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Research Article

Histological, Histochemical and Ultrastructural Studies on the Proventriculus of Ostrich (*Struthio camelus*)

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ABSTRACT

The glandular stomach (proventriculus) of ostrich (*Struthio camelus*) was examined histologically, histochemically and by electron microscope. (6) Adult ostriches of both sexes were used in this study. The proventriculus was a dilated sac and divided into papillary and non-papillary regions. The proventricular wall was consisted of tunica mucosa, submucosa, musculosa and serosa. Its mucosa was thrown into plica and sulci which lined by simple columnar epithelium changed into simple cuboidal at the base of the folds. These epithelial cells reacted strongly to PAS, alcian blue (pH 1)and moderately to aldehyde fuchsine. While, very few granules appeared in the apical part of the columnar cells by Best's carmine stain. The lamina propria was loose connective tissue and housed superficial tubular glands which lined by simple cuboidal epithelium. Histochemically, these cells showed moderate to faint reaction to PAS and Best's carmine. The lamina muscularis mucosa scattered in the deepest part of the lamina propria separating it from the tunica submucosa which composed of dense irregular connective tissue containing compound tubulo-alveolar deep glands. These glands histologically, lined by cuboidal or pyramidal shaped cells which reacted moderately to PAS and Best's carmine. On the other hand, these cells reacted negatively to alcian blue (pH 1) and aldehyde fuchsine. At the ultrastructural level, these glands lined by oxynticopeptic cells, which demarcated into light and dark cells, and enteroendocrine cells which were recognized as a closed type cell. The main difference between the papillary and non-papillary regions were the absence of the submucosal glands from the submucosa of the non-papillary region.

Key words: Ostrich, Proventricullus, Histology, Ultrastructure

INTRODUCTION

Ostrich is reared around the world because it has a significant contribution in meat, egg, feathers and leather production (Lji, 2005). The digestive system of poultry differs from other mammals in their internal structure (Cooper and Mahroze, 2004 and Al-Helali *et al*., 2010). It begins by beak and ends by cloaca or vent (Zaher *et al*., 2012). The stomach is considered the most active and important part of the digestive system of birds (Al-Helali *et al*., 2010). The stomach of ostrich consists of three main chambers: proventriculus (glandular stomach- pars glandularis), ventriculus (muscular stomach- gizzard- pars muscularis) and pyloric region (Tadjalli *et al*., 2011 and Alhomaid and Ali, 2013).

The proventriculus or pars glandularis is the cranial chamber of the stomach. Cranially, it is connected to the oesophagus at the oesophageal proventriculus junction and caudally connected to the gizzard by isthmus (Ahmed *et al*., 2011; Zaher *et al*., 2012; Abumandour, 2014; Helal, 2016 and Zhang and Wang, 2018). The proventriculus has a different or specific shape according to the bird. It is spindle shape in Japanese quail (Ahmed *et al*., 2011 and Helal, 2016), cone shape in pigeon and tubular in duck (Hassan and Moussa, 2012), spindle in *Coturnix coturnix* (Zaher *et al*., 2012), cone shape with circular cross section in cattle egret (Hussein and Rezk, 2016) and dilated sac in ostrich (Zhang and Wang, 2018).

The parts of the stomach of birds have a complementary roles. The proventriculus has a secretory function and the ventriculus has a mechanical function (Zhu, 2015). The proventricular wall contains two types of glands: firstly, superficial glands (tubular glands or simple mucous glands) which are responsible for mucous secretion and the second type is deep glands (gastric glands or compound submucosal glands) which are responsible for mucous, hydrochloric and pepsinogen secretion (Oliveira *et al*., 2008) in ostrich and (Hamdi *et al*., 2013) in *Elanus caeruleus*. The proventriculus of ostrich is consisted of two parts: papillated part and non

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papillated part. The papillated part contains the two types of glands while the non papillated part contains only the superficial type of glands (Tadjalli *et al*., 2011).In addition to the secretion of mucous from superficial glands for the protection of the mucosal surface from the corrosive action of the gastric juices, they also produce koilin which also has a protective effect (Illanes *et al*., 2006 and Oliveira *et al*., 2008).

The paucity of the histological studies of the proventricullus of the ostrich lead us to study the histological, histochemical and ultrastructural features of this region of the ostrich stomach.

MATERIALS AND METHODS

Six (6) apparent healthy sexually mature ostriches of both sexes were used in this study. They were raised and obtained from the farm of Wahet El-naam. All birds are fed manufactured ration and drinking water add-libtum throughout the period of life. The samples were collected frequently all over the previous two year. After cervical dislocation, the stomach were dissected out for gross and histological study. The stomach was washed with normal saline. Parts of the stomach were identified and photographed using digital camera in (Fig. 1). The proventriculus then was separated from the region between the oesophagus and the gizzard.

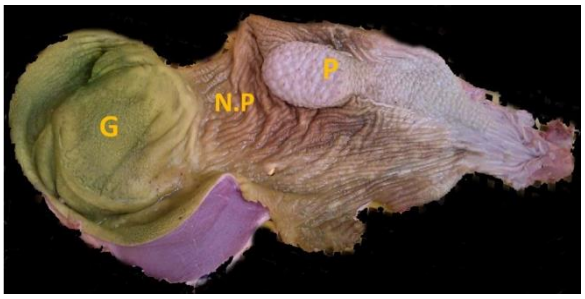

Fig. 1: A photograph showing the internal features of the stomach of ostrich which consisted of papillated part (P) and non papillated part (N.P). Note the gizzard (G).

The samples were obtained from the proventriculus (papillated and non papillated parts), then immersed in 10% neutral buffered formalin and Bouin's solution. Then, the samples dehydrated in ascending grades of ethyl alcohol, cleared in xylene and finally embedded in paraffin wax. Sections were cut about (5-6µm) thick, mounted on clean glass slides and stained using the following methods: Delafield's iron haematoxylin and eosin (H&E) stain: for general tissue structure; Masson's trichrome stain: for demonstration of collagen fibers and smooth muscle fibers; Periodic acid Schiff (PAS) stain: for demonstration of neutral mucopolysaccharides; Alcian blue (pH1): for demonstration of sulphated mucopolysaccharides; Aldehyde fuchsine for: demonstration of sulphated mucosubstance and Best's carmine stain for: demonstration of glycogen.

The aforementioned stains were conducted as outlined by (Bancroft and Stevens, 2010). Tissue sections were examined using Leica Quin 500 analyser computer system (Leica Microsystems, Switzerland) in Faculty of Veterinary Medicine, Cairo University.

Transmission Electron Microscopy (TEM)

Small pieces (1mm) from the obtained samples were fixedin paraformaldehyde- glutraldehyde in phosphate buffer (karnovsky, 1965). Specimens were post fixed in 1% osmium tetraoxide for one hour, washed in 0.1 M phosphate buffer (pH 7.3), dehydrated in ascending grades of ethanol and embedded in Ebon araldite mixture (Mollenhauer, 1964). Semi-thin sections (1µm) were cut and stained with toluidine blue (Richardson *et al*., 1960). Ultrathin sections were cut and stained with uranyl acetate and lead citrate and examined with a JEOL 1010 transmission electron microscope at Faculty of Agriculture, Cairo University, Egypt.

RESULTS

The stomach of ostrich is consisted of three main parts or chambers; the glandular stomach (proventriculus or pars glandularis), the muscular stomach (gizzard or pars muscularis) and the pyloric. Macroscobically, the proventriculus divided into two parts; the papillated part and the non papillated part (Fig. 1). It was connected to the esophagus at esophageal-proventricular junction and connected to the gizzard at proventriculus- gizzard junction. By light microscope, the wall of the proventriculus was consisted of tunica mucosa, tunica submucosa,tunica musculosa and tunica serosa (Fig. 2).

The papillated part

The mucosal surface of the papillated part was invaginated at regular intervals into folds of varying heights. It was highly folded forming parallel folds (plicae) with grooves (sulci) in between. The surface epithelium lining the mucosal folds was consisted of simple columnar cells covered with koilin membrane (Fig. 3 A).

On the ultrastructural level, the lining cells appeared with large oval euchromatic basally situated nucleus with prominent nucleolus and ill developed rough endoplasmic reticulum (Fig. 3 B). The apical part of the cells connected with desmosomal junction and contained secretory granules with variable shapes and densities (Fig. 3 C). The cells toward the base of the fold diminished in their height to become high cuboidal cells with large spherical basal nuclei which contained clumped peripheral heterochromatin (Fig. 3 D).

Histochemically, the epithelial cells reacted strongly to (PAS) (Fig. 4 A), alcian blue stains (pH 1) (Fig. 4 B) and moderately to aldehyde fuchsine stain (Fig. **4** C). While, best's carmine stain showed very few granules in the apical part of the columnar cells (Fig. 4 D). Also, the koilin membrane positive reaction to (PAS) and moderate reaction to alcian blue stains (pH 1) but it showed strong positive reaction to aldehyde fuchsine stain.

The lamina propria occupied the core of the folds. It was consisted of loose connective tissue housing the proprial or mucosal glands. In addition, lamina muscularis appeared as scattered delicate smooth muscle fibers (Fig. 5 A). The proprial glands were lined by cuboidal cells with rounded nuclei. Histochemically, the cells that lined the proprial glands showed faint reaction to PAS and best's carmine (Fig. 5 B & 5 C). Although, there was no reactivity to alcian blue stain (pH 1) and aldehyde fuchsine.

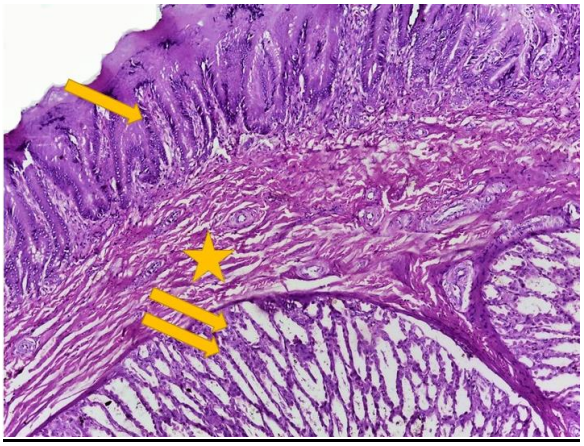

Fig. 2: A photomicrogragh of the papillated part of the proventriculus showing the parallel mucosal folds (arrow) and submucosa (star). Note the submucosal glands (double arrow). H&E stain, x 100.

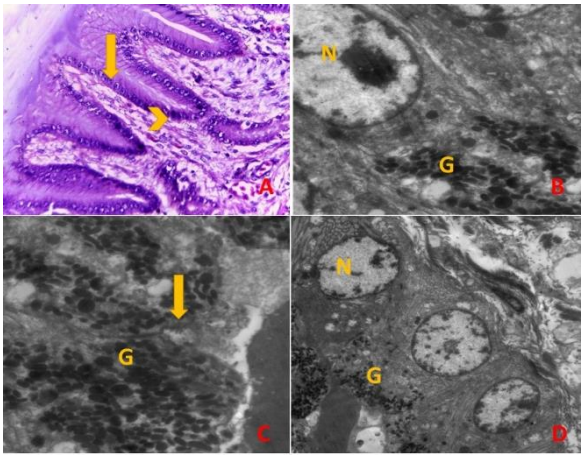

Fig. 3: A photomicrogragh of proventriculus papillated part showing **(A)** the mucosal fold lined by simple columnar cells (arrow) changed to simple cuboidal at the base of the fold (arrow head). H&E stain, x 400, **(B)** T.E.M of the epithelial columnar cells containing oval euchromatic nucleus (N) and the apical granules of different electron density (G). Uranyl acetate and lead citrate stain, x 8000, **(C)** the electron dense granules of various density (G). Note the desmosomal junction between two adjacent cells (arrow). Uranyl acetate and lead citrate stain, x 8000 and **(D)** the cells lining the base of the folds. They are high cuboidal cells with large euchromatic spherical nucleus (N) and apical electron dense granules (G). Uranyl acetate and lead citrate stain, x 4000.

The tunica submucosa was consisted of dense irregular connective tissue (Fig. 6 A). Moreover, this layer contained the proventricular glands (deep glands or submucosal glands) which occupied the greatest part of the proventricular wall. These glands were tubulo-alveolar arranged in the form of rounded or polymorphic lobule (fig. 6B). The glandular units lined by cuboidal cells were tightly attached at the basal part but separated from each other apically by narrow spaces giving the glandular cells a serrated or dentate appearance (Fig. 6C).

The ultrastructural studies to the submucosl glands revealed that each secretory unit was lined by two types of cells oxynticopeptic cells and enteroendocrine cells. The oxynticopeptic cells were two types; light and dark oxynticopeptic cells (Fig. 6D). The light oxynticopeptic

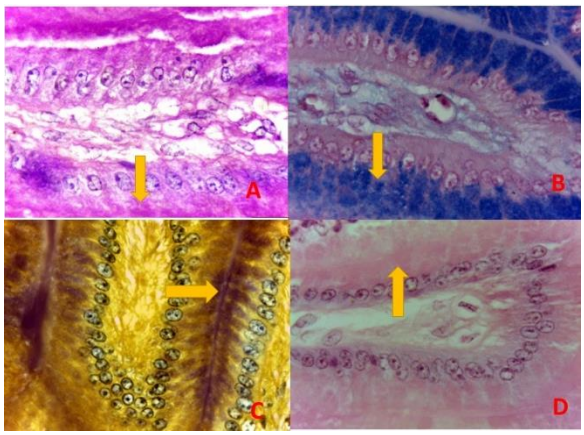

Fig. 4: A photomicrogragh of proventricular mucosal folds of the papillated region showing the histochemical reaction in the apical part of the cells **(A)** strong positive with PAS (arrow). PAS stain, x1000, **(B)** strong positive with alcian blue pH1 (arrow). Alcian blue pH1 stain, x1000, **(C)** mild reaction with aldehyde fuchsine stain (arrow). Aldehyde fuchsin stain, x1000 and **(D)** moderate reaction with best's carmine (arrow). Best's carmine stain, x1000.

cell was high cuboidal or conical in shape with large oval basally situated euchromatic nucleus with prominent nucleolus and numerous mitochondria. The latter appeared spherical and elongated. Moreover, rough endoplasmic reticulum was observed in close association with mitochondria and around the nucleus. Also, smooth endoplasmic reticulum appeared at the apical part of the cell. Very few small dense granules scattered in the cell (Fig. 6E). On the other hand, the dark oxynticopeptic cell was high cuboidal cell with heterochrmatic nucleus. Numerous numbers of large spherical mitochondria and few rough endoplasmic reticulum were associated to them. Also, few smooth endoplasmic reticulum were observed (Fig. 6F). The second type of cells that lined the submucosal gland units were the enteroendocrine cells. They are small cells located between the oxynticopeptic cells and the basement membrane (closed type) (Fig. 6G). These cells contained small oval and rounded granules of different shapes and densities (Fig. 6H).

The cells by histochemical studies showed moderate reaction to PAS and best's carmine but they did not show any reactivity to alcian blue stains (pH 1) and aldehyde fuchsine.

The secretory units were drained by short tertiary ducts which formed by joining of groups of secretory alveoli or tubules. The tertiary ducts opened into wide secretory ducts which formed part of the central cavity. Groups of secondary ducts joined together forming primary ducts which opened on the surface by proventricular papillea. The secondary ductes were lined by psuedostratified columnar epithelium (Fig. 7A), which showed moderate reaction to best's carmine (Fig. 7B). The tunica musculosa consisted of inner circular and outer longitudinal muscle layers.

The non papillated part

The main difference between the papillated and the non papillatedpart was the submucosal deep glands. The latter had no submucosal glands. So, the main components of the submucosal layer of the non papillated part were

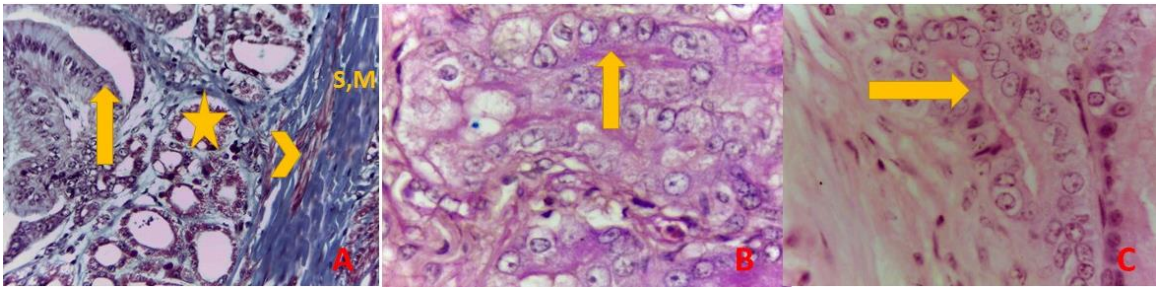

Fig. 5: A photomicrogragh of proventriculus papillated part showing **(A)** the base of the mucosal folds lined by simple cuboidal (arrow), lamina propria containing superficial glands which lined by cuboidal cells (star), delicate smooth muscle lamina muscularis mucosa (arrow head) and dense irregular submucosa (S.M). Masson's trichrome stain, x400, **(B)** showing moderate reaction of proprial glands (arrow) with PAS. PAS stain, x1000 and **(C)** very faint granules in the apical part of cells lined the proprial glands (arrow) with best's carmine. Best's carmine stain, x1000.

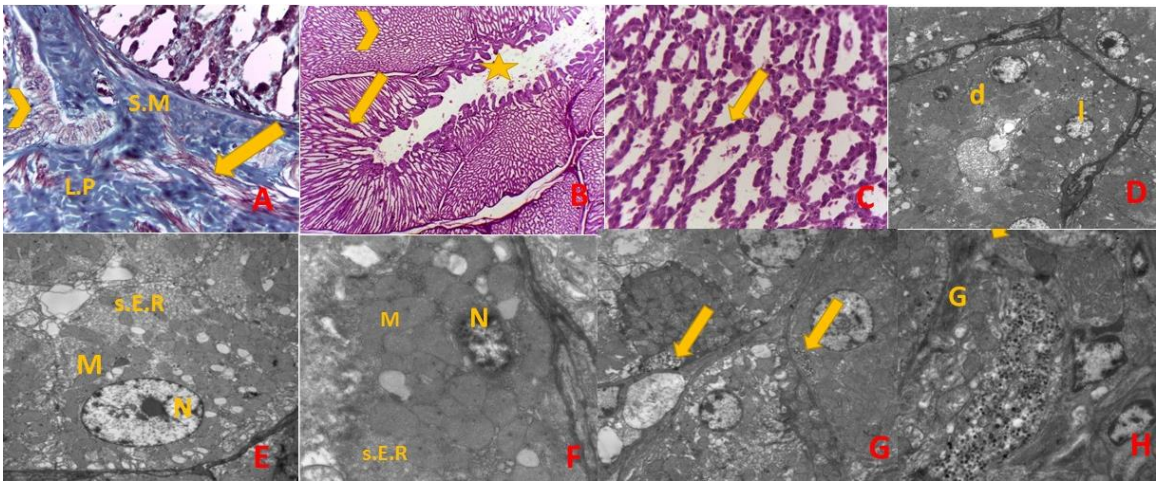

Fig. 6: A photomicrogragh of proventriculuspapillated part showing (A) the lamina muscularis mucosa (arrow) separating the tunica submucosa (S.M) from the lamina propria (L.P) which contained nerve endings (arrow head). Masson's trichrome stain, x400, (B) group of submucosal glands opened together in main central duct (star). Note the alveolar units (arrow head) and tubular units (arrow). H&Estain, x40, (C) alveolar units of submucosal glands lined by high cuboidal cells (arrow). H&Estain, x 400, (D) T.E.M of the submucosal proventricular glands showing alveolar units lined by dark (d) and light (l) oxynticopeptic cells. Uranyl acetate and lead citrate stain, x 2500. (E) T.E.M light oxynticopeptic cell of submucosal proventricular glands showing large oval basally situated nucleus (N), large number of mitochondria (M) and smooth endoplasmic reticulum (sER). Uranyl acetate and lead citrate stain, x 4000, (F) dark oxynticopeptic cell of submucosal proventricular glands showing small dark oval basally situated nucleus (N), large number of mitochondria (M) and smooth endoplasmic reticulum (sER). Uranyl acetate and lead citrate stain, x 4000, (G) T.E.M closed type enteroendocrine cells (arrow). Uranyl acetate and lead citrate stain, x 3000 and (H) enteroendocrine cells (arrow) containing granules of different shapes and electron density. Uranyl acetate and lead citrate stain, x 6000.

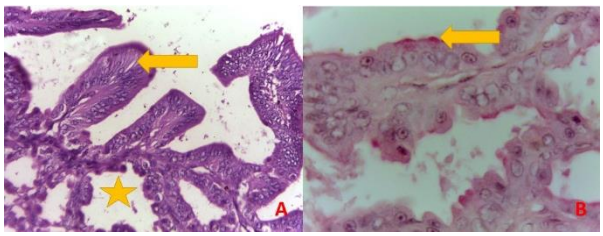

Fig. 7: A photomicrogragh of proventriculus papillated part (A) showing pseudo stratified epithelium lining the secondary duct (arrow). Note the alveoli of submucosal glands (star). H&Estain, x400 and (B) very faint granules in the apical part of cells lined the central duct (arrow) with best's carmine. Best's carmine stain, x1000.

dense irregular connective tissue (Fig. 8A). The mucosa was folded forming short folds (plica) separated by grooves (sulci). These folds were lined by simple columnar epithelium which diminished in height toward the base of the fold to become cuboidal cells (Fig. 8B). Ultrastructurly, the cells lining the mucosal folds appeared with oval basal euchromatic nucleus (Fig. 8C). The apical part of the cells contained high numbers of spherical and oval granules of high electron density.

Histochemically, the cells lining the mucosal fold reacted strongly to alcian blue stains (pH 1) and PAS (Fig. 9A & 9B) and moderately to Best's carmine (Fig. 9C). Very faint reaction appeared in the apical part of the cells that lined the fold by aldehyde fuchsine stain (Fig. 9D). While, the koilin membrane give strong positive reaction to aldehyde fuchsine stain and moderate reaction to PAS and alcian blue stains (pH 1).

The lamina propria was composed of dense irregular connective tissue with collagen fibers, nerve fibers and blood vessels. This lamina also contained simple branched tubular mucosal glands which lined by simple cuboidal cells (Fig. 8B). Histochemically, the apical part of the proprial gland cells showed moderate reaction to alcian blue stains (pH 1) (Fig. 10A) and no reaction appeared by aldehyde fuchsine. Moreover, the apical part of the cells reacted more intensely than the cells of the mucosal fold to PAS and Best's carmine (Fig. 10B & 10C).

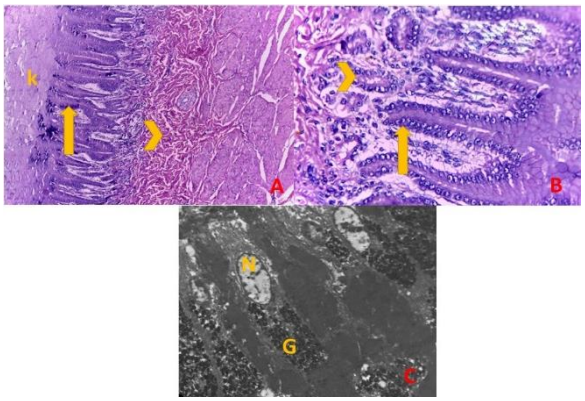

Fig. 8: A photomicrogragh of proventriculus non papillated part showing **(A)** parallel mucosal foldes (arrow) covered by a koilin membrane (K) and dense irregular connective tissue submucosa (arrow head). H&Estain, x40 **(B)** mucosal foldes lined by simple columnar epithelium changed into cuboidal cells in the base of the folds (arrow) and the lamina propria contained superficial proprial glands (arrow head). H&Estain, x400 and **(C)** T.E.M of the columnar cells lining the mucosal fold with large oval euchromatic nucleus (N) and the apical part of the cells containing granules of different shapes and electron densities (G). Uranyl acetate and lead citrate stain, x 3000.

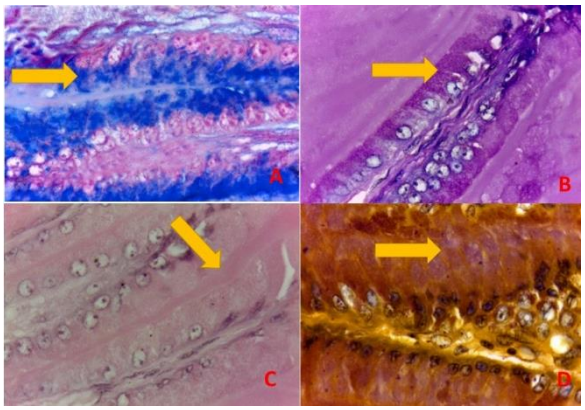

Fig. 9: A photomicrogragh of proventricular mucosal folds of the non papillated region showing the histochemical reaction in the apical part of the cells (A) strong positive with alcian blue pH1 (arrow). Alcian blue pH1 stain, x1000, (B) strong positive with PAS (arrow). PAS stain, x1000, (C) mild reaction with best's carmine (arrow). Best's carmine stain, x1000 and (D) moderate reaction with aldehyde fuchsine stain (arrow). Aldehyde fuchsine stain, x1000.

The lamina muscularis mucosa was ill developed and composed of scattered smooth muscle fibers (Fig. 10D). The tunica musculosa was consisted of inner circular and outer longitudinal muscle fibers between them there were connective tissue, blood vessels and nerve endings.

DISCUSSION

The present study showed that the stomach of ostrich was consisted of three main chambers; proventriculus (pars glandularis, glandular stomach), gizzard (pars muscularis, muscular stomach) and pyloric. This was in agreement with Ahmed *et al*. (2011) in Japanese quail; Zaher *et al*. (2012) in Coturnix coturnix; Helal (2016) in quail; Hussein and Rezk (2016) in cattle egret and Taki-El-Deen (2017) in spur-winged lapwing. The current

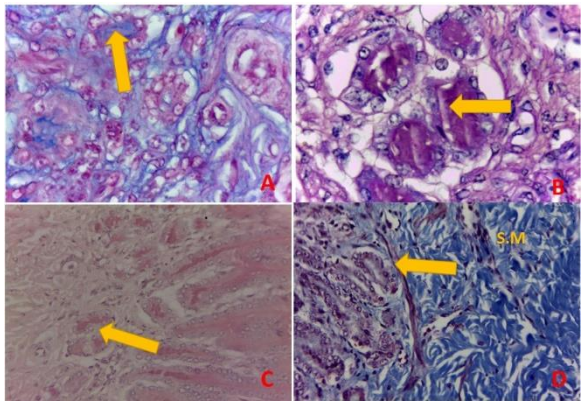

Fig. 10: A photomicrogragh of proventricular non papillated part showing the histochemical reaction in the apical part of the cells lining the superficial proprial glands (A) faint reaction with Alcian blue pH1 stain (arrow). Alcian blue pH1 stain, x1000, (B) strong reaction in the superficial proprial glands (arrow) with PAS stain. PAS stain, x1000, (C) moderate reaction in the apical part of cells lining the base of the folds (arrow) and the proprial glands (arrow head) with best's carmine. Bests carmine stain, x400 and (D) ill developed lamina muscularis mucosa (arrow) and dense irregular connective tissue submucosa (S.M). Masson's trichrome stain, x400.

observation described that the proventriculus was a dilated or distensible sac like similar to that observed by Cooper and Mahroze (2004) and Zhang and Wang (2018). Dissimilar shapes such as spindle shaped proventriculus in Japanese quail (Ahmed *et al*., 2011); *Coturnix coturnix* (Zaher *et al*., 2012) and quail (Helal, 2016), cone shaped proventriculus in pigeon (Hassan and Moussa, 2012) and (Hussein and Rezk, 2016), fusiform in Eurasian hobby (Abumandour, 2014) and tubular shaped proventriculus in duck (Esmael, 2019).

The histological analysis of the wall of the proventriculus revealed that it was consisted of the usual four tunics; tunica mucosa, sub mucosa, musculosa and serosa. Similar observation was reported in Australian passerines (Ogunkoya and Cook, 2009); in Japanese quail (Ahmed *et al*., 2011); in ostrich (Tadjalli *et al*., 2011); in duck and pigeon (Hassan and Moussa, 2012); in broilers (Nasrin *et al*., 2012); in falcon (Abumandour, 2013); in mallard and domestic pigeon (AL- Saffar and Al-Samawy, 2015 & 2016); in cattle egret (Hussein and rezk, 2016)in spur-winged lapwing and (Taki- El- Deen, 2017). Dissimilar observation was observed in black tailed crack as the proventricular wall was consisted of only three layers; tunica mucosa, tunica musculosa and tunica serosa (Zhu, 2015). A unique feature was observed in ostrich; that the mucosa was consisted of papillated and non papillated parts. This observation was recorded by Tadjalli *et al*. (2011) in ostrich.

The mucosa of the proventriculus of the ostrich either papillated or non papillated was consisted of longitudinal parallel folds (plica) with grooves (sulci) in between. Dissimilar shapes of folds were observed in other birds as branched folds in coot bird (Batah *et al*., 2012) and (Esmael, 2019) in duck, anastomosed in *Elanus caeruleus* (Hamdi *et al*., 2013) and finger like in quail (Helal, 2016). The current study showed that the mucosal folds were lined by simple columnar cells. Similar findings were stated in domestic fowl (King and McLelland, 1975 and

Salem, 1985 and 1997); ostrich (Bezuidenhout and Van Aswegenm, 1990 and Tadjalli *et al*., 2011); Japanese quail (Ahmed *et al*., 2011); red jungle fowl (Kadhim *et al*., 2011) and domestic pigeon (AL- Saffar and Al- Samawy, 2016). However, the cells lined the folds decreased in height toward the base of the fold as observed in ostrich (Bezuidenhout and Van Aswegenm, 1990); domestic pigeon and duck (Hassan and Moussa, 2012); *Elanus caeruleus* (Hamdi *et al*., 2013) and black tailed crack (Zhu, 2015). While, the folds were lined by cuboidal cells in partridge (Rossi *et al*., 2005). Hamdi *et al* (2013) thought that the folds presented to enhance the storage capacity and to increase the surface area of the proventriculus.

The histochemical studies of the cells lined the mucosal folds showed strong positive reaction to PAS, alcian blue (pH 1). This result was in accordance with Ahmed *et al.* (2011) Japanese quail; Helal (2016) in quail; Hussein and Rezk (2016) in cattle egret and Das *et al.* (2017) in kadaknath fowl. This confirm the presence of neutral, acidic and sulphated mucus secretory granules in the supra nuclear region of the cells (Suprasert and Fujioka, 1990). The last authors added that this mucus plays a significant role in protecting the stomach from digestive enzymes and pathogens. The mucins formed a gelatinous layer over the mucosal surface and act as a lubricant and a protective barrier to the physical damage (Lacy, 1985). Moreover, the acidic mucins act as anti ulcerogenic while, the neutral mucins assisted in the enzyme secretion (Zhu, 2015). Dissimilar findings were observed in parrots where, the surface epithelial cells did not react to PAS and alcian blue stains (Das *et al*., 2017).

By electron microscope, the surface epithelial cells appeared high columnar cells attached laterally by desmosomes. This was in agreement with Sallam (2001) in falcon; Helal (2016) in quail; Hussein and Rezk (2016) in cattle egret and Esmael (2019) in duck. Sallam (2001) suggested that these junctions for sealing the adjacent cells and preventing the proteolytic effect of acids on the epithelium. In the current study, these columnar cells contained rough endoplasmic reticulum, spherical mitochondria, electron dense granules and basally situated oval euchromatic nuclei similar to that observed by Helal (2016) in quail; Hussein and Rezk (2016) in cattle egret and Esmael (2019) in duck. These structures explained that the nature of the granules were mucins in nature (Sallam, 2001).

The present study revealed that the lamina propria was consisted of loose connective tissue containing blood vessels, lymphatics and nerves. Similar findings were observed in Japanese quail (Ahmed *et al*., 2011); in Eurasian hobby (Abumandour, 2014) and in mallard and domestic pigeon (Al- Saffar and Al- Samawy, 2015 & 2016). While, dense irregular lamina propria was observed in *Coturnix coturnix* (Zaher *et al*., 2012); *Elanus caeruleus* (Hamdi *et al*., 2013) and black-tailed crake (Zhu, 2015). The current study observed superficial tubular glands lined by simple cuboidal cells in the lamina propria. This was in agreement with Tadjalli *et al*. (2011) in ostrich and Hamdi *et al*. (2013) in *Elanus caeruleus*. Al- Saffar and Al- Samawy (2015 & 2016) in mallard and domestic pigeon. While, these glands lined by columnar cells in broilers (Nasrin *et al.*, 2012); Eurasian hobby (Abumandour, 2014) and duck (Esmael, 2019).

Histochemically, the cells lining the proprial glands reacted moderately to PAS and Best's carmine stains but did not show any reaction to alcian blue and aldehyde fuchsine stains. The PAS reaction was similar to that reported by Zhu (2015) in black-tailed crake but the alcian blue reaction was in disagreement with the same author and the same bird.

In this study, the lamina muscularis mucosa appeared as delicate smooth muscle in the deepest part of the lamina propria similar to that reported by Tadjalli *et al*. (2011) in ostrich. On the other hand, in cattle egret this lamina was consisted of longitudinally arranged smooth muscle fibers (Hussein and Rezk, 2016) and African ostrich (Zhang and Wang, 2018). While, it was reported to be circularly arranged layer in duck (Esmael, 2019). However, in Australian passerinesthis lamina was consisted of three orientations of smooth muscle fibers (Ogunkoya and Cook, 2009) and in *Coturnix coturnix* it was consisted of two small smooth muscle layers (Zaher *et al*., 2012).

The present study revealed presence of tubulo alveolar deep proventricular glands in the submucosal layer. This was in agreement with Tadjalli *et al*. (2011) and Zhang and Wang (2018) in ostrich; Batah *et al.* (2012) in coot bird; Abumandour (2014) in Eurasian hobby; Al- Saffer and Al- Samwy (2015 & 2016) in mallard and domestic pigeon and Das *et al.* (2017) in Kadaknath fowl. Meanwhile, these glands located in the lamina propria as reported by Duritis and Mugurecics (2011); in ostrich and Zhu (2015) in Grey-backed shrike. Whereas, Zaher *et al*. (2012) in *Coturnix coturnix* and Wali and Kadhim (2014) in heavy and light line found that these deep glands located between inner and outer layers of lamina muscularis mucosa.

The secretory units of the proventricular glands were lined by cuboidal to pyramidal or low columnar cells similar to that observed in ostrich (Bezuidenhout and Van Aswegen, 1990); pigeon and domestic duck (Hassan and Moussa, 2012) and cattle egret (Hussein and Rezk, 2016).

Histochemically, the cells lining the secretory units showed mild to moderate reaction to PAS similarly as observed in fowl (Imai *et al*., 1991) and (Al- Saffer and Al- Samwy, 2015) in mallard. On the other hand, the cells reacted negatively to this stain in domestic duck (Hassan and Moussa, 2012); heavy and light line (Wali and Kadhim, 2014); black-tailed crake (Zhu, 2015); quail (Helal, 2016); cattle egret (Hussein and Rezk, 2016) and duck (Esmael, 2019). Also, we observed that the secretory cells did not show any reactivity to alcian blue (pH 1) and aldehyde fuchsine. This was in agreement with Helal (2016) in quail and Esmael (2019) in duck.

By electron microscope, this study revealed that the submucosal glands were lined by two types of cells; oxynticopeptic cells and enteroendocrine cells and the oxynticopeptic cells appeared in two forms; light and dark cells. Similar findings were observed in cattle egret (Hussein and Rezk, 2016) and duck (Esmael, 2019). While, Helal (2016) in quail noted that the oxynticopeptic cells was only one cell type; light cells. The light cells were high cuboidal or conical in shape with large oval basally situated euchromatic nucleus with prominent nucleolus. There were large numbers of mitochondria. Rough endoplasmic reticulum was associated with

mitochondria around the nucleus. High numbers of smooth endoplasmic reticulum located at the apical part of the cell and very few small dense granules scattered in the cell. Similar result was observed in cattle egret (Hussein and Rezk, 2016) and duck (Esmael, 2019). The fore mentioned ultrastructure was adapted for secretion of pepsin and hydrochloric acid (Koening and Dabike, 1990) in chicken. The large numbers of mitochondria could be correlated with metabolic activity whichneed active energy for transporting inorganic ions through the process of secretion (Buggs, 1985). The high number of vesicular smooth endoplasmic reticulum was suitable for production of hydrochloric acid (HCl) (Sedar and Friedman, 1960). The other type of cells were the enteroendocrine cells. In ostrich, all the enteroendocrine cells, which observed, were closed type meaning that they located between the basement membrane and the oxynticopeptic cells. Similar type was observed in quail (Helal, 2016); cattle egret (Hussein and Rezk, 2016) and duck (Esmael, 2019). While, both open and closed enteroendocrine cells were observed in falcon (Sallam, 2001).

The non papillated part

The current study showed that the proventriculus of ostrich was consisted of two parts; papillated and non papillated part similar to that stated by Tadjalli *et al*. (2011) in ostrich. The two parts was consisted of the ordinary layers; tunica mucosa, submucosa, musculosa and serosa. The mucosa of the papillated part was folded and also the non papillated part. This was in disagreement with Tadjalli *et al*. (2011) who stated that the non papillated part was consisted of folded and non-folded parts. The lamina propria was consisted of dense irregular connective tissue with blood vessels, nerves and lymphatics and also it characterized by absence of submucosal glands as Tadjalli *et al*. (2011) reported in ostrich.

Conclusions: The stomach of ostrich consisted of papillated and non papillated regions. The papillated part contained tubuloalveolar submucosal glands but the non papillated part lacked those glands.

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