and diseases affecting the species by facilitating the histopathological diagnosis of such diseases affecting fish digestive system.

2. Materials and methods

Freshwater African catfish *C. gariepinus* (Class: Actinopterygii, Order: Siluriformes, Family: Clariidae) of both sexes were used in the current investigation (Figure 1A).

2.1. Collection of the samples

A total number of 40 apparently healthy African catfish adults, ranging from 20 cm to 30 cm in length and from 500 g to 700 g body weight were caught alive from the Nile River at different localities in Beni-Suef Governorate, Egypt. They were transported in plastic aquaria to the laboratory within 2 hours to allow aerial respiration.

2.2. Gross examination

Fifteen fishes were used to demonstrate the gross morphological features of their stomachs. The fishes were sacrificed and a ventral incision was made from the anal opening to the interbranchial membrane. The stomachs

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were examined *in situ* and carefully dissected. Lengths and diameters of the obtained stomachs were measured using a caliber and photographed using a digital camera (Kodak, 12 MP Model: M530 Made in China, 4× optical zoom).

2.2.1. For corrosion casting specimen

The digestive tube was carefully removed outside the body and a canula was fixed in the esophagus. The prepared epoxy (2 parts of polymer A and 1 part of hardener B) was then thoroughly mixed and colored red using rotring ink was injected. The obtained specimen was kept at room temperature for 24 hours to allow curing of the cast, after which it was kept for 3 days in a 3% KOH solution for corrosion.

2.3. Histological and ultrastructural studies

Immediately after fish euthanasia, fresh specimens were carefully collected from the cardiac, fundic, and pyloric regions of the stomach.

2.3.1. Light microscopy

Fifteen fishes were used for the light microscopy. Small pieces of 0.5 cm × 0.5 cm from different portions of the stomach were fixed by immersion in aqueous Bouin's fluid (Bouin's solution has been prepared in the laboratory by the authors: picric acid (Suvchem laboratories chemicals, Mumbai, India e.mail: info@suvchem.com web: www.suvchem.com). Formalin (Biofed pharma industries, Made in Egypt) 3- Glacial acetic acid (Bichem for laboratory fine chemicals, Made in Egypt)) for 24 hours, then dehydrated using ascending grades of ethanol (50–100%), cleared in xylene, embedded in Paraplast (Suvchem laboratories chemicals, Mumbai, India. e.mail: info@suvchem.com, web: www.suvchem.com), sectioned at a thickness of 5 μm, and mounted on clean dry glass slides. The sections were stained with Harris' hematoxylin and eosin (Harris' hematoxylin and Eosin Haematoxylin (Fluka AG, Buchs SG, Made in Switzerland) 2- Eosin yellow Batch No.: 2999 Code No.: E.036688 Made in india Oxford Laboratory Mobile: 400 002 (India) E.mail: oxfordlabchem@Vsnl.net). Some sections were stained with Periodic–acid Schiff reagent (PAS) (Labochemie PTV. LTD, PB No. 2042, Bombay 400 002, India), Alcian blue (AB) (Alcian blue 8GX for microscopy (C.I. No. 74240) oxford laboratory reagent, Mumbai-400 002. Batch no. 8302 Code No.: A-00398), and Crossmon's trichrome (Crossmon's trichrom has been prepared in the laboratory by the authors 1- Dodeca Tungestophospphoric acid reagent (BDH chemicals Ltd Poole, England). 2- light green (Koch-Light Laboratories LTD, CoinBrook Berkks, England)3- acid fuchsin (Labochemie PTV. LTD PB No. 2042 Boombay 400 002 India) stains. Grimelus silver method was used to demonstrate the endocrine cells (argyophilic cells) among the cells of the mucosal epithelium and gastric glands. The above mentioned stains were applied as outlined by Bancroft and Gamble [6].

2.3.2. Transmission electron microscopy

Five fishes were used for transmission electron microscopy. Small pieces of 2.0–3.0 mm from different portions of the stomach were fixed in 4% glutaraldehyde solution in phosphate buffer (pH 7.2–7.4) overnight at 4 °C, then processed to prepare semithin sections (1 μm thick). The obtained sections were stained with toluidine blue (Fluka AG, Buchs SG Made in Switzerland) and examined with the light microscope (LEICA Microsystem CMS GmbH, wetzlar, Germany Model: DM2500 (11888139) Serial No.: 352027 The attached camera manufacturer details are: - LEICA Microsystem CH-9435 Heerbrugg Type: DFC290 HD (12730202) Input: 12V/ 450mA serial No. 390391709 Made in Germany) to select the suitable areas representing the desired observations. Ultrathin sections (600 A in thickness) were mounted on copper grids and stained with 5% uranyle acetate and lead citrate according to Reynolds [41]. Finally, these grids were examined using a JEOL 100S electron microscope (JEOL 100s transmission Electron Microscopy Brand: JEOL MPN: JEM-100s Model: JEM-100S Made in Japan) at the Electron Microscope Unit at the National Cancer Institute, Cairo, Egypt.

2.3.3. Scanning electron microscopy

The whole stomachs obtained from five fresh African catfishes were incised longitudinally to expose their luminal surfaces. Then the tissues were fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.3) and kept for 24 hours at 4 °C. After fixation, the samples were rinsed in 0.2 M phosphate buffer, trimmed into 8–10 mm sections, and postfixed for 2 hours in 1% osmium tetroxide in 0.2 M phosphate buffer (pH 7.3). The tissues were then dehydrated in ascending series of acetone, cleared in isoamyl acetate, and critical point dried with carbon dioxide. The serosal surfaces of the cardiac, fundic, and pyloric portions were mounted on metal stubs with mucosal surface uppermost and the specimens were coated with gold using a vacuum gold coater. The specimens were examined with a JEOL/EO-JSM-6510 LV scanning electron microscope (Jeol JEM-2100 Scanning Electron Microscope Made in Japan) at the Faculty of Science, Beni-Suef University, Egypt.

3. Results

3.1. Gross morphology

The stomach of the adult African catfish (*C. gariepinus)* was a J-shaped muscular sac behind the liver on the left side of the abdominal cavity with an average length of 3.3–4.5 cm. It extended from the esophagus to the proximal intestine. Externally, it had two surfaces: (1) a dorsal surface related to the kidney; and (2) a ventral one related to the proximal intestine and two curvatures starting from the cardia on the right side to the pylorus on the left side (Figures 1B, 1C, and 1E). Internally, the stomach was divided into three distinct regions: (1) cardiac; (2) fundic; and (3) pyloric (Figure 1D).

The cardiac region (right or descending limb) was the initial region of the stomach starting from the esophagus in the form of a cone shape connected distally to the fundic region. It was ventral to the dorsal wall of the body cavity and dorsal to the proximal intestine and visceral surface of the liver. Its inner surface had longitudinal mucosal folds.

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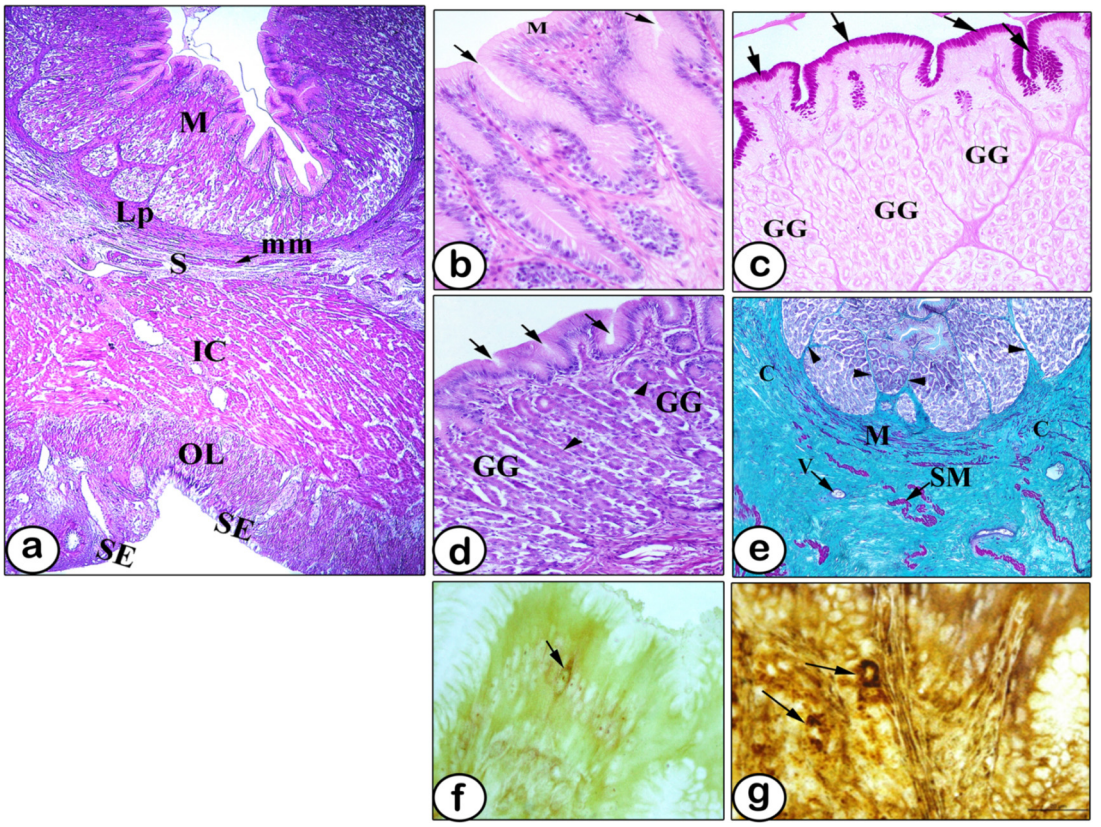

Fig. 2. Light photomicrographs of the cardiac region of African catfish stomach showing: (a) four distinct layers lining the cardiac region, mucosa (M), submucosa (S), muscularis externa composed of inner circular (IC) and outer longitudinal (OL) layers of smooth muscle fibers and serosa (SE). Note, the lamina muscularis mucosa (mm) is situated between lamina propria (Lp) and tunica submucosa (hematoxylin and eosin 40×); (b) cross section of the cardiac region showing cup-shaped mucosal folds (M) lined with simple columnar containing oval basal nuclei. Note: shallow and narrow gastric pits (arrows) between the mucosal folds (hematoxylin and eosin 400×); (c) Periodic–acid Schiff (PAS)-positive mucosubstance present in apical part of mucosal epithelial cells (arrows). Note: weak PAS reaction of the epithelial cells lining the gastric glands (GG; PAS 100×); (d) shallow gastric pits (arrows) lined with high columnar cells with oval basal nuclei. Note: plenty of parallel tubular gastric glands (GG) formed by cuboidal cells and oval basal nuclei (arrow heads; hematoxylin and eosin 200×); (e) collagen bundles (C) run in between the gastric glands and extend to the mucosal folds (arrow heads), longitudinal arranged smooth muscles bundles (M) lie under the bases of the glands forming the muscularis mucosa. Note: the submucosa composed of extensive collagen bundles containing strands of smooth muscles (SM) and many blood vessels (V; Crossmon's trichrom stain 100×); (f) oval shaped enterondocrine cell (arrow) lies between the gastric mucosal cells (Grimelius stain 1000×); (g) enteroendocrine cell (arrows) lies between the cells of the cardiac glands (Grimelius stain 1000×).

The fundic region (middle part) constituted the majority of the stomach; it appeared as a pouch extending caudally and communicated with the other two regions of the stomach. It was situated rostral to the spleen and ventral to the kidney and proximal intestine. Its internal surface was dark brown in color, containing approximately 12 thick longitudinally corrugated mucosal folds.

The pyloric region (left or ascending limb) represented the small terminal portion of the stomach. It was related to the lateral and ventral wall of the body cavity. Externally, it showed a pyloric constriction with the initial part of the proximal intestine. Internally, it appeared paler in color with no visible mucosal folds, and connected with the proximal intestine by a narrow pyloric sphincter.

3.2. Light microscopic examination

Three portions of the stomach—cardiac (anterior), fundic (middle and glandular), and pyloric (posterior and nonglandular)—were observed.

3.2.1. Cardiac region

The cardiac region of the stomach composed of four distinct concentric tunics arranged from inward to outward as follows: (1) mucosa; (2) submucosa; (3) muscularis externa; and (4) serosa (Figure 2A). The tunica mucosa was thrown up into several folds or rugae creating a narrow stellate lumen in the empty stomach. These folds appeared as longitudinally oriented cup-shaped folds of different sizes including the lining surface epithelium and the underlying lamina propria. Its mucosa was lined with high columnar mucous-secreting cells with homogenous faintly stained cytoplasm and basal oval nuclei (Figure 2B). The columnar cells contained apically concentrated secretory granules that strongly reacted positively with PAS (Figure 2C) and negatively with AB stain. Gastric pits (foveolae) were formed by invaginations of the mucosal layer into the underlying lamina propria and continuous with the openings of the gastric glands. These pits appeared short and shallow in depth, lined with tall columnar cells

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representing a continuation of the surface epithelial cells (Figure 2D). Numerous lymphocytes were observed under the epithelial cells lining the gastric mucosa in most examined sections of the cardiac regions.

The lamina propria extending deeply in the core of the mucosal folds contained loose connective tissue with extensive collagen fibers, many blood vessels, and gastric glands (Figure 2E). These gastric glands appeared as simple tubular parallel glands lined with polyhedral-shaped cells and possessed darkly-stained homogenous cytoplasm containing basally situated oval nuclei (Figure 2D). The glandular cells showed weak reaction to PAS (Figure 2C) and negative to AB stains. Enteroendocrine cells (enterochromaffin or argyophilic cells) were demonstrated within the epithelial cells lining the gastric mucosa (Figure 2F) and gastric glands (Figure 2G).

Longitudinally arranged smooth muscle fibers (SMF) situated in between the lamina propria and the tunica submucosa represented the lamina muscularis mucosae. In addition, diffuse strands of SMF were noticed in the tunica submucosa. The muscularis externa represented by inner circular and outer longitudinal SMF with intermuscular connective tissue containing blood vessels and nerves (Figure 2A). Lamina subserosa composed of areolar connective tissue with abundant blood vessels covered with lamina epithelialis serosa composed of mesothelium constituted the tunica serosa of the cardiac region (Figure 2A).

3.2.2. Fundic region

The examined microscopical sections of the fundic region of the stomach showed four concentrically arranged tunics: (1) mucosa; (2) submucosa; (3) muscularis externa; and (4) serosa. The tunica mucosa possessed several broad folds lined with a single layer of columnar epithelial cells containing oval basally located nuclei (Figure 3A) and apical mucosubstances that reacted positively with PAS (Figure 3B) but not to AB. Threads of mucin were observed covering the gastric mucosa secreted by the mucosal epithelial cells. Many depressions were noticed along the mucosal layer into the lamina propria continuous with underlying tubular glands forming the gastric pits. These pits appeared deeper than those observed in the cardiac stomach and their lining epithelium appeared as a continuation of the columnar cells lining the gastric mucosa (Figures 3A and 3C). Numerous enteroendocrine cells were demonstrated within the epithelial cells lining the fundic glands (Figure 3D). Many lymphocytes were seen in the tunica mucosa of the fundic region.

The lamina propria appeared thicker than those of the cardiac stomach and showed extensive collagenic bundles in addition to many blood vessels. These bundles held the fundic glands and penetrated the mucosal folds (Figure 3E). The fundic glands arranged in straight parallel, less coiled tubular manner, perpendicular to the gastric surface mucosa occupying the entire mucosal layer beneath the surface epithelium and continuous with the gastric pits (Figure 3C). These glands were surrounded by layers of connective tissue and lined with oxynticopeptic cells (Figure 3C) and enteroendocrine cells (Figure 3D). Two layers of SMF were situated between the lamina propria and the tunica submucosa forming lamina muscularis mucosa. Small muscular sheaves were intermingled with the connective tissues of the tunica submucosa (Figure 3E). Thick tunica submucosa appeared in between the lamina muscularis mucosa and tunica muscularis externa composed mainly of highly vascularized loose connective tissue containing collagenic fibers and strands of SMF. The tunica muscularis externa was constructed by inner circular and outer longitudinal SMF. The serosa was thin loose connective tissue limited by a single layer of mesothelial cells.

3.2.3. Pyloric region

The pyloric region of the stomach was easily distinguished microscopically by the sudden disappearance of gastric glands, thick long mucosal folds, and wide deep gastric pits (Figure 3A). The epithelial cells lining the gastric mucosa were simple columnar cells with a faintly stained homogenous cytoplasm and basally located oval nuclei. The apical portions of these cells were filled with intensely positive PAS staining (Figure 3B) and negative AB secretions.

The lamina propria appeared thick and showed highly vascularized loose connective tissue rich in collagenic fibers that penetrated the mucosal folds (Figures 3A and 3C). The tunica submucosa was the thinnest layer when compared with that of the cardiac and fundic stomach and composed of vascularized loose connective tissue.

The tunica musculris externa of the pyloric region was the thickest among the other portions of the stomach forming the gastrointestinal sphincter. It composed of three layers: (1) thin inner; and (2) outer longitudinal in addition to (3) thick middle circular layers of SMF (Figure 3C).

A thin serosal layer formed the outer lining of the pyloric stomach, constituted by a thin loose connective tissue lined with a single layer of squamous epithelium (Figure 4d).

3.3. Scanning electron microscopic examination

The stomach showed primary longitudinal mucosal folds along the whole length of the cardiac and fundic parts which left deep furrows in between (Figures 5A and 6A). On the surface of these folds, other secondary mucosal folds irregularly anastomosed with each other leaving discrete pockets in between were detected (Figures 5B, 5C, and 6B). These folds were lined with columnar cells bearing microvilli on their apical surfaces and appeared as a honey comb polyhedral epithelial cells in the form of pentagonal or hexagonal elevations with the presence of gastric pits in between. Extensive mucin droplets appeared as a thin layer overlying the lining mucosa (Figures 5C, 5D , 6C, and 6D). Oval elevations were observed between the secondary mucosal folds representing the luminal surface of the goblet cells (Figures 5C and 6C).

A transverse section of the fundic region showed primary folds with remarked gastric crypts and deep gastric grooves (Figure 6E). These irregularly arranged mucosal folds with prominent gastric pits appeared as narrow concavities that were encircled by rosettes of epithelial cells, oval shaped goblet cells, and extensively scattered mucin droplets on the surface of the epithelial cells. The lining of epithelial cells had short and stubby microridges (Figures 6B–D). The transition area between the fundic and

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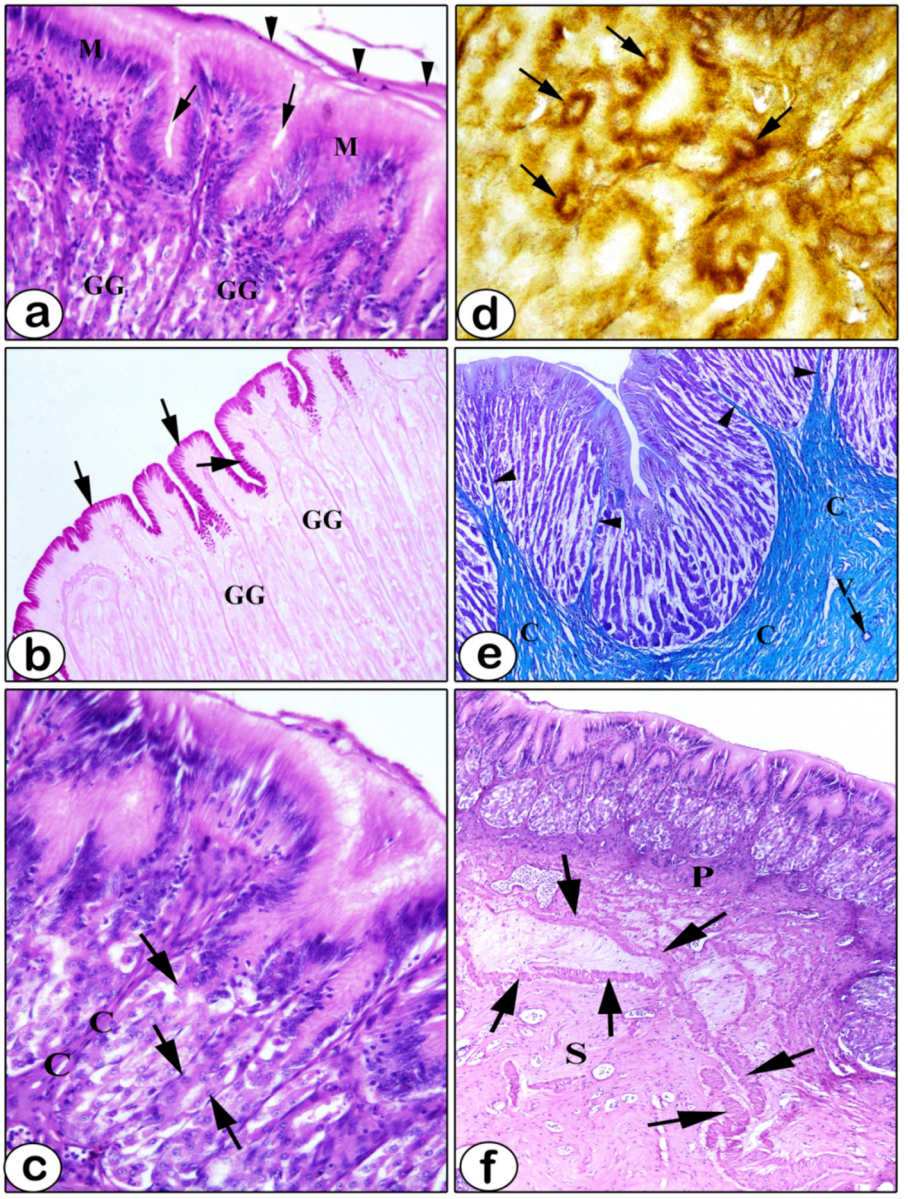

Fig. 3. Light photomicrographs of the fundic region of African catfish stomach showing: (a) broad mucosal folds (M) lined with single layer of columnar cells with oval basal nuclei. Note: wide and deep gastric pits (arrows), gastric glands (GG), and mucous threads adhered to the surface epithelial cells [head arrows; hematoxylin and eosin (H&E) 400×]; (b) apically concentrated Periodic–acid Schiff positive granules (arrows) of the epithelial cells line the mucosal surface epithelial cells and pits. Note: the parallel arranged straight tubular glands (GG; Periodic–acid Schiff 200×); (c) tubular gastric glands formed by a single cell type (oxintopeptic) with central nuclei (arrows). Note: connective tissues (C) derived from lamina propria penetrate between the glands (H&E 200×); (d) a transverse section showing numerous enterochromaffin cells (arrows) within the epithelial cells lining the fundic glands (Grimelius stain 1000×); (e) extensive collagenic bundles (C) containing blood vessel (V) located under the bases of the glands. Note: the tubular glands permeated by many collagen fibers (arrow heads; Crossmon's trichrome stain 100×); (f) a cross section showing double-layered of smooth muscle fibers (arrows) forming the lamina muscularis mucosa and separating the lamina propria (P) from the tunica submucosa (S; H&E 100×).

pyloric regions of the stomach with a prominent shallow groove could be seen (Figure 6F).

A remarkable groove between the terminal part of the pyloric region and the initial part of the proximal intestine was noticed representing the pyloric sphincter (Figure 7A). In addition, deep empty concavities formed by the anastomosis of the pyloric mucosal folds possessing big and stubby microridges were also detected (Figure 7B).

3.4. Transmission electron microscopic examination

The ultrastructural investigation of the three portions of the stomach of catfish showed tall columnar, electron-dense epithelial cells containing numerous apically located secretory granules lining the gastric lumen and pits. The secretory granules appeared homogeneous, elongated in shape with variable sizes, and some of them

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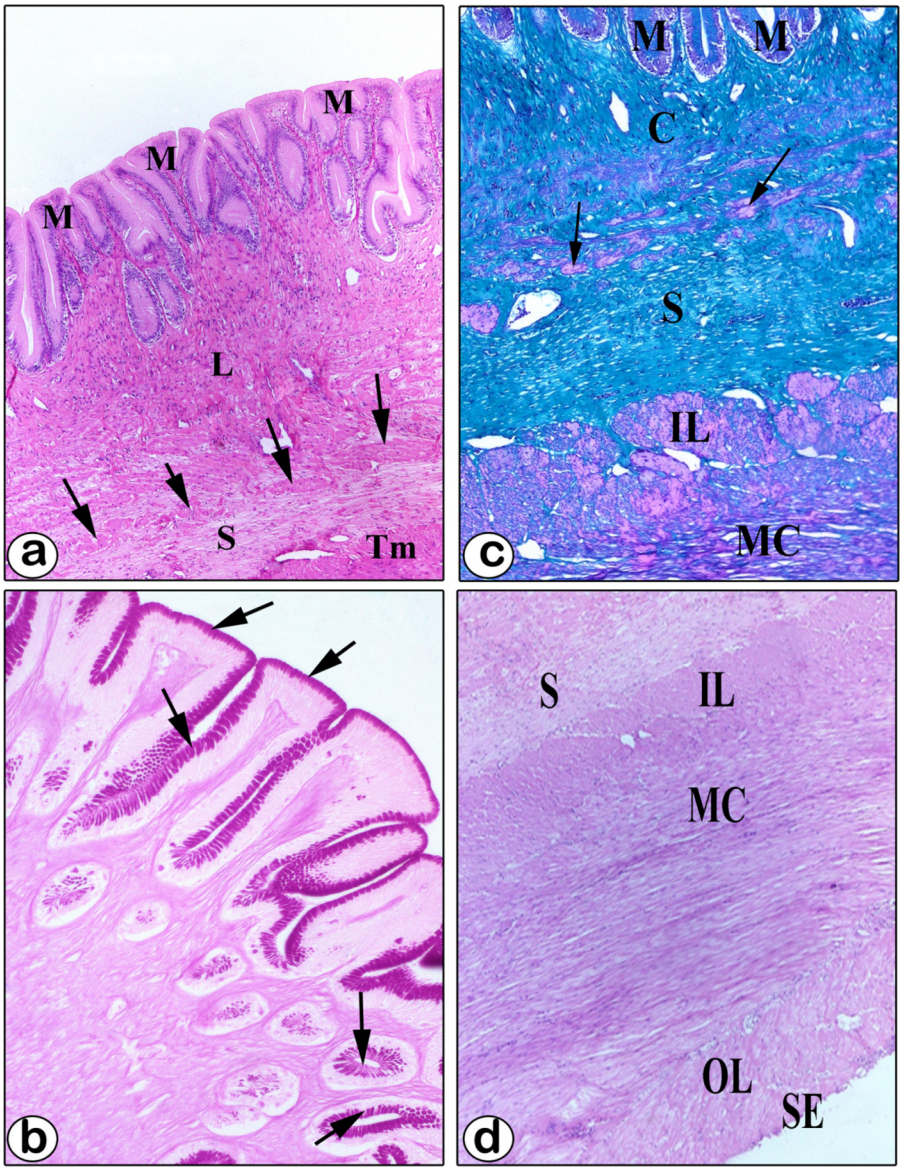

Fig. 4. Light photomicrographs of the pyloric region of African catfish stomach showing: (a) long folded mucosa (M) lined by columnar cells, devoid of gastric gland in a thick lamina propria (L). Note: a layer of smooth muscle fibers (arrows) separated the lamia propria from the thin tunica submucosa (S) and tunica musclosa (Tm; hematoxylin and eosin 100×); (b) a cross section showing intense Periodic–acid Schiff positive reactions (arrows) at the apical portion of the epithelial cells lining the pyloric mucosa (Periodic–acid Schiff 200×); (c) extensive collagen bundles (C) lie in the lamina propria and extend to the core of the mucosal folds (M). Note: lamina muscularis mucosa (arrows), submucosa (S), inner longitudinal (IL), and middle circular (MC) layer of tunica musculosa (Crossmon's trichrom stain 100×); (D) thick tunica musclaris externa composed of inner longitudinal (IL), middle circular (MC), and outer longitudinal (OL) layer of smooth muscle fibers. Note: submucosa (S) and thin serosal coat (SE; hematoxylin and eosin 400×).

were noticed to be fused with the cell membrane. The nuclei of these epithelial cells appeared large, oval, basally located (Figures 8A and 8B), and surrounded by many cytoplasmic organelles, well-developed Golgi apparatus, rough endoplasmic reticulum, and numerous mitochondria. The surface of the gastric epithelial cells exhibited many apically located microvilli.

The cardiac glands showed only one type of cells appeared ultrastructurally polyhedral in shape containing rounded basal vesicular nucleus, many mitochondria, and an extensive network of rough endoplasmic reticulum. The fundic glands were lined with only one cell type—oxynticopeptic cells. The observed oxynticopeptic cells had a polygonal shape and were centrally situated nuclei. Numerous spherical-shaped vesicles of different sizes were distributed in cytoplasm (Figure 8C). The supra nuclear part of their cytoplasm contained a tubulovesicular network composed of numerous smooth small vesicles scattered throughout the cell cytoplasm especially at the apical region in between numerous tubules of rough and smooth types of endoplasmic reticulum as well as many secretory granules (Figure 8D). Numerous mitochondria,

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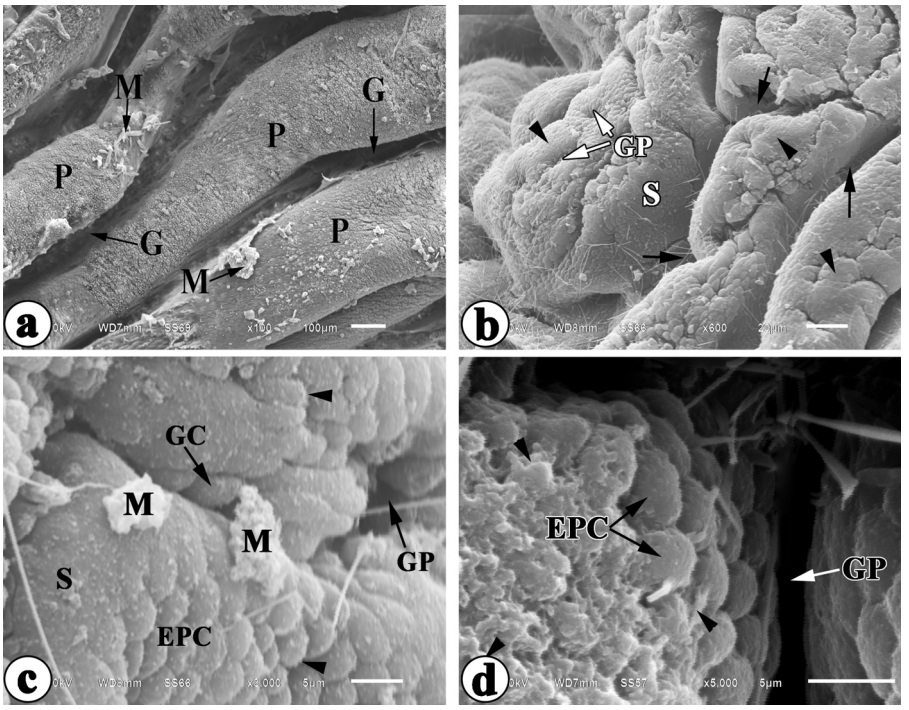

Fig. 5. Scanning electron micrographs of the cardiac region of African catfish stomach: (a) low magnification of the mucosal lining showing numerous primary mucosal folds (P) longitudinally arranged parallel to each other with the presence of grooves in between (G) and scattered mucous secretion on its surface (M; 100×); (b) irregular anastomosis of the secondary mucosal ridges (S) leading to the formation of irregular discrete pockets (black arrows). Note: the presence of microridges (arrow heads) on the secondary mucosal folds and lodging the gastric pits (GP) in between (600×); (c) apical surface of the epithelial cells (EPC) bearing microvilli (arrow heads), oval elevations appeared between the secondary mucosal folds (S) represented the luminal surface of the goblet cells (GC). Note: the accumulated mucin secretion (M) and the presence of the gastric pits (GP) between the columnar epithelial cells (3000×); (d) luminal surface of the columnar EPC appeared as pentagonal and hexagonal elevations with the presence of GP in between and extensive mucin droplets (arrow heads) appeared as thin layer overlying the lining mucosa (5000×). Scale bars: A, 100 μm; B, 20 μm; C and D, 5 μm.

well-developed Golgi apparatus, and lysosomes were located all over the cytoplasm. The lateral cell membranes formed smooth contacts with the neighboring cells and lack any interdigitations.

Concerning the enteroendocrine cells, they appeared in most semithin sections prepared from cardiac, fundic, and pyloric regions. They appeared ultrastructurally as electron-lucent cells scattered throughout the gastric mucosal epithelium. They were rounded in shape with a rounded euchomatic nucleus. Their cytoplasm contained rough endoplasmic reticulum, a few rounded or elongated mitochondria, and numerous scattered secretory granules exhibiting various shapes and sizes (Figure 8E)

4. Discussion

The stomach of the African catfish appeared J-shaped, similar to that in the South American catfish [23], channel catfish [44], and rainbow trout [17], this shape may help in extending the duration that food stays in the stomach, thereby ensuring a greater degree of digestion by gastric juice. Moreover, different shapes of the stomach were reported in other fish species as Y-shaped in *Oreochromis* niloticus [8], Alosa, Anguilla, the true cods, and ocean perch, U-shaped stomach in Salmo, Coregonus, and Clupea, and a straight stomach in Esox [45]. The obtained corrosion cast of the stomach cavity using epoxy polymer and the observed extensive areas of folding using scanning electron microscopy revealed that the stomach is distensible and increases more than its normal size during food consumption; this allows for ingestion of large-sized prey, extends the time that food stays in the organ, and increases the surface area for digestive enzyme activities that allows efficient mixing of food with digestive fluid [30,44].

The histological examination of the stomach confirmed the anatomical observation as the stomach of African catfish composed of three distinct regions: (1) cardiac; (2) fundic; and (3) pyloric. These results were reported in many teleosts [15,37,46].

All examined sections from both cardiac and fundic regions showed that the stomach of the African catfish showed uniform histological features—superficial epithelial cells and simple straight tubular gastric glands in lamina propria surrounded by connective tissue. The presence of these gastric glands in both the cardiac and fundic regions and absence in the pylorus has also been reported in Kingfish [11], *Paralichthy scaliforniaus* [48], yellow catfish [47], and in *Glyptosternum maculatum* [46]. A similar arrangement of gastric glands has been observed in white sturgeon [14], bluefin tuna [25], and in *Mystus vittatus* [10].

Our microscopical investigations of the three portions of the African catfish stomach showed that the epithelial cells lining both the gastric mucosa and pits contain mucosubstances. These mucosubstances intensely reacted with

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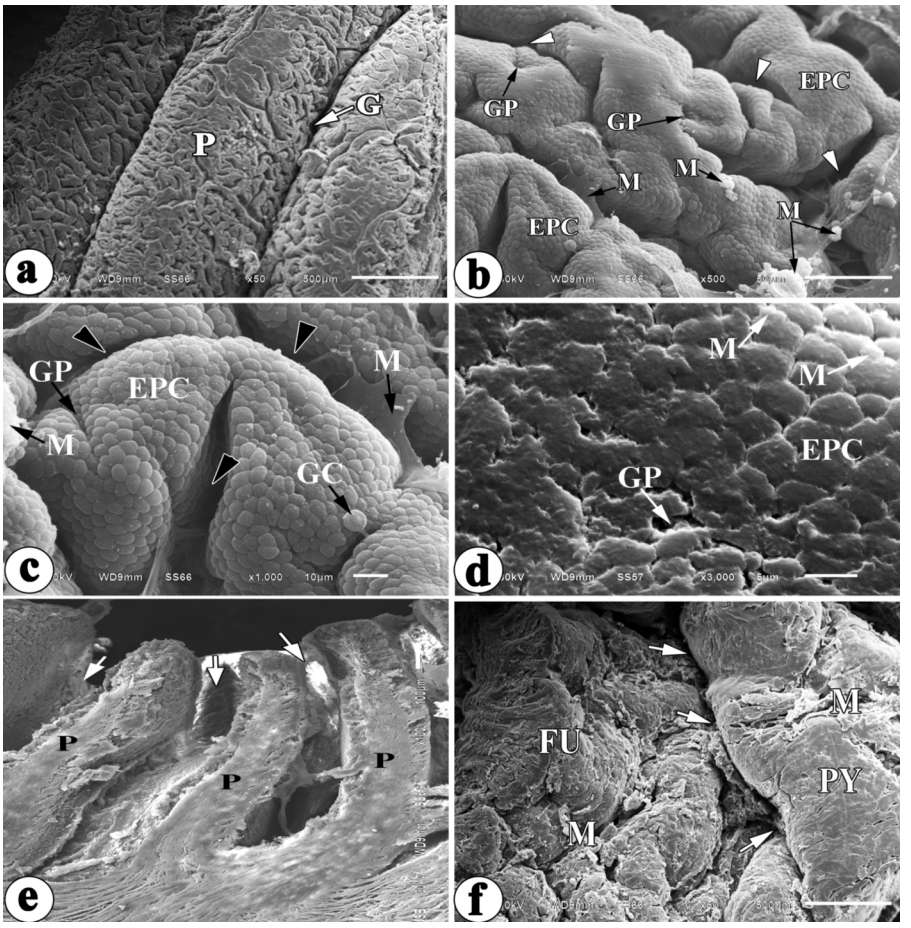

Fig. 6. Scanning electron micrographs of the fundic region of African catfish stomach: (a) low magnification of the mucosal lining showing numerous primary mucosal folds (P) longitudinally arranged with deep furrows (G) in between (50×); (b) irregular arranged secondary mucosal folds and narrow concavities in between them (arrow heads). Note: prominent gastric pits (GP) encircled by rosettes of columnar epithelial cells (EPC), with scattered mucin droplets on their surface (M; 500×); (c) polyhedral (pentagonal or hexagonal) shaped columnar EPC provided with short and stubby microridges. Black arrow heads indicate concavities and prominent GP, M, and oval shaped goblet cells (GC; 1000×); (d) a higher magnification of the luminal surface of the polyhedral columnar EPC and M. Note: the GP surrounded by the EPC (3000×); (e) a transverse section through the damaged surface of the fundic gland region showing primary folds (P), with remarked gastric crypts of the gastric glands (arrows; 100×); (f) low magnification showing the transition area between the fundic (FU) and pyloric (PY) regions of the stomach, with a remarkable shallow groove (solid arrows) could be seen (50×). Scale bars: A, 500 μm; B, 50 μm; C,10 μm; D, 5 μm; E, 100 μm; and F, 500 μm.

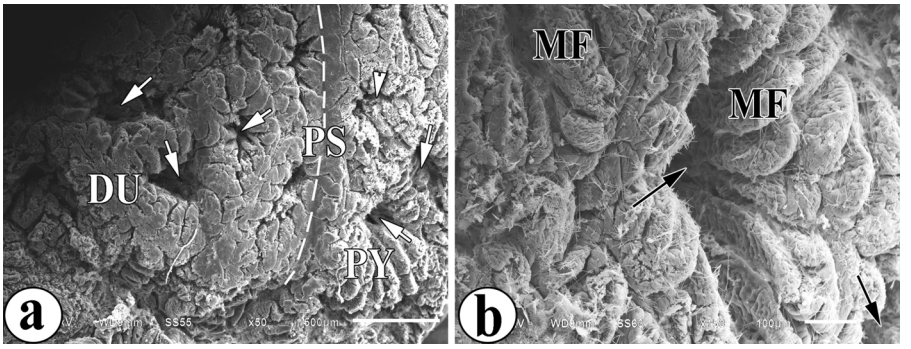

Fig. 7. Scanning electron micrographs of the pyloric region in the African catfish stomach: (a) low magnification showing the terminal part of the pyloric region (PY), and the initial part of the proximal intestine; duodenum (DU), and there is a groove (white dotted line) that indicates the position of the pyloric sphincter (PS) with deep empty concavities (solid arrows) formed by the anastomosis of the mucosal folds (50×); (b) mucosal folds (MF) with big and stubby microridges and deep empty concavities (solid arrows; 100×). Scale bars: A, 500 μm; B, 150 μm.

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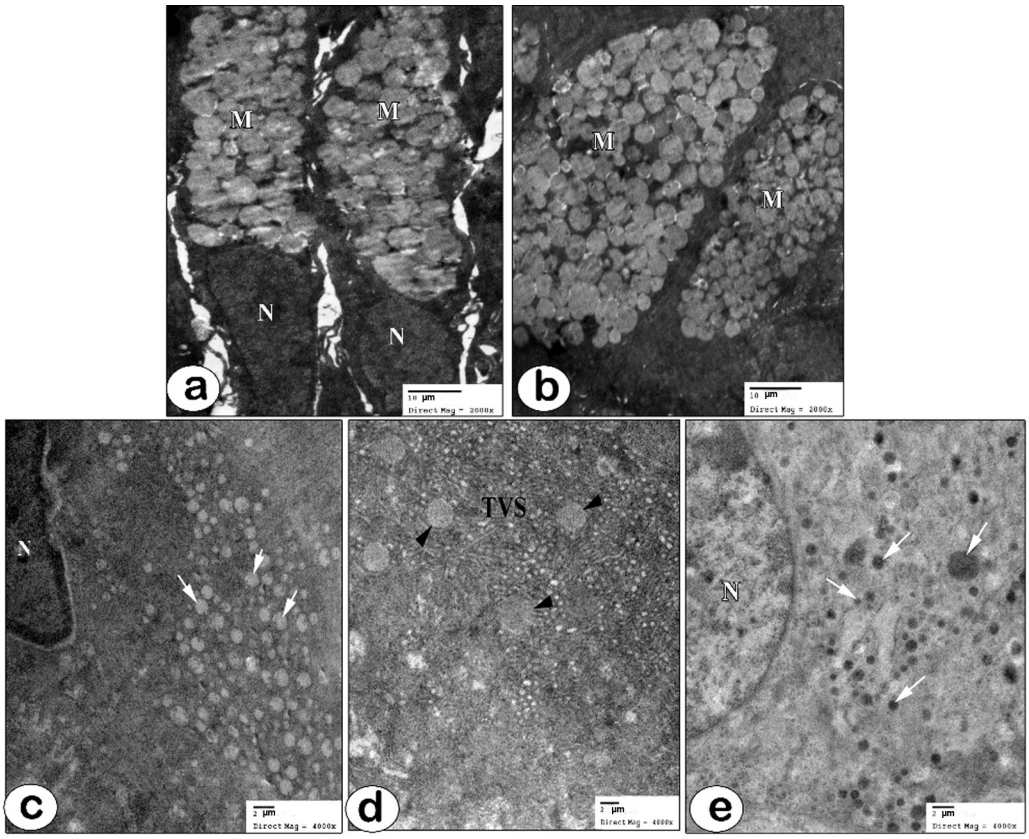

Fig. 8. Electron photomicrographs of the African catfish stomach: (a) cardiac mucosa showing luminal cells contain apically located secretory vesicles (M) occupy most of the cytoplasm and basally situated oval nucleus (N). Uranyl acetate and lead citrate (2000×); (b) gastric mucosal cells of fundic region containing excessive number of apically located secretory vesicles (M) occupy most of the cytoplasm. Note: some of these vesicles fused with the cell membrane. Uranyl acetate and lead citrate (2000×); (c) oxynticopeptic cell containing nucleus (N) and numerous spherical shaped vesicles (arrows) of different sizes. Uranyl acetate and lead citrate (4000×); (d) apical portion of oxynticopeptic cell lines fundic gland showing tubulo-vesicular system (TVS); secretory granules (arrow heads) and extensive network of rough endoplasmic reticulum (arrows). Uranyl acetate and lead citrate (2000×); (e) an endocrine cell lining the fundic gland showing euchromatic nucleus (N) and numerous electron dense secretory granules (arrows) of different sizes. Uranyl acetate and lead citrate (4000×).

PAS but not with AB. This result indicates the presence of only neutral glycoproteins which might be related to the conduction of food, may provide efficient protection against proteolysis and mechanical injury [5,14,28,35], and has buffering effects on high concentration acid contents of the stomach [26]. Moreover, it may also be involved in digestive activity by digestion and absorbtion of easily digested molecules, such as disaccharides and short-chain fatty acids [22]. These mucins also regulate the pH of the gastric fluid, explaining the variations in the pH of the gastric fluid in different species with different diets [30]. However, the neutral mucous may serve to protect the stomach epithelium from auto-digestion processes caused by hydrochloric acid and the enzymes secreted by the gastric gland by forming an adherent mucus gel [18,45] and provides complementation for the degradation [3].

Ultrastructurally, some secretory granules were frequently seen fused with the apical cell membranes; this may be correlated with the emptying of their mucous contents into the gastric lumen via exocytosis process as in *Labeo niloticus* [29], *Oncorhynchus mykiss* [31], and *Anguilla anguilla* [32]. The apical borders of the gastric epithelial cells were observed carrying microvilli which may be correlated to the increase in absorptive activity [4]. Thus, the epithelial cells lining the gastric mucosa with pits rich in neutral mucosubstances, mitochondria-rich cytoplasm, and the carrying microvilli implies that the stomach of the African catfish has strong digestion and absorption abilities.

The fundic glands showed the presence of only one type of secretory cell, called oxynticopeptic cells, but no presence of any kind of glycoproteins. This result is accepted, as pointed out by many authors, because only the gastric glands of mammals have distinct acid-producing parietal cells (oxyntic cells) and zymogenic (chief) cells. In fish, amphibians, and birds, both hydrochloric acid and pepsinogen are assumed to be secreted by only one cell type called oxynticopeptic cells [2,13,21,38,39].

Ultrastructurally, features of both the oxyntic and peptic cells of the mammalian stomach were recorded in oxynticopeptic cells of the fundic region of the catfish stomach; the apical portion of the cytoplasm contains a well-developed vesiculotubular system with many mitochondria which may be involved in the secretion

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of hydrochloric acid. Moreover, these cells possess well-developed rough endoplasmic reticulum and numerous secretory granules which may be involved in the production of pepsinogen [19,31].

In this study, enteroendocrine cells were noticed as being distributed all over the gastric wall between the epithelial cells lining the gastric mucosa and glands. These cells showed a positive reaction to Grimelius stain. Ultrastructurally, these cells showed vesicular nuclei, rough endoplasmic reticulum, and characteristic secretory granules. These endocrine cells may be involved in the secretion of various hormones that play an important role in digestive activities of the gut [24,27,36]. Moreover, they secrete many hormones such as gastrin, somatostatin, and serotonin that might stimulate the glandular cells to increase hydrochloric acid secretion [20].

In agreement with previous literature, the lymphocytes that were observed in the mucosa of the stomach of the species under investigation may play an important role in protecting the fish from pathogenic micro-organisms (*Tilapia nilotica* and mud loach) [30,33].

Extensive collagenous fibers that were noticed in the lamina propria of the three examined portions of the stomach might form a supporting, protective, and strengthened layer as well as keep the gland in position. In addition, powerful gastric musclosa of the carnivorous catfish containing well-developed inner circular and outer longitudinal unstriated muscle fibers and collagenic fibers of the submucosa may be involved in the involuntary contraction of food digestion [2,13,46], increasing the retention time of ingested food in the stomach and increasing the volumetric capacity of the stomach [2]. Moreover, it may also be involved in trituration [9,32] and for supporting and strengthening since the stratum compactum is lacking in the species under investigation [7].

In the pyloric region, a combination of a well-developed musculature consisting of inner and outer longitudinal layers in addition to middle circular layers of SMF and the absence of the pyloric glands may indicate that the primary function of this portion is the mixing of food and pushing it distally [40]. In addition, the absence of gastric glands may be related to the adaptation of this species in reducing the quantity of hydrochloric acid entering the proximal intestine; hence, it might help the alkaline medium to maximize pancreatic enzyme actions in the proximal intestine [42]. The presence of a serosal coat consisting of mesothelial cells and loose connective tissue containing small blood vessels as seen in this study has been reported in most other species of teleosts [4].

5. Conclusions

The present study suggests that the histological features of the stomach of the African catfish were consistent with the feeding habits of a carnivorous fish. This microanatomical structure provides baseline data for further investigative researches, helps fish clinicians in understanding the physiology and pathology of fish, and also aids nutritionists in feed management, preparation, and handling of diets. However, more studies should be carried out for a deeper understanding of the digestion process and nutrient absorption of these fish.

Conflicts of interest

No conflicts of interest have been declared.

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