COMPARATIVE HISTOLOGICAL STUDY OF ESOPHAGUS AND LIVER IN SOME AQUATIC BIRDS

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Abstract

The present work aims to investigate the differences in the structure of liver and esophagus in five species of birds in Egypt having the same food habits. The birds under investigation are: water rail, spotted crake, little crake, common coot and common moorhen. Five birds were obtained from local hunters in Damietta, Egypt all of these birds are belonging to the same family (Rallidae) and all of them are omnivorous, but we found some difference after microscopical examination of liver and esophagus. The birds were dissected, esophagus and liver were fixed and stained for microscopic comparative study. The results revealed that there are some histological differences after microscopical examination to liver and esophagus in five species. In esophagus: Mucous glands are bigger in little crake than that in Rallus aquaticus, muscularis mucosa layer is thicker in Rallus aquaticus and common coot than that in Gallinula chloropus, inner circular in spotted crake is thinner than in Gallinula chloropus, and most thick in common coot. In Liver: Hepatocytes in common coot are polygonal in shape, have rounded nucleus and there are some sinusoids between hepatocords in liver and presence of secondary bronchial cavity in Gallinula chloropus and Rallus aquaticus.

Introduction:

The family Rallidae belongs to the order Gruiformes and includes 17 species of rails and coots. They are mostly small to medium sized marsh or water birds with some terrestrial form (Ali and Ripley, 2009). These birds in general are poor fliers and mostly nest on the ground, in bushes or floating water. The common species include, White breasted water hen (Amaurornisphoenicurus), Common moorhen (Gallinulachloropus), Purple moorhen (Porphyrioporphyrho) and Common coot Fulicaatra are widely distributed throughout the subcontinent, while the remaining have restricted distribution. The rail family (Aves: Rallidae) is globally distributed and extant members occupy niches associated with terrestrial and freshwater habitats. This bird family comprises between 135–148 recognized species in 33–40 genera (Clementset al., 2011) of which 39% are monotypic. The water rail (Rallusaquaticus) is a slender marsh bird of the family Rallidae (order Gruiformes), native to most of Europe and Asia. Its length is about 28 cm (11 inches), and it has a moderately long beak.

The sides of the bird have black and white bands. Water rails are omnivorous, although they mainly feed on animals. These include leeches, worms, gastropods, small crustaceans, spiders, and a wide range of both terrestrial and aquatic insects and their larvae. The little crake Zaporniaparva is a very small waterbird of the family...
Rallidae (Jobling, 2011). Its breeding habitat is reed beds in Europe, mainly in the east, and just into western Asia. This species is migratory, wintering in Africa. These birds’ probe with their bill in mud or shallow water, also picking up food by sight. They mainly eat insects and aquatic animals. Little crakes are very secretive in the breeding season, and are then mostly heard rather than seen. They can be easier to see on migration.

The spotted crake's breeding habitat is marshes and sedge beds across temperate Europe into western Asia. They nest in a dry location in marsh vegetation, laying 6–15 eggs. This species is migratory, wintering in Africa and Pakistan. Spotted crakes are very secretive in the breeding season, and are then mostly heard rather than seen. They are then noisy birds. The coot bird (Fulica atra) is a member of the family Rallidae, and it is about the size of chicken, black or dark grey with yellow lobate feet, and has a very distinctive white spot above the likewise white beak. The coot is an omnivore, and will take a variety of small live prey including the eggs of other water birds, as well as algae, vegetation, seeds and fruit. The common Coot breeds in Eurasia, Australia, and Africa (del Hoyo et al., 2006).

The global population of the species is estimated at about 9 million individuals (Simon and Derek, 2006). It is a common and abundant species in Lithuania, with an estimated breeding population of 20,000–30,000 pairs (Kurlavičius, 2006). The common moorhen is a distinctive species, with dark plumage apart from the white undertail, yellow legs and a red frontal shield. The young are browner and lack the red shield. The frontal shield of the adult has a rounded top and fairly parallel sides; the tailward margin of the red unfeathered area is smooth waving line.

Common Moorhen (Gallinula chloropus) and the Eurasian Coot (Fulica atra) have been recently described in Egypt (El-Morsey et al., 2014). Liver is the largest gland in the body and it can be regarded as the central organ in the maintenance of energy supply, moreover, the liver catalyzes biosynthetic and bio degradative processes and excretes final metabolic products (Dellman and Brown, 1981; Katz, 1992; Whitlow, 2000). Liver has secretary capabilities, this organ also able to excrete, provide, storage, detoxify, metabolize, esterifies and phagocytize. It plays roles as the control center for digestive system it also functions as both endocrine and exocrine gland (Dyce et al., 1988; Whitlow, 2000).

The esophagus in birds is passage for the food (White, 1968; Sisson and Grossman, 1986) observed that the avian esophagus is on the right side of neck (mammal present it was on left side) and it was placed between the pharynx and stomach glandular portion, it was thin and dilatable walls with a diameter relatively larger than of mammals. According to Levin (1984) and Mule (1991) the avian esophagus consist of two parts, cervical and thoracic, while in mammals there was three parts, cervical, thoracic and abdominal. The present study was undertaken to investigate the histological study on esophagus and liver of five aquatic birds: water rail (Rallus aquaticus), spotted crake (Porzana porzana), common moorhen (Gallinula chloropus), common coot (Fulica atra) and common moorhen (Gallinula chloropus), in order to show some difference among them.

Materials and Methods:

Experimental animals:
This study was carried out for five species: water rail (Rallus aquaticus), spotted crake (Porzana porzana), common moorhen (Gallinula chloropus), common coot (Fulica atra) and common moorhen (Gallinula chloropus) were obtained from local hunters in Egypt. The birds were transported to the laboratory of histology.

Histological study:
The birds were dissected, samples (esophagus and liver) were cut for the general histological studies, the samples were drained by saline solution; small pieces of the various segments were fixed rapidly in 10% neutral formalin for 24 hours then kept in a mixture of 70% ethyl alcohol. After fixation, the different parts of samples were dehydrated through ascending grades of alcohol, cleared in xylene and finally embedded in paraffin block. Sections of the different studied samples were cut at thickness of 7 microns. Sections were stained with differential double stained haematoxylin and eosin (Banercof and Gamble, 2002) for general histological structures.

Result and Discussion:
The esophagus consists of four distinct functional tunicae namely; mucosa, submucosa, muscularis and outermost serosa. The esophagus was lined throughout its length with keratinized stratified squamous epithelium. The lamina propria mucosae consist of connective tissue with diffuse lymphatic tissue scattered throughout. Esophageal glands were abundant within the lamina propria mucosae. A well-developed muscularis mucosae separates the lamina
proparia mucosa from the underlying sub mucosa which consists of more dense connective tissue. The musculosa was relatively thick and consists of two layers of smooth muscle cells an inner longitudinal layer and an outer circular layer (Fig. 1A, B, C, D, E).

From the current results, it was revealed that there are some histological differences after microscopical examination to esophagus among five species as: Longitudinal layer (LML) in water rail and Gallinula is thinner than that in little crake, spotted crake and common coot (Fig. 1A, B, C, D, E). Circular layer in water rail is thinner than that in little crake, spotted crake, Gallinulachloropus and common coot (Fig. 1A, B, C, D, E).

Mucous glands are bigger in little crake than that in water rail (Fig. 1A, B). The serosa composed of connective tissue, collagen and elastic fibrous, these results agree with Nagy et al.,(2005), who study the thoracis part in chicken. Histologically the wall of the esophagus in five species is formed from; serosa, musculosa, submucosa and mucosa. This result agrees with that mentioned by Mclelland(1979). The serosa is a thin layer formed of simple squamous epithelium. It is present only in the lower part of the esophagus while a fibrous layer surrounds the upper part. The musculosa consists of two distinct muscle layers; circular and longitudinal. This result agrees with that mentioned by Salem,(1984). Also, Klasing (1999) added that the peristaltic contraction of inner circular and outer longitudinal muscles propel food posteriorly through the esophagus. The mucosal folds are lined by stratified squamous epithelium interrupted by the ducts of the mucosal glands. This observation is similar to that of the fowl (Bradley,1915; Calhoun,1933), and in Tyto alba (Ismail,2000). In Coturnix, mucosa contains esophageal glands which are tubulo-alveolar type. Similar observations were reported by Abd El-Aziz(1984), Salem(1984), El-Bahrawyet al.(1989), El-Bahrawyet al.(1993) and El-Sayyad(1995), and the presence of these mucous glands could be considered as another kind of esophageal adaptation with the nature of food items.

The liver in birds is one of the largest, most important organs in the body. It has numerous functions, as in other vertebrates, including digestive functions, metabolism of proteins, fats, and carbohydrates, synthesizing and secreting bile which contains two bile pigments, bilirubin and biliverdin into the small intestine (Dibner and Richards,2004; Klein and Enders,2007). The avian liver is suspended by peritoneum that is connected with overlying air sac and surrounded by hepatic celomic cavities (Clark,2005; Bailey et al.,1997). The avian liver has two lobes, it's in most avian have the right lobe larger than the left one for example, in pigeon, bustards and ostrich (Bailey et al.,1997; Stornelli,2006; Yoshida et al.,2010). While the two lobes can be of equal size, for example, in Galliformes (Schmidt et al.,2003). However, the left lobe of the domestic fowl divided into the dorsal and ventral parts (Clark,2005; Sivgnanam et al.,2008). The avian liver is covered by a peritoneal layer of mesothelium, under this layer the dense connective tissue. The liver of avian similar to that in mammals but there are some differences such as absent connective tissue septa between lobules except in portal area.

The principal cell of avian liver is the hepatocyte. Avian hepatocytes are polyhedral cells with a large rounded, oval and centrally located nucleus, the sheets of hepatocytes are separated by sinusoids (Whitlow,2000; Yoshida et al.,2010). In both birds and mammals, the sinusoids lined by endothelial cells and Kupffer’s cells, and the perisinusoidal spaces may be linked directly by penetrating traversing the endothelial cells or by intercellular gaps in the sinusoidal linings. The histology picture of the liver in five species was composed of plates of hepatocytes which are usually arranged as two cell thickness between the liver sinusoids, these are hepatic cords, these cells ordered radially around the central vein (Fig. 2A, B, C, D, E). This finding was in agreement with Abdelwahab(1987), in duck, (Melleland,1993) in chicken (Schmidt et al.,2003) and in domestic birds. Abdelwahab (1987) referred that the hepatic cords and blood sinusoids run beside each other in a continuously branching and interlocking structure, this is giving the standard suitable for metabolic interchange between the two systems.

The hepatocytes are polyhedral in shape and it had cytoplasm contain many granules with their ovoid nucleus which had distinct nucleolus. The sinusoids lined by endothelial cells that are nearly flattened in shape with presence of the Kupffer’s cells contract with endothelial cells, last cells had large nucleus with some debris in cytoplasm, the sinusoids continued with the hepatic vein and portal vessels (Fig. 2A, B, C, D, E). The similar findings were previously reported by Wong and Cavey(1992) in chicken and in ostrich.
Fig. (1): Photomicrograph of transverse section of esophagus in: A: *Rallusaquaticus* show longitudinal layer (LML), circular layer (CML), and mucous glands (M). B: *Zaporniaparva* showing longitudinal layer (LML), circular layer (CML), and mucous glands (M). C: *Porzanaporzana* show longitudinal layer (LML) and circular layer (CML). D: *Gallinulachloropus* show longitudinal layer (LML) and circular layer (CML). E: *Fulicaatra* show longitudinal layer (LML) and circular layer (CML). H&E X100.
**Fig. (2):**- Histological section of liver in: A: spotted crake Show 1-Hepatic cell (HC), 2- central vein (CV). B: common coot, show 1-Hepatic cell (HC), 2- central vein (CV), 3- Kupffer’s cells (KC), 4-sinusoid(S). C: in common moorhen shows Kupffer’s cells (KC). D: little crake shows 1-Hepatic cell (HC), 2-Central vein (CV), 3-Kupffer’s (KC). E: water rail shows 1-Hepatic cell (HC), 2-sinusoid (S). H&EX100.

**Conclusion:**
This type of study provides a lot of data for researchers in the different fields, and calls for other studies of the same type. Comparison histological structures among different species, genera and families can help researchers in the field of animal origin and evolution. The hepatic and esophageal histological differential study was carried out in five species; water rail, spotted crake, little crake, common coot and common moorhen. This study was revealed that there are some histological differences after microscopical examination to esophagus and liver.

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