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HISTOLOGICAL, HISTOCHEMICAL AND ELECTRON MICROSCOPICAL STUDIES ON PYLORIC REGION OF BALLADY DUCK *(ANAS PLATYRHNCHOS)*

By

Asma A. Abd El-Salam[1]; Shaymaa H. Mohamed[2]; Ebtihal M. M. EL-Leithy[2] and Saad M.S. El-Gharbawy[2]

[1]Department of Anatomy and Embryology, faculty of Veterinary medicine, Omar El-Mukhtar University, Libya.

[2]Cytology and Histology Department, Faculty of Veterinary Medicine, Cairo University

ABSTRACT

This research was carried on 10 apparent healthy adult ballady ducks *(Anas platyrhynchos)* of both sexes with average age ranged from (6-7) months and average weight ranged from (4.5- 5.5) Kg. Small pieces from longitudinal section of pyloric region were fixed immediately in 10% neutral buffered formalin solution and Bouin's fixatives, processed, stained with Delafield's iron heamatoxylin and eosin (H&E), Masson's trichrome, Periodic acid Schiff (PAS), Alcian blue pH 1, Alcian blue pH 2.5, combined PAS-Alcian blue pH 2.5, Aldehyde fuchsin and Best's carmine stains. In addition, small pieces (1mm) from pyloric region were fixed in paraformaldehyde-glutraldehyde in phosphate buffer and were examined with transmission electron microscope. The pyloric region was a third part of the stomach, it was very small area between the gizzard and duodenum. The mucous membrane of the pyloric region was lined by simple columnar mucous secreting epithelium.The lamina propria contained solitary lymph nodules; supported by smooth muscle fibers of the muscularis mucosa. The surface epithelium displayed strong reaction to PAS, moderate reaction to Alcian blue pH 1, Alcian blue pH 2.5 and Best's carmine stains. Ultrastructurally, five cell types could be observed in the pyloric region;surface epithelial mucous cells, chief, intermediate, basal and enteroendocrine cells. The mucous cells contained round electron dense granules. In addition to the presence of circular mitochondria were found in the cytoplasm in close association with the rough endoplasmic reticulum (rER). Chief cell contained well-developed rER in the perinuclear area, numerous round mitochondria and few electron dense granules. In the intermediate type, perinuclear rER was observed. Basal cells

had few cisternae of rER. However, five types of enteroendocrine cells were identified according to their shape, size of their granules and internal structure.

Key words:

Pyloric, Histology, Electron Microscopy and Ballady duck.

INTRODUCTION

Birds structurally differ from mammals in their various organs; among these is the digestive system **(Al-Helali *et al.,* 2010).** This system is complex in most bird species and differs from other animals **(Zaher *et al*., 2012).**Some of the avian stomach consists of three compartments; namely proventriculus, ventriculus and pyloric part **(Abumandour, 2014)** such as in cattle egret **(Hussein and Rezk, 2016)** and in quail **(Helal, 2016).**The morphology and activities of the digestive system are changed during the bird's development **(Qureshi *et al*., 2017).** The pyloric region is a transitional zone between the gizzard and the duodenum. This zone shows different morphology from the gizzard and duodenum **(Ahmed *et al.,*2011).**However, there is a paucity of histological studies are published on the pyloric region of ducks. So, the present study was carried out to elucidate more light on the morphological, histological structure and some histochemical reactions of pyloric region of adult ballady duck, also to investigate the ultrastructural characteristics of the main cell types of the pylorus.

MATERIALS AND METHODS

10 apparent healthy adult ballady ducks *(Anas platyrhynchos)* of both sexes with average age ranged from (6-7) month and average weight ranged from (4.5- 5) Kg were raised and obtained from the duck farm of the faculty of Agriculture, Cairo University. These ducks were housed with food and water ad libitum. Ducks were slaughtered by euthanasia (injected with sodium pentobarbitone intravenous before being dissected). Parts of the stomach were identified and photographed in situ using digital camera. Locations and relationships of these organs were demonstrated in Figs. (1,2). Contents of the stomach were removed gently by washing with normal saline. After that, the pyloric was separated and small pieces from its longitudinal section were fixed immediately in 10% neutral buffered formalin and Bouin's solutions. The specimens were dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Sections of (4-6µ) thick were obtained, mounted on clean glass slides and stained using the following methods: Delafield's iron heamatoxylin and

eosin (H&E) stain for general tissue structure, Masson's trichrome stain for demonstration of collagenous fibers and smooth muscle fibers, PAS stain for demonstration of glycoprotein (neutral mucopolysaccharides),Alcian blue pH 1 for demonstration of sulphated mucopolysaccharides, Alcian blue pH 2.5 for demonstration of acidic mucopolysaccharides, combined PAS-Alcian blue pH 2.5 for differentiation of neutral and acidic mucopolysaccharides, Aldehyde fuchsin for demonstration of sulphated mucosubstance and elastic fibers and Best's carmine stain for demonstration of glycogen.The aforementioned stains were conducted as outline by **(Bancroft and Stevens, 2010).**Tissue sections were examined under light microscope.

Transmission Electron Microscopy (TEM):

Small pieces (1mm) from pyloric region were fixed in 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. The sections were examined with a JEOL 1010 transmission electron microscope at Regional Center for the Mycology and Biotechnology (RCMB) AL-Azhar University, Cairo, Egypt.

RESULTS

Anatomically: The pyloric region was the third part of the stomach; it was very small area between the gizzard and duodenum. Externally, it was small spindle in shape and internally it emerged from the caudal part of the gizzard Figs. (1, 2).Histologically; the mucous membrane of the pyloric region contained numerous folds; they were irregularly arranged in their lower parts. They were separated from each other by crypts and lined by simple columnar mucous secreting epithelium Fig. (3). the surface epithelium displayed strong reaction to PAS Fig. (4), moderate reaction to Alcian blue pH 1 Fig. (5) and Alcian blue pH 2.5 Fig. (6), strong reaction to combined PAS Alcian blue pH 2.5 Fig. (7), positive reaction to Aldehyde fuchsine stains Fig. (8) and showed moderate reaction to Best's carmine Fig. (9). The lamina propria contained solitary lymph nodules that were surrounded by collagen fibers and supported by smooth muscle fibers of the muscularis mucosa. Collagen fibers were also demonstrated in the very thin tunica submucosa Fig. (10). the lamina propria contained simple tubular glands lined by simple cuboidal epithelium with round, basally situated nuclei Fig. (11).these glands

exhibited a weak positive reaction to PAS stain Fig. (12). Elastic fibers were found in between the glands Fig. (13). ultrastructurally, five cell types could be observed in the pyloric region; surface epithelial mucous cells, chief, intermediate, basal and enteroendocrine cells.

The mucous cells were high columnar in shape with large ovoid nuclei Fig. (14), with a gap junction between them and had variable size round electron dense granules Fig. (15). Circular mitochondria were found in the cytoplasm in close association with the rER Fig. (16). the perinuclear and basal regions of the cytoplasm were occupied by rER Fig. (17). the chief cell had a large irregular nucleus with clumped heterochromatin in the periphery and well developed rER in the perinuclear area. Numerous round mitochondria were detected Fig. (18) and few electron dense granules were also demonstrated Fig. (19). the intermediate cell contained closely packed organelles. The nucleus was large oval in shape and contained two nucleoli with heterochromatin in the periphery. Perinuclear rER, few mitochondria and very few dense granules Figs. (20, 21) were also found. The basal cells appeared ovoid in shape. Their nuclei were large round with prominent nucleoli and marginal heterochromatin. Their cytoplasm had few cisternae of rER Fig. (22).Five types of enteroendocrine cells were identified according to their shape, size of their granules and internal structure.

Type I enteroendocrine cell was pyramidal in shape,contained large ovoid nucleus with condensed peripheral heterochromatin and cytoplasm contained numerous variable size electron dense granules Fig. (23). Type II cell was spindle in shape, had spindle euchromatic nucleus with prominent nucleolus. The cytoplasm in this type contained granules of variable electron density and moderate size Fig. (24). Type III cell appeared ovoid in shape with large ovoid nucleus, the cytoplasm contained polymorphic granules of variable density Fig. (25). Type IV cell appeared pear in shape, had large round euchromatic nucleus with cytoplasm contained few moderate electron dense granules Fig.(26). Type V enteroendocrine cell appeared pyramidal in shape, had large round nucleus with prominent nucleolus.

The cytoplasm contained small size electron dense granules Fig. (27). The tunica muscularis was formed of longitudinal smooth muscle fibers, arranged in an inner thin layer and outer thick layer, while the circular muscle fibers formed the middle layer.

The muscle fibers were intermingled with inter-muscular connective tissue. Tunica serosa covered the pyloric region externally.

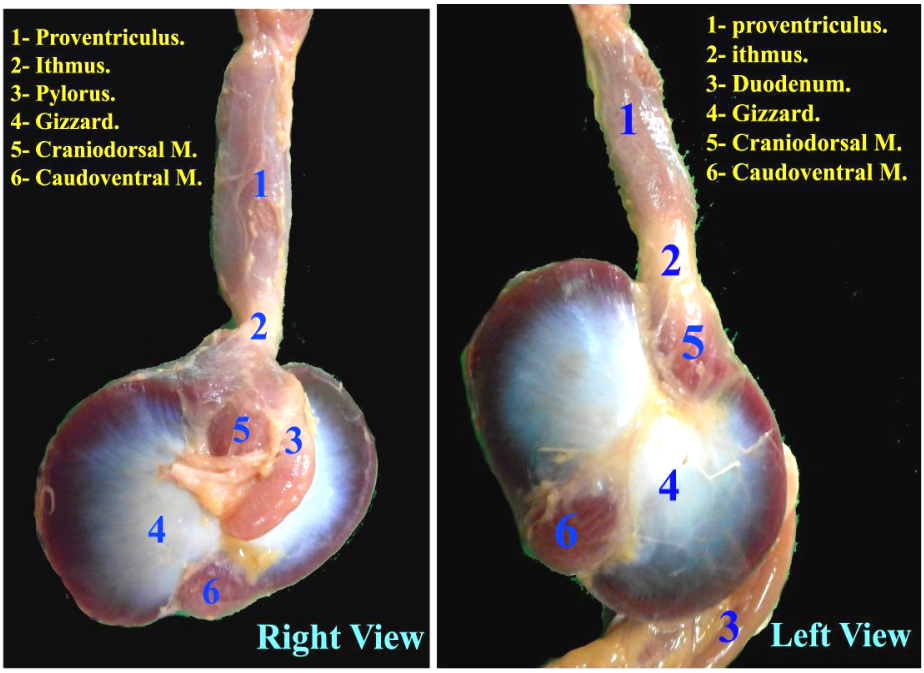

Fig. (1): A photograph showing the external features of the stomach of the ballady duck. M (muscle).

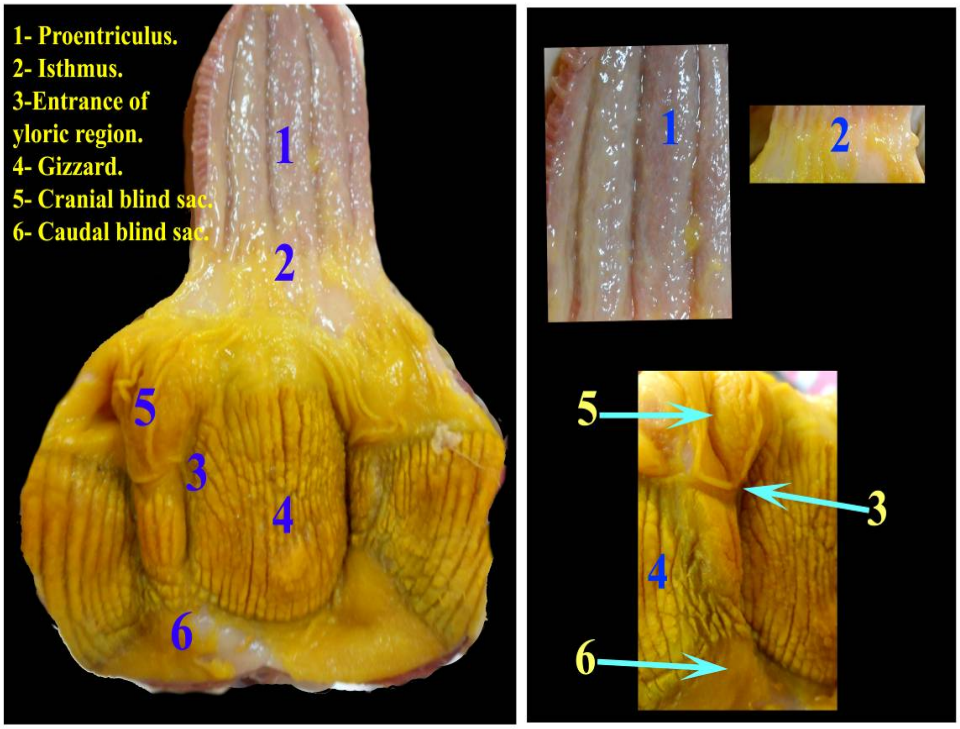

Fig. (2): A photograph showing the internal features of the stomach of the ballady duck.

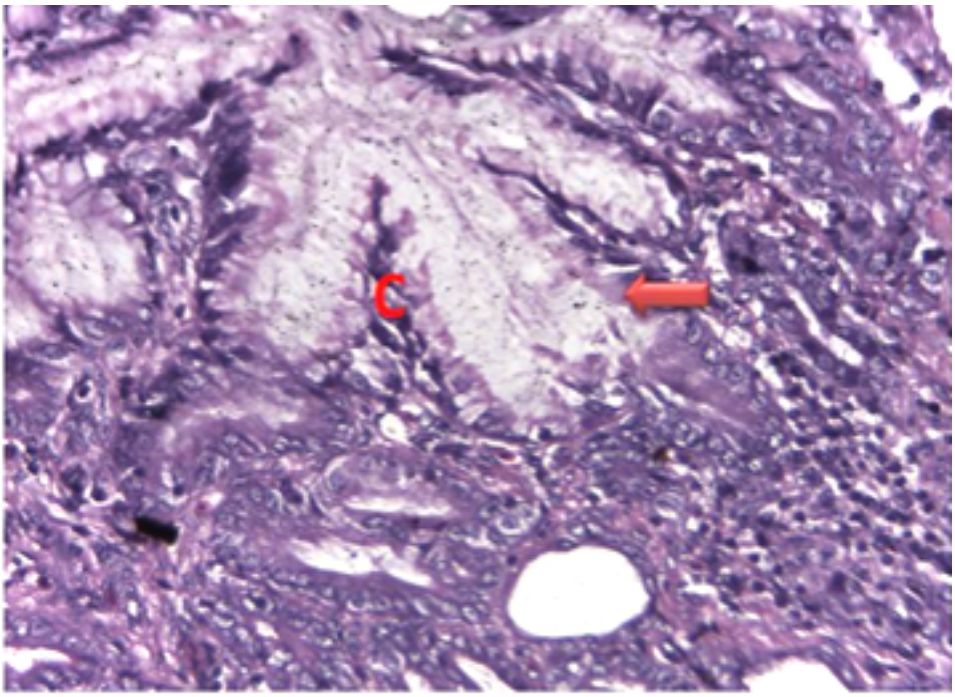

Fig. (3): A photomicrograph of mucosal folds of the pyloric region of female ballady duck showing mucous secreting cell (arrow). Notice crypt (C).**H&E stain, X400.**

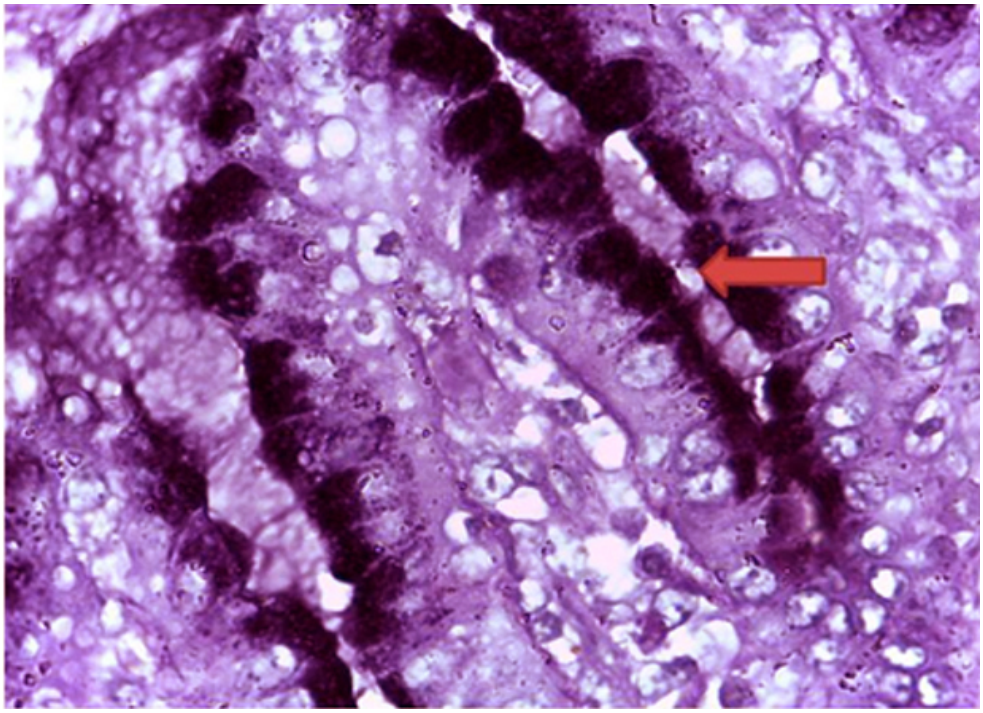

Fig. (4): A photomicrograph of mucosal folds of the pyloric region of female ballady duck showing a strong reaction to PAS in the surface epithelium (arrow). **PAS stain, X1000.**

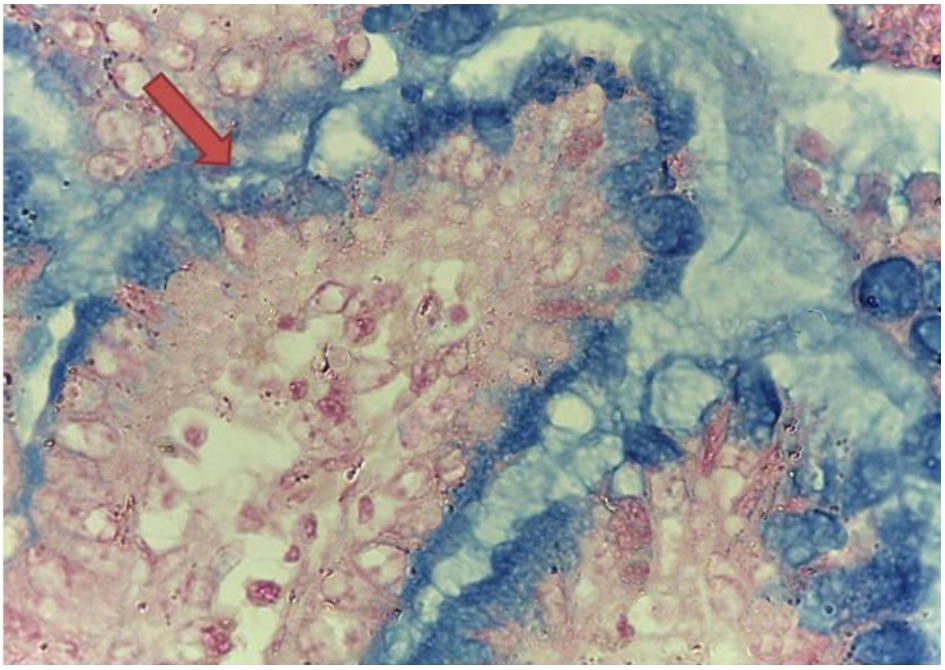

Fig. (5): A photomicrograph of mucosal folds of the pyloric region of female ballady duck showing moderate reaction to alcian blue pH 1 in the surface epithelium (arrow). Alcian blue **pH 1 stains, X1000.**

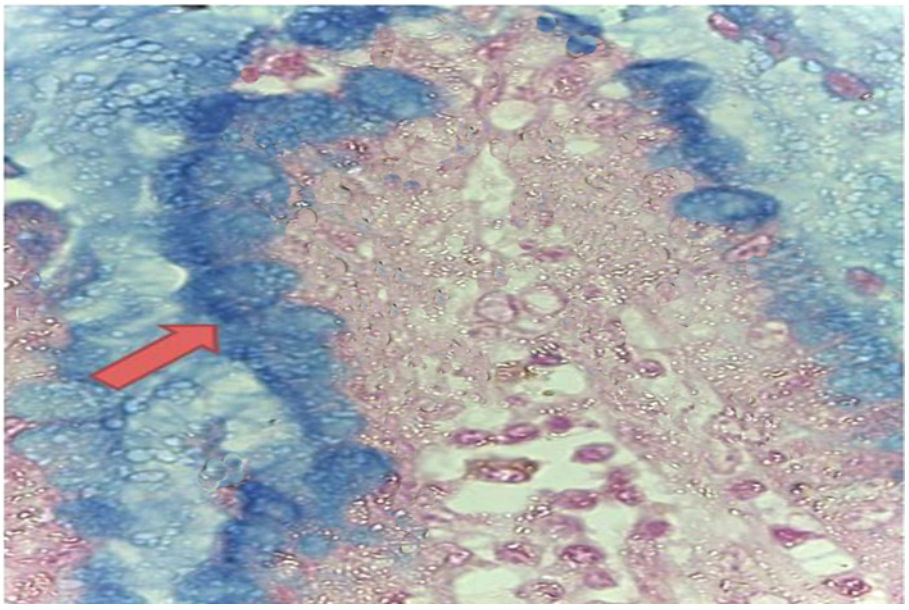

Fig. (6): A photomicrograph of mucosal folds of the pyloric region of female ballady duck showing moderate reaction to Alcian blue pH 2.5 in the surface epithelium (arrow). Alcian blue **pH 2.5 stains, X1000.**

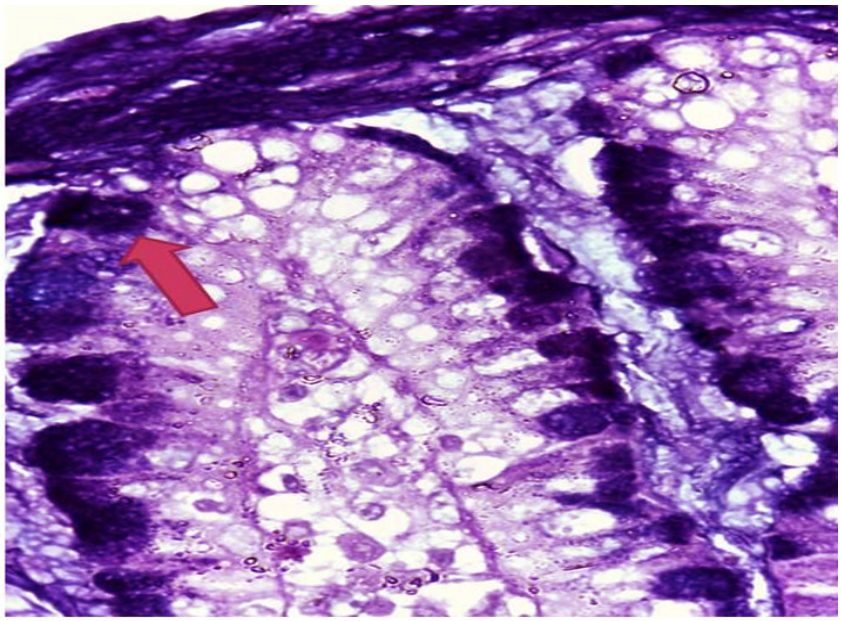

Fig. (7): A photomicrograph of mucosal folds of the pyloric region of female ballady duck showing strong reaction to combined PAS- Alcian blue pH 2.5 in the surface epithelium (arrow). Combined PAS-Alcian blue **pH 2.5 stain, X1000.**

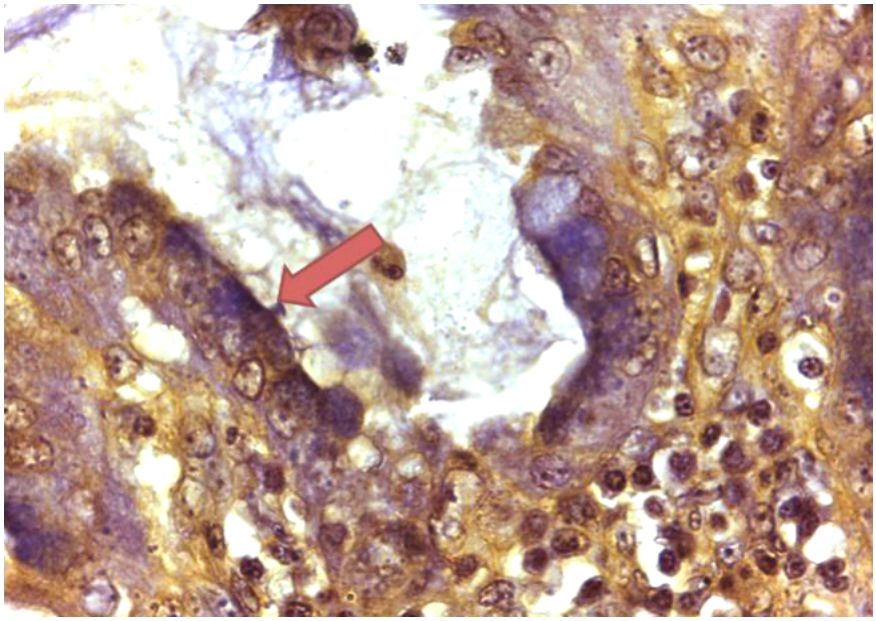

Fig. (8): A photomicrograph of mucosal folds of the pyloric region of female ballady duck showing positive reaction to aldehyde fuchsin in the surface epithelial cells (arrow). Aldehyde fuchsin **stain, X1000.**

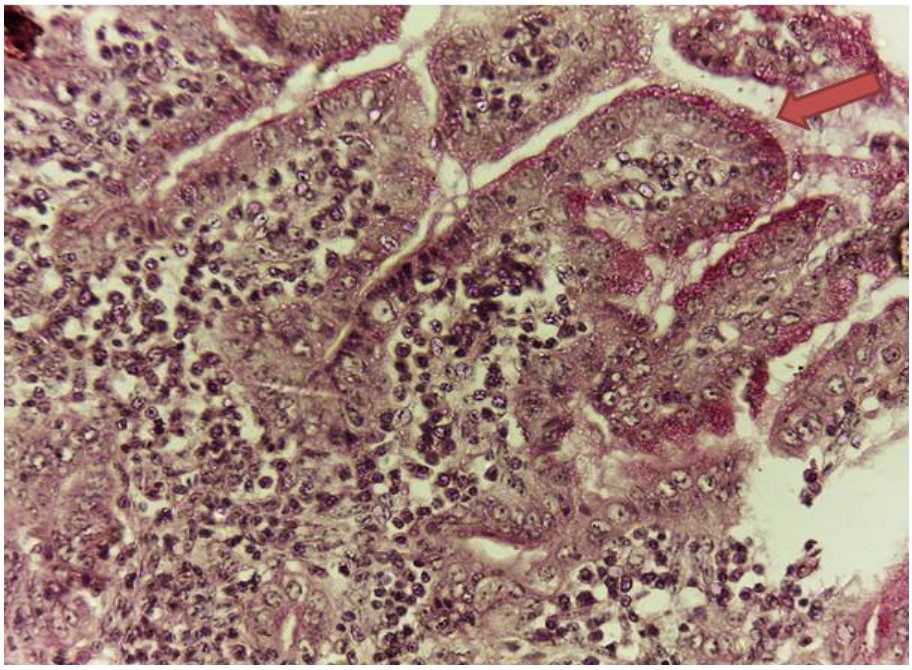

Fig. (9) : A photomicrograph of mucosal folds of the pyloric region of female ballady duck showing the surface epithelium exhibited moderate reaction to Best′s carmine (arrow). Best′s carmine **stain, X400.**

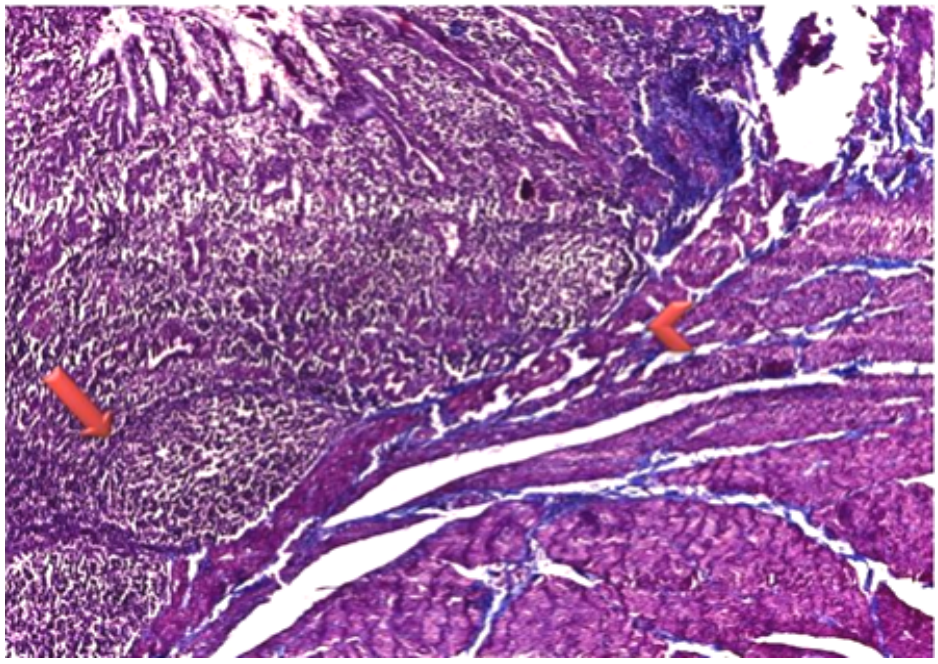

Fig. (10): A photomicrograph of mucosal folds of the pyloric region of male ballady duck showing collagen fibers, solitary lymph nodule (arrow) in the lamina propria surrounded with smooth muscle of lamina muscularis mucosa (arrow head). Masson′s trichrome **stain, X400.**

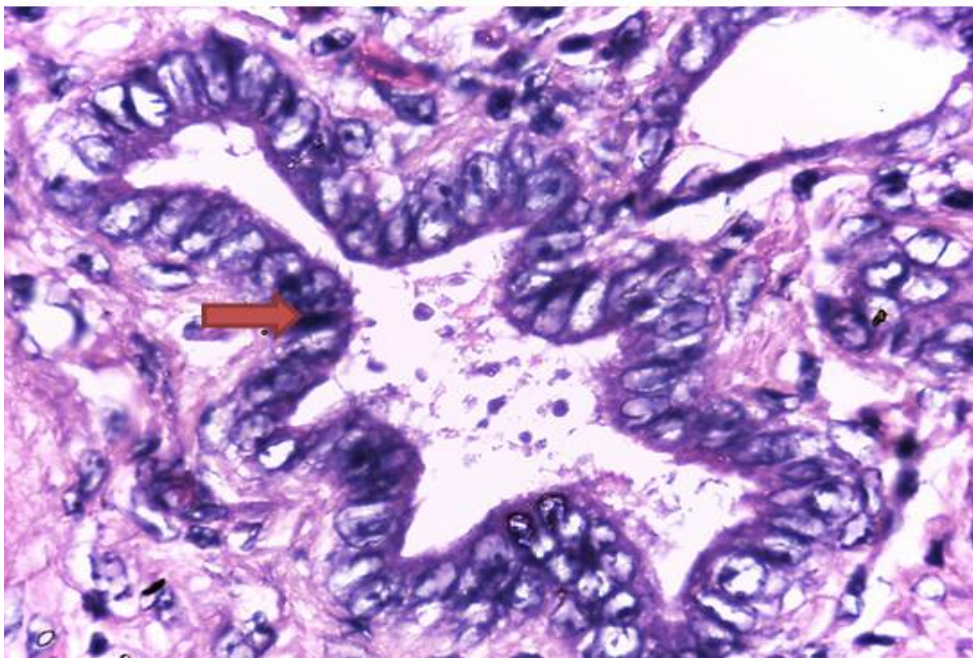

Fig. (11): A photomicrograph of the pyloric region of female ballady duck showing tubular pyloric glands lined with simple cuboidal epithelium (arrow). **H&E stain, X1000.**

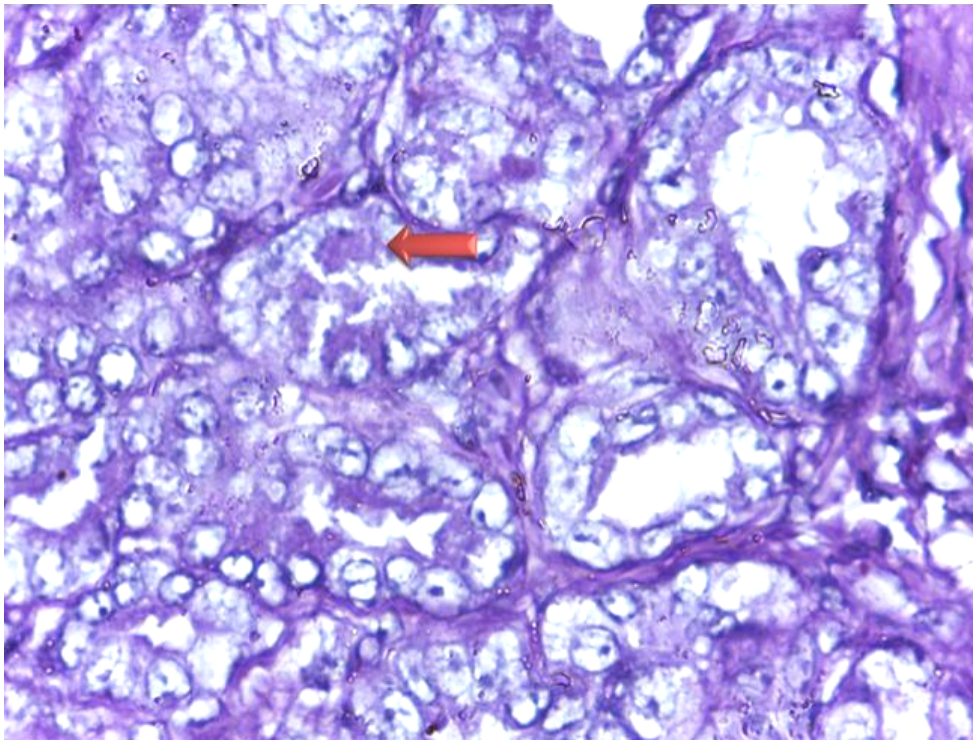

Fig. (12): A photomicrograph of the tubular pyloric gland of female ballady duck showing weak PAS reaction (arrow). PAS **stain, X1000.**

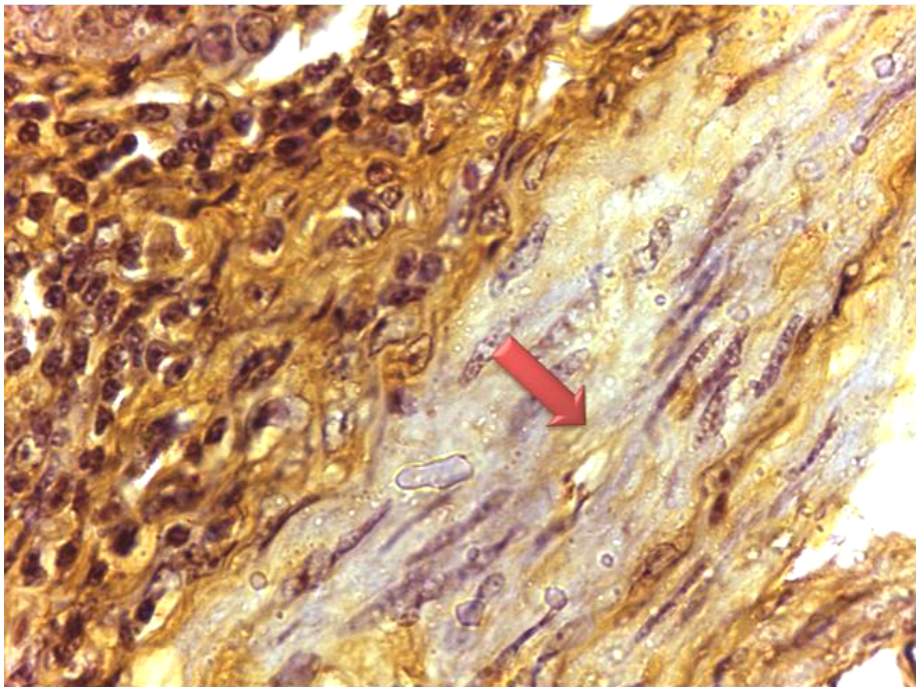

Fig. (13): A photomicrograph of a section in the pyloric gland of female ballady duck showing fine elastic fibers between the tubular pyloric glands (arrow). Aldehyde fuchsin **stain, X1000**.

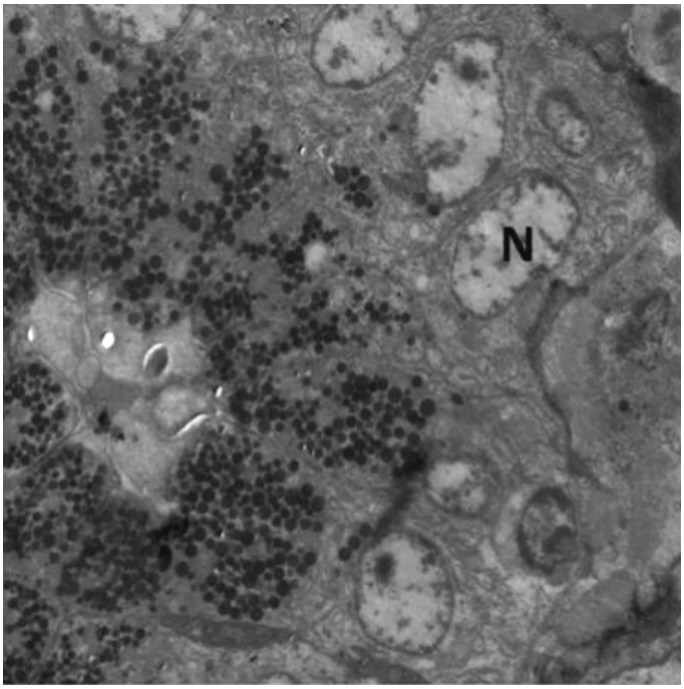

Fig. (14): A transmission electron micrograph of the pyloric region of female ballady duck showing columnar mucous cell with large ovoid nucleus (N). Uranyl acetate and lead **citrate, X1200.**

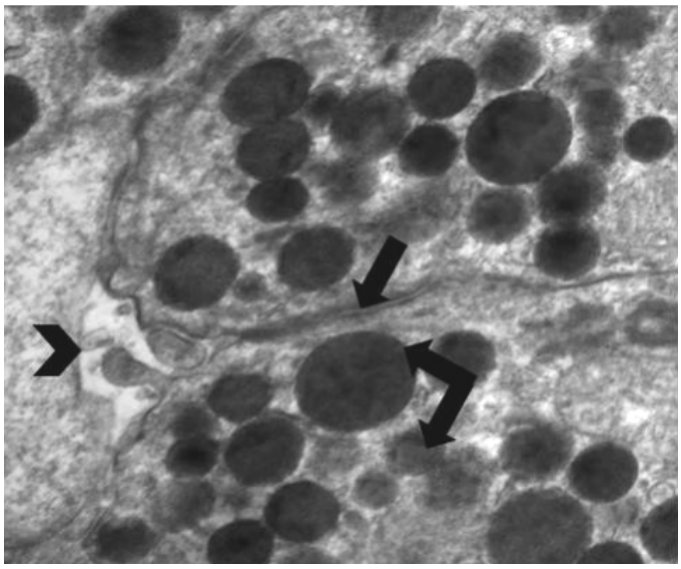

Fig. (15): A transmission electron micrograph of the mucous cell of the pyloric region of female ballady duck showing the gap junction between two columnar cells(arrow) with variable size round electron dense granules (double arrow). Uranyl acetate and Lead **citrate, X10000.**

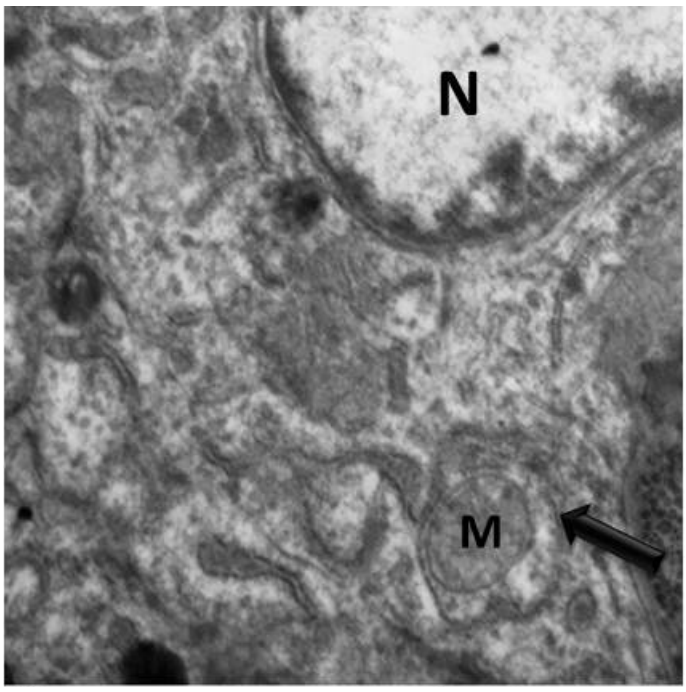

Fig. (16): A transmission electron micrograph of the mucous cell of the pyloric region of female ballady duck showing nucleus (N), mitochondria (M) in close association with rough endoplasmic reticulum (arrow). Uranyl acetate and Lead **citrate, X7500**

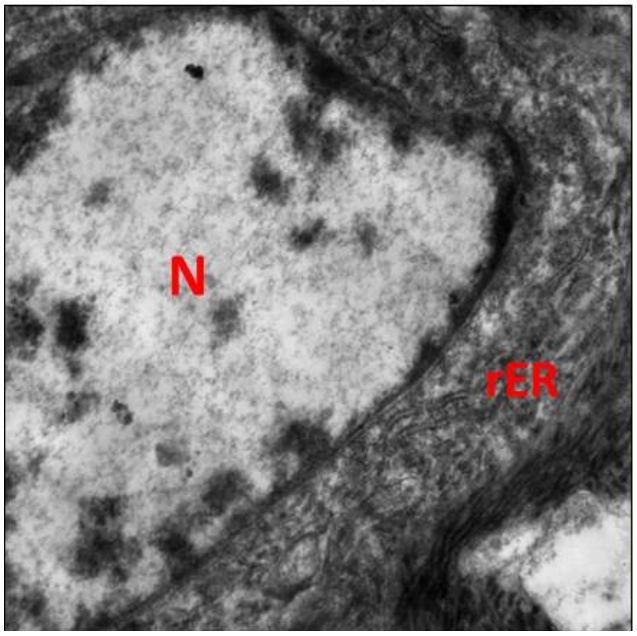

Fig. (17): A transmission electron micrograph of the mucous cell of the pyloric region of female ballady duck showing ovoid nucleus (N) and perinuclear and basal rough endoplasmic reticulum (rER). Uranyl acetate and Lead **X7500.**

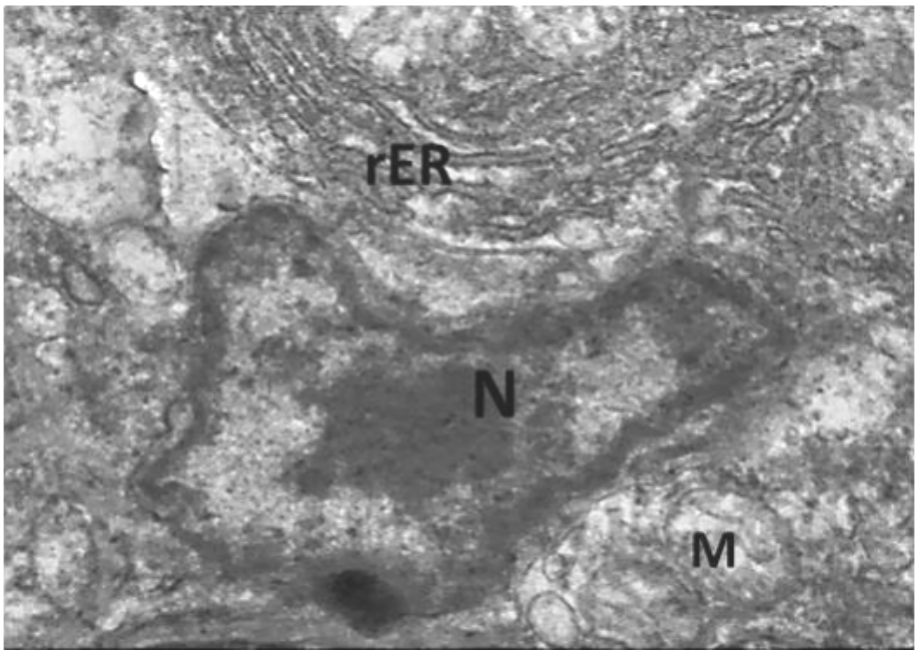

Fig. (18) : A transmission electron micrograph of the chief cell of the pyloric region of female ballady duck showing large irregular nucleus (N) with clumped heterochromatin at the periphery. Well-developed rough endoplasmic reticulum (rER) and round mitochondria (M).Uranyl acetate and Lead **citrate, X7500.**

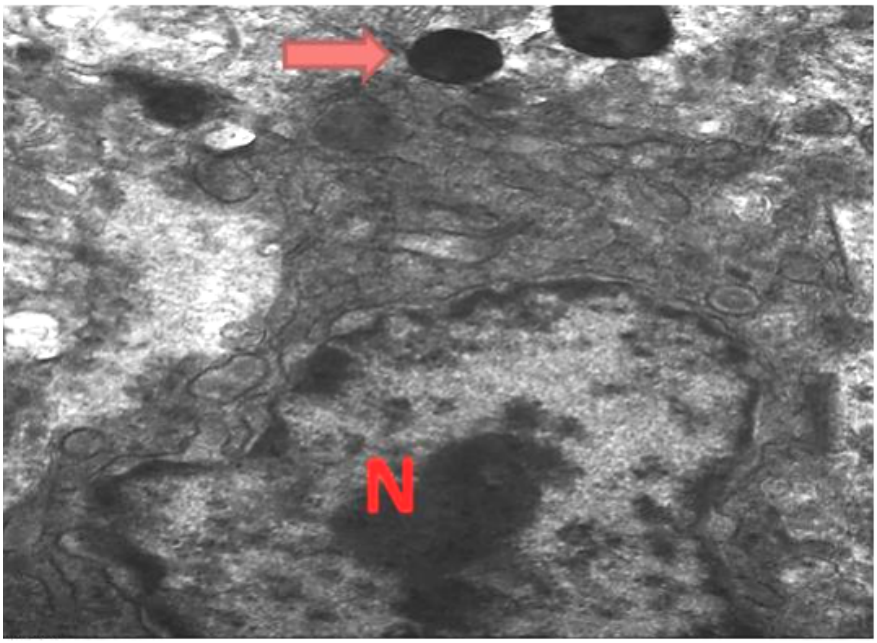

Fig. (19): A transmission electron micrograph of the chief cell of the pyloric region of female ballady duck showing large irregular nucleus (N) with clumped heterochromatin at periphery. Notice the electron dense granules (arrow). Uranyl acetate and Lead **citrate, X6000.**

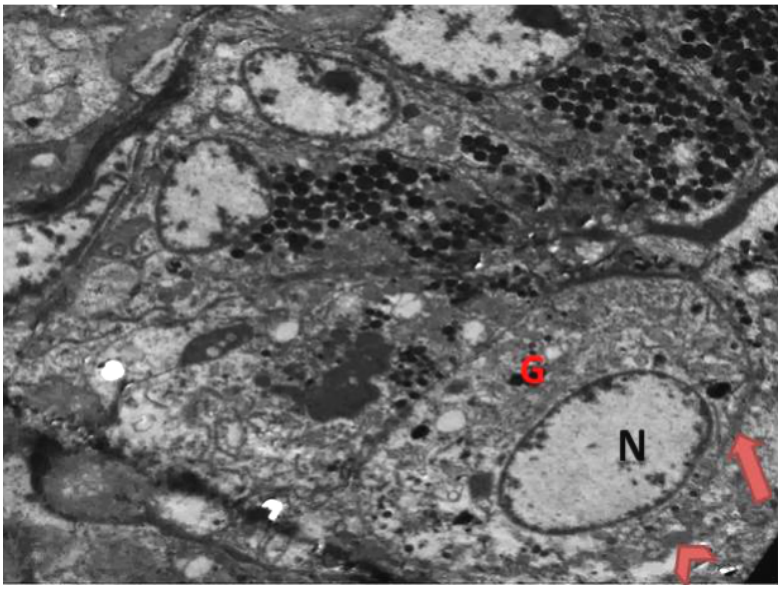

Fig. (20): A transmission electron micrograph of the intermediate cell of the pyloric region of female ballady duck showing oval nucleus (N), perinuclar rough endoplasmic reticulum (arrow) and few electron dense granules (G). Uranyl acetate and Lead **citrate, X1500**

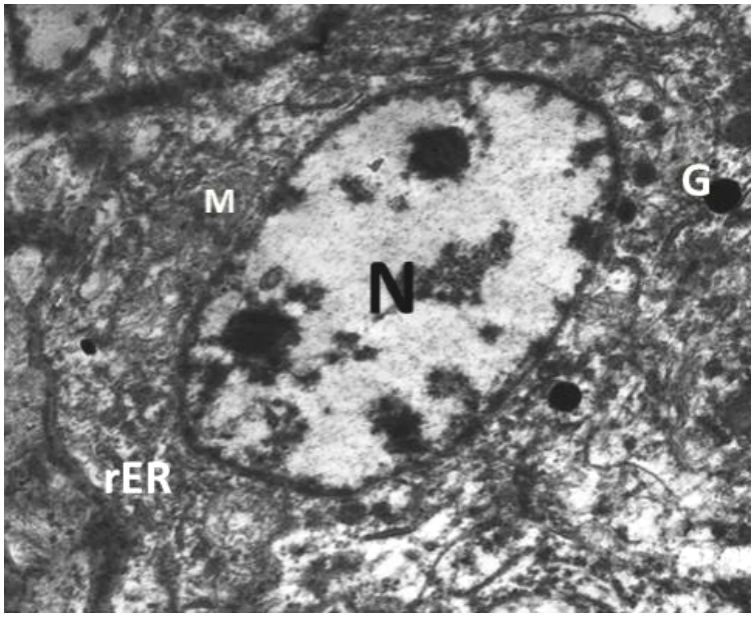

Fig. (21): A transmission electron micrograph of the intermediate cell of the pyloric region of female ballady duck showing oval nucleus (N), perinuclear rough endoplasmic reticulum (rER) and few mitochondria (M), few electron dense granules (G). Uranyl acetate and Lead **citrate, X3000**

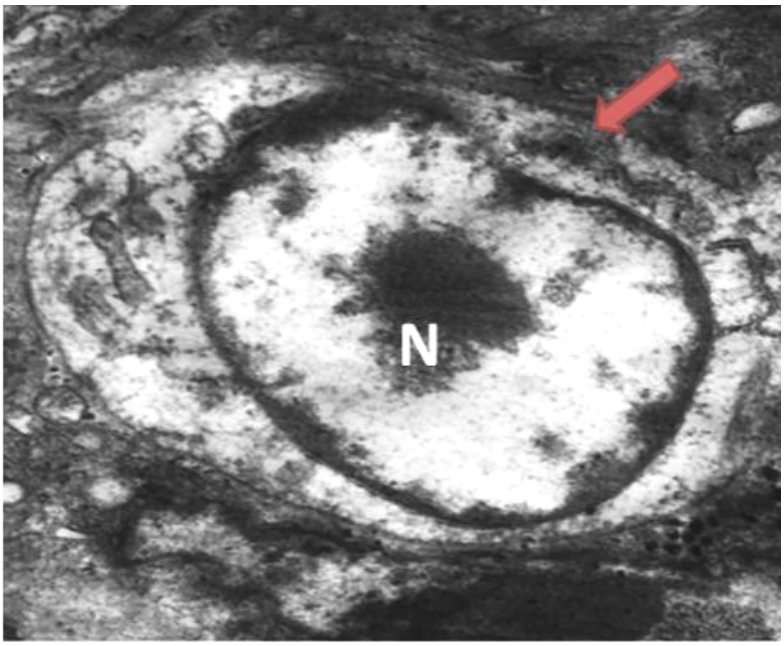

Fig. (22): A transmission electron micrograph of the basal cell of pyloric region of female ballady duck showing large round nucleus with prominent nucleolus (N).Few cisternae of rough endoplasmic reticulum (arrow). Uranyl acetate and Lead **citrate, X5000.**

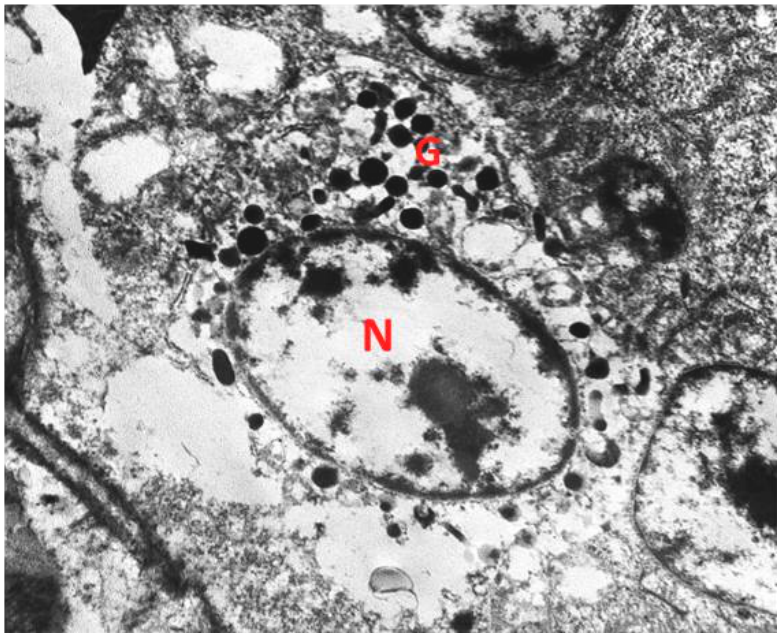

Fig. (23): A transmission electron micrograph of the pyramidal shaped type I enteroendocrine cell of the pyloric region of female ballady duck showing large ovoid nucleus with condensed peripheral heterochromatin (N).Variable size electron dense granules (G). Uranyl acetate and Lead **citrate, X12000.**

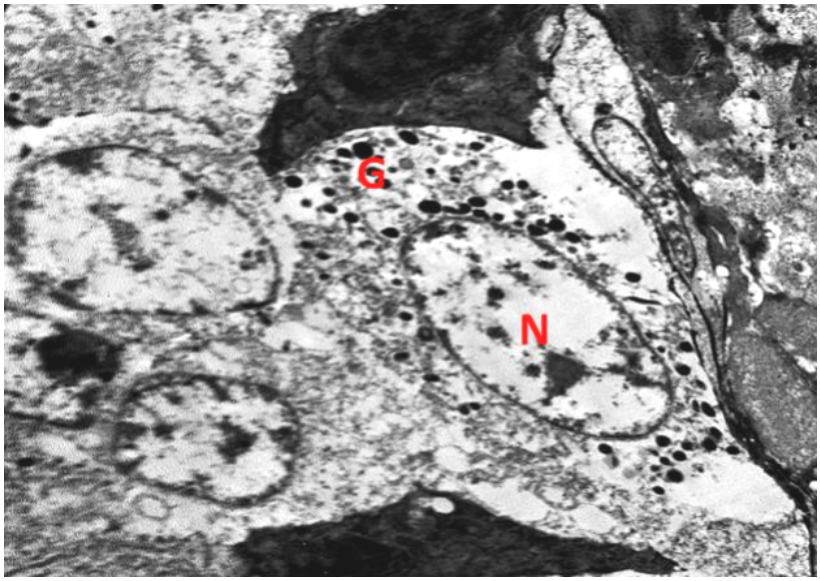

Fig. (24) : A transmission electron micrograph of a spindle shaped type II enteroendocrine cell of the pyloric region of female ballady duck showing spindle euchromatic nucleus with condensed peripheral heterochromatin (N). Granules of moderate size and variable density (G). Uranyl acetate and Lead **citrate, X8000.**

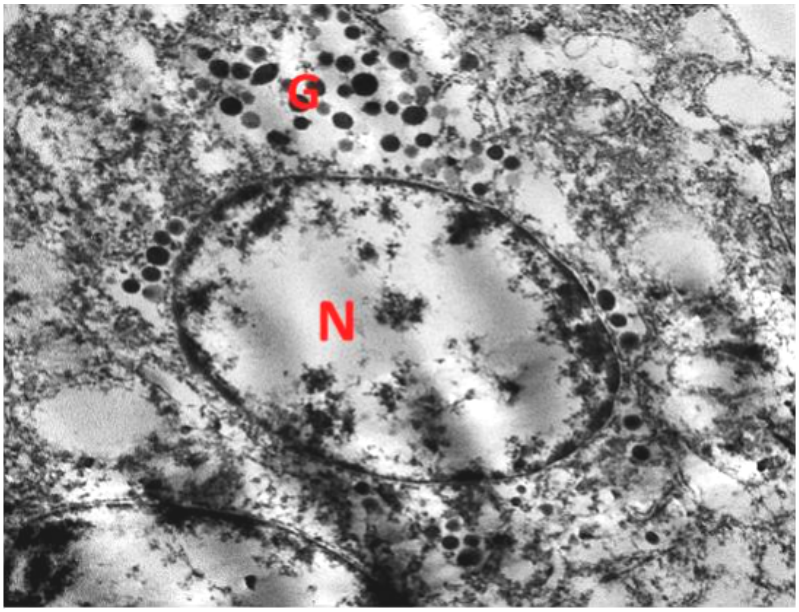

Fig. (25): A transmission electron micrograph of ovoid shaped type III enteroendocrine cell of the pyloric region of female ballady duck showing large ovoid nucleus (N) and polymorphic granules of variable density (G). Uranyl acetate and Lead **citrate, X15000.**

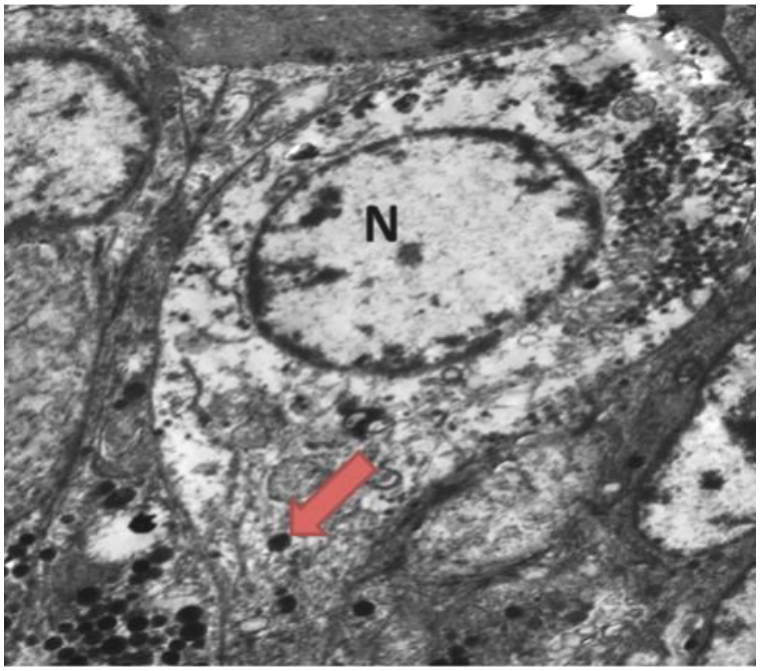

Fig. (26): A transmission electron micrograph of pear shaped type IV enteroendocrine cell of the pyloric region of female ballady duck showing large round euchromatic nucleus (N) and few electron dense granules (arrow). Uranyl acetate and Lead **citrate, X25000.**

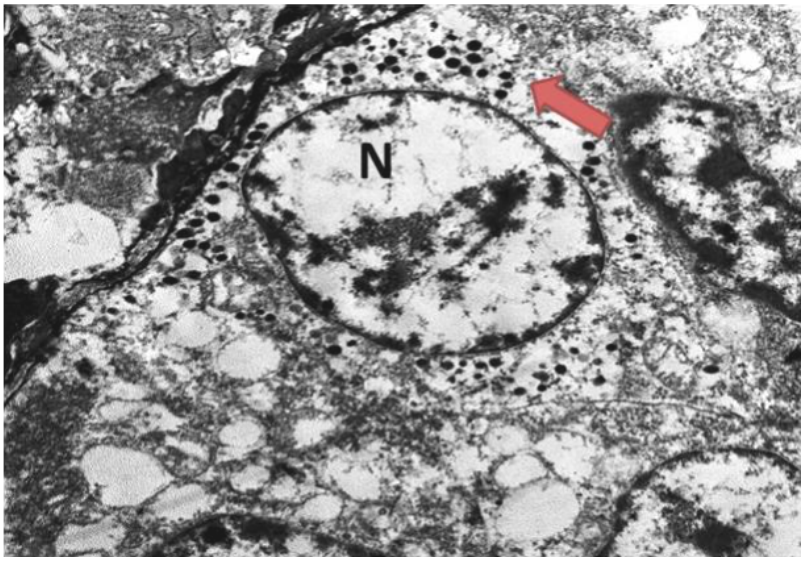

Fig. (27): A transmission electron micrograph of pyramidal shaped type V enteroendocrine cell of the pyloric region of female ballady duck showing large round euchromatic nucleus with prominent nucleolus (N), small size electron dense granules (arrow). Uranyl acetate and Lead **citrate, X10000.**

DISCUSSION

In our study, the pyloric region was the third part of the stomach. The gizzard was separated from the small intestine by pyloric folds, which were thought to regulate the passage of food into the small intestine by slowing the movement of large particles. This agreed with **Hussein and Rezk, (2016)** in cattle egret. The histological observations of the mucous membrane showed numerous folds. These folds were irregularly arranged and separated from each other by crypts lined by columnar epithelium, similar to that reported in Gallus domestics by **McLelland (1979)** and in cattle egret by **Hussein and Rezk (2016)**, while the mucosal folds were present with the villous in pyloric region of fowl **(Aitken, 1958)** and quail **(Ahmed *et al*., 2011 and Helal, 2016)**. **El-Nahla *et al*. (2011)** recorded that such folds were separated from each other by deep pits, which might impede the undigested food. Ultrastructurally, five cell types could be observed in the surface epithelium and pyloric glands; surface epithelial, chief, intermediate, basal and enteroendocrine cells. While, in quail **(Ahmed *et al.*, 2011)** and duck **(Hassan and Moussa,2012)** only two types of cells; chief and basal cells were observed. However, in fowl **(Toner,1964),** in red jungle fowl **(Kadhim *et al*., 2010)** and in quail **(Helal, 2016)** three cell types were demonstrated; mucous, chief and enteroendocrine cells. Meanwhile, in cattle egret **Hussein and Rezk (2016)** mentioned four types of cells;

surface epithelial, chief, and intermediate and basal cells. In this study, the histochemical techniques confirmed a positive reaction of the surface epithelial cells with Alcian blue and PAS stains, which indicated the presence of both acidic and neutral mucopolysaccharides similar to that reported in quail **(Helal, 2016);** in cattle egret **(Hussein and Rezk, 2016)** and in khaki Campbell of duck **(Gedam *et al*., 2018).** According to **Helander (1962) and Toner (1964),** the chief cell was characterized by a fine morphological structure, which was typical for protein secreting cells and in many ways was similar to the zymogenic cells of the exocrine pancreas and mammalian stomach. The granules of the chief cells released their substances into the surface of the cells, which disseminated over the microvilli forming a layer. Finally, these substances spread into the lumen of glands as filaments. **Toner (1964)** mentioned that these filaments accumulate in the upper part of the gland forming a mass**.** Intermediate cells represented phases in differentiation of the chief cells from the basal cells **(Toner, 1964).**The latter cells shared some of the structural features of undifferentiated cells. They were located in the mitotic zone of the glands; therefore, the basal cells might serve as stem cells **(Toner, 1964).** They were also assigned as specific function in the cell renewal and replacement in the glands **(Hill, 1971).** Five types of enteroendocrine cells were found in the pyloric region of duck in this study. **Yamada *et al.* (1980)** also reported these findings in duck. Enteroendocrine cell type I was pyramidal in shape and contained numerous variable size electron dense granules; similar to that reported by **Yamada *et al.* (1980) and Okatomo (1980)** in duck and by **Helal (2016)** in quail who added that type I formed the bulk of endocrine cells and contained granules most of which were empty, had vague, cloud or spot - like materials. Enteroendocrine cells type II were spindle in shape, had granules of variable electron density and moderate size. Similar to type II cells of the pyloric region in quail **(Yamada *et al*., 1978 and Helal, 2016).** In addition, **Yamada *et al.* (1978)** mentioned that these cells were similar to mammalian Glucagon producing cells. Enteroendocrine cells type III appeared ovoid in shape with polymorphic granules of variable density. Similar to type III cells in the pyloric region of quail **(Helal, 2016)** and type V endocrine cells of pyloric region of quail **(Yamada *et al*., 1978).** Enteroendocrine cells type IV were pear in shape, had few moderate electron dense granules. Similar to that found in the type IV of enteroendocrine cells in quail **(Helal, 2016).** Enteroendocrine cells types V were pyramidal in shape, contained small size electron dense granules and similar to type III cells in the pyloric region of quail

(Yamada *et al*., 1978). The lamina propria contained solitary lymph nodules similar to that in quail **(Helal, 2016)** and in khaki Campbell of duck by **Gedam *et al*.(2018)** who suggested that, the significance of this lymphatic tissue; first it was exposed to undigested or partially digested environmental antigens and second it might participate in "B" cell lymphocyte differentiation. The lamina propria also contained simple tubular glands and lined by simple cuboidal epithelium as observed in quail by **Helal (2016).**While, in fowl **(Aitken, 1958)** and in quail **(Ahmed *et al*., 2011)** these glands were lined by simple columnar epithelium. However, the secretion of these glands might be responsible for the formation of keratohyaline layer similar to that speculated in fowl by **Nickel *et al.* (1977) and King and McLelland (1984).**The longitudinal smooth muscle fibers were arranged in an inner thin layer and outer thick layer, while the circular muscle fibers formed the middle layer.

The muscle fibers were mixed with connective tissue. The structure of the serosa of the pyloric region was similar to that of the ventriculus as the same as in cattle egret **(EL-Nahla *et al*., 2011).**

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