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Comparative histological and Histochemical study of the esophagus in two invasive species Acridotheres tristis and Tilapia zillii in Iraq

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***Abstract*---**The current study conducted on two invasive species, the common Myna *Acridotheres tristis* and Tilapia *Tilapia zillii* in Iraq. The study aims to identify the histological and histochemical aspects of the esophagus in both species. Histological sections showed that the esophagus composed of four tunica: mucosa, submucosa, muscularis and adventia. The lumen of these parts displayed numerous long folds, these folds were lined by non-keratinized stratifies squamous epithelium in *A. tristis,* while in *Tilapia zillii* these folds lined by pseudo stratified columnar epithelium. The tunica mucosa showed absence of muscularis mucosa in both species, so the lamina propria was continued with tunica submucosa. In both species, the histochemical sections showed that the submucosal glands with Alcian blue stain contained acidic mucopolysacharrids, meanwhile the sections with PAS stain showed strong neutral secretions within submucosal glands. The middle part of Tilapia esophagus with alcian blue (pH 2.5) stain appeared that the epithelial crypts contained acidic mucopolysacharrids but no reaction within the epithelium, meanwhile with PAS stain showed strong neutral mucopolysacharrids within crypts and weak neutral mucopolysacharrids within epithelium.

***Keywords*---**Acridotheres tristis, Tilapia zillii, esophagus, histology, histochemistry.

Introduction

Invasive species are considered as non-native species that were introduced directly or accidentally through human activity outside their natural habitats, which led to the disruption of the geographical distribution of these species in addition to the dispersal of the natural barriers of many living organisms (Pyšek and Richardson 2006). Invasive species present an opportunity to test the association between selective forces and adaptive morphological traits because

these species can experience rapid changes when introduced to new environments. (Holzapfel, 2006). According to the Fifth National Report to the Convention on Biological Diversity in Iraq considered *A. tristis* and *T. zillii* as invasive species to the Iraqi environment (Haloob, 2014). The *Acridotheres tristis*, has been nominated among 100 of the "World's Worst" invaders by the Invasive Species Specialist Group (Lowe et al 2000). The digestive system in birds generally consists of the oropharyngeal cavity, the gastrointestinal tract, and its glandular appendices. The mouth is connected to the esophagus, which is divided into three parts, which are the cervical esophagus, the crop, and the thoracic esophagus (Shehan 2012; Zhu 2015). The esophagus in birds is a muscular tube that connects the oral pharyngeal cavity from the upper side and the proventriculus from the lower side. Its walls are of a high blood supply, and its inner walls contain continuous longitudinal folds along the esophagus (Taha, 2020). The Esophagus is then attach to the stomach, which transport the food from the mouth to the stomach (Matsuo, 2008).

Tilapia species are invasive fish to Iraqi waters the early records show that *Coptodon zilli* was caught from the Euphrates River near Musaib City, Centre of Iraq (Mohamed, 2020). Now, three tilapia species, *Oreochromis aureus*, *Coptodon zillii (Tilapia)* and *Oreochromis niloticus*, are well established and dominating in different water bodies of Iraqi (Mohamed AR, Abood, 2020). This study aimed to compare the esophagus between two invasive species in Iraq *A. trisris* and *T. zilli*, according to the histological and histochemistry study.

Material and Methods

The birds were obtained from pet stores (Al-Ghazil market in Baghdad), while the fish samples cultured from Euphrates River, and checked for their health status before being euthanize. Esophagus was removed from the dissected animals and placed in 10% formalin solution. Microscopic preparations were made according to (Bancroft, 2008). After fixation, the tissue was dehydrated by transferring through a series of alcohols 70, 80, 90, 100 % cleared with xylene and embedded in paraffin wax (56-58°c). Sections of 5–7 µm in thick-ness were obtained. The sections were then subjected to haematoxylin and eosin (HE) stains for histological features, Masson's trichrome for connective tissue investigation, Periodic Acid Schiff PAS for neutral mucins detection, Alcian blue (pH 2.5) for acidic mucins detection. Photomicrographs of the sections were captured using a digital camera MC2500 attached to microscope Olympus.

Results

The average length of the Myna esophagus in the current study is 6 cm with average diameter is 0.7 cm. Esophagus is a muscle tube with walls have high blood supply. Its inner walls contain continuous longitudinal folds along the esophagus. While, the *T. zillii* esophagus is short, straight tube, passing from the mouth to the cardia of the stomach. The wall of the easophagus in each species composed of four tunica: mucosa, submucosa, muscularis (inner-circular and outer longitudinal) and adventitia. In Myna, the lumen of the upper and middle parts were displayed numerous long folds which formed by submucosa and mucosa. (fig. 1A). These folds were lined by non-keratinized stratified squamous

epithelium, the submucosa layer contains mucosal glands (Esophageal glands). (fig. 1B).While, in Tilapia the esophagus is short, straight tube, passing from the mouth to the cardiac portion of the stomach. The tunica mucosa of anterior part of esophagus was composed of long simple mucosal folds. The mucosa of the esophagus exhibited inner longitudinal fold, and these folds were lined by pseudo stratified columnar epithelium that abundant with non-secretin cells and few of mucous secreting epithelial cells. The folds were supported by thin layer of areolar connective tissue that constructed of lamina propria (fig. 2A & 2B).

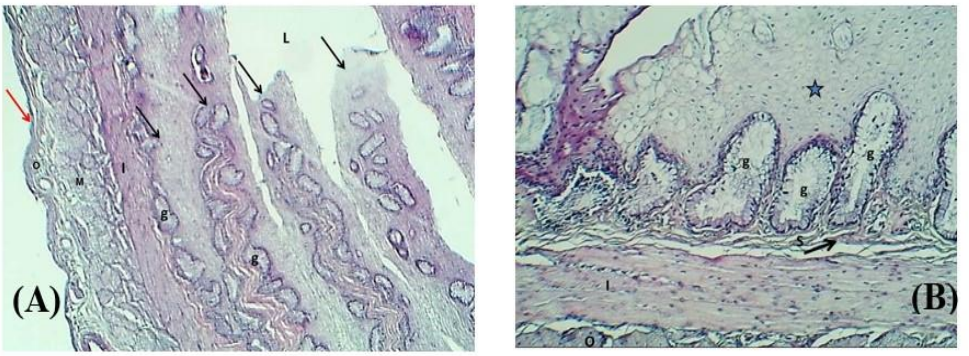

Figure 1: (A) Cross section of esophagus (Bird) shows: long simple mucosal folds (Black arrows), submucosal mucous glands (g), inner circular smooth muscle fibers (I) & middle longitudinal smooth muscle fibers (M) and outer layer of muscularis (O), (A). H&E stain.40x. (B) Shows stratified squamous epithelial cells (Asterisk), submucosal mucous glands (g), collagen bundles (Black arrow) of submucosa -lamina propria (S), inner layer of muscularis (I) & outer layer of muscularis (O). H&E stain.100x.

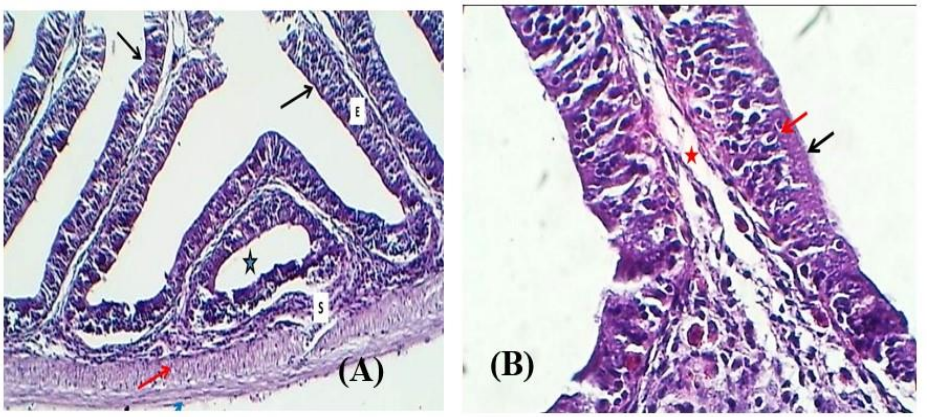

Figure 2: (A) Section of anterior part of esophagus (Fish) shows: simple mucosal folds (Black arrows), anastomosing fold (asterisk), tunica submucosa (S), inner circular muscle fibers (Red arrow) & outer longitudinal layer of tunica muscularis (Blue arrow). H&E stain.100x. (B) Section of esophageal mucosal fold (Fish) shows: pseudo stratified columnar epithelium with mucous secreting cells (Red arrow) and non-secreting cells (Black arrow) and lamina propria (asterisk). H&E stain400x.

In *A. tristis,* the lamina propria was continued with tunica submucosa, lamina propria submucosa was composed of fibrous connective tissue that occupied by huge mucous secreting alveoli (fig. 1B & 3). Also, the *T. zillii* tunica submucosa was continued with lamina propria due to absence of muscular mucosa and composed of loose connective tissue.

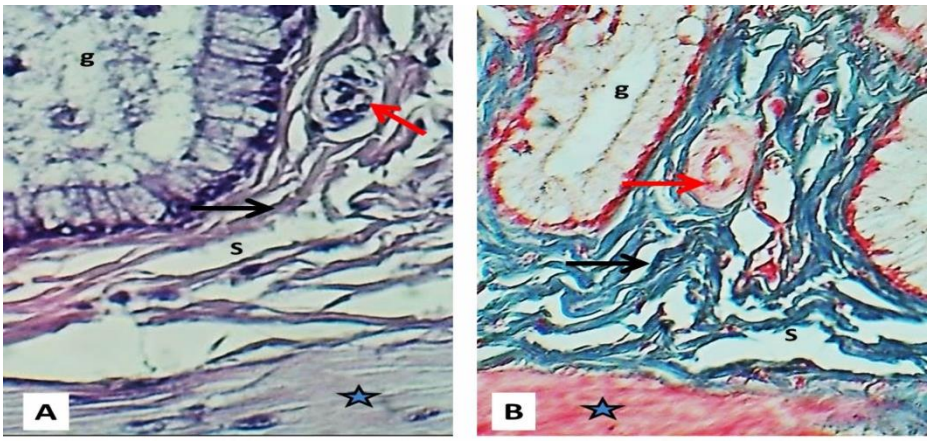

Figure 3: Magnified section of lamina propria – submucosa (S) (Bird) shows: collagen fibers (Black arrows), submucosal mucous glands (g), thick inner circular smooth muscle fibers (asterisks), arterioles (Red arrows), Micrograph (A) H&E stain, Micrograph (B) Massons trichrom stain (400x).

The tunica muscularis of *A. tristis* esophagus was the thickest tunica which composed of three layers: inner circular layer of smooth muscle, thick middle longitudinal layer of smooth muscle and very thin circular outer smooth muscle fibers (fig. 4). The *T. zillii* tunica muscularis was composed of thick inner circular layer of smooth muscle and thin outer longitudinal layer of smooth muscles fibers. The outer most layer of esophagus was the tunica adventitia (fig. 5).

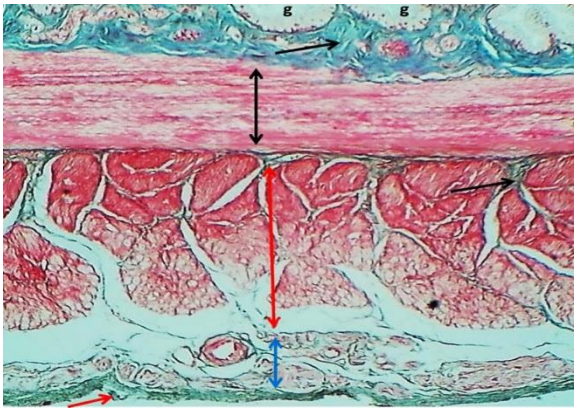

Figure 4: cross section of esophagus (Bird) shows: submucosal mucous glands (g), fibrous connective tissue of lamina propria submucosa (Black arrows), thin inner circular smooth muscle fibers (Black double arrow) & thick middle longitudinal smooth muscle fibers (Red double arrow), thin outer circular smooth muscle fibers (Blue double arrows) & tunica adventitia (Red arrow).

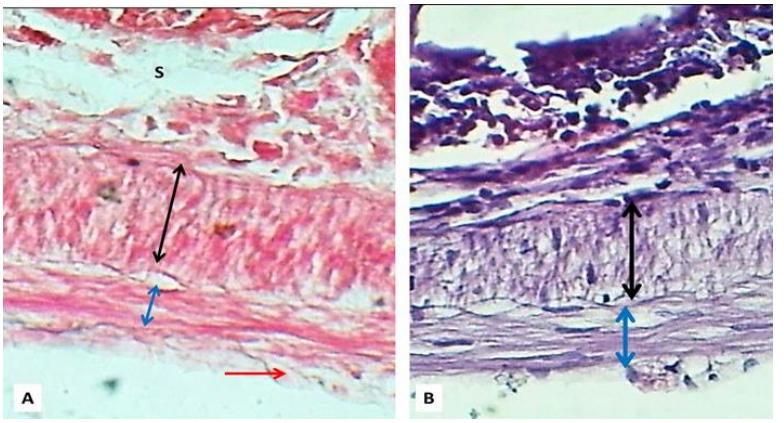

Figure 5: longitudinal section of wall of esophagus (Fish) shows: tunica submucosa (S), thick inner circular layer of smooth muscle fibers (Black double arrow), thin outer longitudinal layer of smooth muscle fibers (Red double arrow) & tunica adventia (Red arrow). Micrograph (A) Massons trichrom stain & (B) H&E stain, 400x.

The histological sections of *A. tristis* esophagus showed that the submucosal glands with Alcian blue stain contained acidic mucopolysacharrids (fig 6), meanwhile the sections with PAS stain showed strong neutral secretions within submucosal glands (fig.6). While the anterior part of *T. zillii* esophagus with PAS stain showed strong neutral mucopolysacharrids within secretory epithelial cells (Goblet cells) and weak neutral secretory products within non-secretory epithelial cells (fig.7).

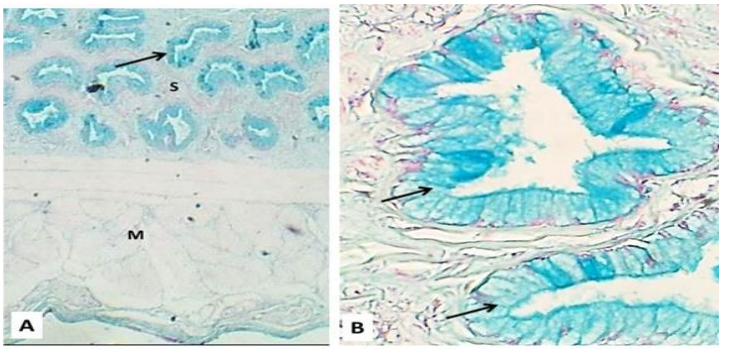

Figure 6: section of esophagus (Bird) shows: acidic mucopolysaccharide within mucous alveoli (Black arrows), submucosa (S) & muscularis (M).Alcian blue (2.5pH) stain. Micrographs (A) 100x & (B) 400x.

Figure 6: section of esophagus (Bird) shows: neutral mucopolysaccharide within mucous alveoli (Black arrows), submucosa (S) & muscularis (M). PAS stain. Micrographs (A) 100x.

Figure 7: section of anterior part of esophagus (Fish) shows: strong neutral mucopolysacharrids within goblet cells (Black arrows) & weak neutral secretory product within epithelial cells (Red arrow). PAS stain. Micrograph (A) 10x0x.

The middle part of *T. zillii* esophagus with alcian blue (pH 2.5) stain the epithelial crypts contained acidic mucopolysacharrids and within the epithelium showed no reaction meanwhile with PAS stain showed strong neutral mucopolysacharrids within crypts and weak neutral mucopolysacharrids within epithelium (fig. 8).

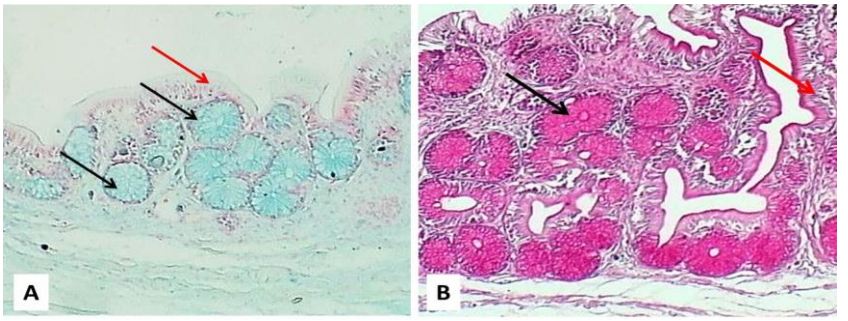

Figure 8: section of middle part of esophagus (Fish) shows: Micrograph (A) showed acidic mucopolysacharrids within epithelial crypts (Black arrows) and no reaction with epithelial cells (Red arrow). Alcian blue stain.100x. Micrograph (B) showed strong neutral mucopolysacharrids within crypts (Black arrow) & weak neutral mucopolysacharrids within epithelium (Red arrow). PAS stain 100x.

Discussion

The esophagus appeared long in *A. tristis* compared with the *T. zillii* esophagus which appeared short, the average length of esophagus is about 6.0 cm, and the average width is about 0.7 cm. The length of the esophagus is related to the nature and the texture of food intake myna needs the long esophagus to moisten the food intake for as long as possible, this result consistent with (Taha, 2020) who studied on zebra finch and starling birds. According to (Evans, 1996) Peristaltic contraction of inner circular and outer longitudinal muscles in the tunica muscularis propels food posteriorly through the esophagus aid in swallowing large food items, the esophagus is expandable as a result of a series of longitudinal folds. This accordion-like arrangement is enriched with mucous glands to provide lubrication. The epithelial lining is thick and cornified for protection against mechanical damage as a result of swallowing food items whole. The presence of large folds of mucosa allows considerable expansion and shrinkage, depending on the amount of contents.

In general the wall of the initial and middle parts of esophagus was composed of four tunica: mucosa, submucosa, muscularis and adventia. These folds were lined by non-keratinized stratified squamous epithelium which showed modification into many mucous alveoli, this result confirm with (AbdElnaeem, 2019) who studied the structure of the esophagus in two birds different in their food behavior Hoopoe and kingfisher and he found an exception that the wall of the esophagus in king fisher lack to submucosal layer. Through the results that appeared, it was shown that the esophagus is a muscular tube in its structure and this corresponds to its function in transporting food from the mouth to the stomach, as the muscular walls help in its movement to push the food and also these walls help it in stretching to accommodate the volume of the swallowed food. The lumen of these parts were displayed numerous long folds which composed of submucosa and mucosa. The function of these folds are to provide elasticity by allowing the esophagus to expand when a bolus enters it. These folds stretch outward through the action of mechanoreceptors, which respond to the increase in pressure, this allows the esophagus to expand (McGuire, 2012).

The tunica mucosa of anterior part of esophagus was composed of long simple mucosal folds which showed anastomosing, as shown by (Okuthe, 2021) the mucosa of the esophagus exhibited inner longitudinal fold, and these folds were lined by pseudo stratified columnar epithelium that abundant with non-secretin cells and few of mucous secreting epithelial cells, The distinct feature of mucosal epithelium as shown by (Okuthe, 2021) it was made up of stratified epithelium consisting of basal cuboidal cells, intermediate columnar mucous cells and superficial layer of flattened cells. The folds were supported by thin layer of areolar connective tissue that constructed of lamina propria.

The sections of esophagus in *A. tristis* showed that the submucosal gland stained with Alcian blue which contain acidic mucopolysacharrids, as well as stain with PAS showed strong neutral secretions within submucosal glands. The main function of submucosal glands secrete mucin and bicarbonate, which help in luminal acid clearance and epithelial protection which consider as one of the most important protection mechanisms for the esophagus from acidic gastric juice (Abdulnour-Nakhoul, 2011). The esophagus of *T. zillii* also showed positive stained at the middle part of esophagus with alcian blue (pH 2.5) stain the epithelial crypts contained acidic mucopolysacharrids and within the epithelium showed no reaction meanwhile with PAS stain showed strong neutral mucopolysacharrids within crypts and weak neutral mucopolysacharrids within epithelium. This explains the staining of cells with Alcian blue and PAS stains, and thus gives an indication of the presence of many of these glands which secrete mucus, which are commensurate with the functional need for them in this part of the digestive tract.

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