

## **Histological changes of kidney and Salt glands in response to over load salty water in Mallard Ducks (*Anas platyrhynchos*)**

Reshag, A.F. Dhyaa, Ab. Abood Maysoon, A. Ahmed

Department of Anatomy, Histology and Embryology /College of Veterinary  
Medicine, University of Baghdad, Baghdad, Iraq.

[E.mail: sabahali503@yahoo.com](mailto:sabahali503@yahoo.com).

### **Abstract**

Present study were carried out on Fifteen adults of the fresh water Mallard ducks, divided into three groups for determinate the histological adaptation of kidneys in absence of salt glands. The results were showed that, the kidneys of birds in group 3 were increased in the size ( $52 \pm 0.8$ cm) in compared to those of control group ( $40 \pm 0.3$ cm). Histologically, showed marked congestion in renal tubules, glomerular congestion, and thickening of bowman capsule. The lining cells of proximal and distal convoluted tubules showed enlarged acidophilic cytoplasm and their nuclei were heterochromic. The collecting tubules were lined with low cuboidal cells, most of them showed degeneration, and increased in their acidic mucus secretion. In group 2, the size of kidneys was ( $42 \pm 0.2$ cm) and in control group was ( $40 \pm 0.1$ cm), the histological results in this group appeared as that of control group. The salt glands of birds in group 2 showed enlargement of these glands, each gland consisted of an array of secretory lobules surrounded by vascular capsule. Each lobe was made up of branched secretory tubules radiate from central canal. The secretory tubule lined with simple cuboidal epithelium. The secretory tubules and central canals drain in a main duct. These histological structures showed mild congestion in blood capillaries. The secretory units at the periphery of glandular lobules appeared hypertrophied. Hematological results showed significant increase in levels of the blood urea in birds of group three ( $24.7 \pm 0.5$  mg/dl), while the results of birds in groups two and control were  $17.9 \pm 0.4$  mg/dl and  $16.12 \pm 0.2$  mg/dl respectively. The present study concluded that, the salt glands in marine bird will be rest and non-functional in bird that watered on fresh water and be activated in salt water intake and the kidney can't play a role of salt gland instead of its absence.

**Key:** Salt gland, histological, *Anas platyrhynchos*

## التغيرات النسيجية في الكليتين والغدد الملحية كاستجابة لفرط ملوحة الماء في البط المحلي

*(Anas platyrhynchos)*

علي فارس رشك ضياء عبد الحسين عبود ميسون عبد القادر احمد

فرع التشريح والانسجة والاجنة / كلية الطب البيطري – جامعة بغداد، بغداد- العراق

## الخلاصة

اجريت الدراسة الحالية على خمسة عشر من البط البالغ، قسمت الطيور الى ثلاثة مجموعات وذلك لدراسة التكيف النسيجي للكلى تحت تأثير شرب الماء المالح في غياب الغدة الملحية. اظهرت النتائج زيادة حجم الكلى  $0.3 \pm 40$  الكلى للطيور في المجموعة الثالثة ( $0.8 \pm 52$  سننيمتر) بالمقارنة إلى طيور مجموعة السيطرة (سننيمتر)، حيث اظهرت احتقان في النبيبات الكلوية مع تآكل محفظة الكبيبات الكلوية. كما اظهرت النبيبات المتوترة الدانية والقاصية تضخم واضحا في خلاياها الظهارية وبدى هبولىها اكثر حامضي. وبدت النبيبات مبطنة بالخلايا المكعبة الواطنة كما وظهرت عليها علامات التآكل الخلوي مع زيادة في إفرازاتها من الجامعة المخاط الحامضي. في طيور المجموعة الثانية كان حجم الكلى  $0.2 \pm 42$  سننيمتر وفي طيور مجموعة السيطرة كان حجم الكلى  $0.1 \pm 40$  سننيمتر. ، نسيجا، اظهرت النتائج أن الكلى بدت كذلك التي في مجموعة السيطرة وان أن كل غدة تتكون من الغدد الملحية في طيور المجموعة الثانية اظهرت كبر حجم هذه الغدد وبيئت النتائج فصوص محاطة بمحفظة ليفية وعائية و كل فص منها مكون من مجموعة فصيصات. كل فصيص مكون من مقترعة تشع من قناة مركزية. الوحدات الإفرازية مبطنة بالظهارة المكعبة نبيبات تشتمل على وحدات افرازية الإفراز الى قنوات مركزية. بين نبيبات الوحدات الإفرازية أوعية دم شعيرية. البسيطة وتصرف النبيبات إفرازاته وظهرت نتائج فحص الدم. الغدية فرط الضخامة الوظيفي الفصيصات اظهرت الوحدات الإفرازية في محيط ملغم / ديسي لتر ( بينما كانت نتائج 0.5 في طيور المجموعة الثالثة ( $24.7 \pm$  زيادة في مستويات يوريا الدم ملغم / ديسي 0.2  $\pm 16.12$  ملغم/ديسي لتر و الطيور في مجموعات الثانية ومجموعة السيطرة 0.4  $\pm 17.9$  لتر) على التوالي. الدراسة الحالية إستنتجت بأن غدد الملحية ستكون في حالة إستراحة وغير وظيفية في الطير دور الذي سقى على الماء العذب ويكون نشطة في حالة شرب الماء المالح وان الكلية لا تستطيع ان تتكيف وتلعب مهم في التخلص من الاملاح في حالة غياب الغدة الملحية

## Introduction

Three processes to osmoregulation in marine birds are filtration of sodium and water from the plasma by the kidneys, reabsorption of filtered water and sodium by cells along the renal tubules, and secretion of sodium by the salt glands. These processes are important as well as the osmoregulatory requirements of birds those habitats of widely disparate salinities,(1). Marine birds possess salt-secreting nasal glands which produce hypertonic solutions of sodium chloride in response to osmotic loads in case of sea water intake. The presence of this gland considered a necessary adaptive tool in birds which live in an environment high salt concentrations,(2)(3). Salt glands are

together with the kidneys, maintain body fluid homeostasis, despite the excess sodium chloride they ingest, (4). The kidneys of birds anatomically divided into three divisions and each histologically contain several lobes and each lobe is divided into lobules which consisted of two zones, the cortex which made up the vast area of the kidney and a small portion being medulla. The cortex and the medulla were arranged in cones of different lengths. It would appear that the avian medullary cones are the majority of nephrons were without a loop of Henle. The proximal tubule formed a thin descending limb of Henle and descended into the medulla. Within the cortex, most nephron tubules were distributed randomly. The glomeruli

occurred most commonly in the peripheral cortex and the majority of distal tubules which were clustered around the intralobular vein (5) (6) (7). The salt glands consist of bilateral glandular lobes, each composed of secretory lobules surrounded by connective tissue capsule. Each lobe is made of a mass of branched secretory tubules arranged into radiating pattern from central canal and enmeshed in one cell layer with blood capillaries. The secretory tubule consists of simple columnar-cuboidal epithelium surrounding a narrow lumen showing round basal cells at their terminal segments. The secretory tubules and central canals drain in a main duct leads to the anterior of the nasal cavity (8) (9).

**This study was aimed to compare the normal histological structure of duck kidney and salt gland to those histological changes in response to over load salt in case the presence and absences of salt glands.**

### Materials and Methods

Fifteen adults of Mallard ducks, involved both sexes were collected from the local markets of Baghdad provinces. The birds were grouped into three main groups (1, 2, and 3). The birds of group 3 were subjected to total surgical removing of both salt glands (locally anesthetized the area of supra orbital region by S/C injection of 0.04ml Lidocaine), then the birds watered on salt water contained NaCl 10 % (1.7mmol/Liter ) for seven days (The water was given in plastic pools and replenished fourth daily). The birds of group 2 were watered on salt water contained the same concentration of NaCl in group 3, but without removing of their salt glands. The

birds of group 1 were watered on fresh water and used as control group. Blood samples were collected from all birds for estimation of blood urea. All birds were killed by decapitation. Both left and right kidneys were quickly removed from each bird of group 3. Both Salt glands and kidneys were quickly removed from each bird of groups 2 and 3 (10). Both salt glands sizes were measured. The kidneys of all groups their overall volumes were measured separately and then immersed in 10% neutral buffered formalin, through a series of graded alcohols, xylene and eventually embedded into paraffin wax. The tissue sections were taken at 4-7  $\mu$ m thickness and stained with Hematoxylin and eosin stain, Hematoxylin & Eosine-phloxine stain and Alcian blue, (11). The data were statistically analyzed with Student t-test using Sigma Stat (version 2.0) statistical software to determine significant differences in size of kidneys and the levels of blood urea among groups (Significance was assumed at  $P < 0.05$  and all results are shown as the Mean  $\pm$  SD).

### Results

The results of present study showed that, the kidneys of birds in group 3 showed increased in the size of kidneys ( $52 \pm 0.8$  cm) in compared to those of control group ( $40 \pm 0.3$  cm). The result showed marked cortical and medullary congestion in renal tubules, Fig. (1). The renal glomeruli showed congestion of capillary tufts and condensation of central glomerulus mesangial cells which had large rounded heterochromic nuclei, and thickening of Bowman capsule. The proximal convoluted (PCT) lining cells

had swelling with intensively acidophilic cytoplasm and their nuclei appeared large and lightly stained (heterochromic) and these tubules showed close lumen. The distal convoluted tubules (DCT) were distended and had acidophilic darkly stained cytoplasm with dilated lumen, Fig. (2). The collecting tubules were lined with low cuboidal cells most of them showed signs of nephritis, and increased in the secretion of acidic mucus, Fig. (3), (4). The results of birds in group 2 showed that, the size of kidneys were ( $42 \pm 0.2$  cm) and in control group the size of kidneys was ( $40 \pm 0.1$  cm). The results showed that, the kidneys appeared as those of control group which consisted of cortex and medulla, the cortex made up the majority of the kidney and composed of large and small renal corpuscles, each renal corpuscle consisted of Bowman's capsule and glomerulus. The proximal convoluted tubules were lined by darkly stained simple cuboidal epithelium with well demarcated brush border and narrow lumen. The distal convoluted tubules and collecting tubules were lined by simple cuboidal epithelium and wide lumen, Fig.(5). Medulla of kidney was composed of thin and thick segment of henles loop and collecting ducts and

these structures were lined by simple cuboidal epithelium, The collecting ducts continued to form the papillary ducts which lined by simple columnar epithelium. But, with less congestion, Fig.(6). The results of salt gland in this group (1) showed that, the paired crescent-shaped salt glands were enlarged, Fig. (7), the bilateral salt glands were consisted of an array of secretory lobules surrounded by richly vascularized connective tissue. Each lobe was made of a mass of branched secretory tubules radiate from central canal and enclosed in one cell layer with blood capillaries. The secretory tubule consists of simple cuboidal epithelium surrounding a narrow lumen showing round basal cells at their terminal segments. The secretory tubules and central canals drain in a main duct leads. These histological structure presented mild congested blood capillaries. The secretory units at the periphery of glandular lobules appeared hypertrophied, Fig. (8),(9). The hematological results were showed increased in levels of the blood urea in birds of group three ( $24.7 \pm 0.5$  mg/dl), while the results of birds in groups two and control were  $17.9 \pm 0.4$  mg/dl and  $16.12 \pm 0.2$  mg/dl respectively.

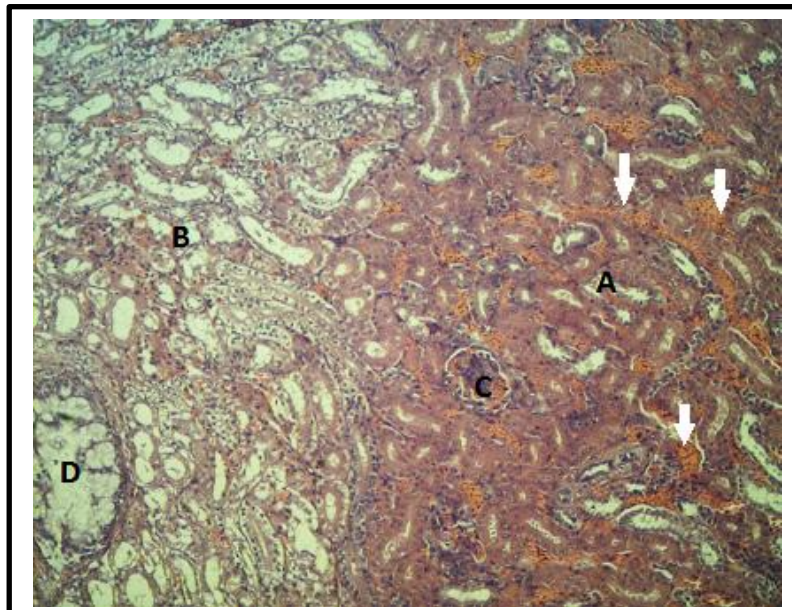


**Figure (1)**

**Histological section of kidney shows:**

- A. Darker cortical region.**
- B. Pale staining medullary core.**
- C. Glomerulus.**
- D. Branch of ureter.**

(White arrows show cortical congestion)  
Hematoxylin &Eosine-phloxine stain 100x.

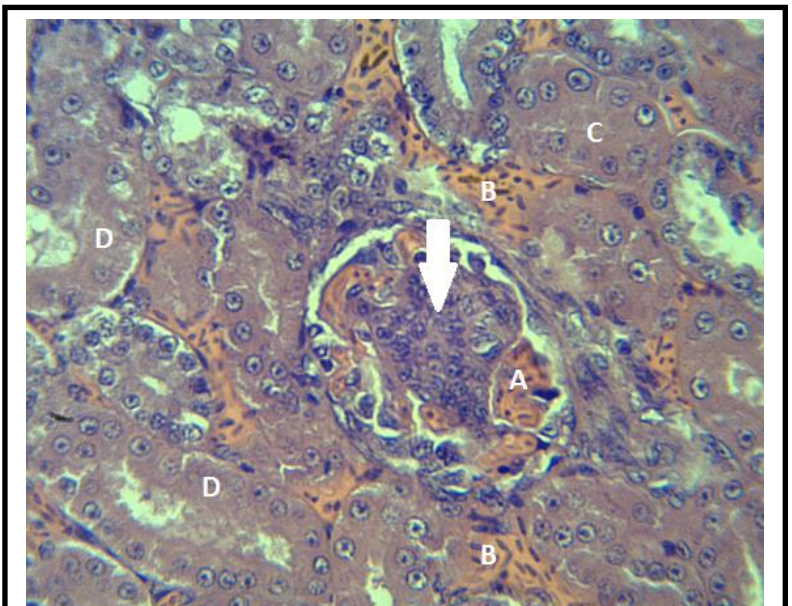
**Figure (2)**

**Histological section of kidney (cortex) shows:**

- A. Congested capillaries tuft.**
- B. Congested capillaries.**
- C. Proximal-convoluted tubules (PCT).**
- D. Distal convoluted tubules.**

(white arrow shows central condense mesangial cells).

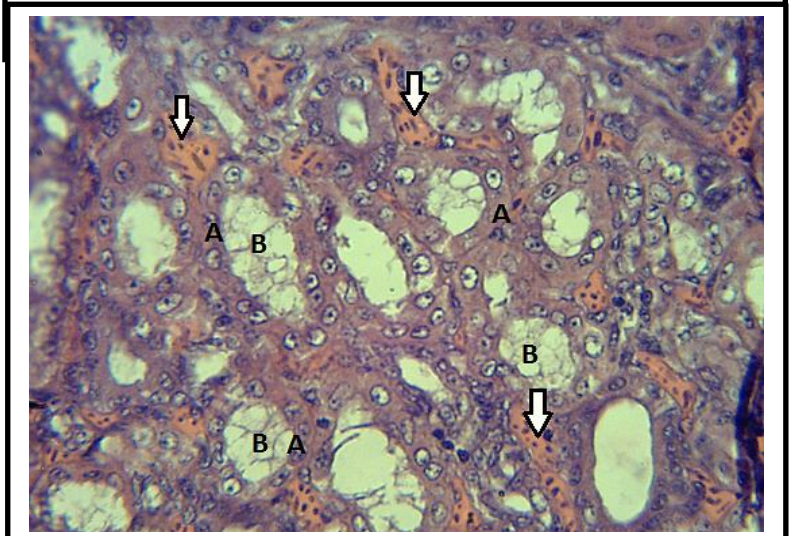
Hematoxylin&Eosine-phloxine stain 400 x.

**Figure (3)**

**Histological section of kidney (medullary core) shows:**

- A. Mucus secreting collecting tubules.**
- B. Mucus secretion within lumen of collecting tubules.**

(White arrows show congestion).  
Hematoxylin &Eosine-phloxine stain. 400 x.



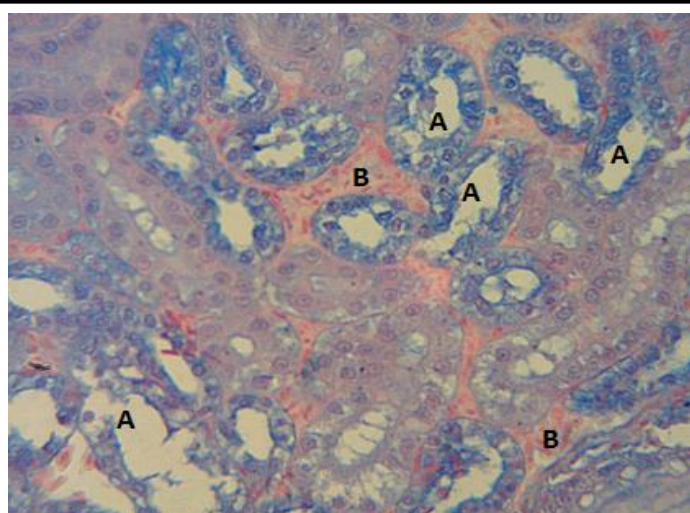


**Figure (4)**

**Histological section of kidney (medullary core) shows:**

- A. Collecting tubules.**
- B. Interstitial congested capillaries.**

**Alcian blue stain. 400 x.**

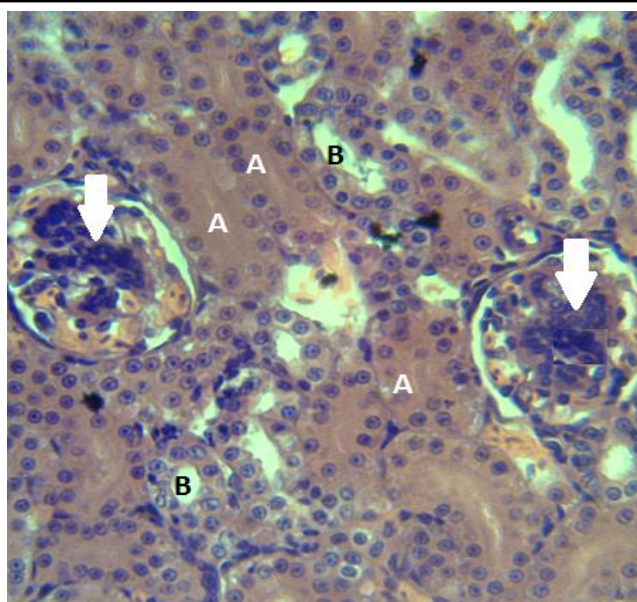
**Figure (5)**

**Histological section of kidney (cortex) shows:**

- A. Proximal convoluted tubules.**
- B. Distal convoluted tubules.**

**(White arrows show central glomerulus mesangial cells)**

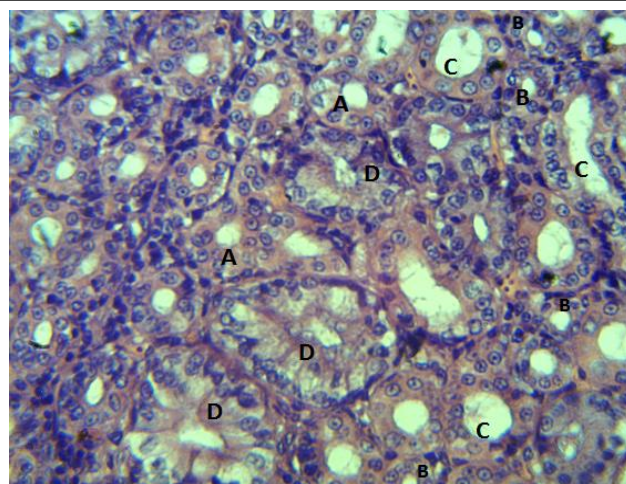
**Hematoxylin & Eosine-phloxine stain. 400 x.**

**Figure (6)**

**Histological section of kidney shows:**

- A. Thick segment of loop of henle.**
- B. Thin segment of loop of henle**
- C. Collecting tubules.**
- D. Papillary ducts.**

**H&E stain. 400 x.**



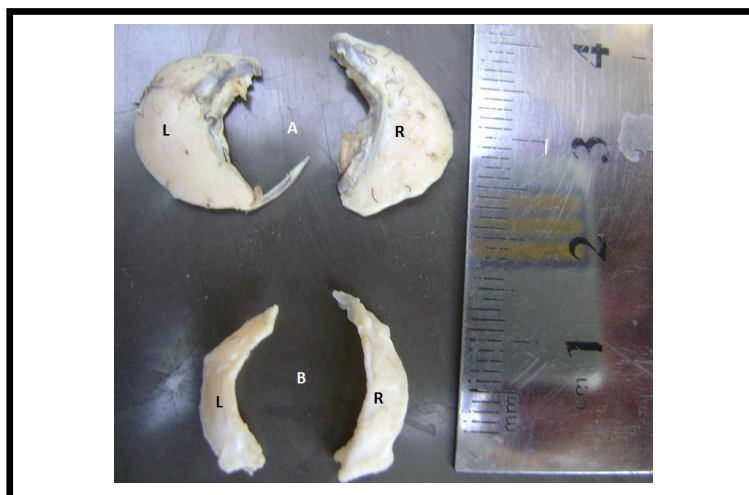
**Figure (7)**

**Anatomical samples of salt gland shows:**

- A. Salt gland of birds in group (2).**
- B. Salt gland of birds in control group.**

**L: show left gland.**

**R: show right gland.**

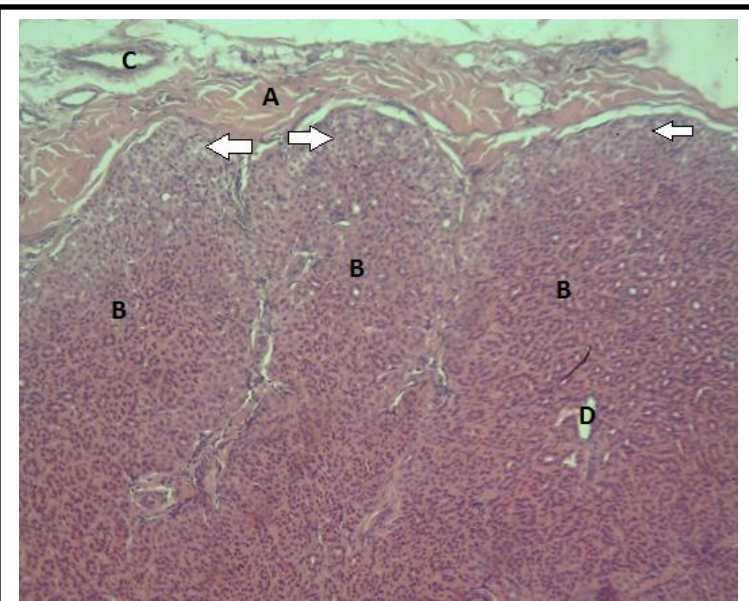
**Figure (8)**

**Histological section of salt gland shows:**

- A. Collagenous capsule.**
- B. Glandular lobules.**
- C. Artery.**
- D. Central main duct.**

**(White arrows show zone of hypertrophied secretory units).**

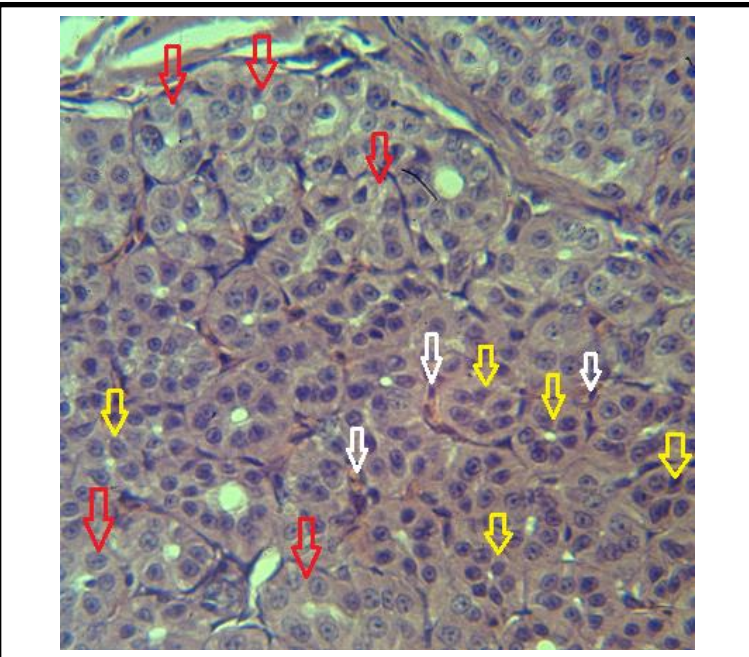
**H&E stain. 200 x.**

**Figure (9)**

**Histological section of salt gland shows:**

- Red arrows: hypertrophied secretory units which lined with simple cuboidal cells of large rounded nuclei.**
- White arrows: capillaries**
- Yellow arrows: normal sized secretory units.**

**H&E stain 400x**





## Discussion

Few of studies have been done to link the morphology of avian kidneys with the ability to concentrate urine (4) (12) when Casotti reported that there were differences in renal anatomy in three species of sparrows that subjected to higher salinity of water. In the present study, the observations were focused on the histological changes and modifications of kidney in case of absent salt gland, the present result showed that the increased in size of kidney in birds of group three that have been removed their salt glands which showed an ability to still a life in case of non-functional salt glands or at removing this glands even they watered on salt water, this result was in compatible with (13) who refer that, the Mallared duck (*Anas platyrhynchos*) had low rate of renal filtration and had low saline tolerance. The present study revealed that, the salt water or acute saline have a little effect on glomerular filtration in presence of salt glands in Mallared duck while the absence of this gland led to significant increase of kidney size, this opinion supported by (4) (14), this result suggests that, the eliminate of water and waste by kidney in abscent of salt glands lead to increased glomerular filtration is consistent with enlarged kidneys. The histological changes in proximal convoluted tubules in birds of group three revealed that, this portion of nephrons responsible for elimination of majority of toxic urate waste, (15). The present result showed increased in secretion of

acidic mucopolysaccharid by collecting tubules which revealed that, these tubules play an important role in elimination of harmful uric acid from kidneys (16). The significantly increased of blood urea in birds in group three suggested that, the removing of these salt glands lead to an increasing the functional load on the kidneys to expelled an excess amount of NaCl at the same time its main responsibility of kidneys birds was to excrete these nitrogenous wastes as uric acid into cloaca birds, this agree with (17). The features of normal histological structure which revealed not marked changes in avian kidney of birds groups two as responses to salt water intake was compatible with (5) (18) . The present result showed hypertrophy of salt glands in case of salt acclimated Mallared, this result suggest that, the Mallared salt glands had excreted NaCl but incorporate with renal excretion, this supported by (19). The salt glands do not function continually as the kidney does, and becoming active only in response to an osmotic load and this result compatible with (20) how show that the salt gland of marine birds is one of the most efficient systems of ionic balance. The present results concluded that, the kidneys of marine birds have a low ability to excreted high concentrations of NaCl and this salt is expelled by the paired nasal glands or supraorbital glands.



## References

1. **Hughes, M. R. (1995).** Responses of gull kidneys and salt glands to NaCl loading. *Can.J. Physiol. Pharmacol.*, 73:1727-1732.
2. **Knut, S.-N. (1960)**The Salt-Secreting Gland of Marine Birds. *American Heart Association*. 21:955-967.
3. **Braun, E. J. (1999).** Integration of organ systems in avian osmoregulation. *J. exp. Zool.*:702-707.
4. **Hughes, M. R. (1980).**Glomerular filtration rate in saline acclimated ducks, gulls and geese. *Comp. Biochem. Physiol.* 65(A): 211-213.
5. **Nabipour, A.; Alishahi, E. and Asadianm, M. (2009).** Some Histological and Physiological Features of Avian Kidney. *J. Appl. Anim. Res.* 36: 195-198.
6. **Lierz, M. (2003).** Avian renal disease: pathogenesis, diagnosis and therapy. *Vet. Clin. Exot. Anim.*, 6:29-55.
7. **Lafi, A. B. (2012).**morphological and histological study for the kidney of Coot Bird (*Fulica atra*).*Bas.J.Vet.Res.*11.(1):128-136.
8. **Mansour I. Al Mansour. (2007).** Anatomy, Histology and Histochemistry of the Salt Glands of the Greater Flamingo *Phoenicopterus ruber roseus* (Aves, Phoenicopteridae). *Saudi J. Biol. Sci.*, 14 (2) 145-152.
9. **Maysoon, A. A. (2011).** A comparative morphometric study of salt gland in some domesticated and wild marine birds. *Bas.J.Vet.Res.*10:128-136.
10. **Wauri, C.N. (1989).** Light microscopic morphometry of the kidneys of fourteen avian species. *J Anat* 162:19–31.
11. **Luna,(1968).**"Manual of Histological Staining Methods of The Armed Forced Institute of pathology". 3rd Ed. McGraw Hill book Co. New York. Pp: 71, 98.
12. **Casotti, G.; Beauchat, C.A. and Braun, E. (1998).** Morphology of the kidney in anectarivorous bird, the Anna's hummingbird *Calypteanna*. *J. Zool. Lond.* 244:175–184.
13. **Darin, C.B. and Maryanne, R.H. (2003).**Comparison of renal and salt gland function in three species of wild ducks.*J.Exp.Biol.*206:3273-3284.
14. **Kalisinsska, F.; Daticzak,A. ;Pierko, M. and Wyscoki, D. (1999).**relationship between kidney mass and body size in some Anseriformes. *Anat. Histol. Embryol.* 28:55-59
15. **Amy, M. B.; James, G.; and Larry, J. R.(2008).** Avian renal proximal tubules epithelium urate secretion is mediated by Mrp4.*Am. J. Regul. Integr. Comp. Physiol.*, 295: R 2024-2033.
16. **Casotti, G. (2001).** Effects of season on kidney morphology in house sparrows. *J. Exp. Biol.*, 204: 1201-1206.

- 17. Goldsteinm, D.L. and Braun, E.J. (1989)** Structure and concentrating ability in the avian kidney. *Am. J. Physiol.* 25:501–509.
- 18. Nishimura, H. (2008).**Urine concentration and avian aquaporin water channels. *Pflugers Arch.*, 456:755-768.
- 19. Bennet, D.C. (2002).** Effect of saline intake on osmotic homeostasis in ducks. Ph.D. thesis, University of British Columbia, Vancouver, Canada. PP.114.
- 20. Jouanin, C. (2000).** Extra renal salt excretion in oceanic birds. *Ann. Pharm. Fr.* 58(5):346-350.