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Histological and Histochemical Insights Into Kidneys in the Sea Gull (*Larus armenicus*)

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Abstract | The study aims to examine the histological and histochemical characteristics of seagull kidneys. For this purpose, a total of 10 healthy birds were investigation. The kidney samples were washed in physiological solution (0.9%) and then preserved for 72 hours in 10% neutral-buffered formaldehyde after the birds had been killed and their kidneys removed. The paraffin procedure was then used to fix the samples. Four stains were applied (H and E, AB-PAS, Masson and Gomori cobalt method). The histological outcome revealed that the kidneys of the seagull have a very delicate capsule. The two kidney lobes, with numerous poorly defined polygonal lobules composed of two parts, the cortex and medulla, were conically arranged. Nephrons are grouped in lobules. Histochemically, a positive PAS-AB satin reaction was observed in the brush surround, the both thin and thick limbs of the loop of Henle, which the endothelial cells of the blood vessels in the tuft, the visceral section (podocyte), the parietal membrane of the capsule of Bowman, the cortex, the cytoplasm of the lining cell epithelium from the distal convoluted ducts, the brush surround, and the cortex and medullary collecting duct. The renal capsule was well-received by the PAS stain. The study concludes that the diet of birds varies with the living environment. The marine birds, native birds, carnivorous birds, and domesticated birds are among the species that have different diets, which cause their kidneys to perform unique, distinct functions compared to those of other species.

Keywords | Histological, Histochemical, Kidney, Sea gull

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INTRODUCTION

The Armenian Gull (*Larus armenicus*) is a migratory bird, classified as an endangered species. It flies in the Caspian Sea and some Balkan countries, and migrates to Iraq from September to April (Allous, 1961). The adult is characterised by a black and red spot at the end of the yellow beak, and it feeds on fish, molluscs, and invertebrates (Habeeb *et al.*, 2021).

In vertebrates, the kidneys are the main organs. Through the intricate system of tubules, they balance the pH, ionic contents, osmolarity, renal secretory volume, and glomerular filtration of bodily fluids. This helps to balance the body fluids and stabilise the internal conditions of vertebrates by controlling osmatic pressure (Martini, 2006).

The symmetrical, paired kidneys of birds are situated in the renal fossa, a depression in the bone of the synsacrum, on either side of the spinal cord. According to research on the kidneys of coots and racing pigeons, each kidney is said to contain three lobes: the cranial, middle, and caudal lobes.

The cortex and the medulla are the two main components of the lobule kidney in certain birds (Nabippour *et al*., 2009; Batah, 2012).

Bird kidneys contain two kinds of nephrons: The reptile type, which are small, lack Loops of Henley, and the mammalian type, which are large and long (Bruun, 1999; Reece, 2004). Both human and reptile renal corpuscles, as well as proximal and distal convoluted tubules, were found in the brain (Abood *et al*., 2014; Reshage *et al*., 2017; Mohsen *et al*., 2024). The Henley loop's thick and thin segments, as well as the characteristic of the mammalian nephron the presence of the Henliyen Loop were found in the secondary area (medulla), whereas the reptile nephron lacks it. In contrast to certain mammalian nephrons, which are juxtamedullary, the majority of reptilian nephrons are found in the cortex; moreover, the majority of reptilian nephrons are concentrated in the cortical peripheral (Cassoti *et al*., 1998). We conducted this study because there was a Lack of studies on the histological, histochemical, and histochemical aspects of Kidneys in seagulls.

MATERIALS AND METHODS

The samples were cleaned and then fixed in 10% neutral buffered formalin for 72 hours. To get histologically healthy birds, the tissues were subsequently subjected to standard histological methods. Al-Basra Market is where the adult sea seagull (*Larus armenicus*) was acquired. The birds were killed and their kidneys removed. The kidney specimens were cleared of the synsacrum with physiological solution (0.9%). Sections (3-5 µm) were cut with the aid of a motorised microtome and stained by Mercury bromophenol blue, Weigert, periodic acid Schiff (PAS), Hematoxylin and Eosin and Masson's trichrome van Gieson (Boncrof and Stevens, 2010; Widhur and Trivedi, 2012). Also, the Gomori cobalt technique of alkaline phosphatase detection (Sigh and Sulochana, 1996).

RESULTS

Histological results

According to the findings of every study, the kidneys are divided into three lobes, each of which contains polygonal lobules surrounding the central vein. Each lobule was separated into a tiny medulla cone and a large cortex. Each lobe of the kidney in a seagull is composed of many poorly distinguished polygonal lobules, surrounded by the interlobular veins on both sides, with an intralobular vein (central vein) in the center. Each lobule is composed of two sections that are grouped in cones and are dispersed randomly throughout the kidney. The narrow part is called the medulla, while the large area is called the cortex (Figure 1A).

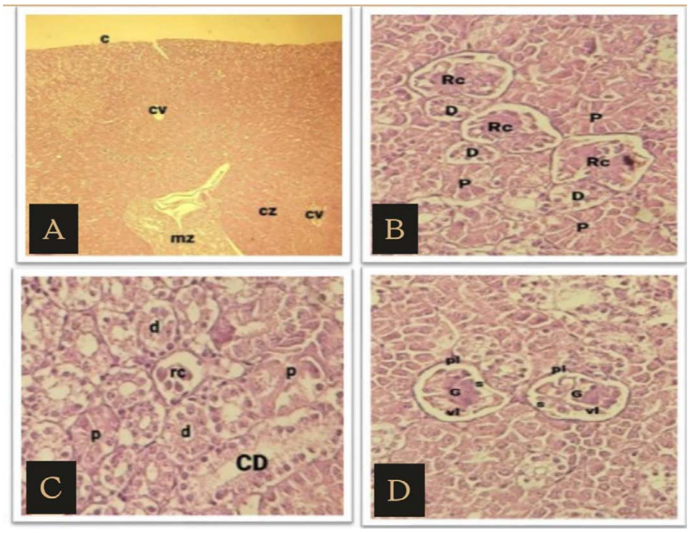

Figure 1: Photomicrograph of kidney showing **A:** (c) capsule, (cv) central vein, (cz) cortex zone, (mz) medulla zone. H&E stain. 40X. **B:** The cortex shows the proximal tubule (p), distal tubule (d) and a mammalian renal corpuscle in a triplet (Rc). H&E stain 400-X. **C:** cortical collecting ducts (CD), proximal tubule (p), distal tubule (d), and cortex (rc) reptile corpuscle. H&E stain. 400 X. **D:** the cortex: mammalian renal corpuscle in group of dual, (G) glomerulus, (pl) parietal layer, (vl) visceral layer, (s) Bowman's space. H&E stain.400X.

Some interstitial tissue is present in the renal parenchyma. Lobules carry a collection of nephrons. Convolutional tubules, loop of Henle, proximal convoluted tubules, distal collecting ducts (cortical and medullary), and renal corpuscle comprise the nephron. There were two different kinds of nephrons in the lobule: The reptilian form, which had a loop of the Henle and few tiny renal corpuscles, and the mammalian type, which had many big renal corpuscles (Figure 1C, D).

The existing findings have shown that both types of corpuscles are scattered haphazardly throughout the periphery to the deep level of the cortex and may occur singly or in close pairs or triplets (Figure 1B, C, D).

Henley loop's thick and thin sections, as well as a medullary collecting duct, were seen in the medulla. In contrast, the cortex showed proximal and distal convoluted tubules as well as a cortical collecting duct. The renal corpuscle is composed of a glomerulus, a small cluster of endothelial-lined capillaries in the center of a Bowman's capsule. The gap between the parietal and visceral layers of epithelium, which consists of podocytes, is called Bowman's gap and forms part of Bowman's capsule (Figure 1D).

Simple, high-cuboidal epithelial cells line the proximal tubule, which has a brush border made of a thick layer of microvilli. The tubule is primarily round in cross-section, while it does include a tiny lumen and occasional elliptical cross-sections. There are many grains and vesicles in the tubule epithelium's apical region (Figure 1B, C).

The collecting duct is composed of cuboidal to columnar cells, with nuclei situated at the bases. There is a pale oval or more rounded nucleus near the basal surface of the cell, and the cell cytoplasm contains an irregular apical surface. The distal tubule is a round-shaped segment with a large lumen lined by cuboidal epithelium with foamy cytoplasm at the basal part. A brush boundary is not seen in the distal tubule epithelium, which ends in small processes that extend to the lumen. The wide diameter of the collecting duct is observed. Low cuboidal epithelium surrounded the loop of Henle's large limbs, whereas flat epithelial cells covered the narrow limbs (Figure 2A).

Histochemical results

There is a very delicate capsule that covers the kidney, composed of connective tissue with collagen fibres (Figure 2B). The cytoplasm of the lined epithelial cells of the brush border, the proximal and distal convoluted ducts, the cortex and the medullary collecting duct, both the thin and thick the limbs of the loop-like structure of Henle, the endothelial cells in the lining of the capillary tuft, the visceral (podocyte) cells, and the parietal layer in the Bowman capsule were all positively stained with PAS-AB satin (Figure 2C, D). The renal capsule responded favorably to PAS stain.

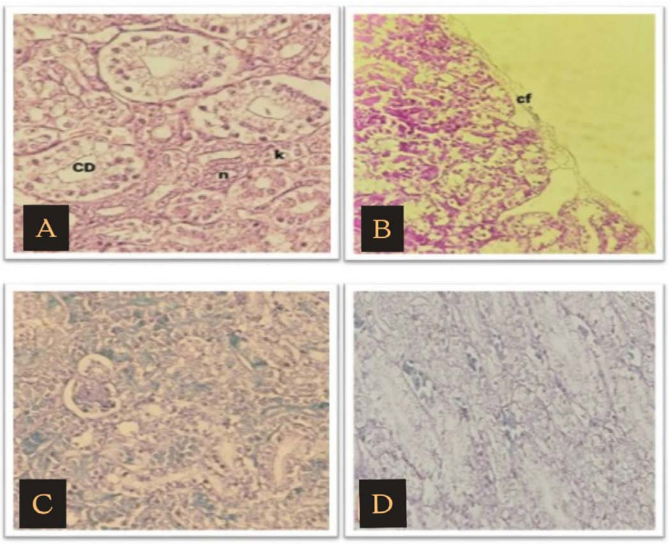

Figure 2: A: A kidney photomicrograph shows the medulla's characteristics as follows: The loop of Henle has (k) thick limbs, (n) thin limbs, and (CD) medullary collecting duct. 400 X H&E stain. **B:** the capsule of the kidney, which consists of connective tissue with collagen fibre (cf). Masson's stain 400X. **C:** Photomicrograph showing mucopolysaccharides of the cortex of the kidney. PAS-AB 400 X. **D:** Photomicrograph showing mucopolysaccharides of the medulla of the kidney. PAS-AB 400 X.

Histoenzymatic results

Alkaline phosphatase currently exhibits a negative activity in the parietal layer of the kidney corpuscle, proximal convoluted duct, and loop of Henle (thick and thin). In contrast, a positive activity is found in the capsule's visceral layer (podocyte), distal convoluted duct (basement membrane and the cytoplasm of the lining cell), cortex collecting duct, and medullary collecting duct (Figure 3A, B, C).

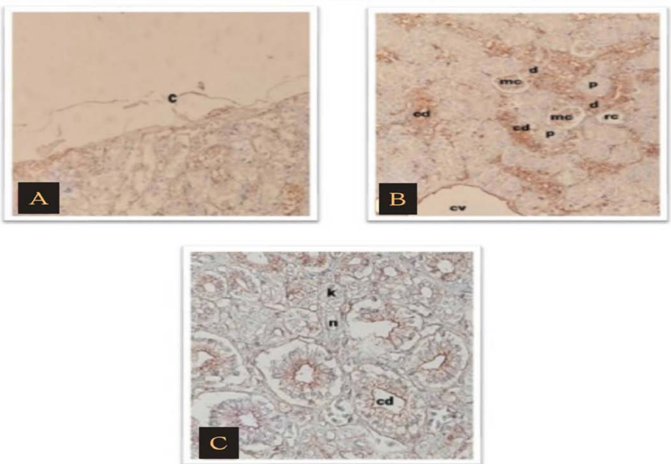

Figure 3: A: A photomicrograph displaying the kidney's histoenzymatic details: (c) capsule. Alkaline Phosphatase 400X. **B:** The photomicrograph displays the histoenzymatic intricacies of the kidney's cortex, including the central vein (cv), cortical collecting ducts (cd), proximal and distal tubules (p, d, d, pc, and rc, or mammalian and reptile corpuscles, respectively. Phosphatase alkaline 400 x. **C:** A photomicrograph displaying the kidney medulla's histoenzymatic details: (cd) the medullary collecting duct; (k) the loop of Henle's thick limbs; and (n) the loop of Henle's thin limbs. Alkaline Phosphatase 400 X.

DISCUSSION

Al-Janabi (2023) demonstrated that the seagull bird has one pair of lobulated kidneys covered by a fragile capsule of connective tissue. However, Alkafagy *et al*. (2019) disagree with this finding in honeyeater birds. In the avian kidneys, the lobular organisation of the liver was observed through the lobular arrangement of the renal cortex (Johnson and Mugaas, 1970). The kidney lobes of a seagull consist of a few poorly differentiated polygonal lobules, containing an intralobular venous (central vein) in the centre. The character has been mentioned in many studies, such as those on great flamingos (Reshag *et al*., 2015); and wild adult starlings (Nicholson, 1982), where Nicholson added that the interlobular veins mark the boundaries of the lobules.

Each lobule consists of a wide cortex and a small medulla. Similar observations were recorded in other bird species.

Warui (1989) observed that the bird species that existed in watery and mild habitat had small size of medulla and significant area of cortex. We found that seagulls contained numerous mammalian and reptilian nephrons. According to Nishimura and Fan (2003), this indicates that birds that drank saltwater needed more reptile nephrons, which limit urine concentrations to release more NaCl and facilitate the activity of supraorbital salt glands.

This agrees with other researchers on honey-eating birds (Casotti and Richardson, 1993), marine birds (Nishimura, 2008), coot birds (Batah, 2012), passer domesticus (Abdulla *et al*., 2014) and breeding emus (Michalek *et al*., 2016). Typically, birds possess the most typical reptile nephron (Carpenter, 2003; Casotti and Braun, 2000).

Reshag *et al*. (2015) revealed that both types of nephrons are located in the middle of the cortical region in great flamingos, even though the intermediate variety of corpuscle was not investigated in this study. In contrast to chickens, which have three different types of nephrons many big and intermediary mammal types and a few reptile types in the medulla Mallard ducks have nephrons located in the zone of intermediate size between the medulla and cortex (Abood *et al*., 2014). The intermediary type of corpuscle was observed in Japanese quail by Mobini and Abdollahi (2016). According to Nishimura and Fan (2003), the mammalian corpuscles provide the basis for concentrated urine, but the reptile corpuscles may limit the kidney's capacity to produce hyperosmotic urine. This finding suggests that many of the intermediate and mammalian corpuscles in birds are linked to their food habits, indicating that these nephrons play a crucial role in water conservation.

The interior architecture of the renal corpuscle is identical to that reported by Naipour *et al*. (2009) and Abood *et al*. (2014). These findings are consistent with those of Abood *et al*. (2014), Michalek *et al*. (2016), and various regions of the nephron, including the loop of Henle, proximal convoluted tubules, distal convoluted tubules, and collecting tubules (cortical and medullary).

Cunningham and Klein (2007) declared that about 70 percent of the total volume of water that has been filtered is absorbed in a proximal tubule in birds owing to the wide membrane infoldings as well as extensive intercellular spaces which are the characteristics of cells with a high capacity of the water and ions reabsorption and which depends on the active reabsorption (Reece, 2004).

In some situations, where the tubule at the distal end absorbs the mineral sodium chloride without reabsorbing water, the nephron may reabsorb water distally together with the medulla collecting ducts (Casotti, 2001). Because of this, the concentration differential is significantly more pronounced across the nephron's tubule. According to the current results, the kidney capsule, which consists of capillary tuft cells, as well as the visceral and parietal layers of the Bowman capsule, all showed positive PAS stain results. This is consistent with findings by El-Daly (2013) in mammal species that a fluid called glomerular filtrates, which is composed of water and electrolytes, can be extruded from plasma due to a neutral pH level of thin the capillary basement membranes (Cunningham and Klein, 2007).

Positive PAS compounds were discovered in the lumen of near-proximal convoluted tubules and the cytoplasm of epithelial cells in the current investigation, indicating the presence of neutral glycoproteins in this area of the nephron. This result tends to support the findings of Brandon *et al*. (2009) in the juvenile American alligator and the discovery of PAS-positive materials in the lumen of proximal convoluted tubules in the rock dove (Reshag *et al*., 2017; Nabipour *et al*., 2009; Mobini and Abdollahi, 2016).

The glomerulus, the inner and parietal layers of the renal corpuscle, the cytoplasm of the innermost epidermal tissues of the tubular convoluted at the proximity and their brush border, the cytoplasm of the lining epithelium cells of the distally convoluted duct, the cytoplasm of the cortical collecting duct, and the cytoplasm of both the thick and thin limbs from the loop of Henle were all included in our study. All of these substances reacted favorably to the Alcian blue stain (pH 2.5). These findings support those of El-Salkh *et al*. (2008) and Nabipour (2008). However, they contradict those of Reshag *et al*. (2017) and Nabipour (2008), who discovered that the ducts used for collecting insectivorous bats did not respond appropriately to the Alcian blue-PAS stain.

The distal convoluted ducts (basement membrane and lining cell cytoplasm), the capsule, the abdominal cavity (podocyte) of the renal corpuscle, and the cortical and medulla or collecting duct all showed positive alkaline phosphatase activity in this investigation. On the other hand, Singh *et al*. (2020) have identified positive activity in the kidneys' capsule, brush border of proximal convoluted tubules, and distal convoluted tubules. Contrary to this, the study of Mishra and Meshram (2019) regarding the organ parts of pecten oculi reported that alkaline phosphatase activity was nearly negative, whereas, in the study of Sivakumar *et al*. (2012) about the kidney the activity of alkaline phosphatase was positive in Guinea fowl, Furthermore, Abdulgader (2023) has revealed that the alkaline phosphatase activity was positive in certain parts of pancreas and gall.

CONCLUSIONS

The histological insights identify that seagull kidneys are formed by three lobes, each lobe was subdivided into cortex and medulla cone. The cortex composed to renal glomerulus, the large glomeruli called mammalian glomeruli which found sub capsular while on the glomeruli were smaller called reputation glomeruli, the glomeruli which surround by Bowman's capsule and center core of massaged cell, other parts of renal capsular proximal and distal convoluted tubules.

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NOVELTY STATEMENT

This is the first study in Iraq to study histological and histochemical characteristics of seagull kidneys.

AUTHOR'S CONTRIBUTION

This study was conducted with the participation of both researchers in the work. Both researchers read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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