



HISTOLOGY AND HISTOCHEMICAL STRUCTURE OF THE STOMACH (PROVENTRICULUS AND VENTRICULUS) IN MOORHEN (*GALLINULA CHLOROPUS*) IN SOUTH IRAQ

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Abstract

Five moorhen (*Gallinula chloropus*) both sex were collected to conduct the current study. They were bought from specific markets at (Al-Basra provinces- Iraq) from the local suppliers. Birds were euthanized prior to its dissection with an intravenous injection of sodium pentobarbitone (140 mg/kg) and dissected to the stomach the specimens were fixed in 10% neutral buffered formalin and Bouin's solution. After well fixation the specimens were dehydrated by grade ethanol and then specimens were cleared in xylene after that embedded in paraffin wax and then the blocks were sectioned at 6 µm thickness and stained with: Mayer's hematoxylin and eosin, Masson trichrome stain, PAS and PAS-alcian blue (AB) (pH 2.5). The histological results of the wall moorhen of stomach revealed the presence of four layers of the typical tubular organ that were (mucosa, submucosa, muscularis and serosa). The mucosa lining with simple cuboidal epi. The underlying lamina propria was constructed of loose connective tissue filled with blood vessels, the submucosa of proventricular it was formed of dense connective tissue containing oval-shaped branched tubular proventriculus glands surrounded by a fibrous capsule. The ventricular of current moorhen the presence of cuticle was similar to other avian species that possessed thick cuticle layer with well-developed muscular stomach. The muscularis appeared as a very thick structure of smooth muscles bundles. In the moorhen, three layers of muscles were distinguished that were thin inner, outer longitudinal and very thick intermediate circular layers. Histochemically, strong PAS-positive reaction in the surface mucous cells of the proventriculus. The connective tissue of the lamina propria showed moderate reaction toward PAS, whereas, submucosal glands gave negative reaction. The cuticle covering in the ventriculus showed positive reaction with PAS stain (purple color). The connective tissue gave the positive reaction with PAS and negative with AB.

Keyword: Histology, Histochemical, Moorhen, Stomach.

Introduction

The poultry production has full a main role between agricultural industries in several parts of the world, Chicken meat production has been increase in all regions with the principal in Asia and South America, The Asia is important the world in poultry meat production, followed by North and Central America which had the lead until 1990 (Daghir, 2008). Poultry meat is the then maximum extensively eaten meat in the world, accounting for about 30% of meat production worldwide (Raloff, 2003). The moorhens are water birds of a size like that of small duck, they live on the riversides, water shelves and among the river plants like reeds and characterized by a red or white color in their foreheads (Steven, 2010). The moorhens are present in the Arab homeland, where they present in morocco, Egypt, sham and extend east to Iraq and Arab gulf till the frontiers of Iran and middle of Asia and most the European countries (Walker, 2009; Jassem *et al.*, 2016). The stomach of birds anatomically composed of two chambers: a cranial chamber (proventriculus) which connect to the esophagus and caudal chamber (ventriculus) which connect with duodenum (Abumandour, 2013). The glandular stomach in chicken characterized by spindle shape which arises directly without any demarcation line from esophagus, while its separated from gizzard by intermediate zone (isthmus) (Jassem *et al.*, 2016; King and McClelland, 1975). In the stomach of Japanese Quail, the gizzard's wall was represented by mucosa revealed branched tubular glands, submucosa, muscular layer and serosa. The mucosa formed folds which were continuous with the tubular glands located in the

underlining lamina propria. Absence of muscularis mucosa and the gizzard's glands were lined by cuboidal cells. The lining epithelium was simple columnar or cuboidal cells characterized by basally located rounded or oval nuclei and basophilic cytoplasm. Apical portions of these lining cells were stained positively by PAS and AB stains. Keratinized laminated coat covered the mucosa which reacted positively toward acid fuchsin after using trichrome stain (Ahmed *et al.*, 2011). The present study was undertaken to study the histology and histochemistry of the stomach (proventricular and ventricular) moorhen (*Gallinula chloropus*).

Materials and Methods

Five moorhen (*Gallinula chloropus*) both sex were collected to conduct the current study. They were bought from specific markets at (Al-Basra provinces- Iraq) from the local suppliers. Birds were housed at animal house of the Veterinary Medicine College/ Al-Muthanaa University in suitable cages. They were fed as well and giving them water ad libitum before their euthanasia and dissection. Birds were euthanized prior to its dissection with an intravenous injection of sodium pentobarbitone (140 mg/kg) (Mitchell and Smith, 1991). Then after, dissected by fixing them on a dissecting board. A mid-line incision was made in the abdominal wall to view the coelomic viscera. The stomach (proventricular and ventricular). The histological aspect of the study, the specimens were fixed in 10% neutral buffered formalin and Bouin's solution. After well fixation the specimens were dehydrated by passing them through a series of ascending grade ethanol each for 2 h and then specimens

were cleared in xylene for 1 to 2 h after that embedded in paraffin wax and then the blocks were sectioned at 6 μm thickness and stained with either one of the following stains: Mayer's hematoxylin and eosin routine stain for general features identification, Masson trichrome stain for the staining of the collagenous and smooth muscle fibers. (Widhi and Trivedi, 2012). The PAS identification of the neutral mucin (Bancroft and Stevens, 2010). For the determination of the acidic mucin, combined PAS-alcian blue (AB) (pH 2.5). Each section was examined under light microscope to study the histological and histochemical characteristics of the stomach.

Results and Discussion

The histological results of the wall moorhen of proventriculus revealed the presence of four layers of the typical tubular organ, that were (mucosa, submucosa, muscularis and serosa) (Fig. 1). The layers which structured the wall of proventriculus was similarly documented in the proventriculus in many avian species such as pigeon (Al-Saffar *et al.*, 2015), African Grey Parrot (*Psittacus erithacus*) and black francolin (*francolinus*) (Al-Saffar *et al.*, 2016), ostrich (*Struthio camelus*) (Cooper and Mahroze, 2004), Japanese quail (Selvan *et al.*, 2008) and Coot bird (Batah *et al.*, 2012). Whereas, in the wall of the proventriculus of Asiatic swift (*Collocalia spp.*), (Marshall and Folley, 1965) observed only three layers in which only mucosa, muscularis and serosa were detected. The mucosa showed longitudinal branched folds that were lined by simple cuboidal epithelium (Fig. 1). The underlying lamina propria was constructed of loose connective tissue filled with blood vessels (Fig. 1,2). The structure of the lamina propria extended inside the folds and possessed simple tubular mucous glands (Fig. 1). These glands were opened into the lumen of the proventriculus via their ducts. The glands were lined with simple cuboidal epithelium and were dispersed at the apical part of the lamina propria. The propria was separated from the underlying submucosa by fibers of smooth muscle called muscularis mucosa. The presence of simple cuboidal epithelial lining of the mucosa in the studied moorhen were disagree to mucosal lining of the most avian species (Samuelson, 2007), pigeon (Al-Saffar *et al.*, 2015) and ostrich (Mina and Paria, 2011). The mucosal glands which were observed lined with simple cuboidal epithelium in moorhen were in accordance with those observed in the same organ of the Coot bird (*Fulica atra*) (Batah *et al.*, 2012). The presence of muscularis mucosa separating markedly the mucosa from the underlying submucosa in the studied moorhen was in a good agreement with those observed in the same organ of the red-gartered coot (*Fulica armillata*) (Espinola and Galliussi, 1990) and the red jungle fowl (Kadhim *et al.*, 2011). Whereas, (Catroxo *et al.*, 1997) found incipient muscularis mucosa in the mucosa of the proventriculus of red-capped cardinal birds and the recent findings of the (Abumandour, 2014) recorded absence of this layer in the mucosa of falcon's proventriculus.

The submucosa prominently, this layer found occupying most of the wall thickness of this organ. It was formed of dense connective tissue containing oval-shaped branched tubular proventriculus glands surrounded by a fibrous capsule (Fig. 1, 2). Such findings were agree in African Grey Parrot (*Psittacus erithacus*) and black francolin (*francolinus*) (Al-Saffar *et al.*, 2016) and not similar with those of (Rocha, 1991) in the burrowing owl (*Speotyto*

cunicularia) whom described these glands as pear and lined by tall columnar epithelium. The glands consists numerous secretory tubules which were lined by cuboidal cells and each tubule continued by one duct opened into the main collecting duct which subsequently opened into luminal surface of the organ. Current findings were similar to those recorded in other birds such as Red-Capped Cardinal (*Paroaria gularis gularis*) (Catroxo *et al.*, 1997) but different to those found in the red jungle fowl (Kadhim *et al.*, 2011). The current findings concerned presence of proventriculus glands were not parallel with those of (Bradly, and Grahame, 1960) and (King and McLelland, 1984) whom referred to the absence of these glands in the submucosa of the proventriculus in chickens.

The muscularis was constructed of two layers, inner thin longitudinal and an outer thick circular layers. Between such layers, fine connective tissue was observed filled with blood vessels (Fig. 1). Differently to current findings, in parrots (Denbow, 2000.) observe only one layer of smooth muscle fibers circularly arranged. In other birds found three layers of smooth muscle bundles constituting the muscular layer such as the red-capped cardinal birds (Catroxo *et al.*, 1997). The layers were inner longitudinal, intermediate circular and an outer longitudinal in which nerves and ganglion cells were distributed. However, in the pigeon proventriculus, (Al-Saffar *et al.*, 2015) found similarly to current studied moorhen two layers but the inner longitudinal layer was well developed that constructs most thickness of the wall of this organ in this bird.

Tunica serosa was constructed of loose connective tissue in which nerves, blood vessels, adipose cells were observed and such structures were covered by a layer of mesothelium (Fig. 1, 2). These findings were similarly observed by (Al-Saffar *et al.*, 2016) in parrot and francolin.

Similarly to the proventriculus, the histological structure of ventriculus also showed the four known layers forming its wall (Fig. 5). Same findings regarding the wall structure were recorded in most avian species such as pigeon (Al-Saffar *et al.*, 2016), African Grey Parrot (*Psittacus erithacus*) and black francolin (*francolinus*) (Al-Saffar *et al.*, 2016), Red-Capped Cardinal (*Paroaria gularis gularis*) (Catroxo *et al.*, 1997) and in guinea fowl (*Numida meleagris*) (Kadhim *et al.*, 2011).

The color and the presence or absence of the cuticle was previously documented in avian species. The previous data in the literatures indicated a relationship between it and the type of food consumed by the bird. As in the current moorhen the presence of cuticle was similar to other avian species that possessed thick cuticle layer with well-developed muscular stomach. In fact many researchers such as (King and McLelland, 1975; Banks, 1993; Gionfriddo and Best, 1996; Bailey and Mensah-brown, 1997), referred to the thickness of the cuticle which is highly correlated with food consumed. They proposed thick cuticle in granivores and a thin in frugivores

The mucosa was constructed by simple cuboidal epithelium characterized by basally located round-shaped nuclei with lightly stained cytoplasm (Fig. 5). Similar epithelial covering observed in the ventriculus of the other species such as in that of the Coot bird (*Fulica atra*) (Batah *et al.*, 2012) and in mallard in which it was simple cuboidal

epithelium (32).but differently in the owl (Kadhim *et al.*, 2011) and pigeon (Al-Saffar *et al.*, 2015).

The lamina propria showed numerous simple tubular glands lined by simple cuboidal cells. The examination of the ventriculus revealed the presence of eosinophilic secretion going away toward the epithelial surface as a strips forming the cuticle (Fig. 5, 6). It spread all over the mucosal surface filling the lumina of the gastric pits as a pinkish thick material. Muscularis mucosa appeared as circularly arranged smooth muscle bundles interrupted by the presence of mucosal glands in the lamina propria. The presence of muscularis mucosa between the mucosa and submucosa in the moorhen appeared dissimilar to previous findings in other birds such as *Codorna nothura* (Fieri, 1984), red-capped cardinal birds (Catroxo *et al.*, 1997), Blue and Yellow macaws (Rodrigues *et al.*, 2012 (and in falcon's gizzard (Abumandour, 2014) in which this layer was absent in their mucosal layer.

The submucosa was composed of abundant dense connective tissue containing blood vessels and nerves (Fig. 5, 6). This outcome in a good agreement with those observed in the Domestic fowl (*Gallus Domesticus*) (Mitchell and Smith, 1991), in Mallard (*Anas Platyrhynchos*) and in pigeon (*Columba livia*) (Al-Saffar *et al.*, 2015) that described connective tissue composition in this tunic.

The muscularis appeared as a very thick structure of smooth muscles bundles. In the moorhen, three layers of muscles were distinguished that were thin inner, outer longitudinal and very thick intermediate circular layers (Fig. 5). There were fine collagenous fibers distributed between their bundles(Fig. 6). The presence of three layers of muscles fibers was in accordance with the findings of African Grey Parrot (*Psittacus erithacus*) and black francolin (*Francolinus francolinus*) (Al-Saffar *et al.*, 2016) and in the pigeon (Al-Saffar *et al.*, 2015) . Conversely, two layers of muscles fibers in the wall of the ventriculus was recorded by (Catroxo *et al.*, 1997; Hamdi *et al.*, 2013; Al-Saffar *et al.*, 2014) in the same organ of red-capped cardinal, Coot bird (*Fulica atra*) most avian species and owl, respectively.

The serosa was constructed of loose connective tissue rich in blood and covered by the mesothelium of simple squamous cells. The structure is commonly observed in many avian species such as Ostrich (*Struthio camelus*) (Bezuidenhout and Vanswegen, 1990), in turkey (El-Zoghby, 2000).

Histochemically, microscopic examination of the proventriculus in moorhen revealed cells in its surface lining of the mucosal folds strongly positive to PAS as the reaction gave rise dark purple coloration. The observed reaction was with the granules located at the supra-nuclear area of these cells which was an indication of the presence of neutral type of mucin. These findings were like to those observed in the proventriculus of the (Al-Saffar *et al.*, 2015). The lamina propria extended between the gastric mucosal glands were weak reacted with same stain. These findings were comparable to those observed by (Hamdi *et al.*, 2013) in the glandular stomach of the black- winged kite (*Elanus caeruleus*). The cells that lined the ducts of the submucosal glands showed in their apical region, PAS positive reaction. The connective tissue and wall of blood vessels of submucosa and serosa give negative reaction with PAS and

smooth muscle fiber in muscularis showed poor staining with PAS (Fig. 3).

On applying the combined PAS-AB (pH 2.5) stain, the mucous cells lining the surface epithelium and gastric pits were strongly reacted giving rise to blue and magenta staining with it. In fact, such reaction indicated the presence of high content of neutral and acidic polysaccharides, respectively. Whereas, the glandular secretion gave the magenta color only (Fig. 4). Similarly recent findings of (Al-Saffar *et al.*, 2015; 26) established in the proventriculus of the quail, abundant neutral and acidic mucopolysaccharides in the gastric glands since they gave red and blue colors with PAS-AB procedure stain. As same as to the above findings, neutral and acid mucopolysaccharides were detected in previous study in the surface lining of the mucosal folds in the proventriculus of the black-winged kite (*Elanus caeruleus*) too (Hamdi *et al.*, 2013).

In the ventriculus (Gizzard), the cuticle covering which was detected in the ventriculus only showed positive reaction to PAS stain (pink color) as it present above their epithelial lining (Fig. 7). Cuticle positive reaction with this stain was similarly observed by (Hunter *et al.*, 2008) in ducks, (Bailey and Mensah-brown, 1997) in the Guinea fowl (*Numida meleagris*), (King and Mclelland, 1984) in quail and (Hamdi *et al.*, 2013) in the ventriculus of the black-winged kite (*Elanus caeruleus*). The earlier one, described the cuticle layer as abrasion-resistant lining membrane present as a covering to the mucosa and extended deeply into the glandular lumina.

The epithelium which lined the mucosal folds in the mucosal layer showed positive reaction with PAS. The secretory material within the lumina of the glandular tubules were negatively reacted with PAS stain. On contrary, in the black-winged kite (*Elanus caeruleus*) (Hamdi *et al.*, 2013) and owl (Al-Saffar *et al.*, 2015), it was recorded strong positive reaction with this stain in the same glandular tubules of the ventriculus as both birds were classified as carnivorous avian species.

The connective tissue in the lamina propria, submucosa and in tunica muscularis showed PAS positive reaction in ventriculus of moorhen. While the smooth muscles fibers which constructed the tunica muscularis of the organ gave rise mild reaction (Fig. 7).

When the combined PAS-AB (pH 2.5) used, the cuticle layer showed pink-colored positive reaction for PAS and negative with Alcian blue in moorhen ventriculus. The PAS-positive cuticle layer was similarly observed in other birds such as Guinea fowl (*Numida meleagris*) (Selvan *et al.*, 2008).

The mucosal simple cuboidal epithelium lining of the surface and gastric pits of the ventriculus tubular glands were stained strongly positive with both parts of the combined PAS-AB (pH 2.5) stain (Fig. 8). It indicated the presence of both neutral and acid mucin, respectively. Such observations were similar to those demonstrated by (Pastor *et al.*, 1988; Imai *et al.*, 1991) in the propria glandular cells in the ventriculus of chicken and fowls, respectively. The presence of neutral and acid mucin may protect the mucosal surface and forms a resistant mucosal barrier in the ventriculus of the birds (Mogilnaia and Bogatyr, 1983). In the ventriculus of black-winged kite which is considered one of the meat eater

birds, (Hamdi *et al.*, 2013) documented similar positive reactions with this combined stain in the mucosa and the gastric crypts, ventriculus tubular glands and the secretory material within the lumina of these glands due to the presence of both neutral and acid mucin. The connective tissue gave the negative reaction with PAS and positive with AB, but the smooth muscle bundles of the tunica muscularis reacted weakly with PAS and negatively with AB (Fig. 8).

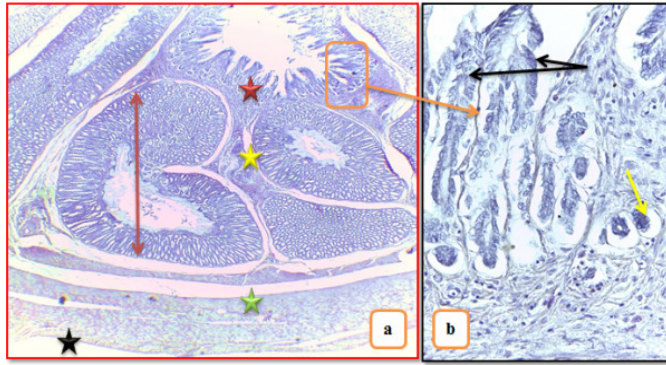


Fig. 1. Microphotography of the proventriculus wall of moorhen showed: mucosa (Brown star), Submucosa (Yellow), Muscularis (Green), serosa (Black), proventricular glands (Red double arrow), epithelial cells in mucosa (Black arrow) and mucosal glands (Yellow arrow). H & E 100 X (a) and 400X (b).

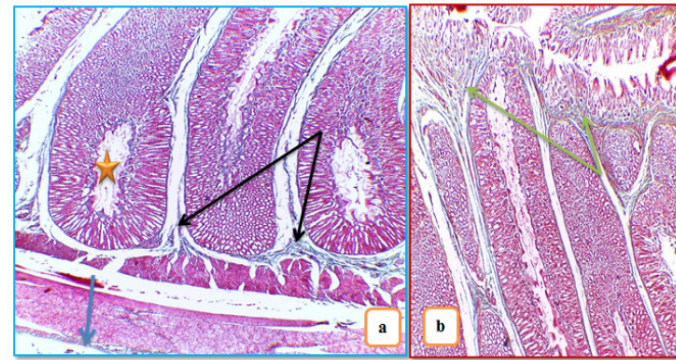


Fig. 2. Microphotography of the proventriculus wall of moorhen showed: Connective tissue in mucosa (Green one head arrow), Connective tissue in submucosa (Black one head arrow) and Connective tissue in serosae (Black one head arrow) Masson Trichrome stain 100 X (a) and 40X (b).

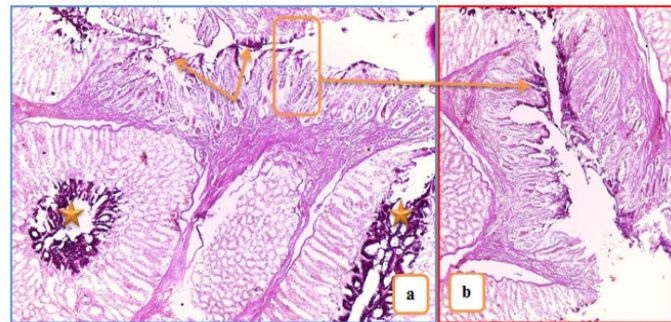


Fig. 3. Microphotography of the proventriculus wall of moorhen showed: Neutral mucin in epithelium (Brown one head arrow) Neutral mucin submucosal glands (Brown star) PAS 100 X (a) and (b).

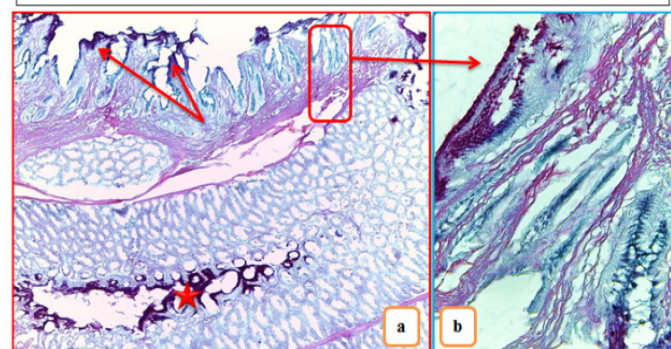


Fig. 4. Microphotography of the proventriculus wall of moorhen showed: Neutral and acidic mucin in epithelium (Red one head arrow) Neutral and acidic mucin submucosal glands (Red star) PAS + AB = pH 2.5 100 X (a) and 400 X (b).

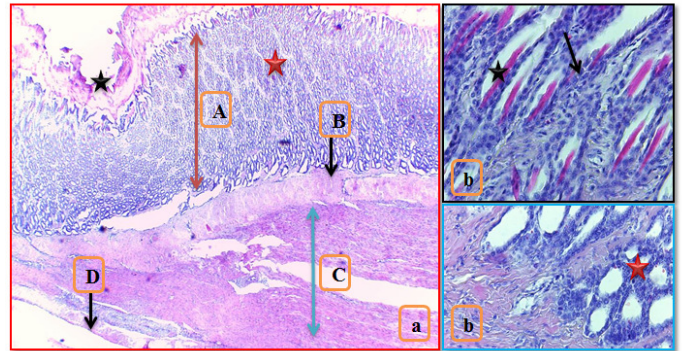


Fig. 5. Microphotography of the Ventriculus wall of moorhen showed: mucosa (A), Submucosa (B), Muscularis (C), serosa (D), mucosal glands (Red star), Cuticle (Black star), epithelial cells in mucosa H & E 100 X (a) and 400X (b), (c).

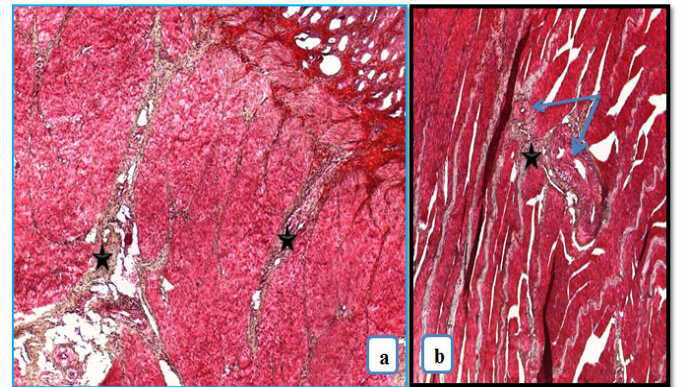


Fig. 6. Microphotography of the Ventriculus wall of moorhen showed: Connective tissue in mucosa (Black star), Blood vessels (Blue one head arrow) Masson Trichrome stain 40 X (a) and 100X (b).

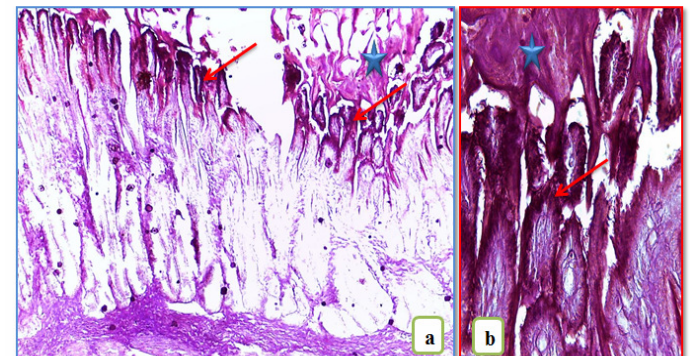


Fig. 7. Microphotography of the Ventriculus wall of moorhen showed: Neutral mucin in epithelium (Red one head arrow) and Cuticle (Blue star) PAS 100 X (a) and X400 (b).

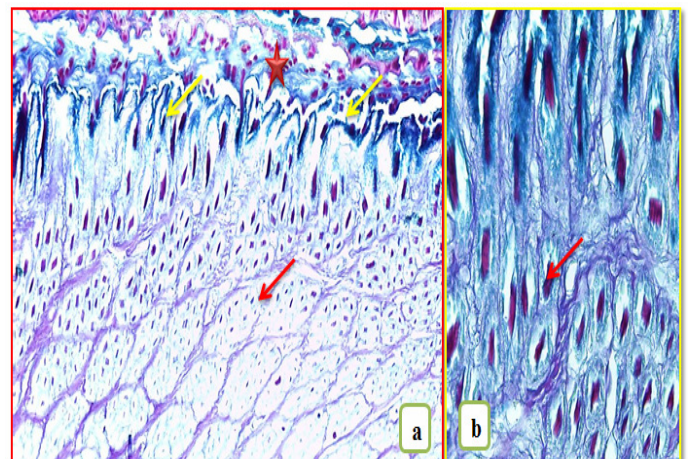


Fig. 8. Microphotography of the Ventriculus wall of moorhen showed: Neutral and acidic mucin in epithelium (Yellow one head arrow) Neutral , acidic mucin mucosal glands (Red one head arrow) and Cuticle (red star) PAS + AB = pH 2.5 100 X (a) and 400 X (b).

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