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Radiographic Findings of Excretory Urography of Avian Kidneys After Nephropathy-Induced in Broiler Chicken as A Model

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Abstract

Background: Although kidneys diseases have a high prevalence, initial recognition and diagnosis of renal disease are difficult and sometimes complicated due to the lack of pathognomonic symptoms and sometimes concurrency with other disease.

Objective: The aim of this study is assessing the Intraosseous excretory urography (IVOU) in order to introduce a new method for evaluation of kidney's function in broilers as a primary model of birds.

Methods: A total of 10 male broiler chickens at the age of 20 days were included and evaluated with plain and serial post-contrast radiography to check the time and concentration of the contrast media (CM) passage from the kidney. In the next step, after the nephropathy induction with gentamicin, plain and serial post-contrast radiography were repeated. After the necropsy, kidney samples were sent for histopathology evaluation, and statistical analysis including the Statistical Package for the *Social Sciences*, *Shapiro-Wilk* test, the *Paired T-Test*, the *Wilcoxon Signed Ranks* test and the *Chi Square test* were used. (p-value ≤ 0.05)

Results: After nephropathy induction, all of the chickens exhibited increase in kidneys size, and in histopathological evaluation, all the samples (30 of 30) showed severe tubular necrosis, and mild glomerulopathy. In the IVEU, the average time to create the maximum concentration of CM, the average time to decrease the CM concentration from the kidney, and the time of the complete clearance of CM indicated a significant incensement after nephropathy compared to the time before it.

Conclusion: Intraosseous excretory urography (IVOU) is a safe contrast imaging modality and can be useful in the assessment of the urinary system in chickens to check the changes in kidney size and function.

Keywords:
avian kidneys, broiler chicken, excretory urography, nephropathy, radiography

Introduction

Kidney diseases in birds are often secondary to other diseases and management disorders or can be associated with multisystemic conditions. (Pollock, 2006) The diseases are classified into two main groups: primary diseases with a low prevalence and secondary diseases with a high prevalence. (Altuzarra et al., 2018; Goellner & Morgan, 2018) Despite the high prevalence of kidney diseases in birds, a combination of kidney diseases with symptoms of other conditions and lack of pathognomonic signs makes it difficult to diagnose. Furthermore, birds have no specific biomarkers for kidney disease. Checking the blood uric acid level and creatinine is an insensitive marker in kidney diseases in birds; therefore, it cannot be considered a reliable diagnostic factor in kidney diseases. (Fajri & Ulum, 2019; Harrison et al., 2006; Holz, 2020; M.-E. Krautwald-Junghanns et al., 1998; McMillan, 1988; Reshag et al., 2016) Although initial recognition and diagnosis of renal disease is complicated in avian medicine, An operational method for the evaluation of kidney function could provide a good chance of treatment for patients (Pollock, 2006) Hence, in many cases, more than one diagnostic method is required to ensure renal involvement and differential diagnosis. (Yamama et al., 2023; Dennis & Bennett, 2000b)

A kidney tissue biopsy provides the most information about kidney conditions among laboratory diagnostic methods and imaging; however, its use in the clinic is limited owing to its invasiveness and complications like bleeding after the biopsy. (McMillan, 1988; Seok et al., 2016) Diagnosis of a renal disorder requires multiple laboratory tests, complete clinical assessments, and diagnostic imaging. (Dennis & Bennett, 2000b) Clinical indications for using diagnostic imaging in kidney evaluation in birds include polydipsia, a long period of lack of access to water, poisoning, severe nutritional problems, polyuria, urine colour change, vomiting, and dehydration.(Echols, 2006; Krautwald-Junghanns et al., 2008; Pollock, 2019; Veladiano et al., 2016; Emadi et al., 2022)

The paired kidneys of birds have a specific anatomic location, located in the retroperitoneal space, one on each side of the vertebral canal, in a fossa ventral to the synsacrum. Each kidney has three lobes: cranial, middle, and caudal.(Deepa et al., 2020; Krautwald-Junghanns et al., 2008) Diagnostic imaging is one of the critical tools for evaluating the diseases of the urinary system in birds, playing a crucial role in avian medicine and exotic animals. It includes various methods such as plain radiograph, contrast media (CM) radiography, nuclear scintigraphy, magnetic resonance imaging (MRI), and Computed tomography (CT-scan). It is often clinically limited to simple methods such as plain radiography and ultrasound, so that other modalities are rarely used. (Krautwald-Junghanns & Konicek, 2020; Krautwald et al., 1992; Pollock, 2006) Since kidneys are located in the synsacral fossa, a plain radiographic assessment of the avian kidney is challenging. For immature animals, in order to remove the effect of growth on kidney sizes, assessing of changes in kidney sizes can be calculated based on the ratio of kidneys length to the length of one the long bones as femur. In the ventrodorsal (VD) view, they are obscured by other parenchymal organs and are visible in the lateral view. In the lateral projection, an enlarged cranial renal division is the most reliable radiographic indicator of renomegaly. The kidney becomes visible in the VD view if its cranial renal division is enlarged. (Konicek & Vetb, 2019; Krautwald et al., 1992; Simoes et al., 2012) Owing to the air sacs of the respiratory system, imaging the kidneys and ureters in healthy birds is typically impossible unless an intracloacal probe is used. In the case of ascites

and organomegaly, ultrasound can be used to evaluate the kidney parenchyma in birds due to air sacs compression. (Kokosinska, 2024; Konicek & Vetb, 2019; Samaniego et al., 2015) Although the CT scan is typically used to detect musculoskeletal or respiratory problems, it can also be utilized to make or confirm a diagnosis of renal disease, But its low accessibility and high cost make it limited to use as a routine clinical examination.(Konicek & Vetb, 2019; Krautwald-Junghanns et al., 2008)
Contrast media (CM) containing iodine helps to diagnose some diseases in mammals and humans; however, it is infrequently used in avian medicine. The kidney and the ureter size, shape, position and function can be evaluated using excretory urography (IVEU). This technique is widely used in both human and veterinary medicine. (FAHMY ABOU ELAZAB & EL-HABASHI, 2015; Pollock, 2006; Schwartz, 2016) The impaired renal function may change the time of the presence of CM in the kidney or ureter. This technique also can assess the ureter function (for example, after ureteroliths are surgically removed). Nevertheless, the absence of knowledge or experience in using this method in the kidney evaluation of birds results in its limited use by clinicians. Iodinated contrast media are said to cause various side effects in men but these effects are rarely reported in avian patients; nephrotoxicity with impairment in renal function is one of the side effects in ionic CM. Although "modern" nonionic, iodine-based contrast agents have fewer side effects, are permitted to be used.(Dennis & Bennett, 2000a; M.-E. Krautwald-Junghanns et al., 1998; M. Krautwald-Junghanns et al., 1998; Schwartz, 2016)
Therefore, considering the high prevalence of kidney diseases, the difficult differential diagnosis with conventional methods, and the critical role of diagnosis time in preventing systemic disorders, it is necessary to employ other diagnostic methods for evaluating kidney function in order to accelerate the diagnosis and treatment. Accordingly, the present study aimed to use diagnostic imaging with the help of contrast material and to introduce new methods that could be used in the practice in order to evaluate kidney function in broilers as a primary model in the evaluation of all birds. This study helped to assess the use of intravascular excretory urography (IVEU) in nephropathy-induced birds in comparison to healthy birds to determine the times of beginning, maximum and end of the nephrographic phase via radiographic examination.

Materials and Methods

Animals:
In this study, 10 male broiler chickens were procured from a commercial poultry farm and then were kept in wire cages under standard management conditions. The chicken were 17 days old, had similar body weights, and were free from any clinical signs.

Preparation:
Ten 17-day-old, male broiler chickens were included in this study. The chickens were acclimated in the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Tehran for three days to adaptation, under 12-hour dark/12-hour light cycles, humidity (55 – 65 percent), and at constant temperature (25± 2°C). The chickens were provided with a commercial diet and sufficient water. This standard condition was implemented during the experiment.

Contrast media administration:

After sedation (*ketamine* 2 mg/kg IM and *Medetomidine* 0.2 mg/kg IM) (Guzman & Beaufrère, 2021), a non-ionic iodine contrast agent (*Iohexol*) was used at a dose of 2 ml/kg in a solution with a concentration of 70- 80% of the contrast agent (300-400 mg iodine/ml). In preparation for urography, the birds were fasted and hydrated, and the contrast medium containing iodine was warmed to body temperature and intraosseous injection was done very slowly. In order to injection, the intraosseous injection in the proximal cancellous bone of tibia was performed in this examination.

Radiography examination before nephropathy induction:

After sedation, the initial plain digital radiographic images of the celomic cavity were obtained in lateral and ventro-dorsal positions using portable x-ray tube unit (model SY-HF-110) and CR digital radiography (*DirectView Kodak* CR system) and exposure factors of 2.6 MAs and 50 kv. Subsequently, after tibial-intraosseous contrast media (CM) injection, radiographs were immediately taken after the administration of the bolus, and at 1, 2, 5, 10, 20 minutes and more until the complete passage of CM from the urinary tract, using the same exposure factors and the same positioning as the survey study.

Nephropathy induction:

To induce the nephropathy, Gentamicin (gentamicin sulfate, 20% *R Caspian-tamin, Iran*) with a dosage of 5 mg/kg intramuscularly every 12 hours for 5 days was used from the age of 22 days. (Fahmy and El-habashi, 2015; Khan et al., 2008; Khorrami et al., 2022)

Radiography examination after nephropathy induction:

After nephropathy induction, the process of plain and post-contrast radiography was repeated for each bird according to the previous descriptions.

Image interpretation:

Plain and post-contrast studies were performed and interpreted for renal size and opacification of the kidneys. Length of kidneys was measured in lateral radiographs by a line from the cranial border of cranial lobe to the caudal border of caudal lobe and parallel to the vertebral column. The specific findings of IOEU included the beginning time of the nephrogram, the maximum CM concentration in kidney parenchyma, the time of decreased CM concentration, and the complete clearance CM of the kidney. Finally, the difference in the size and the CM passage between the two groups were analyzed.

Finally, after euthanizing with the increase dose of anesthesia, kidneys samples from each lobe (cranial, middle and caudal) were collected from the birds and histopathological alternations of the kidney were evaluated using hematoxylin and eosin (H&E) staining in the pathology laboratory.

Analytical statistics:

Statistical analysis was conducted using the Statistical Package for the *Social Sciences*, Version 25 (*IBM Corporation*, *Armonk, NY*). Mean (±SD) was used to describe the samples' characteristics in each group. *Shapiro-Wilk* test was used to check normal distribution, and the *Paired T-Test* and the *Wilcoxon Signed Ranks* test were used to compare the groups before and after the nephropathy induction. Statistical significance was defined as a p-value less than or equal to 0.05.

Since the chickens were in growing age, comparison of kidneys sizes was calculated based on the ratio of kidneys length to the length of femoral bone by using the *Chi Square test*, and the length of femoral bone was measured, by a parallel line to vertebral column, from greater trochanter to distal femoral physis in lateral views.

Results

Pathology assessment of the renal samples, which was conducted blindly, confirmed the pathological changes in the kidney tissue after the nephropathy induction with Gentamicin. According to the report from the 60 samples were prepared from all three kidney lobes, all the samples (30 of 30) exhibited severe tubular necrosis, 2 samples indicated severe inflammation, 27 samples had mild inflammation, and one sample lacked inflammation, and except for one case, all the samples exhibited mild glomerulopathy. (figure 1)

The length of the kidney in radiography:

The comparison of proportion of kidneys length to the femoral bones was not significant, (P>0.05), but significant difference in kidneys length between normal and nephropathy groups was due to nephropathy induction. (P: 0.243 in radiographic examination)

Digital radiography excretory urography

In the post-contrast radiographic examination of the kidneys, the time of the CM entrance into the kidney parenchyma in both groups was 1 minute after the injection. In this respect, no considerable distinction was found between the two groups. In healthy chickens, the average time to create the maximum concentration of CM was 2.30 ± 0.48 min, and the average time to decrease the CM concentration from the kidney was 4.0±80. 42min. The time of the complete clearance of CM was 8.1±8.03 min after the injection, which increased to 4.0±50.84, 10.80±4.04, and 27. 5±7.54 min after the nephropathy induction, respectively (Table 1), indicating significant incensement compared to the time before the injection of Gentamicin (P=0. 01a, P=0. 005a, P<0. 001b, respectively). (figure 2)

Discussion

In this study, the CM passage from the chicken's kidneys parenchyma was evaluated, with radiography, before and after the nephropathy induction to determine the difference in size of kidneys and states of nephrogram between the two groups. Intravenous excretory urography (IVEU) or Intraosseous excretory urography (IOEU) appears to be a secure contrast imaging modality, and can be useful in the assessment of the urinary system in chickens. This technique permits the examination the morphological features of the kidney and ureters as well as the assessment and determination of the time at which urographic phases occur to check the renal function.

IVEU has been a practical imaging method used in the assessment of human and other mammals due to qualification of the kidneys function. The findings in this examination were evaluated based on the number, size, shape, location, and density of the kidneys and urinary tracts, as well as, according to the degree and time of changes in the CM density in nephrogram and pyelogram phases; In humans, in normal kidneys, the nephrogram phase begins 15 seconds after injection and lasts for about 2 minutes.(Goellner & Morgan, 2018; Habing & Byron, 2015). In a study on the kidneys of healthy New Zealand rabbits (Oryctolagus cuniculus) through digital fluoroscopic excretory urography, the average start time of the nephrogram and pyelogram phases were 10 seconds and 1.39 minutes, respectively, and the average time when the contrast material was visible in the bladder was 1.58 minutes. (Altuzarra et al., 2018) In IVEU of cats and dogs' renal arteries become opacified approximately 5-7 second after injection and the nephrogram begins after approximately 10 seconds and lasts up to 2 minutes until the pyelogram phase starts and the nephrogram begins to fade. (Thrall, 2017) despite the anatomical diversity of the urinary system between the mammals and the birds. The findings are compatible with our study observations in birds.

In this examination, features of the urogram phases were reviewed by using digital radiography in control group. In congruency with past studies, the vascular phase was immediately detectable after the beginning of the intravenous or intraosseous contrast administration. Beginning of the nephrogram phase was visible at 1 min after contrast administration. The maximum concentration of CM, beginning of decrease CM opacity of kidney's parenchyma, and complete clearance were identified at about the time of 2.5, 5 and 8 min after CM administration, respectively. This findings are compatible with previous examinations that used iodine compounds to check kidney function in normal birds, that CM was observed in the heart and pulmonary vessels 10seconds after injection, in the kidney -20 50 seconds later, and after 5-2minutes it was entered the cloaca.(Dennis & Bennett, 2000a; Krautwald-Junghanns et al., 2008; Pollock, 2006)

A complex interaction of renal perfusion, normal glomerular filtration, tubular function and absence of urinary tract obstruction is required for normal clearance of CM from the kidney. Alterations to these factors may result in both quantitative and qualitative IVEU image abnormalities. Numerous abnormal patterns of nephrographic opacification and fade-out have been defined in human and veterinary publications and correlated with various pathologies. (Simoes et al., 2012) Furthermore, acute renal diseases, conditions affecting the general renal function, and obstructive disease all create changes in passage time and concentration of CM from the kidney (Krautwald-Junghanns et al., 2008; Seok et al., 2016), being consistent with our findings from IOEU with radiography in chickens after the nephropathy indication in which the beginning of the excretory phases was detected at the same time (at the first minute after injection); but detecting of maximum CM concentration, beginning decrease opacity and complete clearance of CM form kidney's parenchyma took more time than control group (mean times are respectively: 5 min,10 min, and 25.5 min). Previous examinations in mammals also showed that inadequate renal function might result in much slower elimination of the contrast agent, resulting in delayed ureter filling. (Thrall, 2017)Therefore, prolonged elimination times indicate renal insufficiency.(Krautwald-Junghanns & Konicek, 2020; Krautwald-Junghanns et al., 2008)

Recent studies have demonstrated that obstructive diseases are accompanied by changes in nephrographic opacification in IVEU, and this method can also help to find the obstruction site in ureter and post operation evaluations in both mammals and avian classes (Deepa et al., 2020; Habing & Byron, 2015); the method was used in an Amazon parrot to check the peristaltic movements and urethral size. (Dennis & Bennett, 2000b)

Similar to many of the previous examinations, in this study, the kidneys exhibited significant increase size of renal divisions which is visible in lateral view of radiographic examination as bulging of cranial kidney pole as increase kidney size. (Pollock et al., 2016)

Gentamicin is commonly used in birds; however, it has nephropathy effects, that results in kidney enlargement and changes resembling other causes of renal failure. Histopathological finding of our study indicated that all of the samples showed severe tubular necrosis, 2 samples indicated severe congestion, 27 cases mild inflammation, and except for one case, all samples showed mild glomerulopathy. These findings are compatible with previous histopathological findings that revealed marked tubular vacuolar degeneration, focal interstitial mononuclear cells infiltrations, congestion, focal cystic change and tubular necrosis in GEN-treated kidneys. Hence, the cause of this discrepancy is related to an acute disturbance in the function of glomerular infiltration and tubular resorption after the nephropathy induction. Therefore, in birds suffering from glomerular or tubular nephropathy due to kidney dysfunction, renal filtration is affected, affecting the passage of CM in different phases in the kidney. Thus, it can be used in diagnosing and confirming kidney diseases with the help of diagnostic imaging. (Khan et al., 2008)
This study reveals that excretory urography in radiography method, provides images of the kidneys and indicates that IOEU (or IVEU) can play a key role in diagnostic procedures of the urinary system in chickens as a model. Moreover, intravenous/ intraosseous contrast media injection is contraindicated in patients with a history of an adverse reaction to iodinated contrast, dehydration, and severe renal impairments. Consequently, to prevent these complications, free access to water was allowed to ensure proper hydration. (Bertolini, 2017)
The standard and nephropathy characterizations of IOEU described in this study will provide the veterinarian a valuable tool to evaluate renal function and morphologic kidneys features. The results of this study can be used as a primary reference value in the birds; however, standardization of protocols for IOEU is required to facilitate the comparison of the patient results. Moreover, further research is necessary to evaluate the diagnostic efficiency of IOEU in other species of birds with kidney or ureteral disease.

Conclusion

To our knowledge, this is the first study evaluating and comparing the passage of CM in normal and nephropathy-induced kidneys in chickens. Pathology assessment of the renal samples confirmed nephropathy changes after nephropathy-induction; IOEU by using the radiography method indicated that the average time to create the maximum concentration of CM, the average time to decrease the CM concentration from the kidney, the time of the complete clearance of CM indicated and also the kidneys sizes show obvious incensement after nephropathy compared to the time before it.

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متعاقب excretory urographyیافته های رادیوگرافی کلیه در پرندگان توسط ایجاد نفروپاتی القایی در مدل جوجه گوشتی

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چکیده:

زمینه مطالعه: علی رغم شیوع نسبتا بالای بیماری‌های کلیوی در پرندگان، تشخیص اولیه‌ی آن‌ها به دلیل نبود علائم پاتوگونومونیک بسیار سخت بوده و از سوی دیگر همراه شدن بیماری های کلیوی با علائم سایر بیماری ها در پرندگان موجب دشواری تشخیص و تفریق می‌گردد.

هدف: هدف از این مطالعه بررسی Intraosseous excretory urography (IVOU) جهت معرفی روش جدیدی است که بتواند در بررسی عملکرد کلیه در جوجه های گوشتی به عنوان مدل اولیه ی پرنده مورد استفاده قرار گیرد.

روش کار: تعداد 10 عدد جوجه گوشتی در سن 20 روزگی از طریق رادیوگرافی ساده و رادیوگرافی با ماده حاجب، جهت بررسی زمان عبور و غلظت ماده حاجب از کلیه مورد بررسی قرارگرفتند. در مرحله بعدی، پس از القا نفروپاتی به وسیله ی جنتامایسین، تصاویر ساده و با ماده حاجب توسط رادیوگرافی مجددا تکرار گردید و پس از کالبد گشایی، نمونه کلیه جهت بررسی هیستوپاتولوژی ارسال و آنالیز آماری تصاویر با استفاده از آزمون *Wilcoxon Signed Ranks*، *آزمون Paired T-Test*، *آزمون Shapiro-Wilk* و *آزمون Chi Square* انجام شد. ($p\text{-value} \leq 0.05$)

نتایج: تمامی جوجه ها پس از القا نفروپاتی افزایش معنی دار در سایز کلیه ها را نشان دادند و در بررسی هیستوپاتولوژی نیز تمامی نمونه ها (30 مورد از 30 نمونه) نکروز توبولار شدید و گلومرولوپاتی خفیف را نشان میدادند. درIVEU انجام شده نیز در تمامی پرندگان میانگین زمان برای ایجاد حداکثر غلظت در کلیه توسط ماده حاجب، کاهش غلظت ماده حاجب از کلیه و پاکسازی کامل ماده حاجب از کلیه افزایش معنی داری در مقایسه با زمان قبل از القا نفروپاتی نشان دادند.

نتیجه گیری نهایی: Intraosseous excretory urography IVOU یک روش تصویر برداری کم خطر به کمک ماده حاجب است که می تواند در ارزیابی سیستم ادراری جوجه‌های گوشتی به منظور بررسی تغییرات سایز و عملکرد کلیه مورد استفاده قرار گیرد.

کلید واژه ها: کلیه‌ی پرندگان، جوجه گوشتی، اوروگرافی دفعی، نفروپاتی، رادیوگرافی

table

Table 1: Comparison of the time of entrance, persistence and clearance of the CM in the radiography of chickens before and after the nephropathy induction

Variable		Mean ± SD	Z/t	P-value
Maximum CM concentration in RD*	Normal kidney	2.30±0.48	Z=2.53	P=0.01 [a]
	Nephropathy kidney	4.50±0.84		
Decreased CM concentration in RD	Normal kidney	4.80 ± 0.42	Z=2.80	P=0.005 [a]
	Nephropathy kidney	10.80±4.04		
Complete clearance of CM in RD	Normal kidney	8.80 ±1.03	Z=2.80	P< 0.001 [b]
	Nephropathy kidney	27.50 ± 7.54		

a: Wilcoxon Signed Ranks test

b: Paired T-Test

*Radiography

Figures

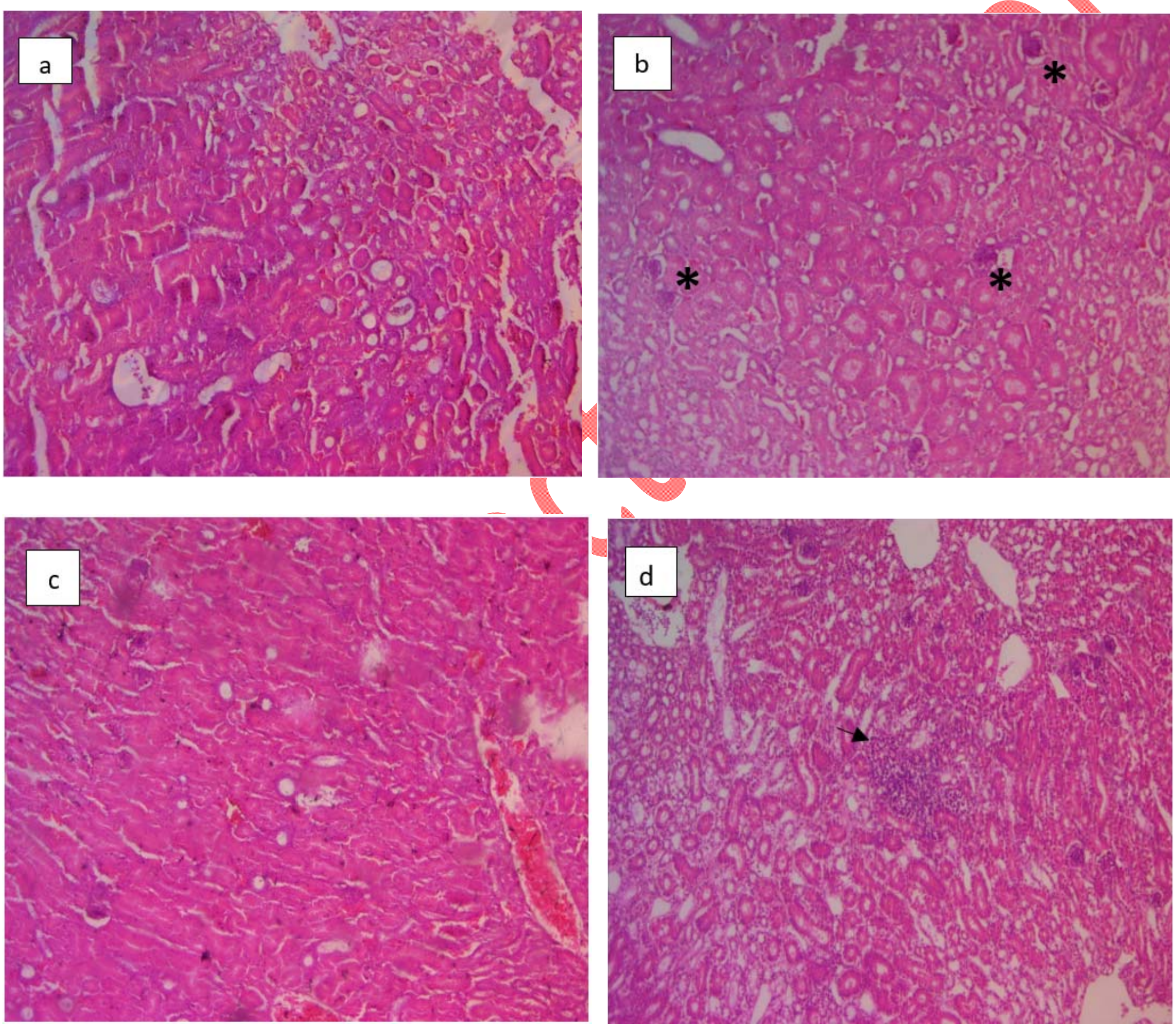

Figure 1: H&E images of tubular changes in different kidneys lobe after nephropathy induction. (a) Tubular necrosis in cranial lobe. (b) tubular necrosis, tubular dilation and spheroid bodies (asterisks) are visible in middle lobe. (c) severe tubular necrosis in distal lobe. (b) tubular necrosis and lymphoid aggregation (arrow) in distal lobe. 100x

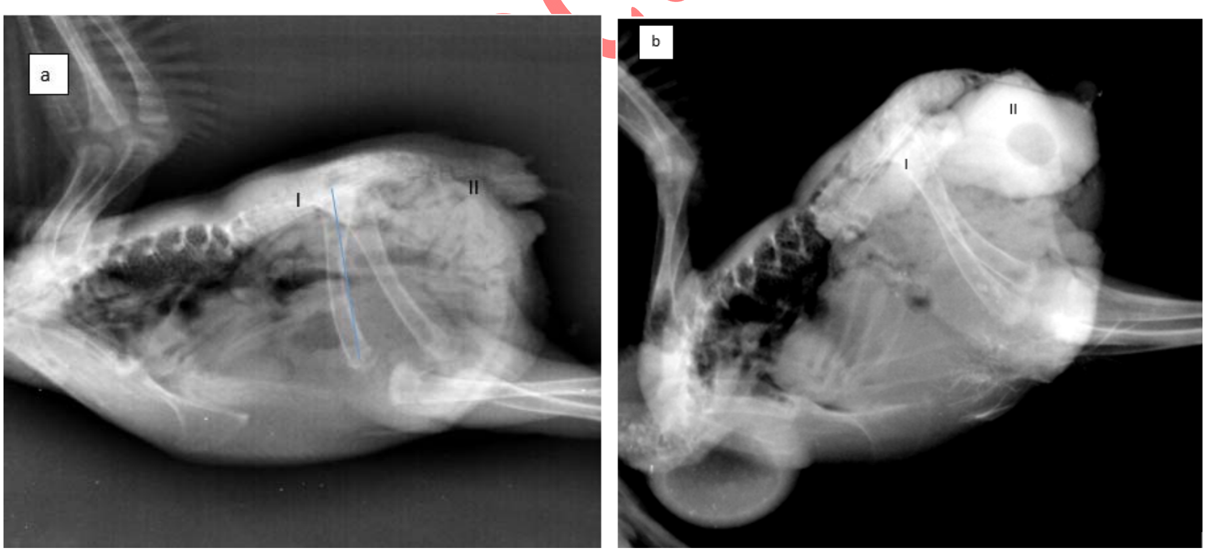

Figure 2: Plain and post-contrast radiography examination from normal kidney parenchyma (a, b) and after the nephropathy induction (c, d). In plain radiographs (a, c), normal opacity of the kidney is visible; In post-contrast radiographs after 5 min of CM injection (b, d), CM results in the increased opacity of the kidney's parenchyma and cloaca. Kidney (I), Cloaca (II). Blue line in figure (a) shows landmarks for femoral length from greater trochanter to distal physis. Orange line in figure (c) shows landmarks for kidney size from cranial pole to caudal pole of kidney and parallel to the vertebral column.