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Some Histological and Scanning Electron Microscopic Studies on The Gizzard of Turkey

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ABSTRACT

This research was designed to study the histological characteristics of gizzard in turkey by light and scanning electron microscopes. The study was performed on ten healthy mature birds of turkey. The specimens were immediately removed after slaughter, gently cleaned and fixed in 10% buffered neutral formalin then processed for light and scanning microscopic studies. The histological observations revealed that as other species of birds the turkey gizzard was consisted of four tunics; mucosa, sumbucosa, musculosa and serosa. Thick layer of cuticle was lined the gizzard mucosa which contains gastric pits. The lamina epithelialis lined with simple cuboidal epithelium, the lamina propria contained simple tubular glands which arranged in groups. These glands are lined by simple cuboidal epithelium. The inner part of these tubular glands contains an eosinophilic secretion. PAS positive material distribute nearly in almost region. It was within the lumen of the glands, within the cells lining the surface and crypts, Also, within the deep glandular cells**.** The submucosa consists of connective tissue formed mainly from collagen fibers**.** The tunica muscularis was very thick formed from parallel bundles of smooth muscle fibers, these bundles separated by thin layer of connective tissue. Scanning electron microscopic observations revealed that the surface of the koilin cuticle was very thick and appeared in form of clusters separated by grooves resembling the cracked ground appearance. These clusters were in the form of rodlets.

1. INTRODUCTION

Turkeys are found to be of high economic value in our life as it is an important source of food in many parts of the world. Turkey meat has nutritional properties which make it almost ideal raw material for rational nutrition.

The alimentary canal of birds is physiologically totally different from alternative animals. It is a double-ended open tube starting at the beak and finishing at the vent (Zaher et al., 2012). The bird can take advantage of a wide variety of food due to presence of proventriculus and gizzard. Also, the digestive system of bird helps to supply conditions for flight; it's overall less length as compared to it of mammals, lack of teeth, light-weight beak, high metabolic rate. (Harndi et al., 2013).

The bird's stomach composed of two components the proventriculus and the ventriculus or gizzard. The two parts separated by an intermediate zone (Dyce, 2002). The ventriculus would have the mechanical operate of trituration of food (Sturkie, 2000).

The gizzard is found posteriorly within the abdomen. It is characterized by presenting a secretion plate on the inner surface and a well-developed muscle layer. Each characteristic represents associate adaptation to grind grains (Catroxo et al., 1997; Aksoy & Cinar 2009; Liman et al. 2010).

The membrane lining the ventriculus appeared as wavy lines running parallel to the surface. It is "horny" and appeared to consider that it was absolutely a keratinous substance. (Calhoun, 1954) reported that the ventriculus cuticle is keratohyalin, keratinoid and keratin-like. However, later researchers (Eglitis and Knouff, 1962; Akester, 1986) indicate that the gizzard membrane is a mucoprotein complex and its nomenclature was differed (Akester, 1986) but it is now referred to as the gastric cuticle (Baumel et al. 1993).

Little studies have demonstrated on turkey gizzard so the essential point of this work was to study the histological structure of gizzard by light and scanning electron microscope.

2. MATERIALS AND METHODS

2.1. Tissue preparation for Light microscopically studies:

This study has been conducted on ten adult turkeys of either sex from farms in Sharkia governorate. The specimens were removed after slaughter, cleaned with normal saline to remove any debris then fixed in 10% buffered neutral formalin, the tissue samples were routine histological processing. The slides stained by Harris's Hematoxylin and Eosin (H & E) stain to demonstrate general structure. Van Gieson stain: to detect collagen and muscle fibers**.** Periodic acid Schiff technique for neutral and some acidic mucopolysaccharides. The methods of processing and staining were adopted after **(Bancroft and Gamble, 2008)**.

2.2. Tissue preparation for scanning electron microscopy (Bozzola and Russel, 1999).

The specimen were fixed in 2.5% glutaraldehyde in 0.1 m sodium phosphate buffer, PH 7.3, then the specimens were washed in in 0.1 m sodium phosphate buffer, PH 7.3 for 10 minutes each and post fixed in 1% buffered osmium tetroxide for 2 hours at 4°C, the specimens were again washed in0.1 m sodium phosphate buffer, PH 7.3 and then in distilled water (2 times for 5 min each), dehydrated in alcohol and dried to the critical point. The specimens were mounted in metal base, sputtered with gold in an Emitech K550 sputter apparatus analysed and photographed under LEO 435 VP and JEOL JSM 5200 scanning electron microscope in Faculty of Medicine Tanta University**.**

3. RESULTS

3.1. Histological structure:

In the turkey, the gizzard was consisted of four tunics, the tunica mucosa, tunica submucosa, tunica muscularis and the tunica serosa. Upon the surface of tunica mucosa there was a thick layer of cuticle that may be detached throughout the preparation of histological section. The mucosa contains gastric pits.

The lamina epithelialis of this organ lined with simple cuboidal epithelium, the surface epithelium invaginate toward the lamina propria**.** Lamina propria contained simple tubular glands that produce a material called the gastric cuticle. These glands are lined by simple cuboidal epithelium. The inner part of these tubular glands contains an eosinophilic secretion, along with the cuticle (Fig. 1).

The cuticle showed a positive reaction to PAS stain. The reaction of PAS positive material distribute nearly in almost region. It was within the lumen of the glands, within the cells lining the surface and crypts, within the deep glandular cells**.** Also, within the submuocosa and between the smooth muscle bundles of tunica musculosa (Fig. 2).

No muscularis mucosa was observed. Propria submucosa in the gizzard of turkey consists of connective tissue formed mainly from collagen fibers**.** The tunica muscularis was very thick formed from parallel bundles of smooth muscle fibers, these bundles separated thin layer of collagen fibers. Well-developed blood vessels were observed within tunica musculosa. Tunica serosa was formed from connective tissue covered with mesothelium (Fig. 3).

3.2. Scanning microscopic observation:

The surface of the koilin cuticle was very thick and composed by plates which present in form of clusters separated by grooves resembling the cracked ground appearance. (Fig.4). In higher magnification the cuticle plates were in the form of rodlets (Fig. 5). The side view showed the layers of gizzard, superfecially the thick cuticle, underneath the tubular glands then the thick tunica musculosa (Fig. 6).

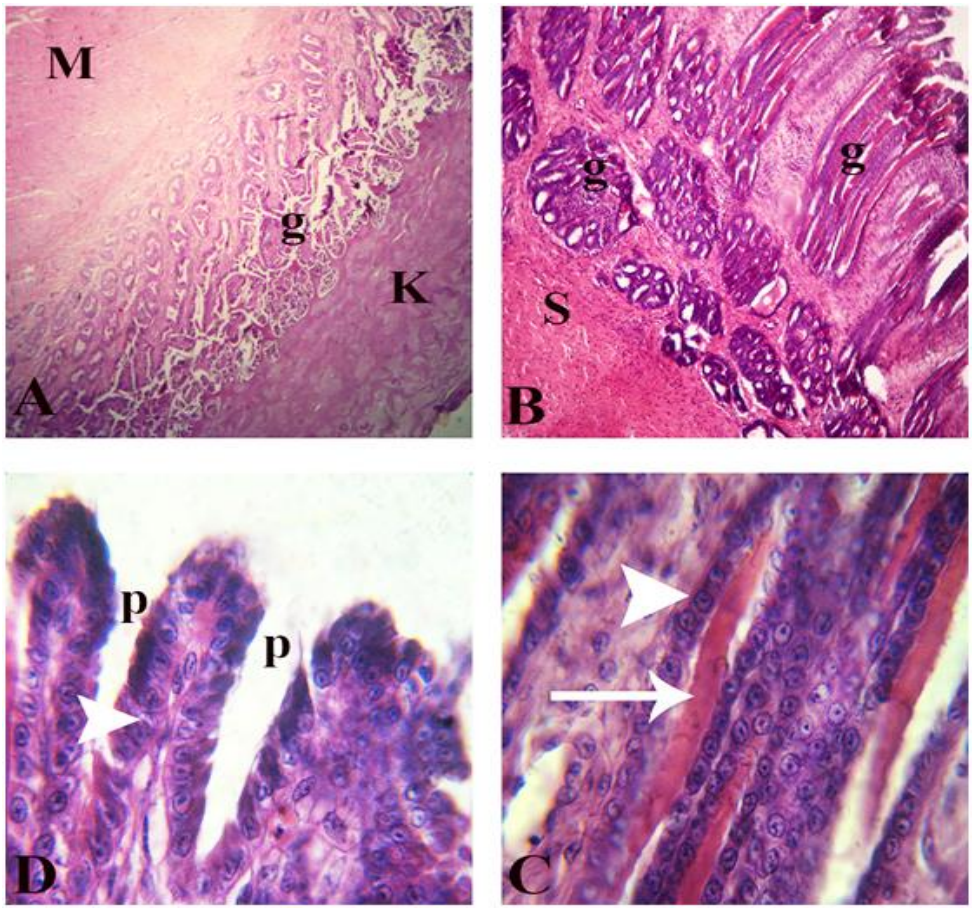

Fig.1. Photomicrograph of turkey gizzard. (A) showing thick koilin cuticle (K), lamina propria containing the mucosal glands (g), thick tunica musculosa (M). (B) showing the simple tubular glands (g) and summucosa (S). (C) showing the glands are lined by simple cuboidal epithelium (arrow head) and contains of eosinophilic secretion (arrow) . (D) showing the gastric pit (p) and the lining epithelium of surface epithelium (arrow head). Stain: H&E. (x. 100in A&B, x. 1000in C&D).

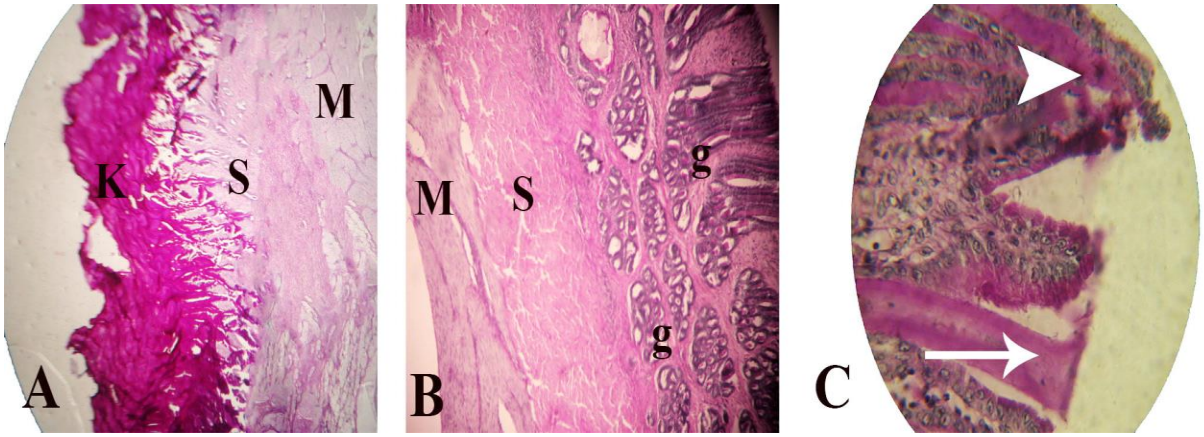

Fig. 2. Photomicrograph of turkey gizzard. (A) showing PAS positive reaction in the koilin cuticle (K). (B) the reaction of PAS within the deep glandular cells (g), within the submuocosa (S) and between the smooth muscle bundles of tunica muculosa (M). (C) showing the reaction within the cells lining the surface (arrow head) and crypts (arrow). PAS stain. (x. 100 in A&B, x. 1000 in C)

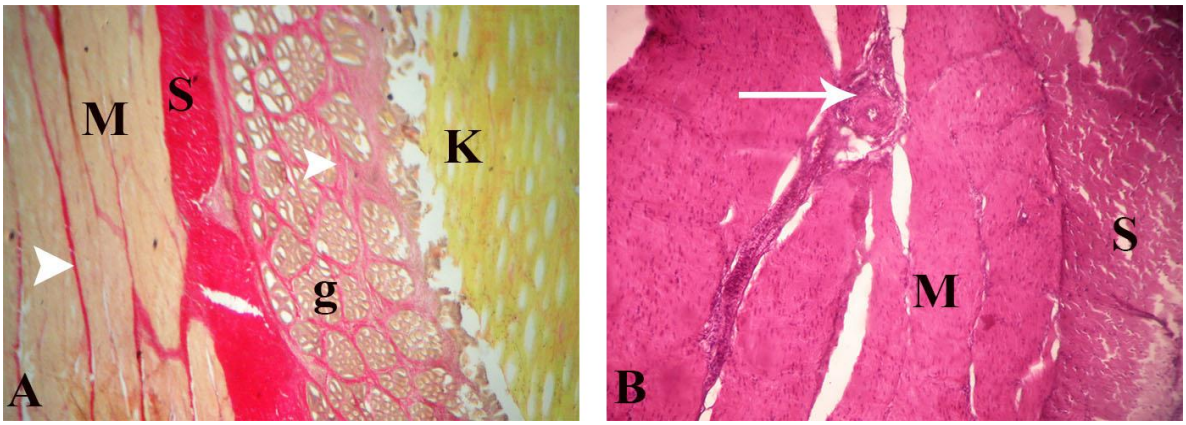

Fig. 3. Photomicrograph of turkey gizzard. (A) showing cuticle (K), gland lobules of mucosa (g) separated by collagen fibers (arrow head), submucosa (S), muscle bundles of musculosa (M) separated by thin layer of collagen fibers (arrow head). (B) showing the submucosa (S), thick musculosa (M) contains well developed blood vessels (arrow). Stain: Van Gieson stain in A, x. 100. Stain: H&E in B, x. 100

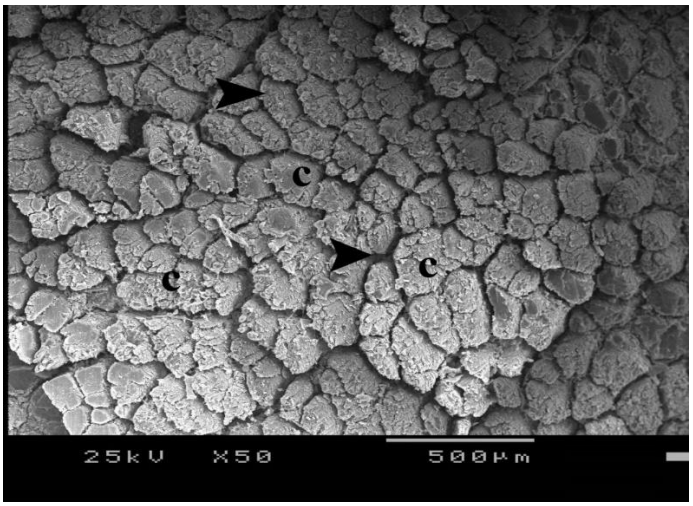

Fig.4. Scanning electron micrograph of turkey gizzard showing the cuticle membrane consists of clusters (c) of plates separated by grooves (arrow heads).

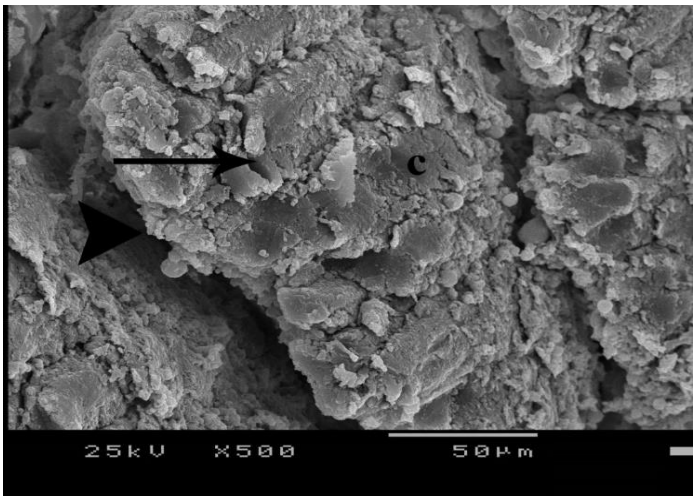

Fig.5. Scanning electron micrograph of turkey gizzard showing the rodlets (arrow) of hard cuticle which found in clusters(c) separated by grooves (arrow head).

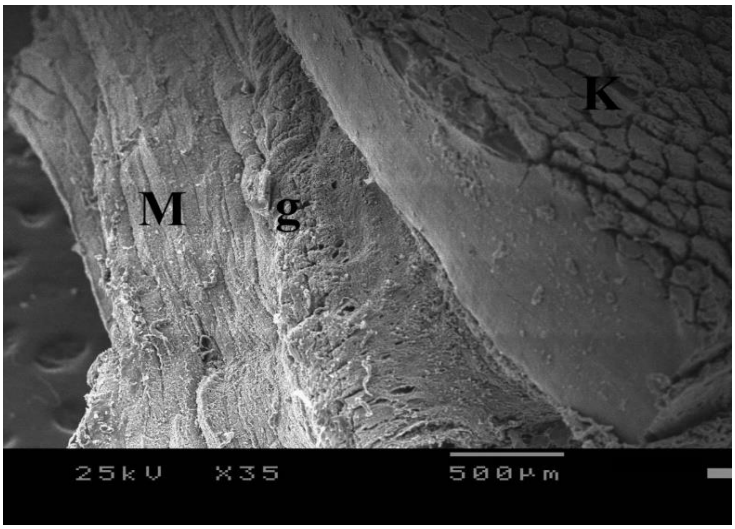

Fig. 6. Scanning electron micrograph of turkey gizzard showing the side view of gizzard formed from cuticle koilin membrane (k), tubular glands (g) and thick tunica musculosa (M).

4. DISCUSSION

Gizzard is a very important organ in birds because it permits the mechanical reduction of tough material through powerful muscular development**. (**Nasrin et al., 2012) stated that the gizzard was an extremely muscular organ liable for grinding and macerating the ingesta.

As mentioned previously in other researches, the turkey gizzard consists of four tunics, (Rodrigues et al., 2012) In maccaws, (Batah et al., 2012) in coot bird, (Nasrin et al., 2012) in broilers, (Selvan et al., 2008) in fowl.

In turkey gizzard the mucosa has simple cuboidal epithelium and the lamina propria contains simple tubular glands that produce a material called the cuticle (Akster, 1986; Batah et al., 2012; Nasrin et al., 2012).

(Qureshi et al., 2017) mentioned that gizzard was lined internally by yellowish layer of cuticle secreted by gizzard glands.

In the present study the mucosal surface was coated by koilin cuticle, , this cuticle may be not observed as it has been destroyed during the preparation of the histological sections, These results are similar to the findings of (Rodrigues et al., 2012) in macaws.

The koilin cuticle which is a secretary layer above the mucosa, showed a positive reaction to PAS. The surface epithelium was PAS positive; these results are in line with the findings of (Selvan et al., 2008) and (Ahmed et al., 2011) in quail.

Turkey gizzard contains gastric pits in the mucosa these gastric pits lead to gizzard tubular glands lined by cuboidal epithelium and stained positively to PAS. The gizzard tubular glands located in the lamina propria, these results are in line with (Ahmed et al., 2011), (Rossi et al., 2005) and (Akester, 1986).

Similar to the finding was mentioned by (Nasrin et al., 2012) the results obtained in this study revealed that propria submucosa in the gizzard consist of connective tissue formed mainly from collagen fibers. On the other hands submucosa was not observed in the gizzard of coot bird (Batah et al., 2012) due to confused of mucosal layer with muscularis layer.

(Qureshi et al., 2017) mentioned the lamina propria contained many tubular glands that were the protruding lamellae of the glandular cells making elongated crypts. The simple glandular tubules and the simple tubular glands open into the shallow crypts.

The gizzard of turkey confirmed that all the components of the gizzard were PAS positive these results are in line with Gaikwad et al. (2002) in chicken and disagree with the finding of (Selvan et al., 2008) and (Demirbağ et al., 2015) who observed that PAS positive material was present in the lumen of the glands and in the cells lining the surface and crypts but the deep glandular cells were found to lack mucin.

The inner part of the tubular glands contains an eosinophilic secretion, located along with the cuticle, which was also observed by (Catroxo et al., 1997).

The finding showed that the turkey gizzard have not contained the muscularis mucosae, these results are in accordance with (Catroxo et al., 1997) in red-capped cardinal and (Ahmed et al., 2011) in quail. This character allows the tubular gizzard glands to penetrate the lamina propria more deeply and to become more active in secreting the keratinized layer. (Jain, 1976) found less developed muscularis mucosae in the ring-necked parakeet (fruit bird) while in the macaw (Rodrigues et al., 2012) were found only single layer.

Ahmed et al. (2011) mentioned that the tubular glands lined mostly by chief cells which stained basophilic, similar to fowl (Toner, 1964). These cells are suggested to secrete photolytic enzyme, pepsin.

In this study the tunica muscularis of turkey gizzard is very thick, and consists of parallel bundles of smooth muscle fibers as (Nasrin et al., 2012) and (Rodrigues et al., 2012) while (Qureshi et al., 2017), (Batah et al., 2012) and (Samuelson, 2007) reported that the tunica muscularis consists of two layers internal circular and external longitudinal.

Starck et al. (2003) in quails described that muscles of gizzard consisted of smooth muscles which were separated by layers of connective tissue giving an onion structure shape to gizzard muscles. Mathias and Abdul Rahman (2003) mentioned that thickness of gizzard muscles were approximately 180.52 mm in two weeks old Japanese quails.

In turkey gizzard the serosal layer is covered with mesothlium and rich with blood vessels and these results similar to that observed in chicken (Caceci, 2006).

(Gosomji et al., 2017) revealed that the epithelial lining of the primordial ventriculus exposed the tubular glands which later became canalized from 23 th day and gain prominence at the 27th day indicate that starting from the 19th day there could slight functionality of the ventriculus agreeing with the findings of Soliman et al. (2015) that observed in quail from the 12th day and by 14 th day it has become canalized.

The present work revealed that in turkey the cuticle appeared in form of plates present in form of clusters separated by grooves which resemble the cracked ground appearance; this is similar to (El-Ghazali, 2013) in pigeon. In macaw's ventricle (Rodrigues et al., 2012) demonstrated the presence of a hard and thick gastric cuticle observed macroscopically and few folds of mucosa of dense connective tissue band.

(El-Ghazali, 2013) also mentioned that in duck the koilin cuticle has longitudinal folds separated by grooves, and in goose each fold had a honey comb appearance. All types of folds of koilin membrane were formed of groups of clusters of rodlets of hard koilin simulating the finding of (Akester, 1986) in domestic fowl. However the layers of the macaw's koilin cuticle (Rodrigues et al., 2012) only display koilin plates and desquamated dead epithelial cells.

The cuticle which differs according the bird species, bird habits and food consumed, in which (Akester, 1986, Catroxo et al., 1997, Dyce et al, 2002). In the Eurasian Hobby (Abumandour, 2014), found that, over a small area of the mucous membrane of ventriculus, which was situated at the region around the pyloric opening only was a small weak cuticle layer while the remainder of the luminal surface not have cuticle membrane. (Gionfriddo and Best, 1996) refer to that the thickness of the cuticle was highly correlated with food consumed; thin in frugivorous and nectarivorous while thick in granivorous,

CONCLUSION. The gizzard which is the muscular portion of bird's stomach is very important; its thick cuticle and thick musculosa perform functions which are important in the process of digestion. The difference in the structure of turkey gizzard may be attributed to the diet and its alimentary habits.

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