Anatomical and histological study of the kidneys and salt glands in great flamingos (*Phoenicopterus* roseus)

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Summary

This study was designed to explain the anatomy and histology of kidneys and salt glands in Great Flamingos. Eight adult healthy Great Flamingos of both sexes have been used. The results showed that the kidneys in Great Flamingos consist of three separated lobes. The right kidney was longer 8.9±0.3 mm than the left kidney 8.4±0.4mm. The ratio of the kidneys weight to the total body weight was 0.39%. The salt gland was very large crescent shape, and occupied the supraorbital fossa and has 20.8±0.2mm long and 3.9±0.2mm in diameter. The volume of cortex was 60-70% and the medulla was 30-40%. Within the middle region of cortex there were numerous large corpuscles (mammalian type) and few of small corpuscles (reptilian type). There was a variation between the means diameters of mammalian type 59±1µm and reptilian type 42±0.9µm. The proximal and distal convoluted tubules and collecting ducts were lined by simple cuboidal epithelium and their means diameters were 46±0.9, 44±1 and 55.1±0.7µm, respectively. Within medulla the thick and thin segments were arranged at the peripheral zone of medullary cone while the collecting ducts were at the central part of cone and all were lined with simple cuboidal epithelium except thin segments were lined with squamous cells. The salt gland were consisted of lobules surrounded by thick connective tissue capsule and each has mass of branched tubuloacinar secretory unites. The latter were made up by single layer of cuboidal cells. The tubules lead into central duct lined by double layer of cuboidal cells while the main duct lined with stratified cuboidal epithelium. The secretory units of salt gland gave negative reaction to PAS and combined AB (pH 2.5) and PAS stains and this indicate the absence of neutral mucosubstances. The results concluded that the kidneys in great flamingo was small size organ with low relative weight in compare to birds size and the salt glands was active organ.

Keywords: Kidneys of flamingos, Salt gland, Great Flamingos.

Introduction

Flamingos are migratory birds. They feed on shellfish and algae. There are six species worldwide and only 1 species in Iraq (1). This species travels from Siberia and visits Iraqi marshes in winter which once home to millions of migratory birds including flamingos. Kidney and salt gland are the organs those regulate the body fluids and mineral composition thus they necessary to maintain the homeostasis and expelled the metabolic waste products. All marine birds and waterfowl have salt glands, but they well developed in Cormorants, Herring-gull, Gannets, Pelicans, Albatrosses and Penguins (2 and 3). Birds have little ability to produce concentrated urine (4 and 5), thus the salt gland function providing an extra renal function specially for the excretion of sodium chloride while bird consume large amount of salt than its more ability of renal excretion (6 and 7). This study has designed to explain the anatomy and histology of both organs in this bird.

Materials and Methods

Eight adult healthy Great Flamingos of both sexes (four males and four females) have been used for this study. All birds have been collected from local markets of Mesan governorate. Before birds scarifying their body weights were taken. Birds have been euthanized by intravenous injection of an overdose of Phenobarbital-sodium through the wing vein and then they were sacrificed. The samples of kidneys and salt glands were removed out from the body. Morphometrical measurements including length, width, absolute and relative weight of kidneys and salt glands have been estimated. The kidneys and salt glands have been fixed in 10% formalin and prepared for routine histological paraffin technique and the tissue sections of

kidney have been stained with Hematoxylin and Eosin–phloxine stain, while section of salt gland have been stained with H and E stain and for detection the glycogen and neutral mucopolysaccharid the sections have stained with combined Alcian blue (AB) (2.5 pH) and Periodic Acid-Schiff stain (PAS) to distinction between acidic and neutral mucopolysaccharid (8).The measurements of renal corpuscles, diameter of renal tubules and the volumes of the cortex and medulla were done with oculometer.

$$\textbf{index of relative kidney weight} = (\textbf{weight of kidney})/(\textbf{body eight})$$

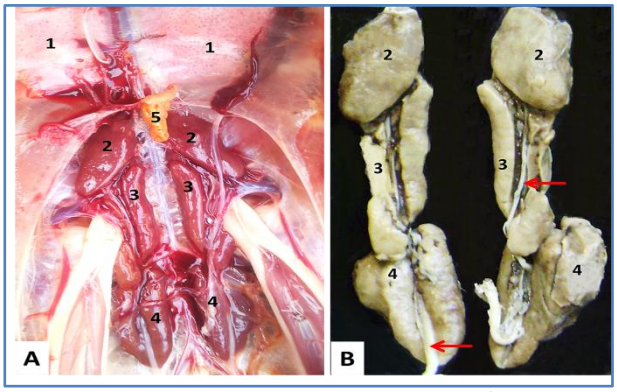
Results and Discussion

Anatomically, the results showed that the anatomical location of both kidneys extended from the caudal aspect of the lungs untill the end of the synsacrum and occupied the synsacral fossa; such a result was recorded in avian species by (9 and 10). Each kidney consisted of three separated lobes: these were cranial, middle and caudal (Fig. 1) similar lobes were previously recorded by (11 and 12), but in most passerine the kidney possess fused middle and caudal renal lobes (13). In another aspect in herons, puffins and penguins those possess connected caudal renal lobes across the midline (10). The present findings showed that the cranial lobe was oval in shaped and bent in oblique position. The middle lobe was narrow elongated in shape while the caudal lobe appeared the largest and irregular in shape (Fig. 1) this result was parallel with result of (12) in Mallard duck and this suggested that the shapes of kidneys lobes were relatively similar in marine birds and differed from those of chick. The color of kidney was reddish to brown. The mean length and width of the kidneys lobes, total length of kidney and absolute weight in addition to their percentage to the total body weight are all showed in (Table, 1).

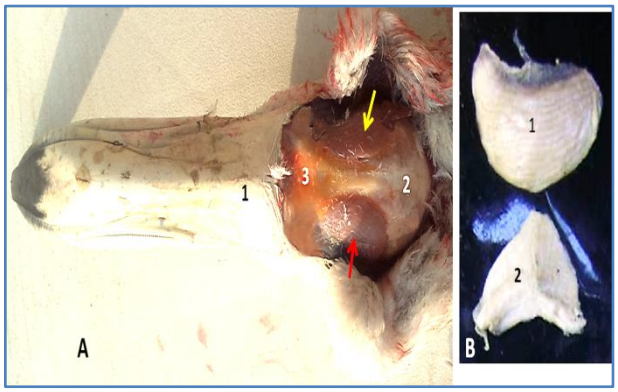
The morphometrical result showed that the right kidney was relatively taller than left one. This result was in parallel with (14) who mentioned that the left kidney in laying hens tends to be longer than the right kidney. The relative weight of the kidneys was lower than that recorded by (15 and 16) who indicated that the avian kidneys comprise 1-2.6% of body weight, similarly was lower than those in Harrier (*Circus aueroginosus*) and Mallard duck (12). The present findings suggested that the relative kidney weight didn't affect by the active salt glands and this disagree with (16) who mentioned that the kidney mass is relatively larger in those birds with active salt glands. The salt gland of the great flamingos' was large crescent shape, brown in color and occupied the supraorbital fossa. Anteriorly the salt gland was bordered by frontal bone, laterally the gland was bordered by the dorsal border of orbit and posteriorly the salt gland reached the occipital bone (Fig. 2), similar finding were mentioned by (17) in Moorhen, (18) in some wild and domestic birds and (19) in Kentish Plover. The slat gland of great flamingos has measured 20.8±0.2 mm in length and 3.9±0.2 mm in diameter, while the weight was 0.56±0.3 g, these values were similar relatively to those salt gland in domestic duck (20). The values of present study were lower than those recorded by (17) in Moorhen, (19) in Kentish Plover. In most birds the size of salt gland is depends on the salt consumption and the hyperplasia response is considered normal in some species (4). The salt gland hyperplasia could be induced in aquatic birds by adding high levels of sodium chloride into the drinking water, but not in chickens (5). In general, the birds which intake little salt have small salt glands and when they intake high level of salt, there is rapid and profound hyperplasia and hypertrophy response greatly and enhanced salt-secretory capacity within 1 to 7 days (4 and 21).

Histologically, the kidneys have divided into lobules. Each lobule showed an outer cortex and inner medulla (Medullary cone) (Fig.3). Within cortex, there were two types of renal corpuscles: numerous large corpuscles (mammalian type) which characterized by presence of loop Henle and few of small corpuscles (reptilian type) which lacked loop Henle such types of corpuscles has recorded by (12) in Mallard. The present findings showed that both types of corpuscles have distributed in the middle of cortex region (Fig. 3), but in Mallard duck both types are located at the intermediated zone between the cortex and medulla (12). Each renal corpuscle has composed of glomerulus which surrounded by

Bowman's capsule and consisted of central core of mesangial cells those surrounded by podocytes (Fig. 4) (22). The proximal convoluted tubule displayed eosinophlic cytoplasm and narrow lumen while the distal convoluted tubules in addition to collecting duct were showed wide lumen and less eosinophlic cytoplasm, all have lined by simple cuboidal epithelium and their mean diameters were 46±0.9, 44±1 and 55.1±0.7µm, respectively. Medulla consisted of thin and thick segment of loop Henle in addition to the collecting ducts. The thick and thin segments have arranged at the peripheral zone of medullary cone while the collecting duct were restricted at the central part of cone (Fig. 5).

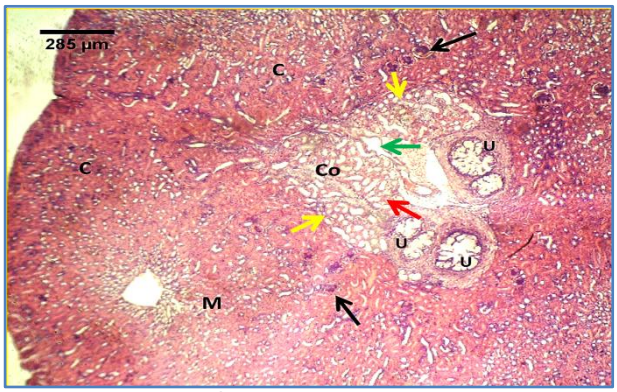


Figure, 1: Micrograph (A): coelomic cavity of Flamingos shows: Lungs (1), kidneys cranial lobes (2), kidneys middle lobes (3) and kidneys caudal lobes (4). Micrograph (B): Left and right kidneys were removed out of coelomic cavity shows: cranial lobes (2), middle lobes (3) caudal lobes (4) and ureter (red arrows).

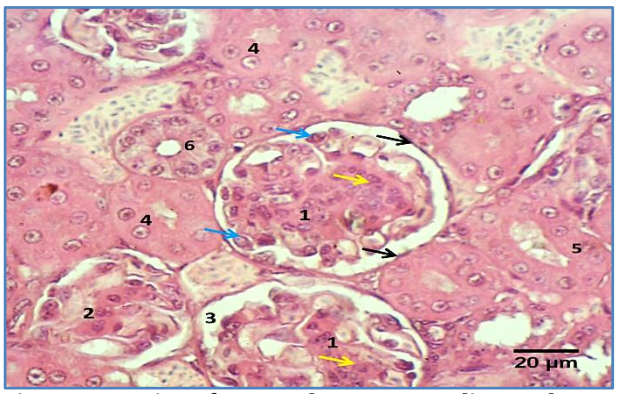


Figure, 2: The salt glands of greater flamingos, (A) Dorsal view of head region shows: beak (1), occipital bone (2), frontal bone, (3), left and right lobes of salt glands (arrows). (B) shows right lobe of salt gland (1) and left lobe of salt gland (2).

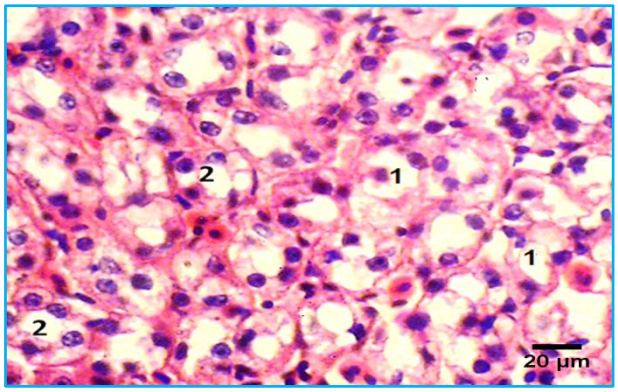
This result is incomparable with those of wet and arid zone honeyeaters birds (22), and with passerines in which renal tubules are arranged only in the superficial areas of the medulla (23), whereas in sparrows the medullary cones form an outer ring of thick limbs of Henle which surround an inner ring of the collecting ducts, which in turn surround a central core of thin limbs Henle (9 and 24). The latter indicated that this separation is attributed to theory on the production of a concentrated urine in bird and this theory showed that the sodium chloride is transported actively from the ascending limb of loop of Henle and passively into the descending limb. A close association between the descending and ascending limbs may facilitate the exchange of sodium chloride. In this manner a single solute maintains a high medullary interstitial osmolality essential for water reabsorption along the distal nephron. The current study showed that proximal, distal and collecting tubules were lined with simple cuboidal epithelium except the thin segments were lined with squamous cells (Fig. 5).



Figure, 3: section of kidney: shows cortex (C), medulla (M), branches of ureter (U), medullary cone (Co), renal corpuscles (Black arrows), zone of thin and thick segments of loop of Henle (Yellow arrows), collecting tubules (Red arrows), and wide vein (Green arrows). H and E– phloxine stain.

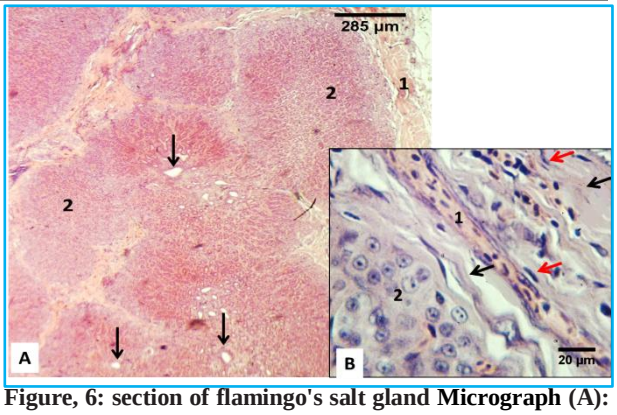


Figure, 4: section of cortex shows: mammalian nephrons (1), reptilian nephrons (2), bowman space (3), proximal convoluted tubules (4), distal convoluted tubule (5), parietal layer of Bowman capsule (Black arrows), nuclei of podocytes (Blue arrows) and nuclei of mesangial cells (Yellow arrows) H and E phloxine stain.

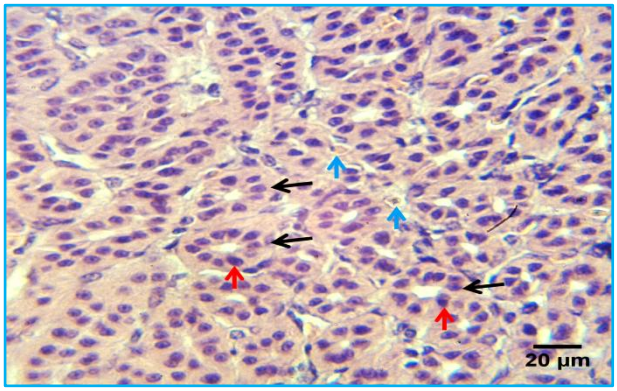


Figure, 5: The medullary cone shows: Thin segment of loop of Henle (1) and thick segment of loop of Henle (2). (H and E-phloxine stain).

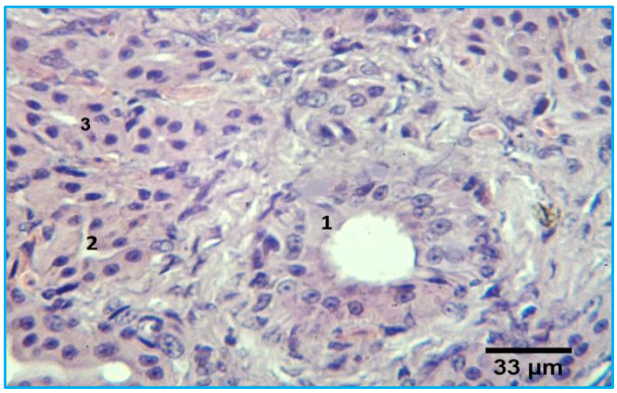
In great flamingos the volume of cortex was ranged from 60-70% and the medulla was 30-40% and this range exceeded those recorded in pervious study by (12) who indicted that in mallard duck the volume of cortex ranged from 50-52% and the medulla was 48-50%, this result gave suggestion that the time has spent by marine bird in wet environment affected this volume, also (25) found that the kidneys of wet zone honeyeaters contained a higher proportion of cortex whilst arid zone honeyeaters contained a higher proportion of medulla. The salt gland were composed of lobules and surrounded by thick capsule which made up by bundles of vascular collagenous connective tissue (Fig.6) (26).The glandular capsule has sent thin septa into glandular parenchyma and these septa were carried blood vessels into secretory units (Fig.6) such observation were mentioned by (17, 18, 19 and 27). Each glandular lobule has composed of a mass of branched tubuloacinar secretory unites which made up by single layer of cuboidal cells that surrounded narrow lumen. These cuboidal cells at the ends of acini have showed mitotic activities (Fig.6 and 7) and this findings were similar to those of (18) in different domestic and wild birds but, was different with those in Kentish Plover (19) in which the simple cells type which lined branched tubuloacinar unites. This suggested that there is one type of cells in the secretory units of branched tubuloacinar secretory and the mitotic activities at the acinus part to supply the tubuloacinar secretory unites with new generation of secretory cells that replaced the lost cells, this suggestion was in good agreement with recorded of (28) who reported only one type of secretory cells in salt gland.



Figure, 6: section of flamingo's salt gland Micrograph (A): Collagenous capsule (1), lobules (2), and central duct (Arrows) H and E stain. Micrograph (B): magnification section of capsule region shows: blood vessels (1), acini with mitotic activities (2), collagen bundles (Black arrows) fibrocytes (yellow arrows) H and E stain.



Figure, 7: Transverse section tubuloacinar glands shows secretory unites (Black arrows) and nucli of cuboidal cells (Red arrows), blood vessels. (H and E) stain.

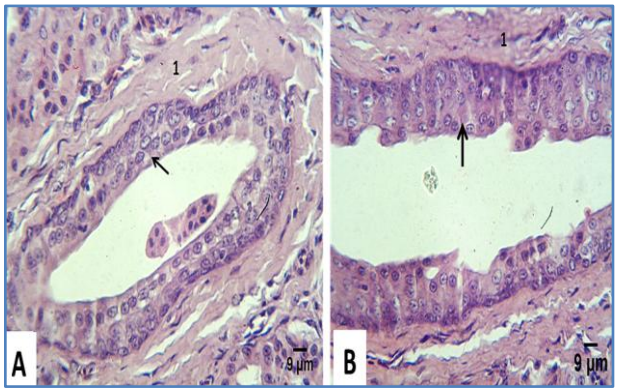


Figure, 8: Transverse section salt glands shows: striated duct (1), intercalated duct secretory unite (3). (H and E) stain.

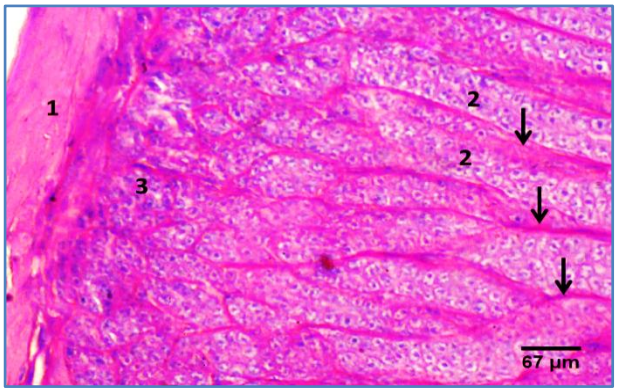
On other hand this feature has reported by (29) in nasal glands from both the fresh watered and saline-fed ducks there is an increase in the amount of glandular tissue. Each secretory tubules lead into inter calated duct that drained their secretion into striated duct and the later lead into the central duct of lobule which lined by double layer of cuboidal cells. The striated duct were lined with tall

cuboidal epithelial cells, while the intercalated duct lined with lower cuboidal cells (Fig. 8).

The main duct of each glandular lobes were lined with stratified cuboidal epithelium and surrounded by collagenous connective tissue (Fig. 9), such observation has recorded by (18 and 19) in Kentish Plover. The great flamingo feeds on fish, aquatic insects and seeds thus this type of feeding might lead to ingest large amount of salts, consequently the histological structure of flamingos salt gland showed best developed and functional organ as in birds which live in fresh water and this and this findings was in accordance with observation of (29, 30 and 31) in fresh water birds, also the actively salt glands have been reported in penguins and the mallard duck (29 and 31).



Figure, 9: Flamingo's salt gland. Micrograph (A): Transverse section of central ducts shows: double layer of cuboidal cells (Black arrows), tubular part of secretory units (Red arrows), and collagenous connective tissue (1). Micrograph (B): Transverse section of the main duct shows: stratified cuboidal epithelium (black arrow) and collagenous connective tissue (1). (H and E) stain.



Figure, 10: Flamingo's salt gland shows: Collagenous capsule (1), branched tubuloacinar lobules (2), acini (3) and septa (Arrows), combined (AB-PAS) stain.

The secretory units of flamingos' salt gland showed negative reaction to both PAS stain and combined AB (2.5pH), PAS stains (Fig.10) this result was indicating the absence of neutral and acidic mucopolysaccharides that the PAS stain confirm to (19 and 27) in Kentish Plover. The PAS negative reaction of secretary cells must be presumed to be no glycogens content in these cells, while the result was positive to AB (pH 2.5) by (19) in Kentish Plover. The present results have concluded that the kidneys in great flamingo was small size organ with low relative weight in compare to birds size and the salt glands was active organ.

Table, 1: The length, width of flamingo's kidney lobes, total length of kidney, total kidney weight, total body weight and the ratio. Mean±SD

Kidneys lobes	Right kidney		Left kidney	
	Length cm Mean ±SD	Width mm Mean ±SD	Length mm Mean ±SD	Width mm Mean ±SD
Cranial lobes	2.7±0.1	1.4±0.1	2.5±0.2	1.3±0.3
Middle lobes	3.4±0.1	1.1±0.2	3.3±0.2	1.1±0.1
Caudal lobes	3.2±0.2	1.9±0.3	3.1±0.3	1.8±0.2
Total length mm Mean±SD	8.9±0.3		8.4±0.4	
Total Kidney weight/gm	19.2±0.3			
Total body weight/kg	4.9			
Ratio	0.39%			

References

1. Mayr, G. (2004). Morphological evidence for sister group relationship between flamingos (Phoenicopteridae) and grebes (Podicipedidae). Zoolo. J. Linnean Soc., 140 (2): 157-169.
2. Chapman (1978).The body fluids and their functions. New York; St Martins press. Pp: 52-53.
3. Allous, E.B. (1961).Birds of Iraq. 1st ed. Al- Rabette Press. Baghdad. Pp; 27-29.
4. Shuttleworth, T. J. and Hildebrandt, J. P. (1999). Vertebrate salt glands: short and long-term regulation of function. J. Exp. Zoo., 283: 689-701.
5. Riddell, C. and Roepke, D. (1991). Inflammation of the nasal gland in domestic turkeys. Avian Dis., 35: 982-985.
6. Buggiania, S.S. and Rindi, S. (1980). Lead toxicosis and salt glands in domestic ducks. Bull Environ Contam. Toxicol., 24: 152-155.

7. Hughes, M. R. (1994). Responses of gull kidneys and salt glands to NaCl loading. Can. J. Physiol. Pharmacol., Pp: 1727-1732.
8. Bancroft, J. D. and Marilyn, G. (2008). Theory and practice of histological techniqes. 6th ed. London, Elsevier Limited. Pp: 168-173.
9. Canny, C. (1998). Gross anatomy and imaging of the avian and reptilian urinary system. Sem. Avian Exot. Pet. Med., 7(2): 72-80.
10. Nabipour, A.; Alishahi, E. and Asadian, M. (2009).Some Histological and Physiological Features of Avian Kidney. J. Appl. Anim. Res., 36: 195-198.
11. Batah, A. L. (2012). Morphological and Histological Study for the Kidneys of Coot Birds (Fulica atra). Bas. J. Vet. Res., 11: 128-136.
12. Dhyaa, Ab. Abood; Ali, F. R; Azhar, S. K. and Myson, A. A. (2014). Comparative anatomical and histological features of the kidney in harrier (*Circus aueroginosus*), chicken (*Gallus domesticus*) and mallard duck (*Anas platyrhynchos*). The Iraqi J. Vet. Med., 38 (1): 017 – 003.
13. Casotti, G. and Braun, E. J. (2000). Renal anatomy in sparrows from different environments. J. Morphol., 243(3):283-91.
14. Wideman, R.F. (1988). Avian kidney anatomy and physiology. Poult. Biol., 1: 133-176.
15. Hughes, M. R. (1995). Responses of gull kidneys and salt glands to NaCl loading. Can. J. Physiol. Pharmacol. 73: 1727-1732.
16. Frazier, D. L.; Jones, M. P. and Orosz, S. E. (1995). Pharmacokinetic considerations of the renal system in birds: part I. Anatomic and physiologic principles of allometric scaling. J. Avian Med. Surg., 9: 92-103.
17. Hussein, A. K; Hussein, A. J. and Mustafaa, S. (2006). Topographical and histological study of the salt gland of the moorhen (*Gallinula choropus*). Marsh Bulletin; 2: 93-98.
18. Maysoon, A. A. (2012). Comparative morphometric study of salt gland in some domesticated and wild marine birds. Bas. J. Vet. Res., 11(1): 349-354.
19. Bashir, M. J. (2009). Microanatomy and histochemistry of the salt glands of the kentish plover (*Charadrius alexandrines*). J. Biol. Sci., 9(3):75-80.
20. Mirhish, S. M. (2004). Anatomical, histological and ultrastructural study of salt gland on domestic duck (mallard) *Anas platyrhnchos*, M.sc. Thesis, College of Veterinary Med. University of Baghdad Iraq.
21. Butler, D. G.; Youson, J. H. and Campolin, E. (1991). Configuration of the medial and lateral segments of the duck (*Anas platyrhynchos*) salt glands. J. Morphol., 207: 201-210.
22. Casotti, G. and Richardson, K. C. (1993). A qualitative analysis of the kidney structure of Meliphagid honeyeaters from wet and arid environments. J. Anat. 182: 239-247.
23. Braun, E. J. And Reimer, P. R. (1988). Structure of avian loop of Henle as related to countercurrent multiplier system. Am. J. Physiol., Pp: 255, 500, 512.
24. Nishimura, H.; Koseki, C.; Lmai, M. and Braun, E. J. (1989). The thin descending limb of Henle of the quail. Am. J. Physiol., 257(6): 994-1002.
25. Casotti, G. and Richardson, K. C. (1992). A stereological analysis of kidney structure of honeyeater birds (Meliphagidae) inhabiting either arid or wet environments. J. Anat. 180: 281-288.
26. Donna, L. O. and Daniel, M. A. (2002). Avian salt gland. Institute of Creation Research. Pp: 129-130.
27. Benson, G. K. and Phillip, J. G. (1964). Observations on the histological structure of the supraorbital (nasal) glands from saline-fed and freshwater-fed domestic ducks (*Anas platyrhynchus*). J. Anat., 98(4): 571-578.
28. Gills, F. B. (1995). Ornithology. 2nd.New York. W.H. Freeman and Company. Pp: 142-143.
29. Schmidt-Nielsen, K. and W. L. Sladen, (1958). Nasal salt secretion in the Humboldt penguin. Nature. 181: 1217-1218.
30. Gray, D. A.; Downing, C. and Sayed, N. (1997). Endogenous plasma atrial natriuretic peptide and the control of salt gland function in the Pekin duck. Am. J. Physiol. Regul. Integrat. Comp. Physiol., 273:1080-1085.

31. Hossler, F. E. and Oslon, K. R. (1990). Microvasculature of the nasal salt gland of the duckling, Anas platyrhynchos: Quantitative responses to osmotic adaptation and deadaptation studied with vascular corrosion casting. J. Exp. Zool., 254: 237-247.

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الخلاصة

صُممت هذه الدراسة لمعرفة التكوين الشكلي والنسجي للكليتين والغدد الملحية في النحام الكبير. وقد استعمل ثمانية طيور بالغة وسليمة ومن كلا الجنسين. أظهرت النتائج أن الكليتين في طيور النحام الكبير تتكون من ثلاثة فصوص مفصولة فصلاً كاملاً عن بعضها. الكلية اليمنى أطول 0.3±8.9 ملم من الكلية اليسرى 0.4±8.4 ملم. وإن نسبة وزن الكليتين لمجموع وزن الجسم الكلي منخفضة وقد بلغت 0.39٪. الغدة الملحية ذات شكل هلالي كبير، ويبلغ طوله 0.2±20.8 ملم وبلغ قطرها 0.2±3.9 ملم. وحجم القشرة 60-70٪ والنخاع 30-40 ٪ من متن الفص. تحوي المنطقة الوسطى من منطقة القشرة على كبيبات كلوية كبيرة من نوع الثدييات وعدد قليل من الكبيبات كلوية صغيرة من نوع الزواحف. وكان هناك تباين بين متوسط أقطار نوع الكبيبات الكلوية الثدييات 1±59 ميكرون و نوع الزواحف 0.9±42 ميكرون. النبيبات الملتوية الدانية والقاصية والقنوات الجامعة مبطنة بظهارة مكعبة وبلغ متوسط قطرها 46± 0.9 ميكرون، 1±44 ميكرون و 0.7±55.1 ميكرون على التوالي. داخل منطقة النخاع انتظمت الأنابيب السميكة والنحيفة لعروة هنلي في المنطقة الطرفية من مخروط النخاعية في حين كانت القنوات الجامعة في الجزء الأوسط من مخروط وجميعها تبطن بظهارة مكعبة بسيطة عدا الجزء النحيف كانت تبطن بالخلايا الحرشفية. تكونت الغدة الملحية من فصيصات وتحيط بها محفظة سميكة من النسيج الضام ويتكون كل فصيص من كتلة من وحدات إفرازية أنبوبية متفرعة عنيبية والتي تتكون بدورها من صف واحدة من الخلايا مكعبة. الأنابيب تؤدي إلى قناة مركزية والتي تبطن بطبقة مزدوجة من الخلايا المكعبة في حين تبطن القناة الرئيسية بظهارة مكعبة مطبقية. اظهرت الوحدات إفرازية للغدة تفاعلاً سلبياً لصبغات كاشف الشفت الايودي واليشيان الازرق وهذا يشير إلى غياب الواد المخاطية المتعادلة التفاعل. استنتج من الدراسة ان الكليتين في طيور النحام الصغير صغيرة الحجم والوزن بالمقارنة مع حجم الطير الكبير ويمتلك غدة ملحية نشطة.

الكلمات المفتاحية: كلية طير النحام، الغدد الملحية، النحام الكبير.