Effect of Urinary System Diseases on Some Constituents in Blood and Urine of Cattle

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For Ph.D. Degree
in
VETERINARY MEDICAL SCIENCE
(Clinical laboratory Diagnosis)

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2022

بسم الله الرحمن الرحيم

وَمَنْ يَتّقِ اللّهَ يَجْعَلْ لَهُ مَخْرَجًا * وَيَرْزُقْهُ مِنْ حَيْثُ
لَا يَحْتَسِبُ وَمَنْ يَتَوَكّلْ عَلَى اللّهِ فَهُوَ حَسْبُهُ إِنّ
اللّهَ بَالِغُ أَمْرِهِ قَدْ جَعَلَ اللّهُ لِكُلّ شَيْءٍ قَدْرًا

صدق الله العظيم

(سورة الطلاق: ٢-٣)

Dedication

To the soul of my teacher Prof. Dr. Ahmed Abdel-Fatah Amer

To the soul of my mother
To the soul of my father

ACKNOWLEDGMENT

I would like to start by thanking almighty Allah for everything that ALLAH gives to me.

I would like to express my deepest gratitude and appreciation to Late **Prof. Dr. Ahmed Abdel-Fatah Amer**, Professor of Internal Medicine and Clinical Laboratory Diagnosis, Faculty of Veterinary Medicine, Assiut University for helpful guidance and valuable advice and I ask ALLAH to forgive and ruthless him.

I would like to express my deepest gratitude and deep appreciation to **Prof. Dr. Mahmoud Rushdi Abd Ellah**, Professor of Clinical Laboratory Diagnosis, and Head of Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University for his keen supervision, helpful guidance, valuable advice, helpful suggestion, fruitful discussion, encouragement and unlimited help and support throughout the course of this study.

I would like to express my deepest gratitude and deep appreciation to **Prof. Dr. Sary Khaleel Abdel-Ghaffar,** Professor of Pathology and Clinical Pathology Faculty of veterinary medicine, Assiut University, and Dean for the Faculty of Veterinary Medicine, Badr University in Assiut, for his keen supervision, unending advice, valuable guidance, fruitful discussion, continuous encouragement, unlimited help and support throughout the course of this study.

I would like to express my deepest gratitude and appreciation to **Prof. Dr. Bedeir Ibrahim Agag,** Professor of Biochemistry, Animal Health Research Institute, Dokki, for his keen supervision, helpful guidance, valuable advice, helpful suggestion, encouragement, and for facilitating the collection of samples.

Many thanks to **Prof. Dr**. **Mohammed Abdel-Salam Mohammed,** Prof. of biochemistry and nutritional deficiency diseases, Animal Health Research Institute, Giza; and head manager of animal health research laboratory, Assiut. for his helpful guidance، his support and for providing facilities for the completion of this study.

My best thanks are also extended to staff members of the Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University for their support and for providing facilities for the completion of this study.

CONTENTS

List of Tables

List of Figures

INTRODUCTION

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The kidney consists of specialized morphological structures with specific functions. These functional units comprise glomeruli that co-localize with afferent and efferent arterioles, which form the Bowman's capsule, this compartment is a key for the filtration of blood (Brenner, 2004)

The initiating causes of urinary system diseases are highly variable, including drug toxicity, inflammation, oxidative stress, hyperuricemia, dyslipidemia, autoimmune diseases, and urinary tract infections (Tanner *et al*., 2012; Jabarpour *et al*., 2019 and Ejaz *et al*., 2019).

Diseases of the bladder and urethra are more common and more important than diseases of the kidneys in farm animals. Occasionally, renal insufficiency develops as a sequel to diseases such as pyelonephritis, embolic nephritis, amyloidosis, and nephrosis. Knowledge of the physiology of urinary secretion and excretion is required to properly understand disease processes in the urinary tract (Radostits *et al*. 2006).

Oxidative stress, a disturbance in the complex pro-/antioxidant balance, is widely recognized as a critical component of the pathogenesis and progression of urinary system diseases (Che *et al*., 2014 and Daenen *et al*., 2019). Due to its high metabolism, the kidney is extremely vulnerable to oxidative damage, and several experiments have shown that oxidative stress can cause/accelerate both disease progression and complications (Scholze *et al*., 2016 and Daenen *et al*., 2019). Despite several experimental and clinical studies having explored the intricate mechanisms between urinary system diseases and oxidative imbalance, the pathophysiological mechanisms of organ damage have not been clarified (Pellegrino *et al*., 2019). The details of

renal function and renal failure in farm animals have received only limited study (Radostits *et al*. 2006).

Aims of the work:

1. Evaluating the value of blood biochemical and urine analysis in diagnosis of urinary tract affections in cattle.
2. Investigating the effect of urinary tract affections in cattle on blood oxidative stress.

Review of Literature

REVIEW OF LITERATURE

The urinary tract consists of the bladder, ureters, and kidneys, and is an essential organ system for filtration and excretion of waste products and maintaining systemic homeostasis (Radostits *et al*., 2006).

The kidney consists of specialized morphological structures with specific functions. These functional units comprise glomeruli that co-localize with afferent and efferent arterioles, which form the Bowman's capsule. This compartment is a key for the filtration of blood, and the glomeruli membrane is surrounded externally by podocytes that constitute a protective layer in addition to the glomerular endothelial cells and smooth muscles cells located on the inner surface of the vessels. Mesangial cells regulate capillary flow which directly influences the glomerular filtration rate (GFR). Afferent and efferent arterioles constrict and dilate under various stimuli and regulate glomerular blood flow. The diameter of the efferent arterioles is smaller than the afferent arterioles, thus creating background resistance to blood flow. Hydraulic pressure of the renal circulation drops progressively along intra-renal micro-circulation. (Brenner, 2004)

The pathophysiology of urinary system diseases involves two mechanisms: the initial mechanism of the specific underlying etiology (as immune complex glomerulonephritis or the exposure to toxins in some renal tubules (and interstitial disease) and a series of progressive mechanisms involving the hyperfiltration and hypertrophy of the remaining viable nephrons. In addition, inflammation causes epithelial–mesenchymal transitions in renal tubular cells that move away from the basal membrane and form new interstitial fibroblasts that lead to tissue fibrosis. Interstitial fibrosis seems to drive further nephron injury through

the promotion of renal ischemia. Remaining viable nephrons lose the ability to perform autoregulation, resulting in systemic hypertension, which will ultimately be more damaging to the glomerulus and worsen urinary system diseases progression (Schnaper *et al*., 2017).

Diseases of the bladder and urethra are more common and more important than diseases of the kidneys in farm animals. Occasionally, renal insufficiency develops as a sequel to diseases such as pyelonephritis, embolic nephritis, amyloidosis and nephrosis. A knowledge of the physiology of urinary secretion and excretion is required to properly understand disease processes in the urinary tract. The principles of renal insufficiency presented here are primarily extrapolated from research in other species, particularly human medicine. Although, in general, these principles probably apply to farm animals, the details of renal function and renal failure in farm animals have received only limited study (Radostits *et al*., 2006).

In chronic kidney diseases mostly characterized by proteinuria and inflammatory conditions, cytoskeleton (extracellular matrix) integrity is impaired, that leads to podocyte foot process effacement (Krishnan *et al*., 2018).

The urinary tract is constantly exposed to microorganisms that inhabit the gastrointestinal tract, but generally the urinary tract resists infection by gut microorganisms. This resistance to infection is mainly ascribed to the versatility of the innate immune defences in the urinary tract, as the adaptive immune responses are limited, particularly when only the lower urinary tract is infected. In recent years, as the strengths and weaknesses of the immune system of the urinary tract have emerged and as the virulence attributes of uropathogens are recognized, several potentially

effective and unconventional strategies to contain or prevent urinary tract infections have emerged (Abraham and Miao, 2015).

Urinary tract infection (UTI)

Glomerulonephritis can occur as a primary disease or as a component of diseases affecting several body systems. In primary glomerulonephritis, the disease involves only the kidney, predominantly affecting the glomeruli although the inflammatory process extends to affect the surrounding interstitial tissue and blood vessels (Radostits *et al*., 2006).

Pyelonephritis is an inflammation of the renal pelvis and renal parenchyma, which is often accompanied by ureteritis and cystitis (Confer and Panciera, 1995).

According to Confer and Panciera (1995), a common cause of pyelonephritis in cattle is an ascending infection, often associated with the reflux of infected urine from the ureters and urinary bladder into the renal pelvis. It has also been reported that catheterization of the urinary bladder may induce pyelonephritis (Van Metre and Divers, 2002).

Rebhun *et al*. (1989) described the clinical signs of pyelonephritis in cattle, which in acute cases may include anorexia, decreased milk production, fever and colic, and in chronic cases weight loss. Strangury, polyuria, an arched stance, swishing of the tail and gross haematuria or pyuria may occur. Organisms that have been associated with the disease in cattle are *Escherichia coli, Arcanobacterium pyogenes, Corynebacterium renale, Corynebacterium pilosum, Staphylococcus aureus, Streptococcus, Enterococccus, Proteus, Klebsiella* and *Pseudomonas species*.

Yeruham *et al*. (2006) reported that cystitis, urethritis and pyelonephritis in cattle most commonly result from ascending urinary tract infection with *Corynebacterium renale, Corynebacterium pilosum* or *Escherichia coli*. Depression, muscle wasting, weakness and frequent urine dribbling were the main characteristics of UTI in calves. Affected cows showed weight loss and an abrupt reduction in feed intake and milk production.

Pathology of the urinary system

Diseases of the kidneys are as complex as its structure that damage the three basic morphologic components; glomeruli, tubules, and interstitium. Further, some components appear to be more vulnerable to specific forms of renal injury: for example, tubular and interstitial diseases are more likely to be caused by toxic or infectious agents. In spite of, other diseases damage more than one structure. Chronic renal disease can be able to destruct all three components of the kidney, culminating in chronic renal failure, and what has been known, end stage contracted kidney. Renal diseases have received much less attention in cattle than in some species and there is still a lack of knowing in this field (Constable *et al*., 2017).

Katsoulos *et al*. (2020) found that renal lesions were detected in 37 out of 53 animals that examined after slaughter. Eleven cattle had interstitial nephritis (IN), 7 had glomerulonephritis (GN), and 19 had both lesions (IGN). In all cases, histopathologic changes were mild.

El-Mashad *et al*. (2019) detected the comparative pathological affections in kidneys of cattle that were collected from El-basatin abattoir in Cairo-Egypt. Microscopically, the highest incidence of interstitial nephritis was found to be 17.39%, followed by glomerulonephritis

21.73%; circulatory disturbances were 15.94%; suppurative nephritis were 11.59%; amyloidosis was 7.24%. Moreover, parasite, stones, polycystic kidney, hydronephrosis and acute necrotic nephritis were observed in few cases of cattle as 2.89%, 7.24%, 2.89%, 8.69% and 4.34% respectively. Furthermore, at histopathological examination of the kidneys slight to severe mononuclear cell infiltrations were commonly observed. Haemorrhage and connective tissue proliferations were also seen. Neutrophil leucocyte infiltrations caused by pyelonephritis were observed in some cases and multiple stone formations were found in these cases. Calcium deposits and eosinophilic material were found in the medullary tubules and pelvic lumens in some kidneys. Whereas sand-like material accumulation seen in urinary bladders in some cases, no obstruction was observed in the urinary canal in this study.

El-Mashad *et al*. (2019) performed a pathological study on kidneys affections in slaughtered cattle. Inflammatory conditions in the kidneys were a common finding in both cattle. However, parasitic infestation is not a common finding in renal lesion. The authors found that in hydronephrosis, the kidneys were swollen and enlarged. Histopathologically, the kidneys showed degenerative changes in the glomerular tuft manifested by vesiculation of the cytoplasm of endothelial cells lining of the glomerular tuft capillaries. Moreover, segmentation of the glomerular tuft with periglomerular mononuclear cell infiltration were also detected. Occasionally, the lumen of large numbers of renal tubules contained proteinaceous eosinophilic material. Cystic dilatations of some of the renal tubules were also seen. Membranous glomerulonephritis was identified grossly as the kidneys were enlarged, pale, with smooth nonadherent surface. Microscopically, the glomeruli showed severe thickening and hyalinization of the Bowman's capsule and the basement

membrane of the glomerular tuft. Shrinkage and segmentation of the glomerular tuft were also seen.

Urine analysis

Urine content is affected by diet, water intake, activity, and body temperature. Morning urine is more concentrated, and is more likely to contain cells, casts, and abnormal constituents, but it is unaffected by activity or feeding. Normal urine can range from clear to dark yellow. Darker urine is usually, but not always, more concentrated. Some foods can affect the color of urine, such as carrots, blackberries, beets, and rhubarb. Turbidity or cloudiness indicates the presence of crystals, cells, mucus, bacteria, casts, or fluids from the reproductive tract. Test strips are a simple way to evaluate multiple parameters including specific gravity, pH, protein, glucose, ketone, bilirubin, urobilinogen, leukocytes, nitrite, and hemoglobin (Whalan, 2015).

Urinalysis is an important laboratory test that can be readily performed in veterinary practice and is considered part of a minimum database. It is useful in documenting various types of urinary tract diseases and may provide information about other systemic diseases, such as liver failure and hemolysis (Callens *et al*., 2015).

Unlike blood analysis, urinalysis is a simple, safe, and noninvasive method to investigate health status; it creates no discomfort, poses no health-related risks, and has no direct side effects. Besides diagnosing specific kidney diseases or failures, the main indications for urinalysis in dairy cows are the detection and monitoring of energy balance and periparturient metabolic diseases, as highlighted by recent research. Moreover, urine macro-mineral analysis is considered a very useful tool to

gauge acid-base balances; urinalysis is more accurate than blood analysis in reflecting the attempts of the kidneys to stabilize the serum acid-base status, which is subject to strict homeostatic control. Thus, urinalysis could be a useful tool to troubleshoot and monitor metabolic problems, but information on values in healthy cows and the influence of external factors is essential for the correct evaluation of results (Constable *et al*., 2017).

The urinalysis is a diagnostic test may be crucial for the early identification of numerous metabolic diseases and can allow for early intervention or prevention. The urinalysis should be considered an essential part of every diagnostic workup for ill patients, but also as essential part of the general wellness examination and should be performed regardless of the patient's age or current health status (Piech and Wycislo, 2019).

Although overlooked by some practitioners, a complete urinalysis is often considered to be the single most important diagnostic test by many veterinary specialists. In addition to the identification of urinary tract disorders, such as bacterial cystitis, protein-losing nephropathy, and transitional cell carcinoma, a urinalysis can aid in the diagnosis of nonurinary tract disorders. Endocrinopathies such as diabetes mellitus and other systemic disorders such as intravascular hemolysis can often be diagnosed through urine evaluation (Piech and Wycislo, 2019).

Chemical analysis of urine is most performed semi-quantitatively by use of reagent dipstick systems. The results of individual reagent pads on dipstick are typically graded on scales provided by the manufacturer. These strips can either be visually interpreted or analyzed by automated methods, which most commonly implement reflectance photometry.4 Several recent studies reported good agreement between visual estimation

and automatic measurements for most parameters. In general, urine dipstick is reliable for the measurement of urine pH, glucose, ketones, bilirubin, occult blood, and protein. They are considered unreliable for measurement of urine specific gravity and leukocytes, which should be assessed by refractometry and a microscopic sediment examination, respectively. Nitrite and urobilinogen measurements are also not typically reported in veterinary medicine (Piech and Wycislo, 2019).

A complete urinalysis consists of the assessment of color and clarity, measurement of urine specific gravity (USG), chemical analysis of urine, and microscopic examination of a urine sediment. It is important to remember that the results of these tests should be interpreted together rather than in isolation, considering pertinent patient clinical history and other physical examination findings. Urine should be at room temperature before evaluation to ensure accurate results and should be analyzed within 60 minutes of collection to avoid temperature and time-dependent effects on crystal formation (Parrah et al., 2013 and Piech and Wycislo, 2019).

Normal urine contains only traces of protein due to normal leakage. Protein that passes through the glomeruli is reabsorbed in the tubules. It is normal to find brief proteinuria in newborn animals, during estrus, at parturition, or following exercise; otherwise, proteinuria is considered pathologic (Whalan, 2015).

The normal composition of urinary protein is about 40% of albumin, 40% of tissue proteins originating from renal and other urogenital tissues, 15% of immunoglobulins and their fragments, and remaining 5% of other plasma proteins. Healthy individuals are known to excrete protein in their urine. Abnormalities may occur both in the quantity and in the composition of urinary proteins. Several systemic and primary renal

diseases may affect one or more glomerular structures and thereby increase the effective permeability of the glomeruar capillary wall to proteins. Proteinuria following a renal damage has been studied most intensively and is still regarded as one of the most sensitive markers for the pathologic conditions of the kidney (D'amico and Bazzi, 2003; Grauer, 2011)

Tests have been developed for the detection of proteinuria, including the dipstick colorimetric test as a first-line screening test, the sulfosalicylic turbidimetric test or the Heller reaction, and the urine protein:creatinine (UPC) ratio (Garry *et al*., 1990; Gaspari *et al*., 2006; Grauer, 2007; Grauer. 2011).

Apart from urinary tract infections such as cystitis and pyelonephritis, there are sporadic reports of clinical conditions with renal involvement associated with proteinuria in cattle, all due to glomerulonephritis. In addition, proteinuria due to lymphocytic and neutrophilic glomerulonephritis with degenerated tubular epithelium has been observed in cattle infected with *Pasteurella multocida* B as well as after active immunization with the Salmonella Typhimurium core antigen vaccine (Murray and Sharpe, 2009; Annas *et al*., 2015; Bernier *et al*., 2018)

In cattle, the assessment of proteinuria, as one of the indicators for renal disease, cannot be performed accurately at the farm; urine dipsticks give false-positive results for proteins due to the alkaline pH of urine. Therefore, proteinuria measurements should be performed using either semiquantitative methods, like a sulfosalicylic acid test or other quantitative laboratory methods (Divers, 2008 and Nourmohammadzadeh et al., 2010).

Katsoulos *et al*. (2020) found that serum biochemical profiles did not show any suspicion of subclinical renal disease in cattle; all animals had serum albumin, BUN, and creatinine concentrations within the reference intervals, and urine pH values were >7.5. This is associated with mild renal pathology confirming the subclinical disease course in these animals. The authors added that, proteinuria was associated with interstitial nephritis in the majority of cases. However, it has been reported that proteinuria could result in cases of interstitial nephritis as excessive protein concentrations in the glomerular filtrate contribute to interstitial inflammation, fibrosis, and cell death. Therefore, it can be assumed that functional or prerenal proteinuria or undetectable structural damage might have increased the amount of protein in the glomerular filtrate, in turn, causing interstitial nephritis.

Urine reference values of cattle, as well as the composition of the bovine urinary proteome have been previously reported (Bathla *et al*., 2015; Rawat *et al*., 2016; Isani *et al*., 2018; Ihedioha *et al*., 2019; Herman *et al*., 2019 and Ferlizza *et al*., 2020).

Several studies had reported that cattle urine samples had an alkaline pH, between 8 and 9 (Herman *et al*., 2019; Ihedioha *et al*., 2019; Ferlizza *et al*., 2020).

Ihedioha *et al*. (2019) performed a cross-sectional study and evaluated kidney function and urinary analytes in cattle presented for slaughter at Nsukka abattoir, Enugu State, Nigeria. Serum creatinine evaluation of the 133 cattle showed that 7.5% (10 cattle out of 133) had renal impairment (serum creatinine above 2 mg/dl). There was no significant association ($p > 0.05$) between renal impairment and age or sex. Out of the sampled cattle, 11 (8.3%) had positive urine bilirubin levels, while none (0%) had

urobilinogen in urine, and 5 (3.8%) were positive for ketonuria. Only 6 (4.5%) were positive for urine glucose, but 113 (92.5%) were positive for urine proteins. The cattle sampled had urine pH ranging from 6 to 9, and specific gravity ranging from 1.000 to 1.030 and of all the sampled cattle, 6 (4.5%) were positive for nitrite. The authors concluded that based on the serum creatinine level, which is a known marker of kidney function, 7.5% of cattle sampled had renal impairment.

Blood contamination within a urine sample may also contribute to proteinuria. However, several studies on urinary blood contamination found that significant proteinuria (21 or greater) on the urinary dipstick pad often does not occur until the urine sample is visibly pink. Interestingly, yellow urine that contains blood (but is not visibly pink in color) may still have a significantly elevated urine protein to creatinine ratio compared with yellow urine without blood contamination, which may or may not result in a clinically relevant increase in urine protein to creatinine ratio. If proteinuria is identified via dipstrip methods in a patient with an inactive sediment, it is recommended to repeat a full urinalysis and, if proteinuria is still present, to perform more quantitative tests such as a urine protein to creatinine ratio (Vaden *et al*., 2004 and Vientós-Plotts *et al*., 2018).

A variety of oxidation products are found in urine and thought to mirror local and systemic oxidative stress (Kirschbaum, 2001). Acute terms of various diseases accompany many inflammatory conditions and influence the endogenous antioxidant enzyme activities. Urinary tract infection (UTI) may cause an oxidative stress, and the antioxidant enzymes measured quantitatively were depleted in response to oxidative stress (Kurutas *et al*., 2005).

Kirschbaum (2001) reported that total antioxidant activity was lower in cases with acute renal disease compared to those of control urine specimens. UTI may cause oxidative stress by consuming urinary antioxidant enzymes and it is possible to say that urinary antioxidant enzymes are not enough to prevent the oxidative stress in UTI (Kurutas *et al*., 2005). The authors declared that overproduction of free radicals generated during infection may lead to the low levels of antioxidant enzymes.

Abd Ellah *et al*. (2012) studied urine biochemical constituents in camel cystitis. The authors found that urine analysis for cases with acute cystitis revealed the presence of traces of protein, microscopical examination revealed increases number of RBCS and pus cells/high power field (HPF). Urine from cases with chronic cystitis was whitish, turbid and with strong uriniferous odour, specific gravity was increased, with the presence of protein and blood. Microscopical examination revealed the presence of clumps of transitional epithelial cells, increase number of RBCs and pus cells/HPF. Urine biochemical findings showed a significant increase in urinary gamma glutamyl transferase (GGT) activity in the acute cystitis group compared with the control and chronic cystitis groups, which may be attributed to the acute inflammatory reaction in the urinary tract during acute cystitis. However, urine urea and creatinine levels showed insignificant changes in the acute and chronic cystitis groups.

Kaneko *et al*. (2008) specified that kidney parenchymal damage may cause a change in urinary enzyme activity but not in serum activity that changes only when the lesion has already invaded most of the kidney's parenchyma.

Urine analysis for urinary tract infection (UTI) in camels was studied by El-Deeb and Buczinski (2015). The results revealed the presence of protein. The microscopical examination of urine revealed hematuria and pyuria. The isolated bacteria were E. coli, Corynebacterium renale and mixed bacterial culture with different types of bacteria including *Corynebacterium* with other bacteria as *Staphylococci, Streptococci* and *Proteus*.

Cattle urine contains a variety of nitrogenous constituents, but quantitative information on urinary N composition is limited. The dominant form of N in urine is urea. The urea-N concentration varied between 2.1 and 19.2 g/l and represented from 52.1% to 93.5% of the total N. Diets fed in excess of protein requirement generally result in high concentrations of urea in blood and urine, and urea-N as a fraction of total urinary N generally also increases with dietary protein supply. Urea is formed mainly in the liver as a means of detoxification of NH_3 present in the systemic circulation. In beef and dairy cattle, net urea-N release by the liver accounts for on average 0.65 of increments in N intake. Renal urea reabsorption and consequently urea concentration in urine is actively regulated by means of urea transporters. Urea excretion by the kidneys is not just controlled by the concentration of urea in plasma, but also by physiological status of the animal. Urea is an important osmolite in the renal reabsorption of water. A rise in urea reabsorption to increase renal osmotic pressure and water absorption from the renal filtrate will increase plasma urea levels and may explain the increased plasma urea levels in dehydrated cattle (Dijkstra *et al*., 2013).

Serum biochemical constituents

Over the last few years, routine biochemical profiles in dairy cattle have become useful diagnostic and prognostic tools for the assessment of productive and reproductive performances of livestock and herd animal welfare (Constable *et al*., 2017).

Braun *et al*. (2008) described the biochemical findings of 17 cattle with pyelonephritis. The most frequent abnormal biochemical finding was an increase in the serum concentrations of total protein, fibrinogen, urea and creatinine.

Abd Ellah *et al*. (2012) studied serum biochemical constituents in camel cystitis. Serum biochemical results of the chronic cystitis group showed significant increases in serum total proteins and globulins and decrease in albumin compared with the control and acute cystitis group. On the other hand, gamma glutamyl transferase (GGT), aspartate aminotransferase (AST) activities and blood urea nitrogen (BUN) and creatinine concentrations did not change between groups.

Serum creatinine levels below the reference range may be due to muscle wasting, while serum creatinine level above reference range may be due to renal failure or dysfunction (Niraj *et al*., 2009). On the other hand, serum urea level below the reference range in the sampled cattle may be due to portosystemic shunt or hepatic failure, and animal receiving low-protein diet, while serum urea above reference range in the cattle sampled may be due to renal dysfunction, gastrointestinal hemorrhage, dehydration, and drugs (Otter, 2013).

El-Deeb and Buczinski (2015) determined the concentration of total protein, albumin, globulin, blood urea nitrogen (BUN) and creatinine in

dromedary camels suffering UTI. The authors found that concentrations of total protein and globulin increased but albumin decreased in diseased camels when compared to healthy ones. On the other hand, concentrations of blood urea nitrogen (BUN) and creatinine did not differ in camels suffering UTI from healthy camels.

Vysakh *et al*. (2017) studied the biochemical changes in disease progression of acute pyelonephritis in experimental rat model. The blood urea nitrogen (BUN) and creatinine level was increased significantly during 12 h post infection as compared to the normal. The BUN and creatinine level in 7^{th} day post infection group was increased significantly as compared to the12 h post infection group. The total protein was decreased significantly in 12 h post infection group as compared to the normal. The 7^{th} day post infection group also showed significant decrease in level of total protein as compared to 12 h post infection group. AST, ALP, ALT activities were significantly increased in kidney tissues of 12 h post infection group as compared with the control group. There were significant increases in AST, ALP, ALT activities in the 7^{th} day post infection group as compared to the12 h post infection group.

Aliyu *et al*. (2017) reported the biochemical and pathologic changes in the kidneys of cachectic Zebu cattle presented to the abattoir for slaughter. The cachectic group showed slightly higher concentrations of creatinine and higher concentrations of urea than the non-cachectic group and urinalysis revealed no aciduria, ketonuria or pyuria in both cachectic and non-cachectic cattle. Similarly, cachectic cattle had increased alkaline phosphatase, ALT and AST activities compared to the non-cachectic animals whose values of these enzymes did not vary significantly. Postmortem examination of the carcasses revealed smooth spherical grayish-brown colored uroliths (stones) in the kidneys of 11 (15%) of the

cachectic cattle. The uroliths weighed between 200-700 mg, with a diameter of 5-10 cm. Histopathologically, there was intra glomerular cellular infiltration (predominantly lymphocytes and macrophages) for both cachectic and non-cachectic cattle. The cachectic cattle also showed obliterated Bowman's space and moderate congestion. Nephritis was also observed in the cachectic cattle.

Gosselin *et al*. (2018) reported that consistent clinicopathological abnormalities in calves with glomerulonephritis presented for clinical evaluation, which included hyperkalemia, hyperphosphatemia, hypoproteinemia, hypoalbuminemia, hyperbilirubinemia, increased creatine phosphokinase activity, and proteinuria.

Ihedioha *et al*. (2019) evaluated kidney function and urinary analytes in cattle presented for slaughter at Nsukka abattoir, Enugu State, Nigeria. Serum creatinine evaluation of the 133 cattle showed that 7.5% (10 cattle out of 133) had renal impairment (serum creatinine above 2 mg/dl). There was no significant association ($p > 0.05$) between renal impairment and age or sex. The cattle sampled had urine pH ranging from 6 to 9, and specific gravity ranging from 1.000 to 1.030 and of all the sampled cattle, 6 (4.5%) were positive for nitrite.

Elgioushy *et al*. (2020) noticed that urinary tract dysfunction due to aflatoxicosis in cattle revealed significant increases in serum alanine amino transferase, aspartate amino transferase, alkaline phosphatase activities, creatinine. Moreover, a significant decrease in total protein was also seen.

Free radicals and antioxidants in diseases of the urinary system

Free radicals are identified as molecules having one or more unpaired electrons in their outer orbits, the name radicals mean unstable. Free radicals gain the stability by attracting electrons from neighboring molecules as proteins, enzymes, lipids, or amino acids (Abd Ellah, 2013b). They are highly reactive substances produced continuously during metabolic processes and participating mainly in physiological events such as immune response, metabolism of unsaturated fatty acids, and inflammatory reactions. The most important free radicals include superoxide anion, hydroxyl radical, and hypochlorous acid (Abd Ellah, 2010).

In cells, these radicals can act as oxidants or reductants by losing or accepting a single electron, and they are continuously produced by the organism's normal use of oxygen (Lobo *et al*., 2010). Free radicals include reactive radical and nonradical derivatives of oxygen (ROS) and nitrogen (RNS) that are collectively called reactive oxygen nitrogen species (RONS) (Powers *et al*., 2011).

Generally, sources of free radicals are oxidative phosphorylation through the mitochondrial electron transport chain, cytochrome P_{450} enzymes, oxidase enzymes like NADPH oxidases. Furthermore, capillaries of the glomerular and tubular cells, circulating leucocytes and platelets represent important sources for production of free radicals (Wardle, 2005). It has long been recognized that reactive oxygen species (ROS) are harmful for cells, mainly because they lead to structural and functional impairments of lipids, proteins, and nucleic acids (Abd Ellah, 2013c).

Oxidative stress occurs when there is an imbalance between the production of free radical species and the antioxidant ability to neutralize their harmful effects (Salisbury and Bronas, 2015).

The generation of reactive oxygen nitrogen species (RONS) is a physiological process and, at moderate or low levels, RONS are important molecules involved in several cellular signaling pathways, in the extraction of energy from organic molecules, in immune defense, in mitogenic response, and in redox regulation (Genestra, 2007). An excess production or a decreased scavenging of RONS has been implicated in aging and age-related diseases (Venkataraman *et al*., 2013).

The endogenous sources of RONS include different subcellular organelles, such as mitochondria, peroxisomes, and endoplasmic reticulum, where oxygen consumption is high (Phaniendra *et al*., 2015). NADPH oxidase (nicotinamide adenine dinucleotide phosphate oxidase) is a prevalent source of the superoxide radical, which is formed by the addition of one electron leak from the electron transport system during cellular respiration to the molecular oxygen. Most of the superoxide dismutates into hydrogen peroxide (H_2O_2) through superoxide dismutase (SOD) (Genestra, 2007).

Endogenous or exogenous RONS can damage biologically relevant molecules with consequent cell damage and homeostatic disruption (Zampelas and Micha, 2015). Among them, lipids, carbohydrates, nucleic acids, and proteins are the major targets, and their oxidative modification can also be used as markers of oxidative stress (Frijhoff *et al*., 2015).

Hydrogen peroxide (H_2O_2) is a neutral molecule because it has no unpaired electrons, but it can form the most reactive and dangerous

radical, the hydroxyl radical (•OH), through a Fenton or Haber–Weiss reaction. Hydroxyl radicals mainly react with phospholipids in cell membranes and proteins. In activated neutrophils, in the presence of chloride and myeloperoxidase, H_2O_2 can be converted into hypochlorous acid that can react with DNA and produce pyrimidine oxidation products and add chloride to DNA bases (Kulcharyk *et al*., 2001). Another important determinant in the cellular redox equilibrium is nitric oxide (NO).

In mammals, NO can be generated by three main isoforms of nitric oxide synthase (NOS): endothelial NOS, which is related to vasodilation and vascular regulation; neuronal NOS, which is linked to cellular signaling; and inducible NOS, which is activated in response to various endotoxin or cytokine signals (Adams *et al*., 2015).

The most important cellular defense mechanism is represented by antioxidant systems. The cells contain important antioxidant defense mechanisms that protect against free radical toxicity and include both endogenous and exogenous molecules. Endogenous antioxidants (naturally generated in situ) include enzymatic and nonenzymatic molecules. The primary enzymatic scavengers are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) (Darenskaya *et al*., 2018).

Scholze *et al*. (2016) demonstrated that knowledge on causes and consequences of oxidative stress in chronic kidney diseases (CKD) is rapidly expanding. Many gaps, however, remain, calling for additional research efforts to unravel its pathogenesis and to test therapeutic approaches. No simple antioxidant solutions are at hand and solid clinical outcome data both for biomarker research and for interventions studies are necessary. Moreover, when analyzing oxidative stress related molecular

changes and the impact of antioxidant interventions, important variables such as CKD stage, genetic background, current therapies, and comorbidities should be accounted for.

In many experimental studies, the participation of ROS in glomerular damage was confirmed by measuring the products of oxidant injury and antioxidants levels in renal tissue and urine (Wojcicka and Beltowski, 2001). Many toxic chemicals induce nephrotoxicity through the generation of ROS as suggested by Somani *et al*. (2000). In addition, tissue deposition of immune complexes can induce an acute inflammatory response resulting in tissue injury.

Many research studies had been reported that oxidative stress mediates a wide range of renal injuries, which ranging from acute renal failure (Shah, 2001), obstructive nephropathy (Klahr, 2001), glomerular damage and chronic renal failure (Handelman *et al*., 2001).

Studies in models of acute renal failure (ARF) have generated evidence that ROS production occurs during ischemia/reperfusion (Greene and Paller, 1991). Reactive oxygen species are involved in the pathogenesis of toxic, ischemic, immunologically mediated renal injury (Baude and Ardaillou 1993) and chronic renal failure (CRF). The increased production of ROS in CRF may be related to metabolic consequences of the uremia such as decreased production of NADPH (Yawata and Jacob, 1975) and GSH-Px activity (Schiavon *et al*., 1994) and lowered vitamin E level (Yalcin *et al*., 1989).

Some studies considered oxidative stress in CRF as an important source of morbidity and mortality, through their involvement in the pathogenesis of malnutrition (Galle *et al*., 2003), anemia (Taccone-

Gallucci *et al*., 1999), and increased risk of carcinogenesis (Vamvakas *et al*., 1998).

Abundant evidence indicates that the toxic radical nitric oxide (NO), formed by activation of the inducible nitric oxide synthase, plays an important role in host defense to bacterial infections, including UTI. The major source of NO production during UTI is from inflammatory cells, especially neutrophils, and from the uroepithelial cells that are known to orchestrate the innate immune response during UTI (Svensson *et al*., 2018). Several studies have shown that higher concentrations of NO may have cytotoxic effects on uroepithelial cells, underlining that NO production in the urinary tract needs to be tightly regulated to avoid host tissue damage (Svensson *et al*., 2018).

In pathological states like inflammation, inducible NOS (iNOS) is responsible for NO production. Moreover, NO-induced oxidative stress has been shown to positively correlate with glomerular damage (Snijder *et al*., 2013). This, in turn, may trigger inflammatory response due to the activation of redox-sensitive proinflammatory transcription factors and signal transduction pathways that may eventually result in apoptosis and further progression of kidney injury (Ozbek, 2012; Shirazi *et al*., 2019).

Oxidative stress in renal diseases

Studying the oxidative stress in diseases of the urinary system in animals are lacking compared with similar research in human. Further studies are required to elucidate the oxidative status in urinary system diseases in relation to the productive and reproductive capacity of different animal species (Abd Ellah, 2013a).

Templar *et al*. (1999) have shown that MDA levels are significantly raised in patients with glomerulonephritis, regardless of serum creatinine, which suggests that there is oxidative injury independent of any possible MDA retention due to renal impairment.

Maciel *et al*. (2006) have reported a positive correlation between serum levels of nitric oxide and serum creatinine levels in patients with UTI infection due to leptospirosis.

Abd Ellah *et al*. (2012) studied serum biochemical constituents including the antioxidant status in camel cystitis. They found a significant decrease in serum vitamin C level in the chronic cystitis group compared with acute cystitis and control groups. A significant decrease was also noticed in beta-carotene and α-tocopherol in acute and chronic cystitis groups compared with the control group. The authors concluded that the most important biochemical changes in camels with acute and chronic cystitis are decreasing serum α-tocopherol and β-carotene. On the other hand, biochemical findings in chronic cystitis are hyperproteinaemia, hyperglobulinaemia and decrease serum vitamin C level.

El-Deeb and Buczinski (2015) provided reliable biochemical evidence for the generation of circulating oxidative stress as detected by enhanced lipid peroxidation (serum MDA and erythrocytic MDA) and decreased serum levels of the enzymatic (SOD, CAT) and non-enzymatic (GSH) antioxidant markers in dromedary camels suffering UTI. The concentrations of MDA were significantly ($P < 0.0001$) higher in diseased camels when compared to healthy ones. Moreover, catalase, superoxide dismutase and glutathione levels were significantly ($P < 0.0001$) lower in diseased camels when matched with the same levels in control group. The authors found that globulin, total protein, and MDA were the most

accurate in predicting treatment outcome in camels with UTI. While catalase, fibrinogen and blood urea nitrogen were moderate in predicting treatment outcome in diseased camels.

Vysakh *et al*. (2017) studied the oxidative biomarkers in disease progression of acute pyelonephritis in experimental rat model. The antioxidant enzymes status in rat kidney (SOD, CAT, GPx, GR) were significantly decreased during 12 h post infection group as compared with the control group. There were significant decreases in SOD, CAT, GPx, GR levels in the 7^{th} day post infection group as compared to the 12h post infection group. GSH level was significantly decreased at 12 h post infection group as compared to the normal group. The significantly decreased level of GSH was observed in the 7^{th} day post infection group as compared to the 12 h group. Malondialdehyde level was significantly increased in kidney tissues of 12 h post infection group as compared to the normal group. The MDA level was increased significantly in the 7^{th} day infection group as compared to the 12h group.

By immunoperoxidase staining of MDA antibody, Gumasta *et al* (2019) studied the effect of cadmium toxicity on kidney of cattle. Results showed that the affected kidney expressed oxidative stress and MDA accumulation in the form of brown colored reaction which confined up to the cytoplasm of cells in the kidney tissues. This reaction was quite prominent in tissues with high cadmium concentration as compared to the low cadmium concentration.

Miyata *et al*. (2019) reported changes in endogenous antioxidants in various animal due to urinary dysfunction caused by bladder outlet obstruction, experiments. The antioxidant activities of CAT, GSH, and SOD were significantly lower than those in the control in many of these

experiments. Several reports have shown that plasma total antioxidant capacity (TAC), which reflects the cumulative effect of all antioxidants in a fluid sample, was decreased in urinary dysfunction in rabbit model. Based on this evidence, there is a general agreement that antioxidative activity is decreased by urinary dysfunction.

Elgioushy *et al*. (2020) noticed that urinary tract dysfunction due to aflatoxicosis in cattle revealed a significant increase in catalase and malondialdehyde levels and a significant decrease in reduced glutathione (GSH). Serum NO showed a non-significant decrease in affected cattle when compared with control ones.

Soleimani *et al*. (2016) found high significant difference between case and control groups for TAC and MDA values, respectively that were represented by the lower serum level of TAC while the higher serum level of MDA in cases compared with controls might be attributed to UTI-induced OS.

Rodrigues *et al*. (2018) found the involvement of inflammatory response in the pathogenesis of acute kidney injury in a rat model, as confirmed by increased renal tissue mRNA expression of TNF-α, IL-1β, and TGF-β1. These molecules play significant roles in the pathogenesis of sepsis, which is correlated with multiple organ failure, immunosuppression. Animals with septic acute kidney injury showed significant increase in NO content after the sepsis process. They also observed that acute kidney injury and sepsis augmented the oxidative stress in the kidney, indicated by higher values of MDA and nitrite levels, respectively.

MATERIALS AND METHODS

MATERIALS AND METHODS

Animals:

A total number of 97 male cattle (18 - 20 months old) were selected from animals slaughtered in Mallawy slaughterhouse (El-Minia governorate, Egypt) and included in this study during the period from June 2018 to December 2019.

Clinical examination

Animals were inspected before slaughtering for presence of any abnormal clinical signs according to Kerr (2002).

Blood sampling

Blood samples were collected from each animal by jugular vein puncture before slaughtering. Two types of blood samples were collected:

a. Whole blood samples were collected in vacutainer tubes containing EDTA as anticoagulant for separation of plasma (Coles, 1986) and used for measuring plasma Malondialdehyde (MDA) level.
b. Blood samples were collected in plain vacutainer tubes without anticoagulant for separation of serum (Coles, 1986) and used for measuring serum biochemical parameters.

Postmortem inspection

After slaughtering, animals were inspected carefully by the meat inspectors. Animals that showing postmortem pathological lesions in other organs rather than the urinary system were excluded from the study.

Urine and tissue sampling

a. Urine samples were collected directly from the bladder using a disposable 10-ml syringe and preserved in ice bag until analysis.
b. The kidneys and urinary bladder were collected from each slaughtered animal in sterile bags. They preserved in ice bags until the

macroscopic investigations and the histopathological sampling in the laboratory.

c. Tissue specimens from the kidney and bladder were preserved in formalin 10% for histopathological examination.

Methods:

Serum Biochemical Parameters:

Blood serum was used for the determination of total proteins (g/dl), albumin (g/dl), globulins (g/dl), gamma glutamyl transferase (GGT, U/l), blood urea nitrogen (mg/dl), creatinine (mg/dl), alkaline phosphatase (ALP, U/l), using commercial kits (Spectrum Diagnostics and Diamond Diagnostics, Cairo, Egypt) according to the manufacturer instructions.

Oxidative stress parameters:

Blood serum total antioxidant capacity (TAC, mM/L), plasma malodialdehyde (MDA, nmol/ml) and serum nitric oxide (NO, μmol/L) were measured colormetrically by means of commercial test kits (Biodiagnostics; Dokki, Giza, Egypt) according to the manufacturer instructions.

Urine analysis:

Urine samples were divided into two parts. The first was examined physically and chemically using test strips (Medi-Test Combi 10® SGL, Macherey- Nagel, Germany) and microscopically according to Coles (1986). The other part was used for bacteriological examination.

Bacteriological examination:

Urine samples were cultured bacteriologically on blood agar, nutrient agar, and MacConkey agar for 48 h at 37 ˚C. The isolated bacteria were identified using a VITEK2 Compact (BioMerieux, Marcy-l'E´ toile, France) according to the manufacturer instructions.

Histopathological examinations:

Samples from the kidney and bladder were fixed in 10% neutral buffer formalin and routinelly processed and sectioned at 5 um. The sections

stained with hematoxylin and eosin stain (H & E stain) for histopathological examination according to Bancroft et al. (1982). The histopathological examination done by light microscopy (CX31; Olympus, Tokyo Japan) and then photographed using a digital camera (Toupview, LCMos10000KPA, china) in the Photomicrograph Lab. at the Department of Pathology & Clinical Pathology, Faculty of Veterinary Medicine, Assiut University.

Statistical analysis

Statistical analysis was done by using SPSS program for windows, V. 21 (SPSS, Chicago, IL, USA) by the analysis of variance (ANOVA) Student's t-test was used for comparing data between the two groups. Data were tabulated as mean ± standard error. Significant difference was set at $P < 0.05$.

RESULTS

RESULTS

Cattle subjected to study (n.=97) were classified into different groups based on the gross and histopathological examination of the kidneys and urinary bladder. The detected urinary system diseases included: Nephrosis (n.=23), Glomerulonephritis and interstitial nephritis (n.= 23), Nephrolithiasis (n.=23), and cystitis (n.=5). Animals that showed no abnormal clinical findings and no histopathological affections were considered as the control group (n.23).

I. Clinical findings

No abnormal clinical signs were recorded in any of the observed pathological affections.

II. Serum biochemical analysis

Blood serum biochemical analysis in cattle suffering from nephrosis are presented in Table 1 and Figs. 1-5. There were significant increases in serum urea ($p = 0.03$) and creatinine ($P < 0.001$) in diseased cattle when compared with the control. There were nonsignificant differences in total protein, albumin, globulin, alkaline phosphatase and γGT between the diseased cattle and the control. There was a significant increase in serum MDA ($p = 0.003$), and significant decreases in serum TAC ($p = 0.02$) and NO ($p = 0.01$) in diseased cattle when compared with the control.

Blood serum biochemical analysis in cattle suffering from glumrulonephritis and interstitial nephritis are presented in Table 2 and Figs. 6-9. There was a significant increase in serum urea ($p = 0.01$) and creatinine ($P < 0.001$) in diseased cattle when compared with the control. There were nonsignificant differences in total protein, albumin, globulin,

alkaline phosphatase and γGT between the diseased cattle when compared with the control. There was a nonsignificant change in serum MDA ($p = 0.07$), and significant decrease in serum TAC ($p = 0.05$) and NO ($P < 0.001$) in diseased cattle when compared with the control.

Blood serum biochemical analysis in cattle suffering from cystitis are presented in Table 3 and Figs. 11-15. There was a non-significant change in blood urea ($p = 0.54$) but serum creatinine increased ($P < 0.001$) in diseased cattle when compared with the control. There were nonsignificant differences in total protein, alkaline phosphatase and γGT between the diseased cattle and the control, but serum albumin and globulins decreased ($p = 0.01$) in diseased cattle when compared with the control. There was a significant increase in serum MDA ($p = 0.005$), significant decrease in serum TAC ($p = 0.02$) and significant increase in NO ($p = 0.02$) in diseased cattle when compared with the control.

Blood serum biochemical analysis in cattle suffering from urolithiasis are presented in Table 4 and Figs. 16-18. There was a significant decrease in serum urea ($p = 0.02$) and a significant increase in creatinine ($P < 0.001$) in diseased cattle when compared with the control. There were nonsignificant differences in the mean values of total protein, albumin, globulin, alkaline phosphatase and γGT between the diseased cattle and the control.

III. Urine analysis

Results of physical examination of urine samples are shown in Table 5. Urine color was yellow in nephrosis, white to yellow in glomerulonephritis and interstitial nephritis, white in cystitis, white to dark yellow in nephrolithiasis, and yellow in the control group. Urine odor was

uriniferous in cases of nephrosis, putrid and ammoniacal in some cases of glomerulonephritis and interstitial nephritis, putrid in cases of cystitis, ammoniacal in cases of nephrolithiasis, and uriniferous in the control group. Foam was positive in all groups. Urine was cloudy to turbid in all groups.

Chemical examination of urine samples of examined cases is shown in Table 6. pH ranged from 7-8 for cases of nephrosis, glomerulonephritis and interstitial nephritis, cystitis, and in the control group, whereas it recorded 6 in cases of nephrolithiasis. Urobilinogen was normal in all groups. Nitrite was positive in cases of nephrosis and cystitis, but it was negative in the other groups. Protein was high in positivity in cases of nephrosis and in other groups. Blood was positive in cases of nephrosis and cystitis, but it was negative in the other groups. Glucose and ketone bodies were negative in all the studied groups.

Microscopic examination of urine sediment is shown in Table 7. Affected cases showed increased numbers of WBC, RBCs (hematuria and pyuria) and epithelial cells than the control group. Crystals in the affected cases was amorphous phosphate and triple phosphate.

Types and number of bacterial isolates in urine are shown in Table 8. *E. coli* was found in 18 cases, *Corynebacterium renale* was found in 25 cases, *Staphylococcus* sp. was found in 9 cases, *Streptococcus* sp. was found in 6 cases, and *Micrococcus* was found in 16 cases.

The relation between the results of bacterial isolation and urinary disorders in diseased cattle cases is shown in Table 9. *E. coli* was found in 5 cases of nephrosis, 4 cases of glomerulonephritis and interstitial nephritis, 7 cases of cystitis and 2 cases of nephrolithiasis.

Corynebacterium renale was found in 7 cases of nephrosis, 8 cases of glomerulonephritis and interstitial nephritis, 8 cases of cystitis and 2 cases of nephrolithiasis. *Staphylococcus* sp. was found in 3 cases of nephrosis, 2 cases of glomerulonephritis and interstitial nephritis, 3 cases of cystitis and 1 case of nephrolithiasis. *Streptococcus* sp. was found in 3 cases of nephrosis, 1 case of glomerulonephritis and interstitial nephritis, 2 cases of cystitis and no case of nephrolithiasis. *Micrococcus* was found in 5 cases of nephrosis, 8 cases of glomerulonephritis and interstitial nephritis, 3 cases of cystitis and no case of nephrolithiasis.

Table 1: Blood serum biochemical analysis in control and cattle suffered from nephrosis.

Parameters \ Groups	Control	Diseased*	P- value
Urea (mg/dl)	27.20±1.09	32.56±2.20*	0.03
Creatinine (mg/dl)	1.32±0.04	1.75±0.07**	<.0001
Total protein (g/dL)	6.08±0.10	6.20±0.15	0.51
Albumin (g/dL)	3.41±0.06	3.33±0.12	0.52
Globulins (g/dL)	2.67±0.13	2.88±0.14	0.28
Alkaline phosphatase (U/L)	189.46±5.29	197.39±12.57	0.56
γGT (U/L)	22.34±0.45	23.35±1.17	0.42
MDA (nmol/ml)	2.48±0.32	5.61±0.69**	0.003
TAC (mM/L)	0.46±0.04	0.28±0.05**	0.02
Nitric oxide (µmol/L)	7.81±0.44	4.05±1.15**	0.01

Data are presented as Mean±SE.
γGT: Gamma Glutamyl transferase; MDA: Malondialdehyde; TAC: Total antioxidant capacity. *: P<0.05; **: P<0.01.
*: animals suffered from Nephrosis

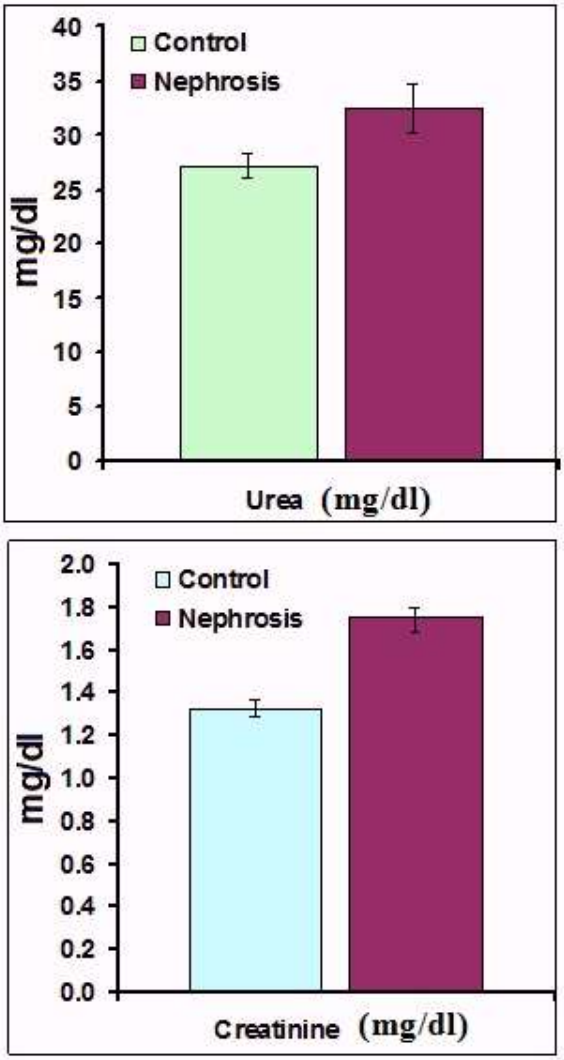

Fig 1: Blood urea and creatinine in control and cattle suffered from nephrosis.

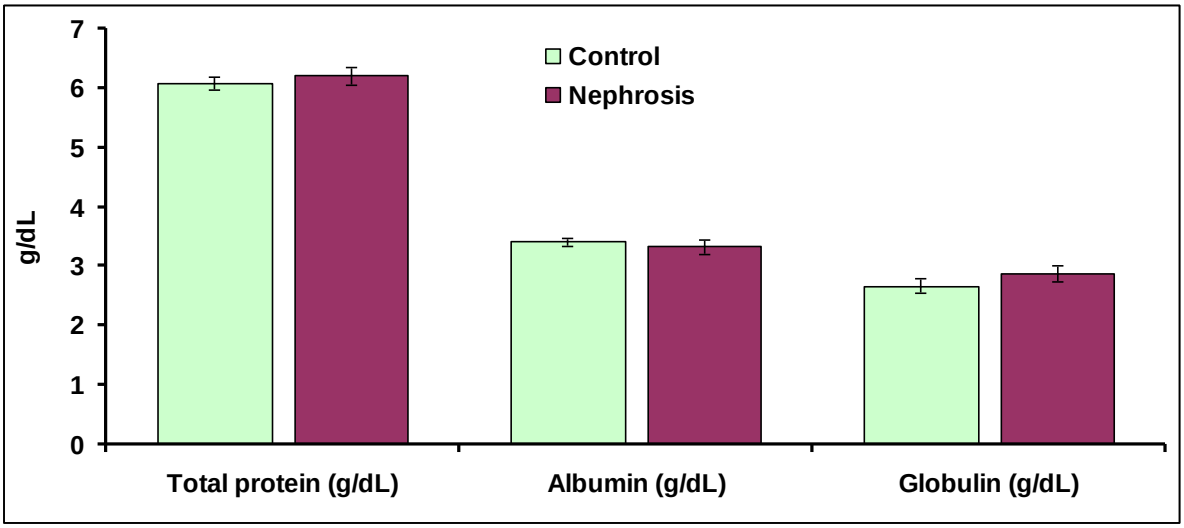

Fig. 2: Blood serum proteins in control and cattle suffered from nephrosis.

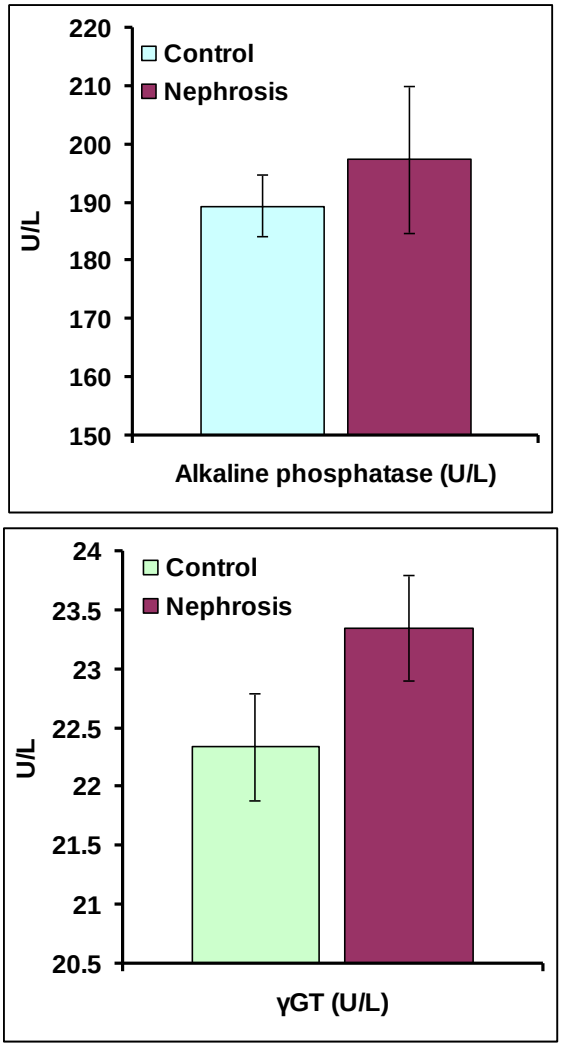

Fig. 3: Mean values (± SE) of serum alkaline phosphatase and γGT in healthy (control) and cattle suffering from nephrosis.

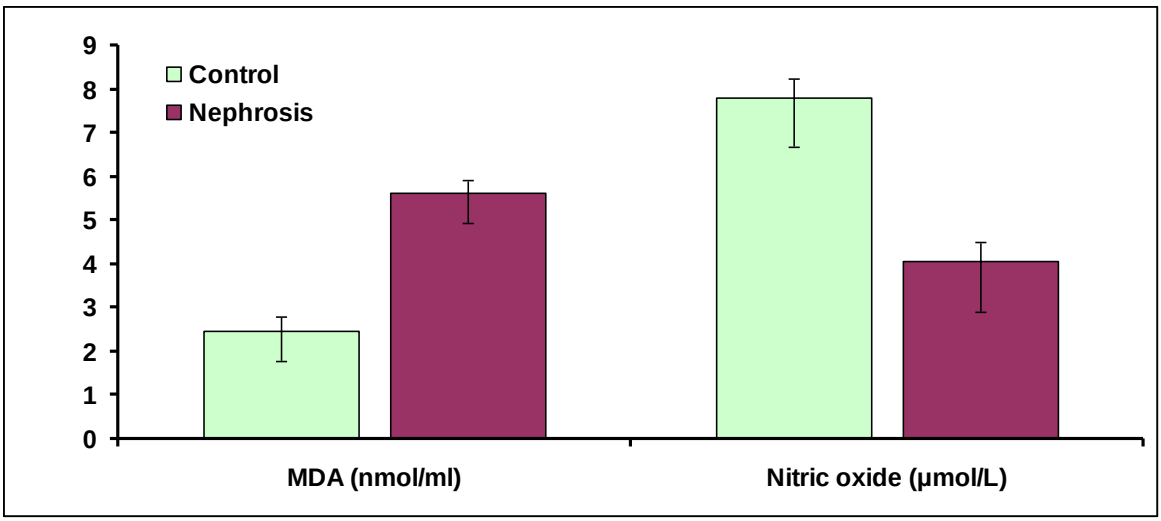

Fig. 4: Serum MDA and NO level in control and cattle suffered from nephrosis.

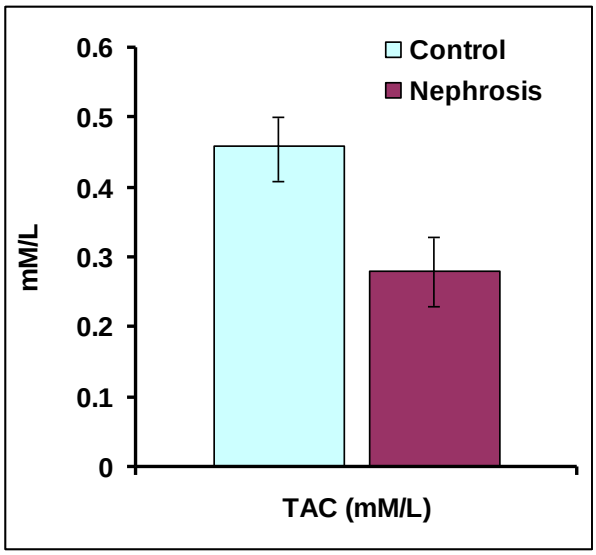

Fig. 5: Serum TAC in control and cattle suffered from nephrosis.

Table 2: Serum biochemical analysis in control and cattle suffering from glomerulonephritis and interstitial nephritis.

Groups / Parameters	Control	Diseased*	P- value
Urea (mg/dl)	27.20±1.09	33.66±2.12**	0.01
Creatinine (mgldl)	1.32±0.04	1.84±0.03**	<.0001
Total protein (g/dL)	6.08±0.10	6.07±0.17	0.97
Albumin (g/dL)	3.41±0.06	3.30±0.05	0.13
Globulin (g/dL)	2.67±0.13	2.78±0.18	0.62
Alkaline phosphatase (U/L)	189.46±5.29	192.06±11.28	0.84
γGT (U/L)	22.34±0.45	21.60±0.98	0.50
MDA (nmol/ml)	2.48±0.32	6.32±1.96	0.07
TAC (mM/L)	0.46±0.04	0.31±0.05*	0.05
Nitric oxide (µmol/L)	7.81±0.44[a]	2.92±0.20[b]	0.0002

Data are presented as Mean±SE.

γGT: Gamma Glutamyl transferase; MDA: Malondialdehyde; TAC: Total antioxidant capacity. *: P<0.05; **: P<0.01.

*: Animals suffered from glomerulonephritis and interstitial nephritis

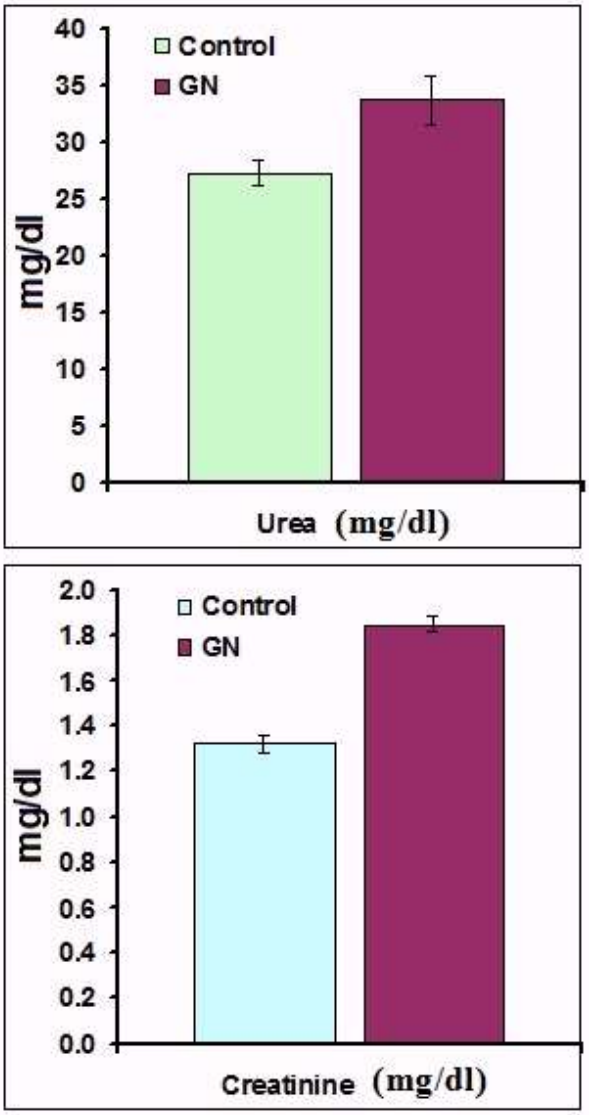

Fig. 6: Blood urea and creatinine levels in control and cattle suffered from glomerulonephritis and interstitial nephritis.

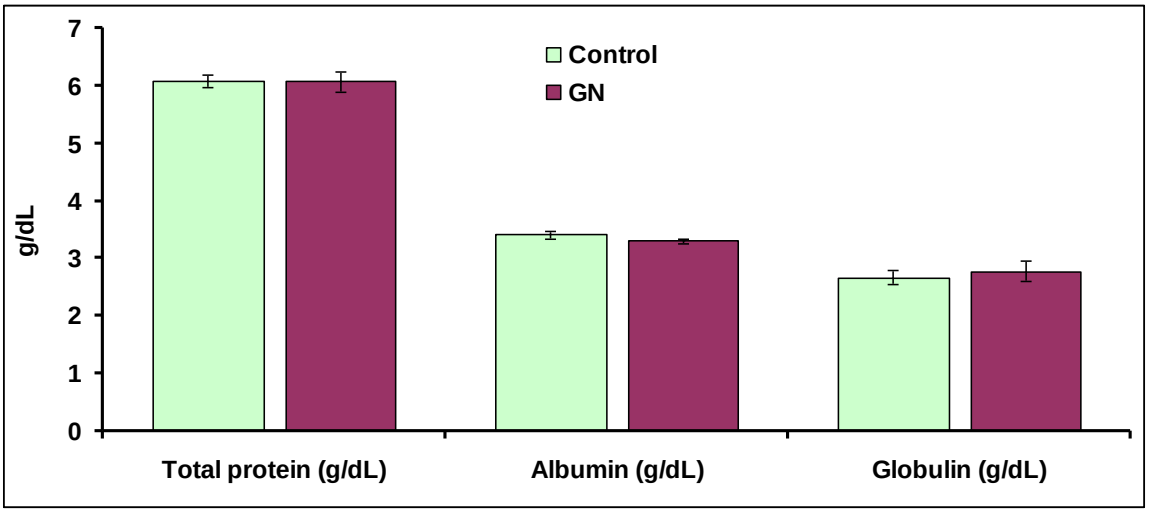

Fig. 7: Serum proteins in control and cattle suffered from glomerulonephritis and interstitial nephritis.

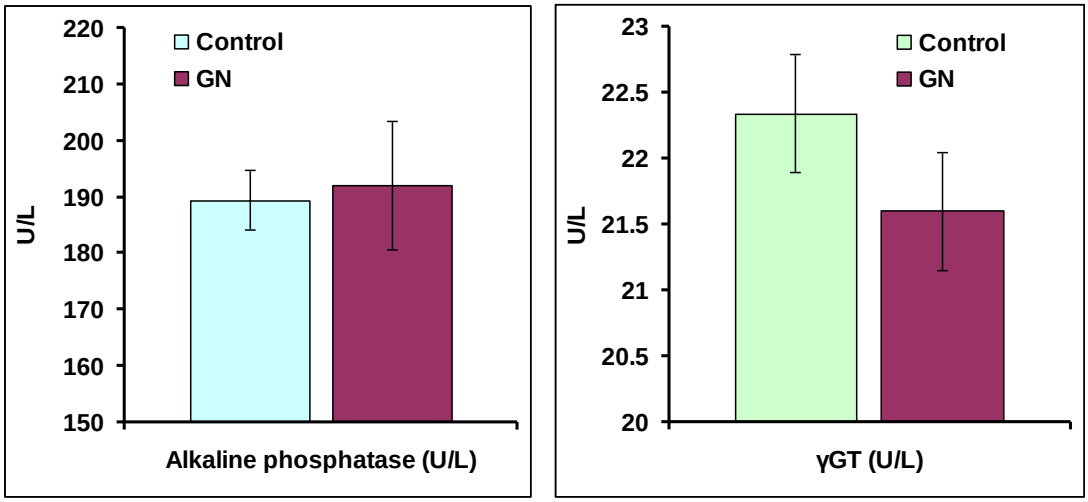

Fig. 8: Serum alkaline phosphatase and γGT activities in control and cattle suffered from glomerulonephritis and interstitial nephritis.

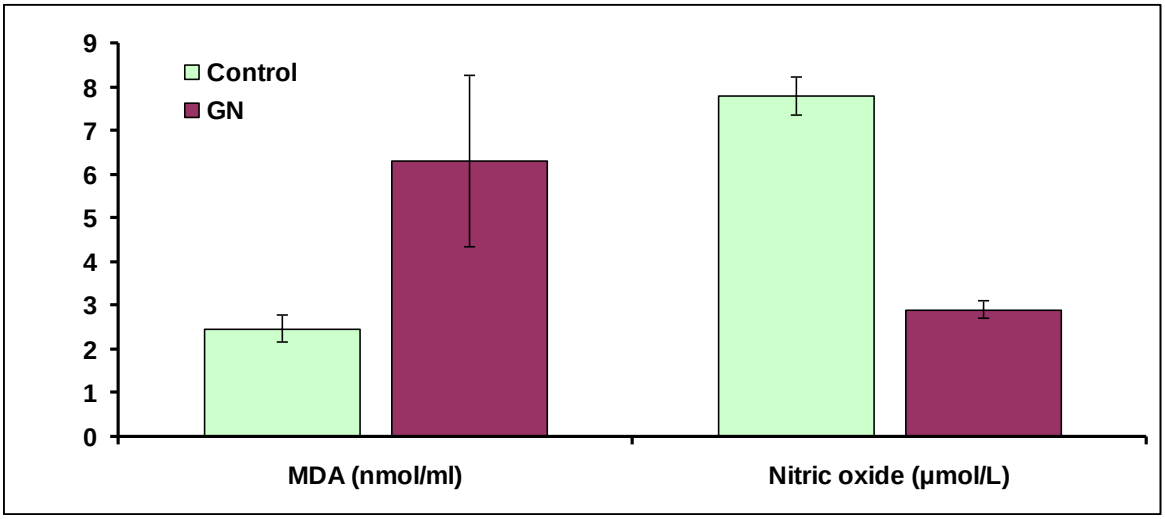

Fig. 9: Serum MDA and NO levels in control and cattle suffered from glomerulonephritis and interstitial nephritis.

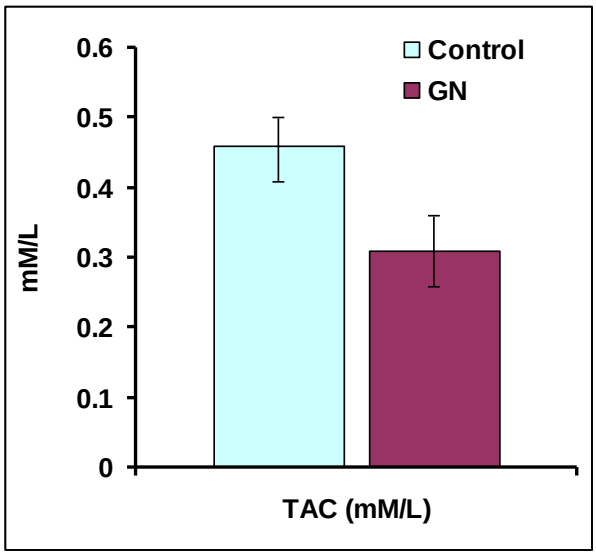

Fig. 10: Serum TAC in control and cattle suffered from glomerulonephritis and interstitial nephritis.

Table 3: Serum biochemical analysis in control and cattle suffered from cystitis.

Groups / Parameters	Control	Diseased*	P- value
Urea (mg/dl)	27.20±1.09	28.40±1.60	0.54
Creatinine (mg/dl)	1.32±0.04	1.80±0.04**	<.0001
Total protein (g/dL)	6.08±0.10	6.37±0.15	0.12
Albumin (g/dL)	3.41±0.06	3.12±0.10**	0.01
Globulin (g/dL)	2.67±0.13	3.24±0.16**	0.01
Alkaline phosphatase (U/L)	189.46±5.29	194.42±8.34	0.62
γGT (U/L)	22.34±0.45	21.57±0.64	0.33
MDA (nmol/ml)	2.48±0.32	7.81±1.56**	0.005
TAC (mM/L)	0.46±0.04	0.28±0.05*	0.02
Nitric oxide (µmol/L)	7.81±0.44	22.90±6.20*	0.02

Data are presented as Mean±SE.
γGT: Gamma Glutamyl transferase; MDA: Malondialdehyde; TAC: Total antioxidant capacity. *: P<0.05; **: P<0.01.
*: Animals suffered from cystitis.

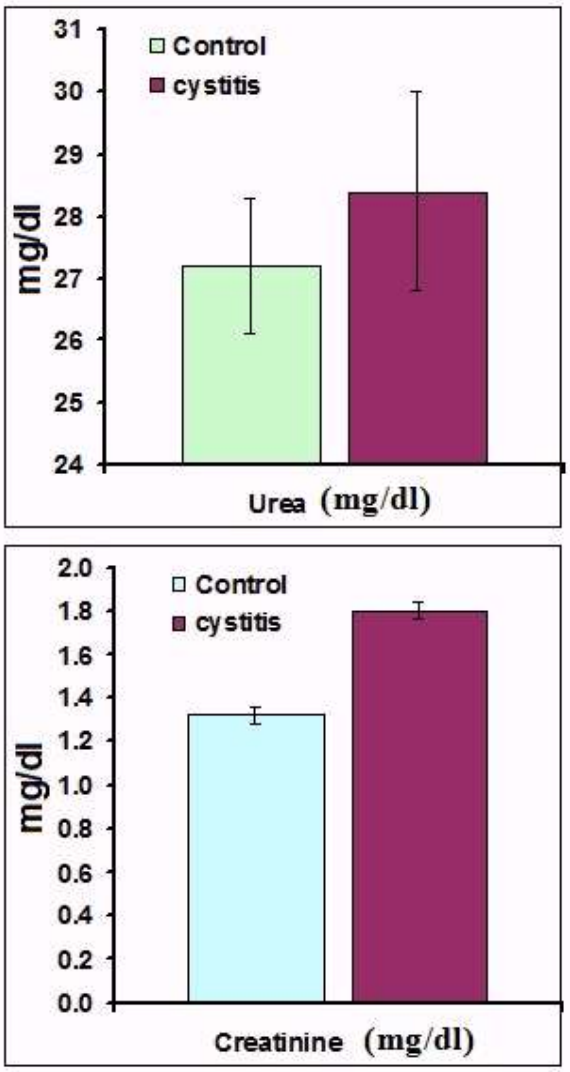

Fig. 11: Blood urea and creatinine levels in control and cattle suffered from cystitis.

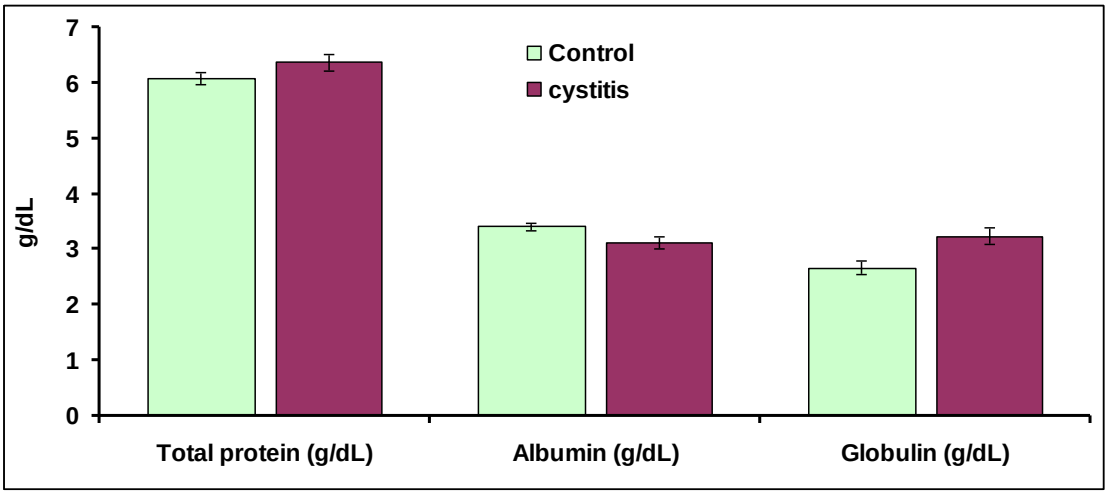

Fig. 12: Serum proteins in control and cattle suffered from cystitis.

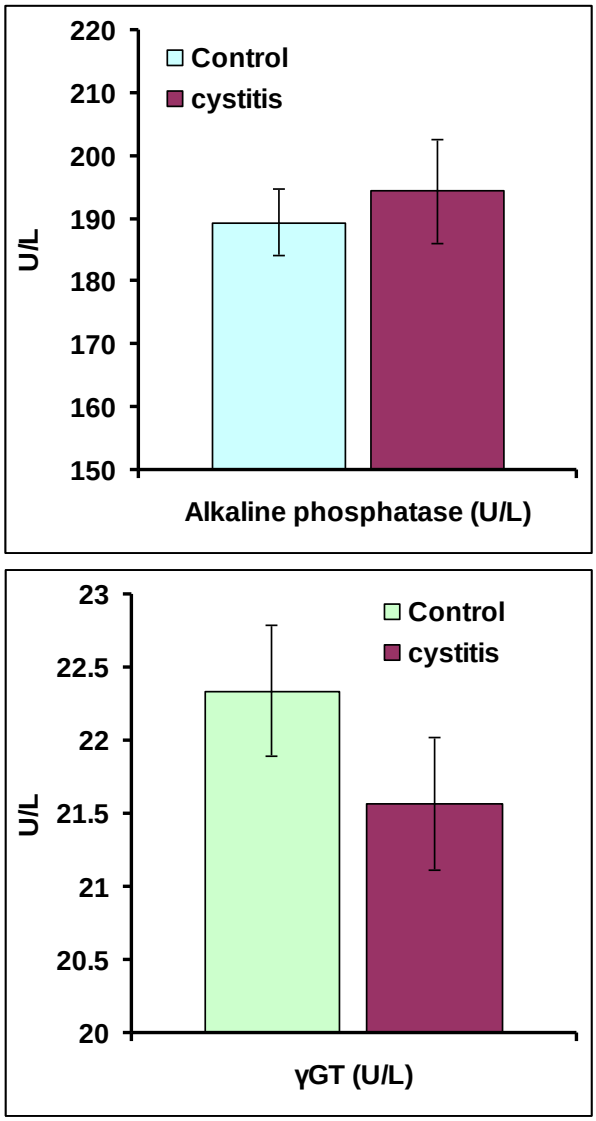

Fig. 13: Serum alkaline phosphatase and γGT activities in control and cattle suffered from cystitis.

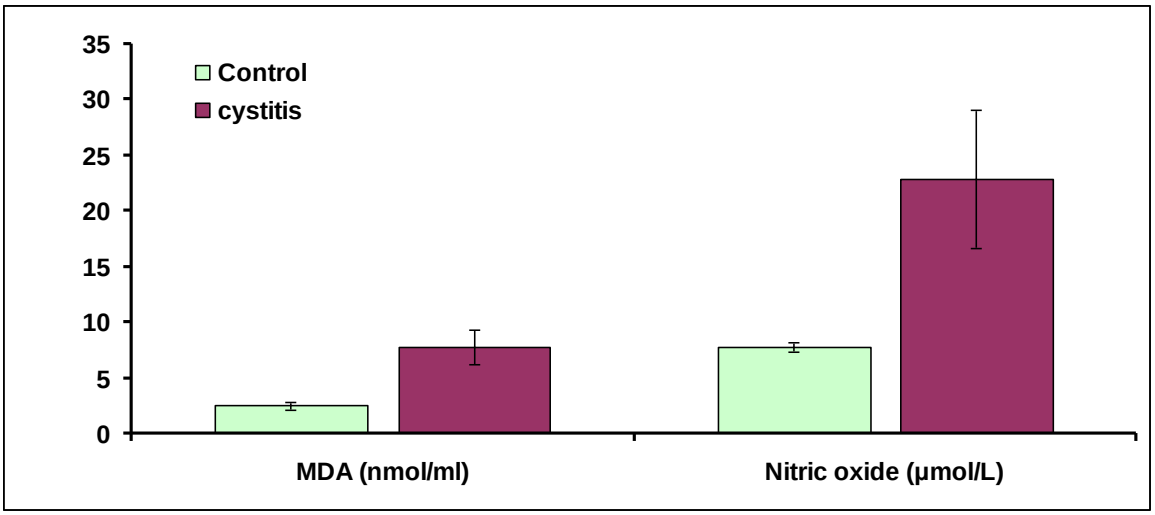

Fig. 14: Serum MDA and NO in control and cattle suffered from cystitis.

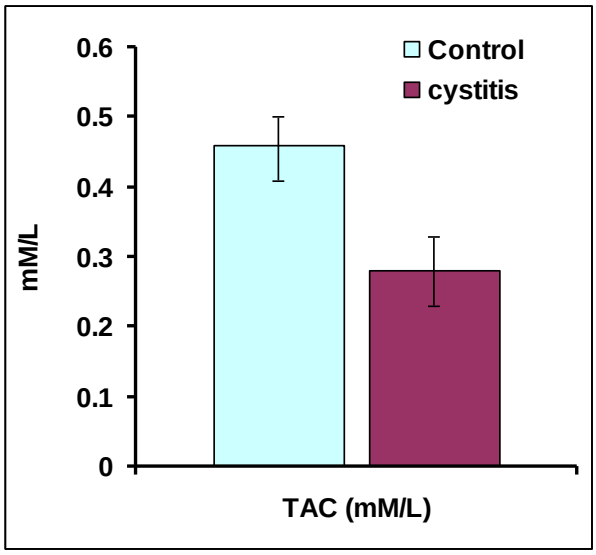

Fig. 15: Serum TAC level in control and cattle suffered from cystitis.

Table 4: Serum biochemical analysis in control and cattle suffering from urolithiasis.

Parameters \ Groups	Control	Diseased*	P- value
Urea (mg/dl)	27.20±1.09	21.46±0.16*	0.02
Creatinine (mg/dl)	1.32±0.04	1.82±0.04**	<.0001
Total protein (g/dL)	6.08±0.10	5.80±0.13	0.22
Albumin (g/dL)	3.41±0.06	3.04±0.32	0.06
Globulin (g/dL)	2.67±0.13	2.76±0.30	0.77
Alkaline phosphatase (U/L)	189.46±5.29	204.28±15.57	0.28
γGT (U/L)	22.34±0.45	21.96±0.30	0.70
MDA (nmol/ml)	2.48±0.32	-	-
TAC (mM/L)	0.46±0.04	-	-
Nitric oxide (µmol/L)	7.81±0.44	-	-

Data are presented as Mean±SE.

γGT: Gamma Glutamyl transferase; MDA: Malondialdehyde; TAC: Total antioxidant capacity. *: P<0.05; **: P<0.01.

*: Animals suffered from urolithiasis

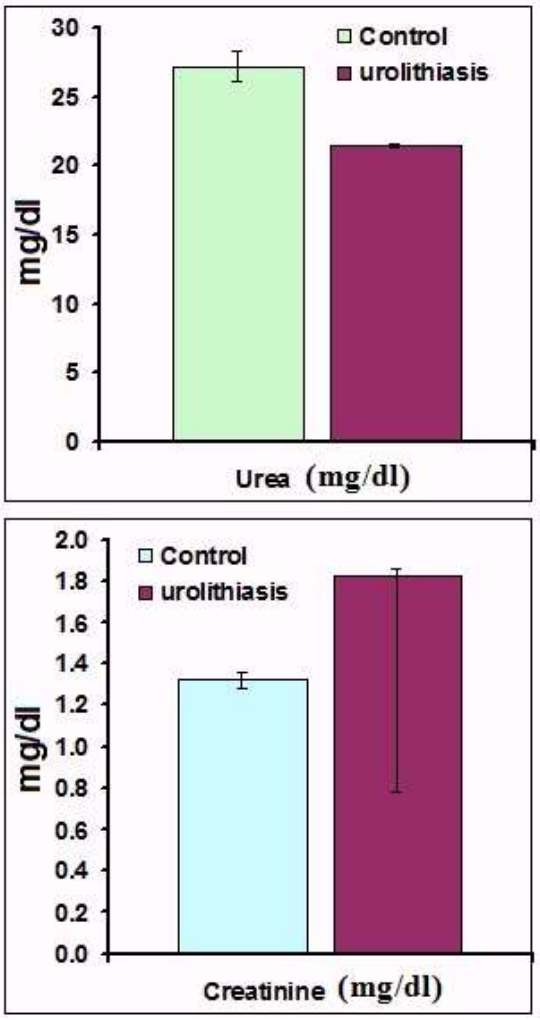

Fig. 16: Blood urea and creatinine in control and cattle suffered from urolithiasis.

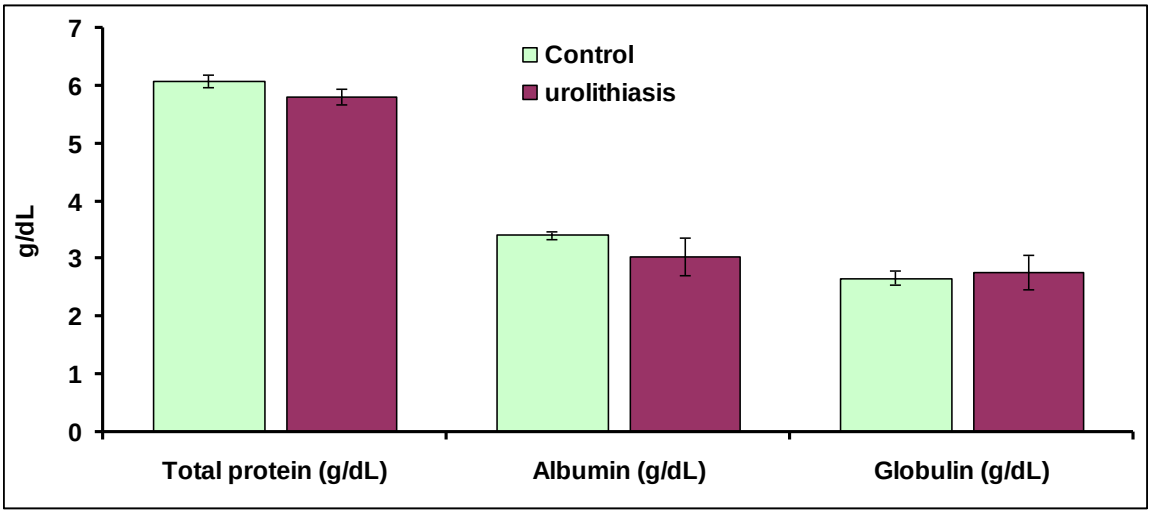

Fig. 17: Serum proteins in control and cattle suffered from urolithiasis.

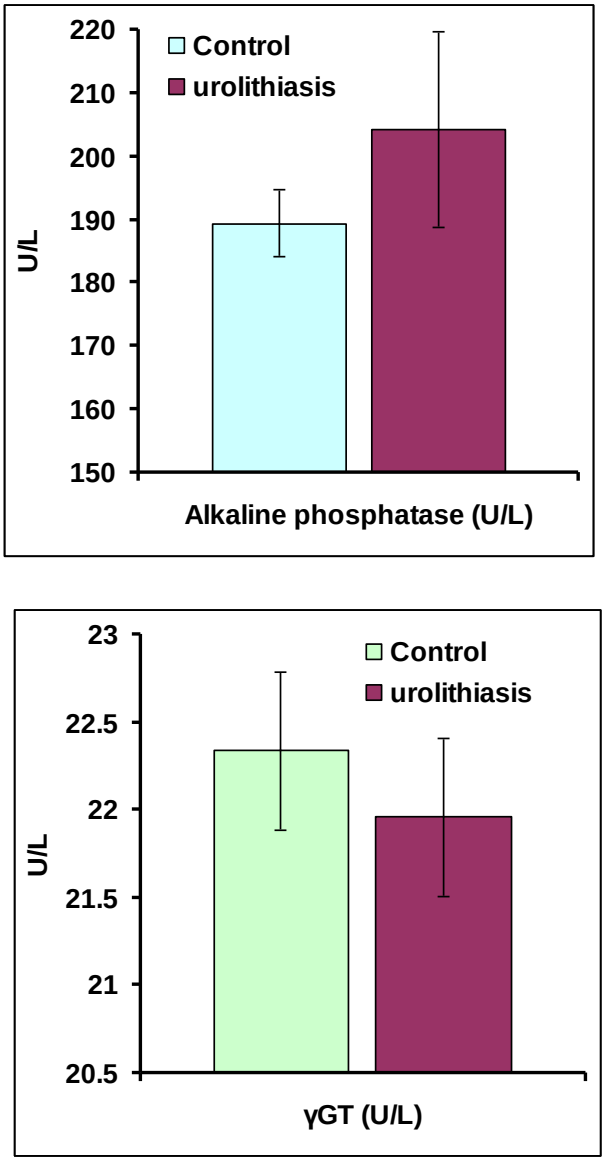

Fig. 18: Serum alkaline phosphatase and γGT activities in control and cattle suffering from urolithiasis.

Table 5: Results of physical examination of urine from the examined cases.

Physical Exam. / Diseases	No.	Color	Odor	Foam	Transparency
Nephrosis	23	yellow	Uriniferous	-ve	cloudy to turbid
Glomerulonephritis and interstitial nephritis	23	Whitish to yellow	Putrid and some cases ammoniacal	-ve	Cloudy to turbid
Cystitis	23	Whitish	Putrid	-ve	Clear to cloudy
Nephrolithiasis	5	Whitish to dark yellow	Ammoniacal	-ve	Cloudy to turbid
Control	23	Yellow	Uriniferous	-ve	Clear

No.= Number of cases

Table 6: Results of chemical examination of urine of the examined cases.

Chemical Exam. / Diseases	No. of cases	pH	Urobilinogen	Nitrite	Protein	RBCs	Glucose	Ketone Bodies
Nephrosis	23	7–8	normal	Positive	+ve	+ve	-ve	-ve
Glomerulonephritis and interstitial nephritis	23	7–8	normal	Negative	+ve	-ve	-ve	-ve
cystitis	23	7– 8	normal	Positive	+ve	+ve	-ve	-ve
Nephrolithiasis	5	6	normal	Negative	+ve	+ve	-ve	-ve
Control	23	8	normal	Negative	-ve	-ve	-ve	-ve

Table 7: Microscopic examination of urine sediment of the examined cases.

Microscopic Exam. / Disease	No. of cases	RBCs/HPF	WBCs/HPF	Epithelial cells/HPF	Crystals	Casts
Nephrosis	23	30 - 40	15 – 20	5 – 10	Amorphous phosphate, Triple phosphate.	-
Glomerulonephritis and interstitial nephritis	23	8 – 10	10 – 12	3 – 8	Amorphous phosphate, Triple phosphate.	-
Cystitis	23	8 – 15	20 – 25	6 – 12	Amorphous phosphate, Triple phosphate.	-
Nephrolithiasis	5	12 – 15	4 – 6	5 – 7	Amorphous phosphate, Calcium oxalate.	-
Control	23	3 – 5	2 – 4	1 – 3	-	-

Table 8: Types and number of single bacterial isolates from urine of the examined cases.

Type of isolates	Number
E. coli	18
Corynebacterium renale	25
Staph. sp.	9
Strep. sp.	6
Micrococcus	16

Table 9: The relation between the results of bacterial isolation and urinary disorders in diseased cattle.

Lesions	Bacterial isolates				
	E. coli	*Corynebacterium renale*	*Staph. sp.*	*Strep. sp.*	*Micrococcus*
Nephrosis	5	7	3	3	5
Glomerulonephritis and interstitial nephritis	4	8	2	1	8
Cystitis	7	8	3	2	3
Nephrolithiasis	2	2	1	-	-

IV. Histopathological findings:

IV.1. Kidney:

The following pathological lesions were observed in the examined kidneys. These include :

IV.1.A. Nephrosis

Twenty-three animals were affected with nephrosis (23.7 % of all diseased cases .

Grossly:

The kidneys were diffusely pale in color and somewhat enlarged.

Microscopically:

The affected kidneys showed massive degeneration and sloughing of the renal tubular epithelium (Fig. 19) with appearance of sloughed degenerated epithelial cell casts in the renal tubules in some cases (Fig. 20). In, other cases the affected kidneys showed multiple hemorrhages (Fig. 21). In some cases, kidneys showed massive degeneration and sloughing of the renal tubular epithelium in the renal medulla (Fig. 22) and (Fig. 23). In other cases, the renal tubular epithelium undergoes necrosis, and the necrobiosis which may involve some renal glomeruli (Fig. 24). In some cases, kidneys showed necrosis in both renal tubules and glomeruli associated with hemorrhage (Fig. 25).

IV.1.B. Nephritis:

IV.1.B.1. Focal interstitial nephritis:

Twelve animals were affected with focal interstitial nephritis (12.3 % of all diseased cases).

Grossly:

the kidneys showed small multiple white foci distributed all over the renal capsule and in the renal parenchyma

Microscopically:

The affected kidneys showed multiple focal areas of cellular reaction associated with necrosis of the glomeruli and renal tubules (Figs. 26 & 27). The cellular reaction was of mononuclear type such as monocytes and lymphocytes (Fig. 28). The focal cellular reaction was observed either in the interstitial tissue at the renal cortex (Fig. 29), renal medulla (Fig. 30) or appear as periglumerular infiltration (Fig. 31). Segmentation of glomerular tuft was also seen in some cases (Fig. 32).

IV.1.B.2. Glomerulonephritis:

Eleven animals were affected with glomerulonephritis (11.3% of all diseased cases.

Grossly:

The kidneys were darkly congested and enlarged in size.

Microscopically:

The affected kidneys showed a marked swelling of the glomerular tuft, filling the glomerular corpuscles and the glomeruli became more prominent (Fig. 33). The swelling in the tuft of the capillaries in the glomeruli was due to proliferation of the cellular components as endothelial cell lining, epithelial cells covering as well as the mesangial cells leading to the hypercellularity appearance (Fig. 34).

The reaction in the glomeruli was associated with congestion of the glomerular tuft and interstitial capillaries as well as degeneration of the renal tubular epithelium (Fig. 35).

IV.1.C. Renal calculi:

Five animals were affected with renal calculi (5.1 % of all diseased cases).

Grossly:

Small calculi (stones) were observed in the renal pelvis and renal medulla.

Microscopically:

Multiple small calculi of microscopic dystrophic calcification (nidus) were observed mainly in the renal medulla (Fig. 36-38). The calculi were associated with congestion of the interstitial blood vessels and degeneration of the surrounding tubular structures (Fig. 39). Some cases showed severe necrosis in the renal parenchyma surrounding the calculus (Fig. 39). The renal calculi took the basophilic staining (blue discoloration) due to its acid radicals (phosphates, carbonates, etc). (Fig. 39).

IV.2. Urinary bladder:

The following pathological lesions were observed in the examined urinary bladder.

IV.2.A. Cystitis:

Twenty-three animals were affected with chronic cystitis (23.7 % of all diseased cases).

Grossly:

the wall of the bladder was extremely thickened with a marked mucosal corrugation.

Microscopically:

The wall of some affected urinary bladder showed diffuse thickening of due to diffuse monocytic and lymphocytic cellular reaction associated with connective tissue proliferation. The cellular reaction was seen either in the subepithelial tissues or deeper in the lamina propria (Figs. 40 and 41).

Other affected urinary bladder showed necrosis and sloughing of the transitional epithelial lining (Fig. 42). The blood vessels in the lamina propria and subepithelial tissue appeared congested in association with thickening of the tissue in the lamina propria due to interstitial edema (Fig. 43).

The wall of other cases showed multiple polypoid formation in the bladder mucosa with epithelial covering and fibrovascular core (Fig. 44). These changes were usually associated with focal cellular reaction either in the core of the polyps or in the thickened lamina propria (Fig. 45).

The wall of some cases showed Polypoid Cystitis associated with appearance of hyperplasia of the bladder epithelium with diffuse monocytic and lymphocytic cell reaction in the subepithelial tissue of the bladder mucosa (Fig. 46).

Other affected urinary bladder showed follicular cystitis associated with appearance of focal cellular reaction in the lamina propria as well as congestion of the blood vessels and edema in the bladder wall (Fig. 47).

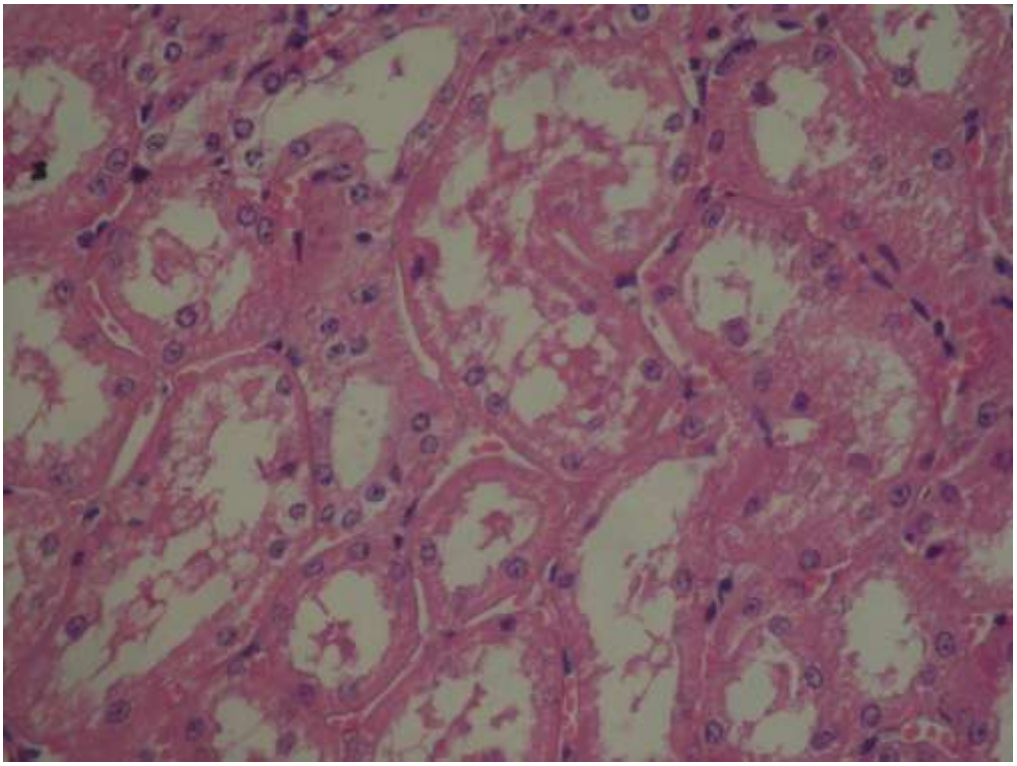

Fig. 19: Kidney from cattle showing nephrosis in cattle showing massive degeneration and sloughing of renal tubular epithelium. H&E. 10×40.

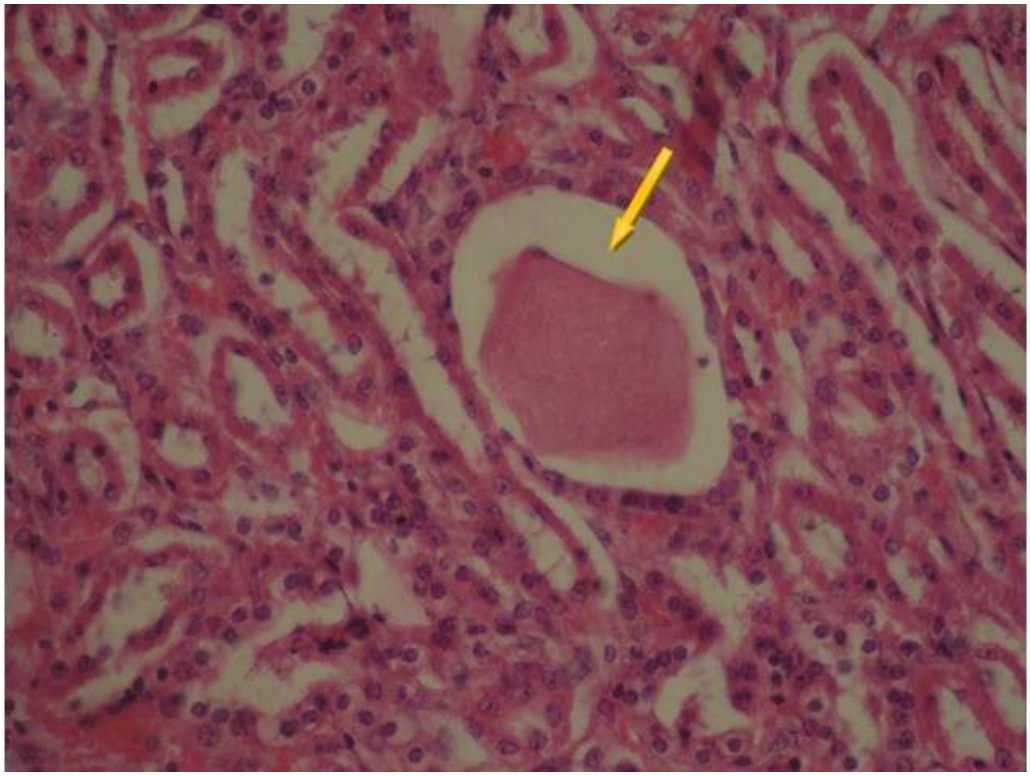

Fig. 20: kidney of cattle showing nephrosis with appearance of degenerated epithelial cell casts in the renal tubules (arrow). H&E. 10×40.

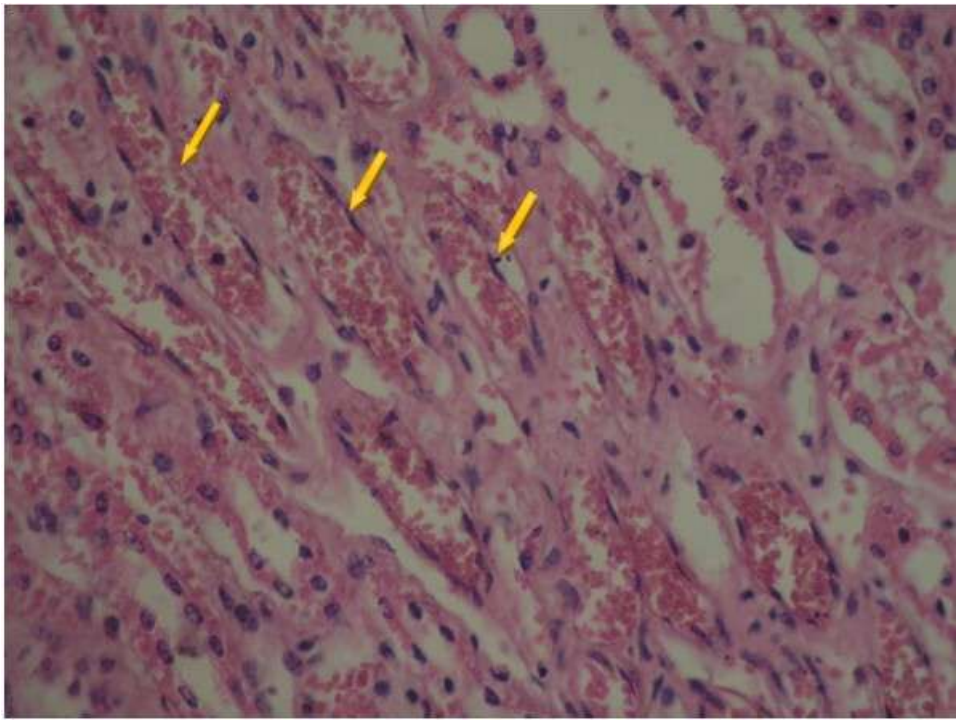

Fig. 21: kidney of cattle showing nephrosis with appearance of multiple hemorrhages (arrows). H&E. 10×40.

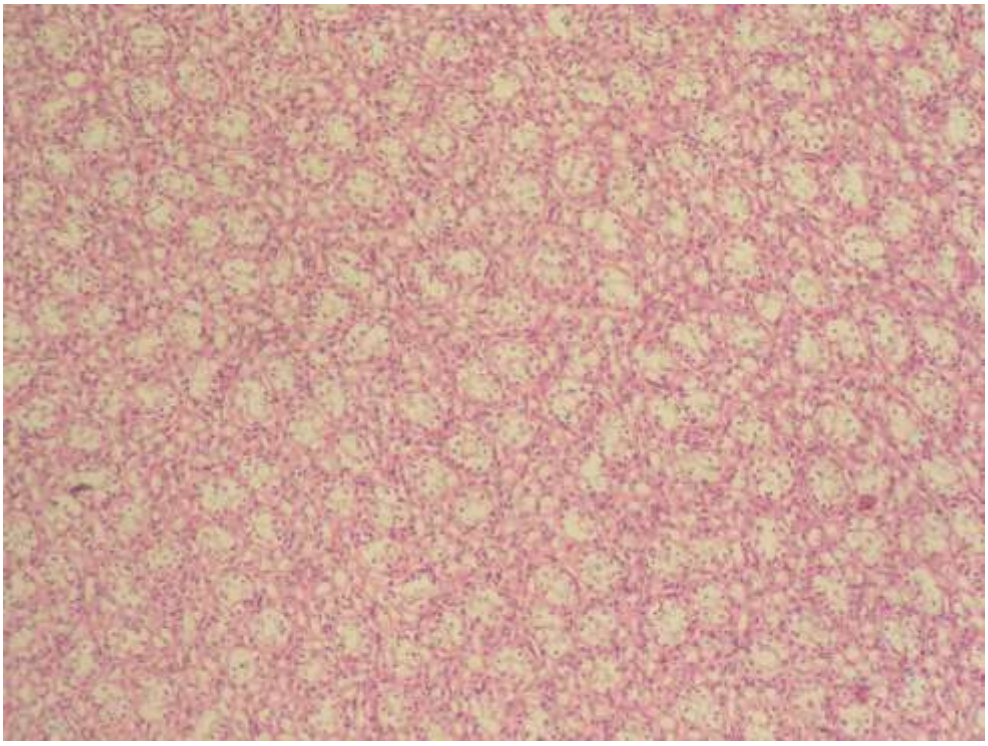

Fig. 22: Kidney of cattle showing nephrosis with massive degeneration and sloughing of renal tubular epithelium in the renal medulla. H&E. 10×10.

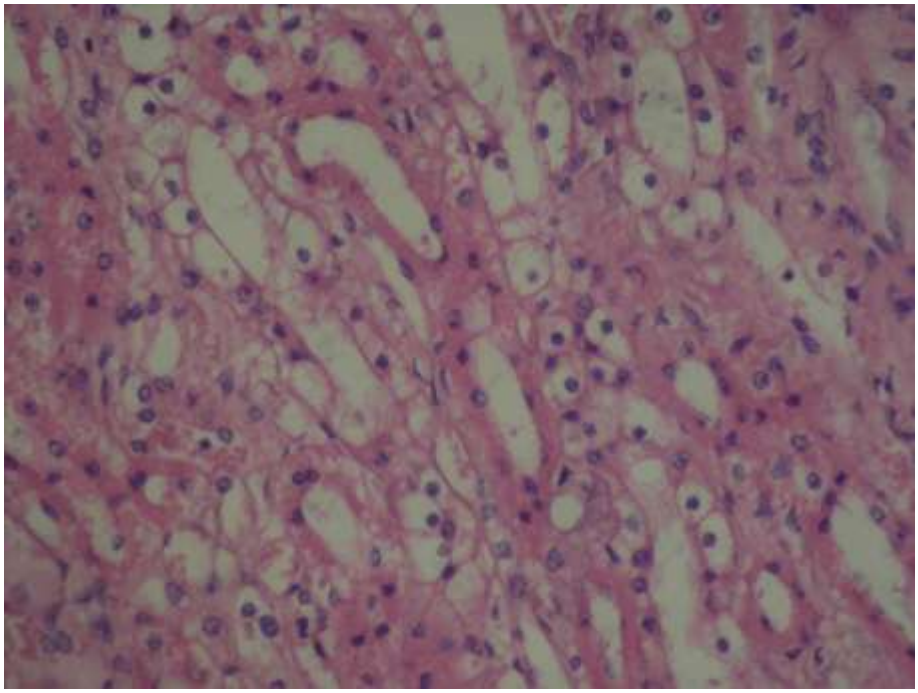

Fig. 23: High magnification of nephrosis in cattle showing massive degeneration and sloughing of renal tubular epithelium in the renal medulla. H&E. 10×40.

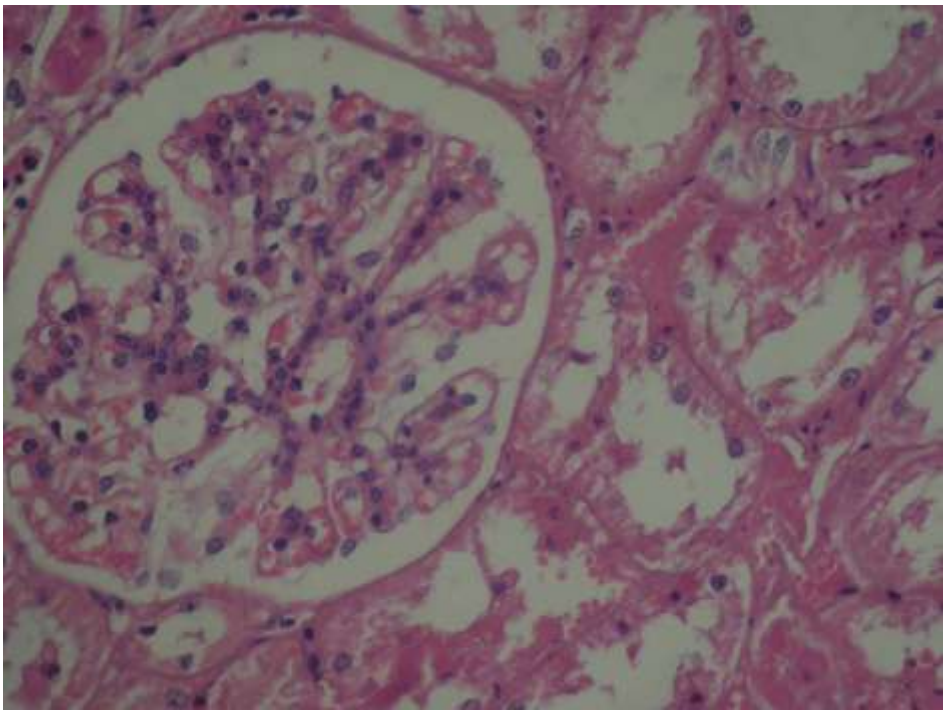

Fig. 24: High magnification of nephrosis in cattle showing necrosis in both renal tubules and glomeruli. H&E. 10×40.

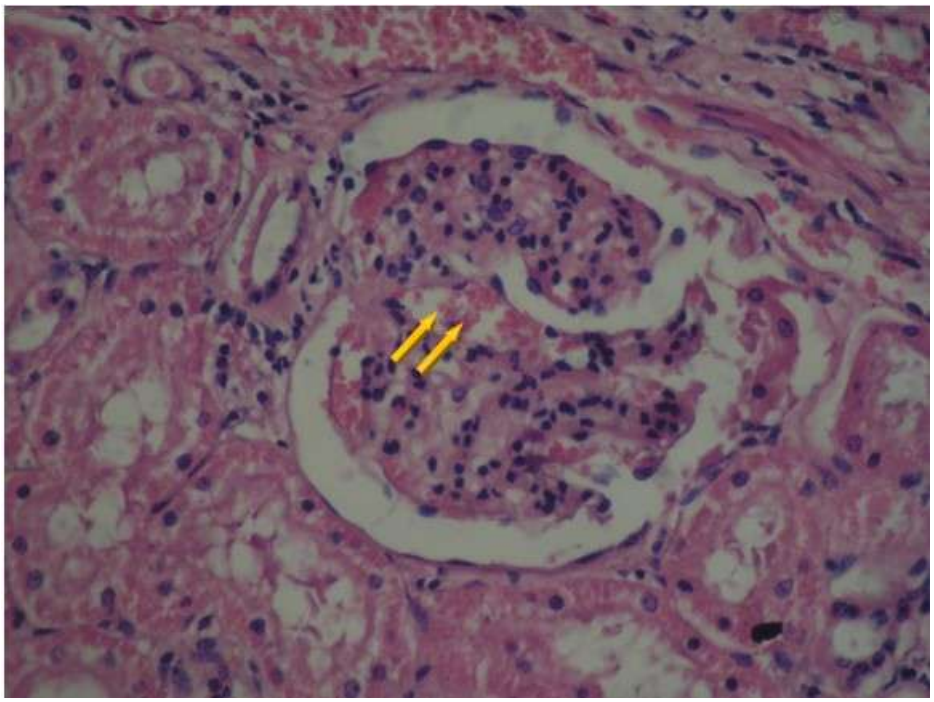

Fig. 25: High magnification of nephrosis in cattle showing necrosis in both renal tubules and glomeruli associated with hemorrhage (arrows). H&E. 10×40.

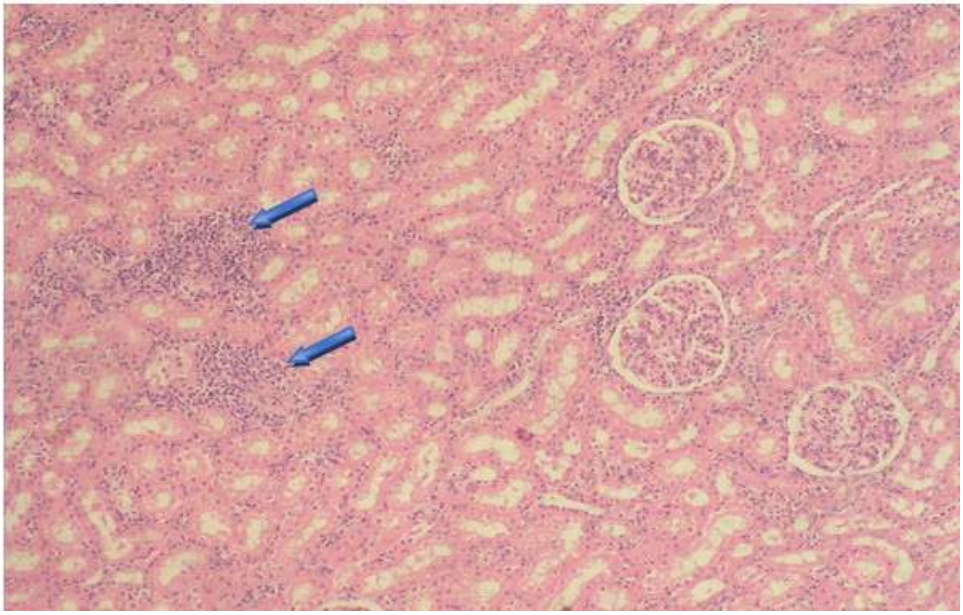

Fig. 26: Focal interstitial nephritis in cattle showing multiple focal areas of cellular reaction (arrows) with necrobiosis of the glomeruli and renal tubules. H&E.10×10.

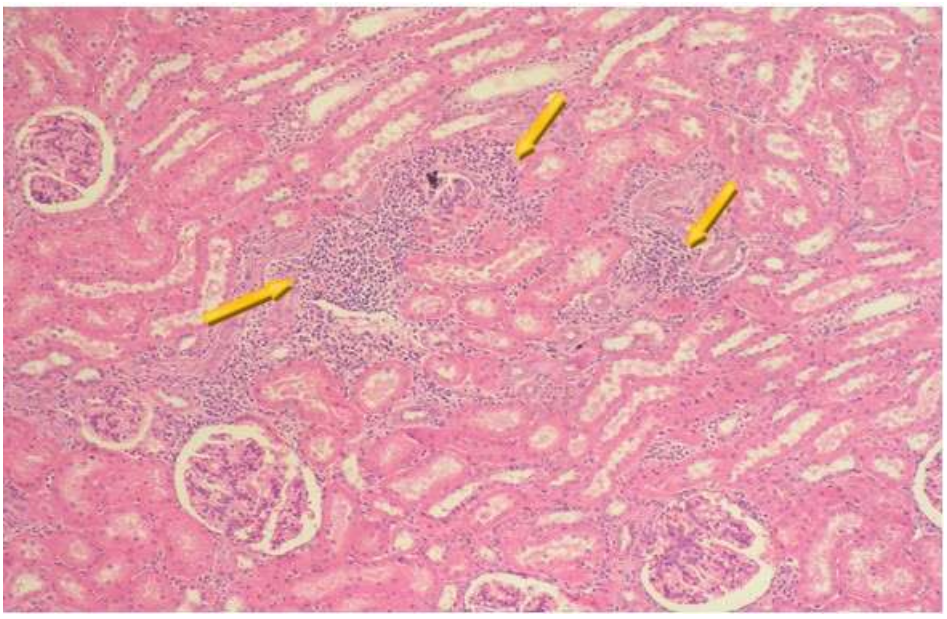

Fig. 27: Kidney of cattle showing focal interstitial nephritis with appearance of multiple focal areas of cellular reaction (arrows) with necrobiosis of the glomeruli and renal tubules.H&E.10×10.

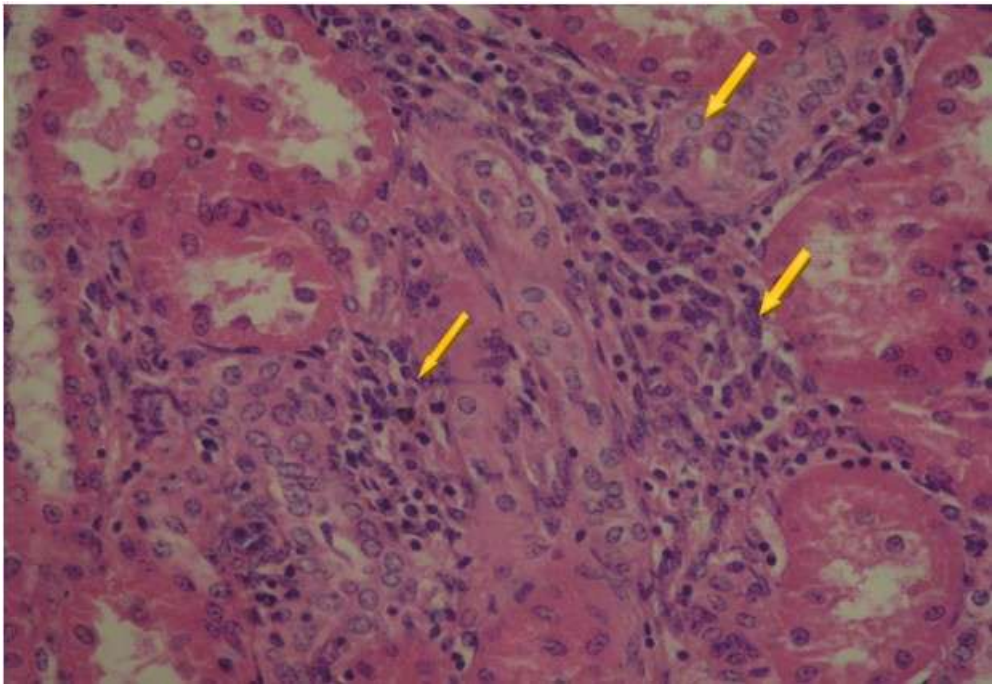

Fig. 28: Focal interstitial nephritis showing focal mononuclear reaction in the renal cortex (arrows) as well as tubular degeneration.H&E.10×40.

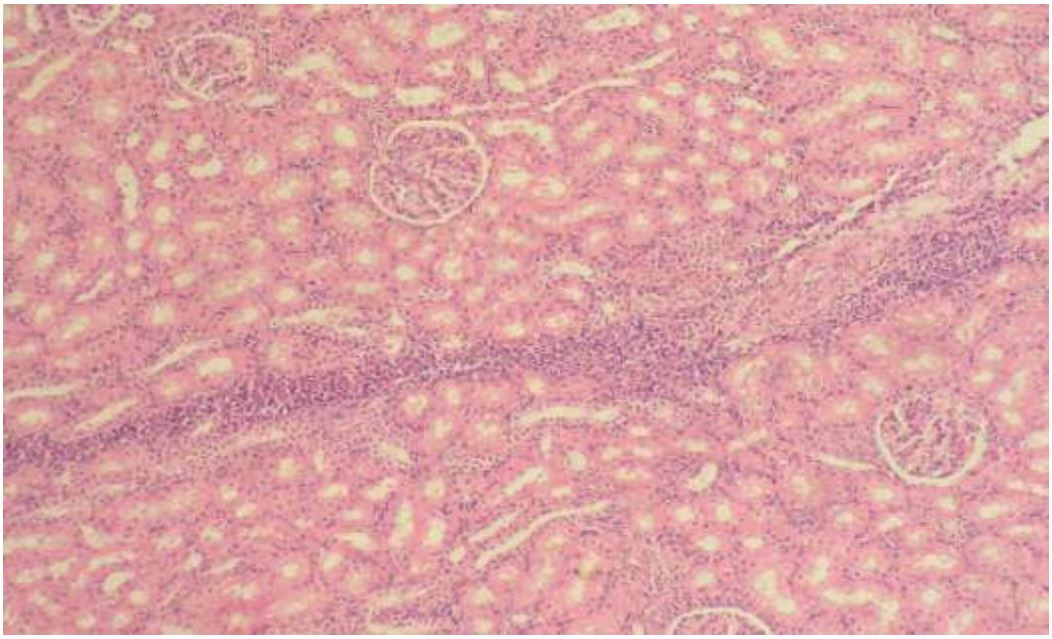

Fig. 29: Kidney of cattle showing focal interstitial nephritis in cattle with appearance of sheets of mononuclear cells in the renal cortex. H&E.10×10.

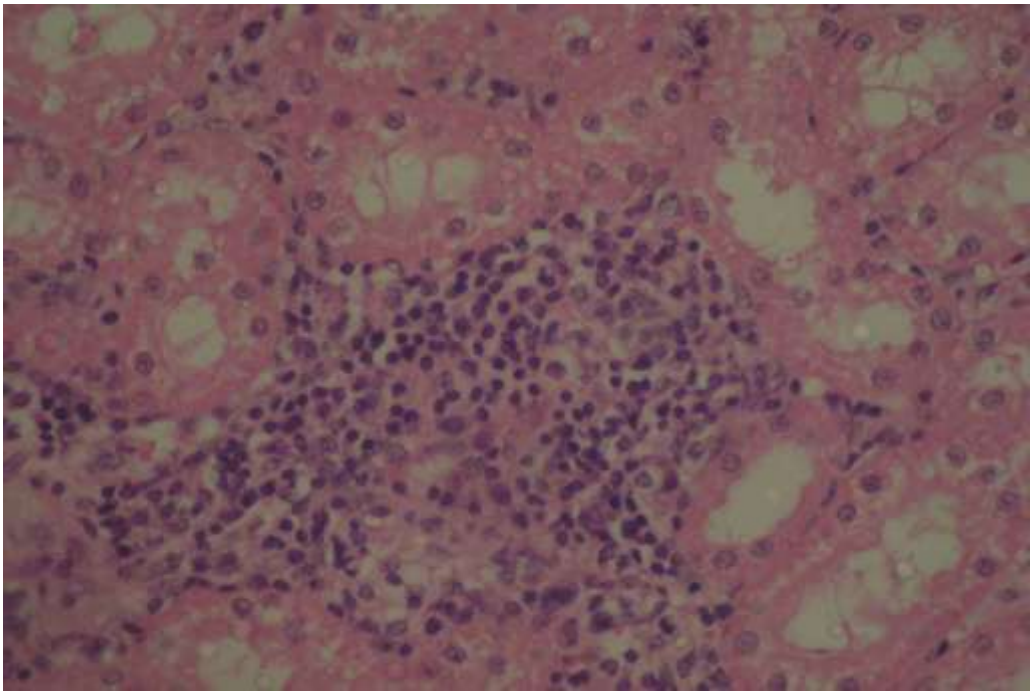

Fig. 30: High magnification of focal interstitial nephritis in cattle showing focal aggregation of mononuclear cells in the renal medulla. H&E.10×40.

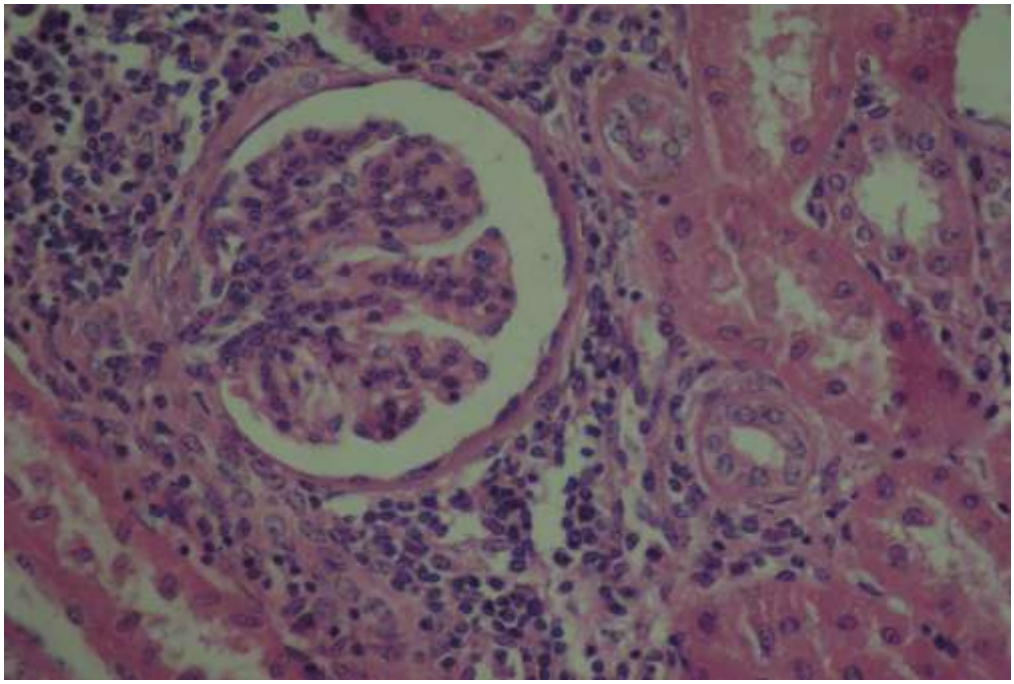

Fig. 31: High magnification of focal interstitial nephritis in cattle showing periglomerular infiltration of mononuclear cells. H&E.10×40.

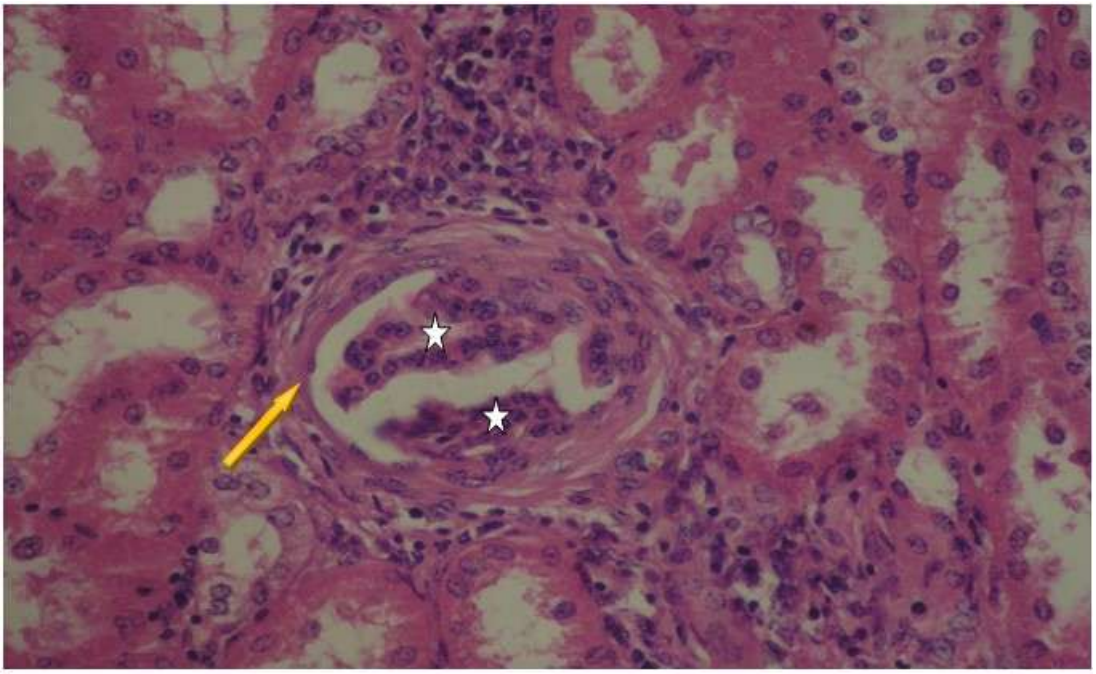

Fig. 32: High magnification of interstitial nephritis in cattle showing appearance of focal areas of cellular reaction associated with segmentation and necrosis of the glomerular tuft (Stars) and periglomerular fibrosis (arrow). H&E.10×40.

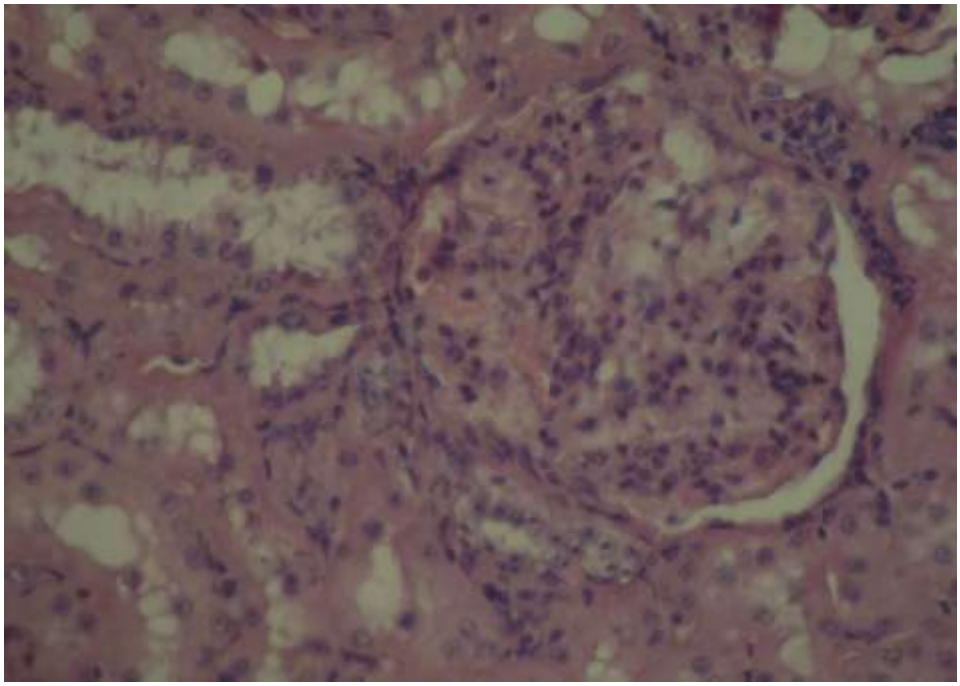

Fig. 33: Kidney from cattle showing glomerulonephritis with swelling of the glomerular tuft, filling the glomerular corpuscles. H&E. 10×40.

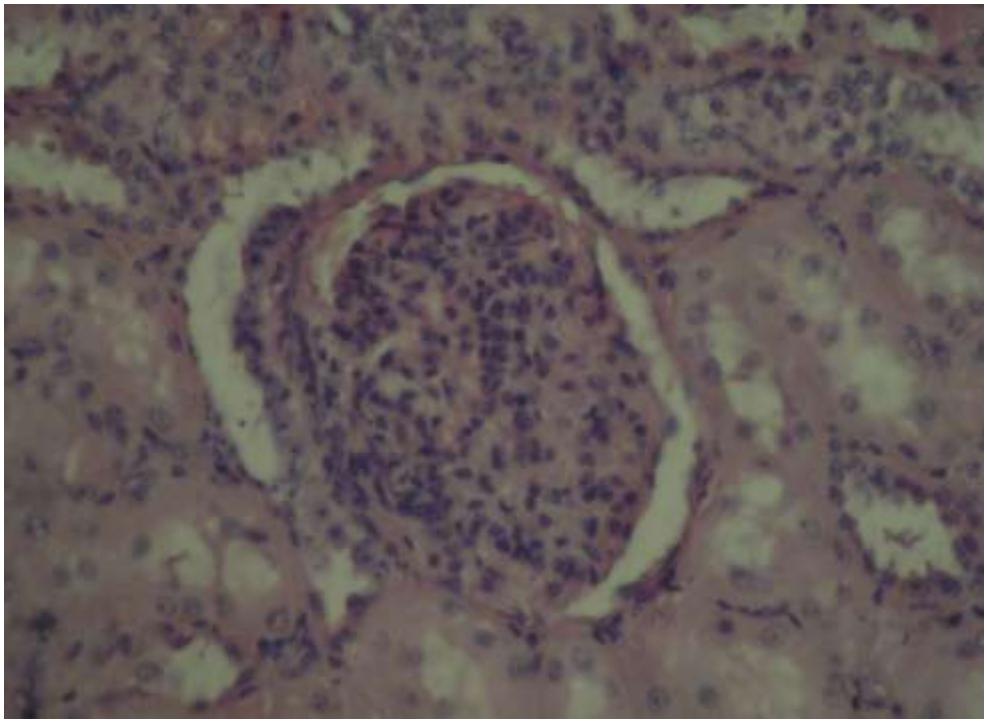

Fig. 34: Kidney from cattle showing glumerulonephritis with hypercellularity of the glomerular tuft. H&E.10×40.

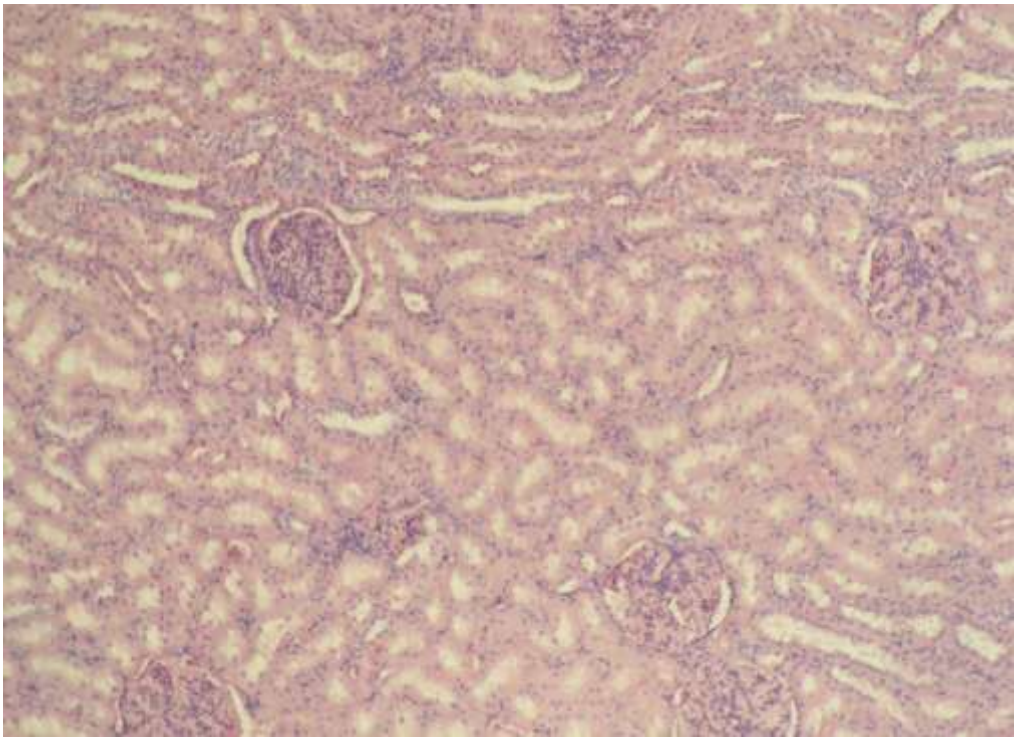

Fig. 35: Kidney from cattle showing glomerulonephritis with congestion of the glumerular and interstitial capillaries as well as degeneration of the tubular epithelium. H&E.10×10.

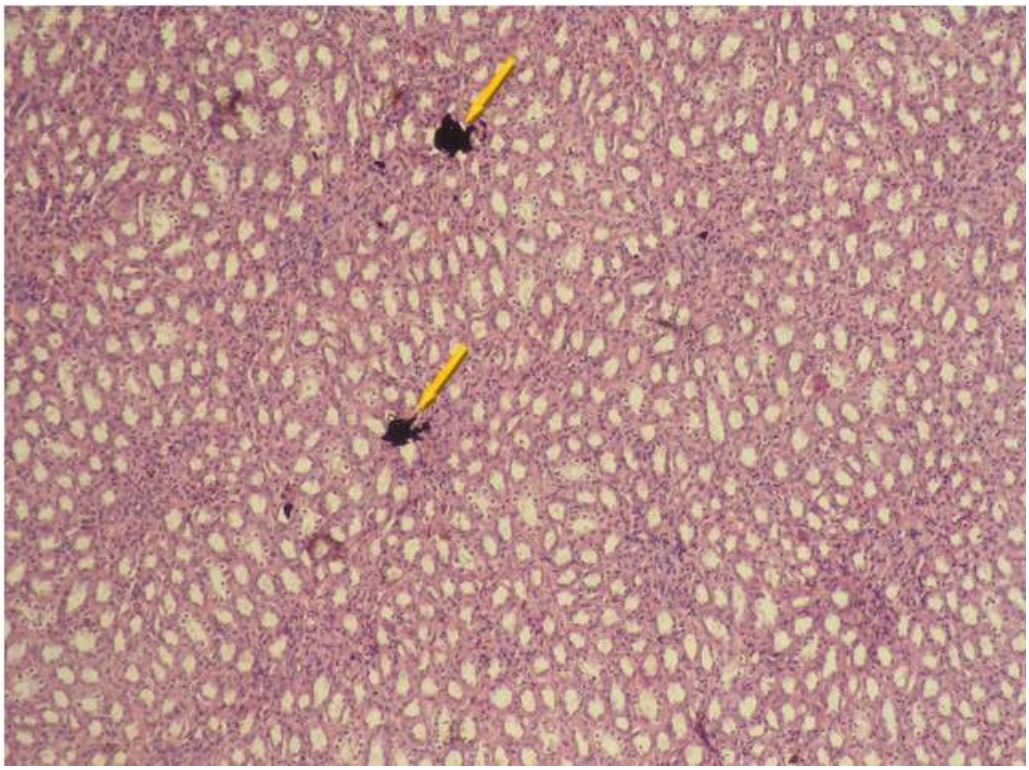

Fig. 36: Kidney of cattle showing multiple microscopic dystrophic calcification (nidus) in the renal medulla (Arrows). H&E.10×10.

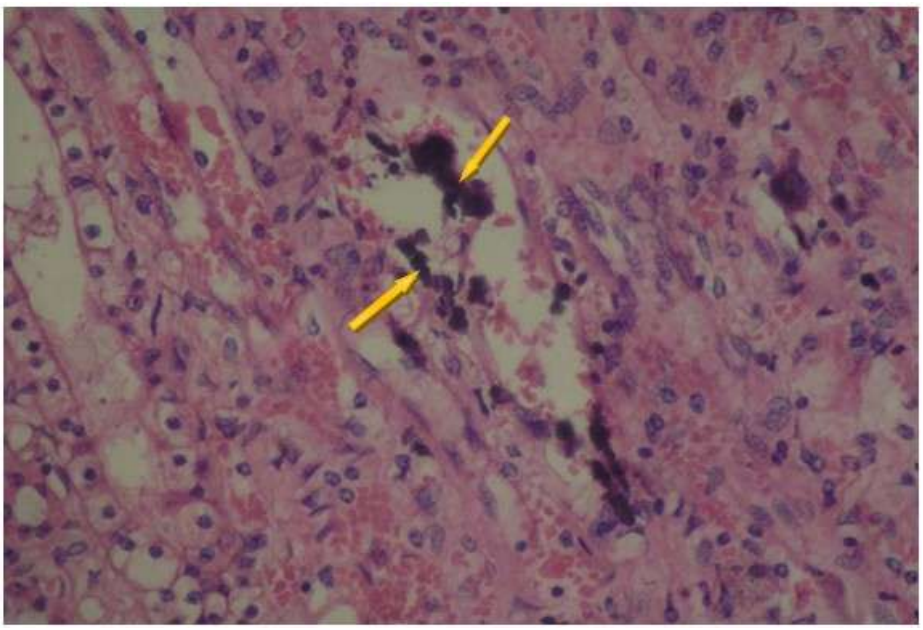

Fig. 37: Dystrophic calcification in the renal medulla in the kidney of cattle (Arrows). Note the congestion of surrounding blood vesseles and necrobiosis in the tubular epithelial cells.H&E.10×40.

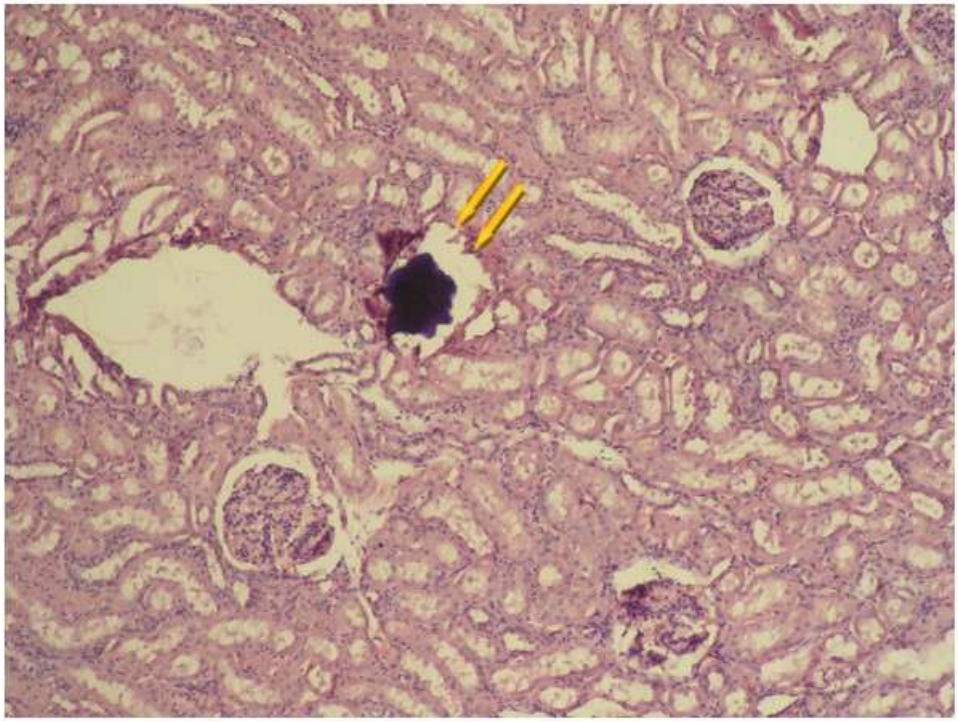

Fig. 38: Dystrophic calcification in the kidney of cattle with sever necrosis of the serrounding tissues (arrows). H&E.10×40.

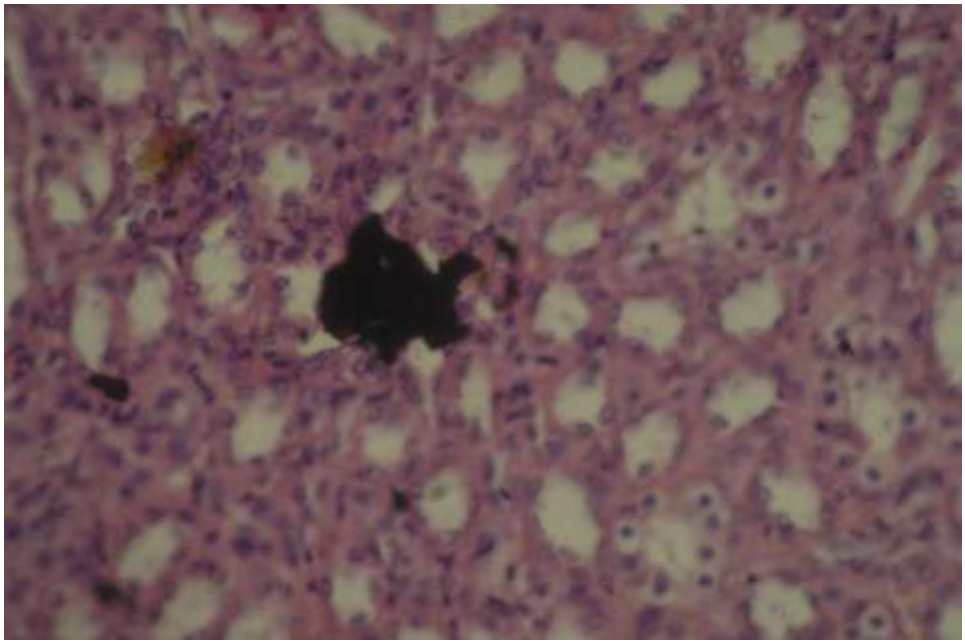

Fig. 39: Miroscopic renal calculi in the kidney of cattle, the renal calculi taking the basophilic staining (bluish discolouration) observed in the renal medulla. The calculi surrounded with cellular reaction.H&E.10×40.

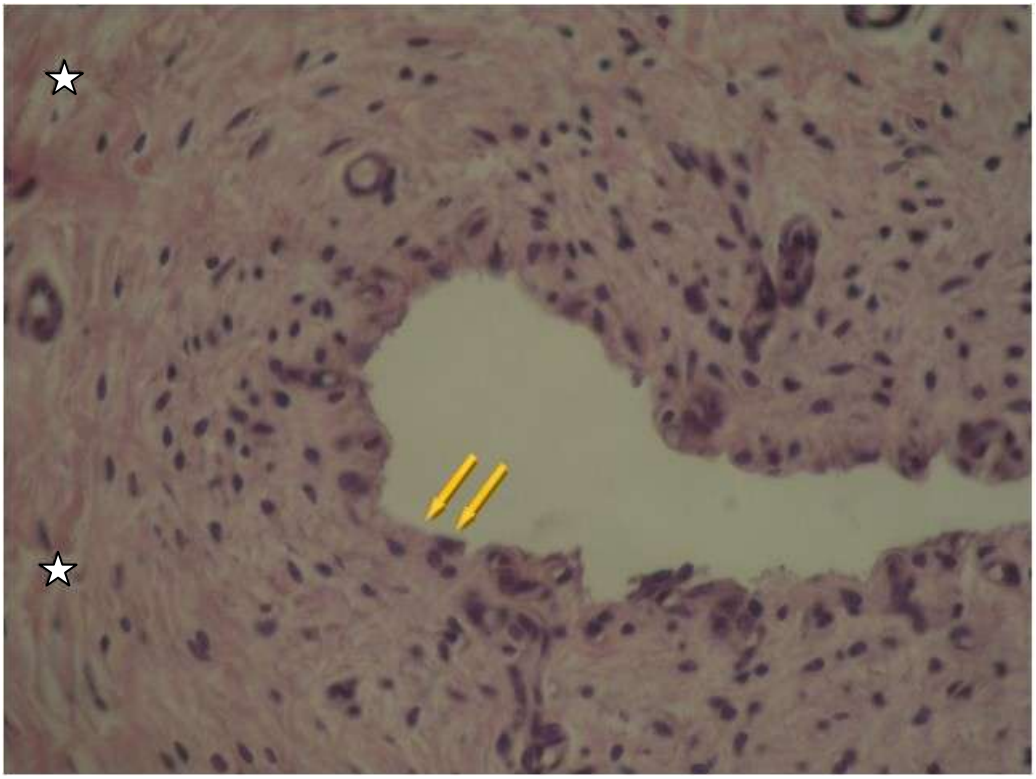

Fig. 40: Diffuse cystitis in cattle showing edema in the lamina propria (Stars) as well as necrosis and sloughing of the epithelial linning the mucosa (Arrows). H&E. 10×40.

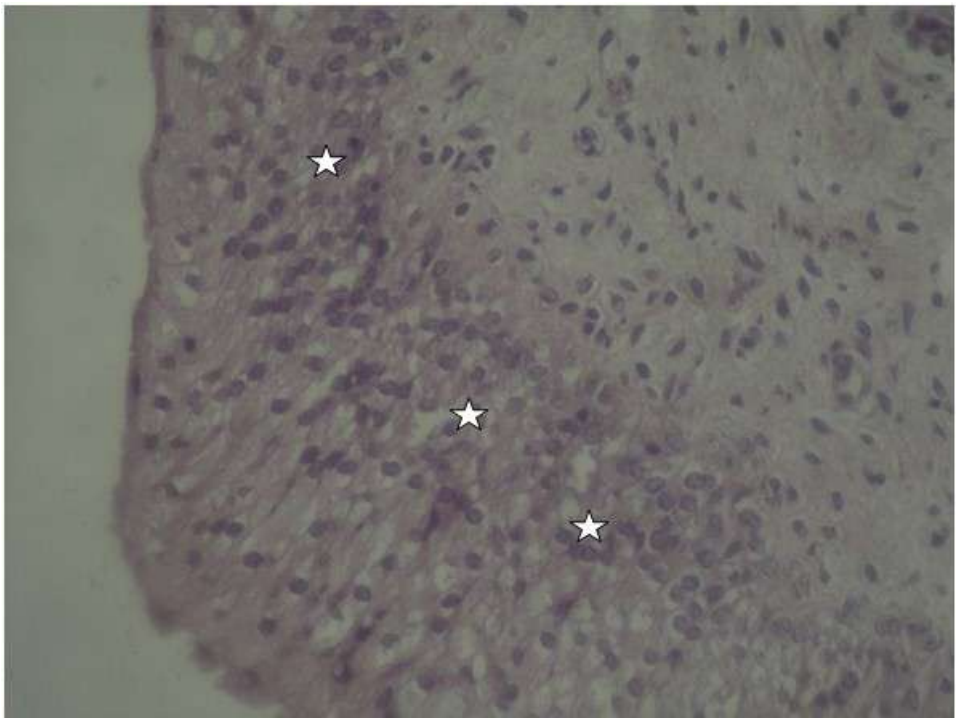

Fig. 41: Diffuse cystitis in cattle showing diffuse cellular reaction in the bladde wall (Stars). H&E. 10×40.

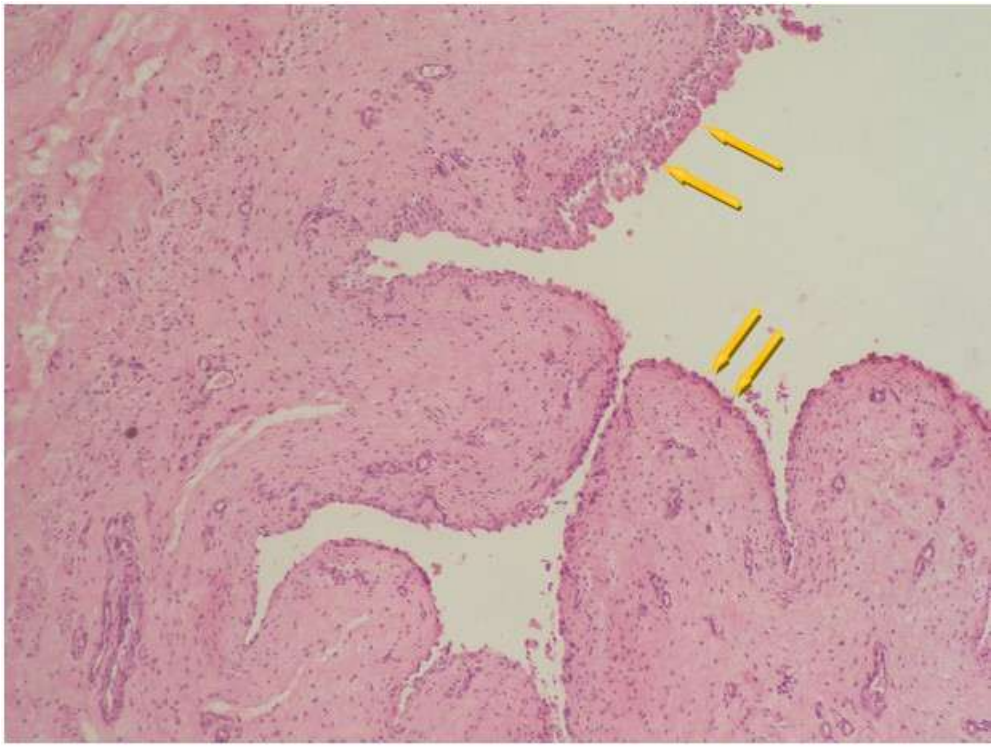

Fig. 42: Diffuse Cystitis in cattle showing necrobiosis and sloughing of the transitional epithelial cell linning (Arrows). H&E. 10×10.

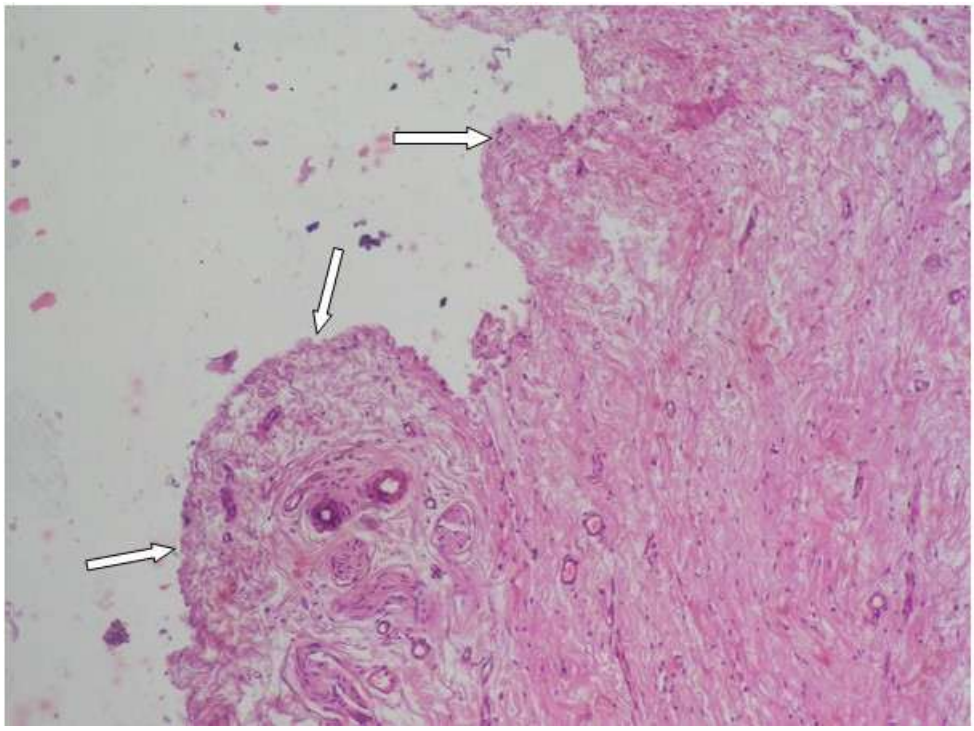

Fig. 43: Diffuse Cystitis in cattle showing diffuse necrosis in the entire wall of the bladder and sloughing of the transitional epithelial cell linning (Arrows). H&E. 10×10.

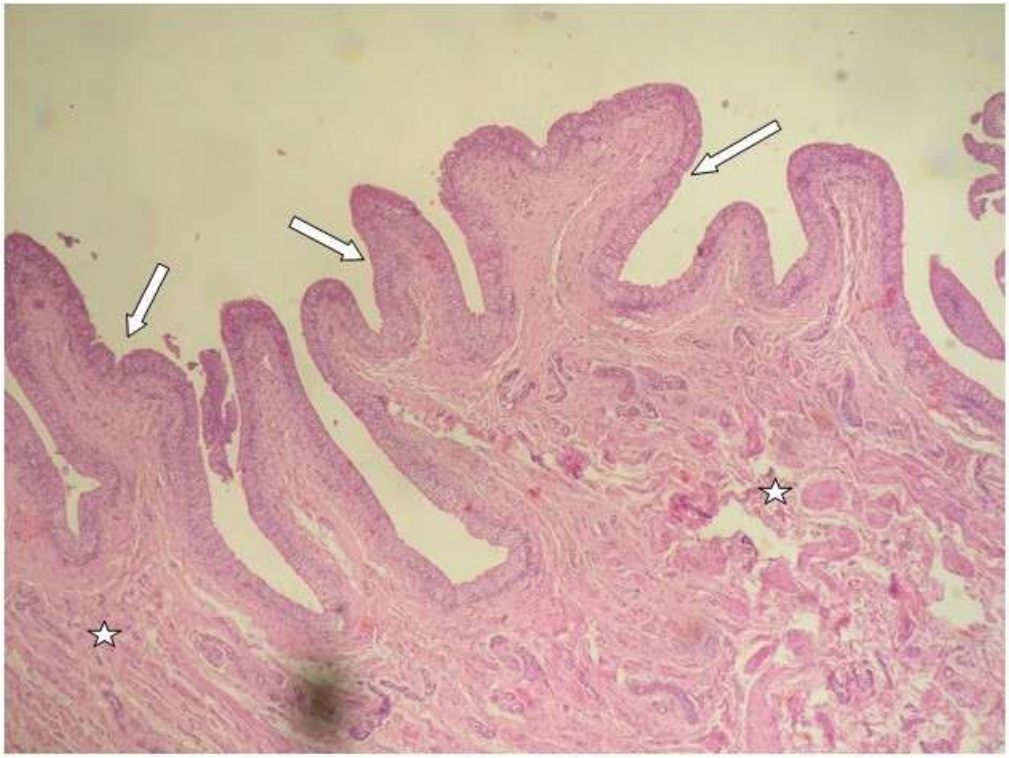

Fig. 44: Polypoid Cystitis in cattle showing polypoid formation (Arrows) of the bladder mucosa with fibrovascular wall (Stars). H&E. 10×10.

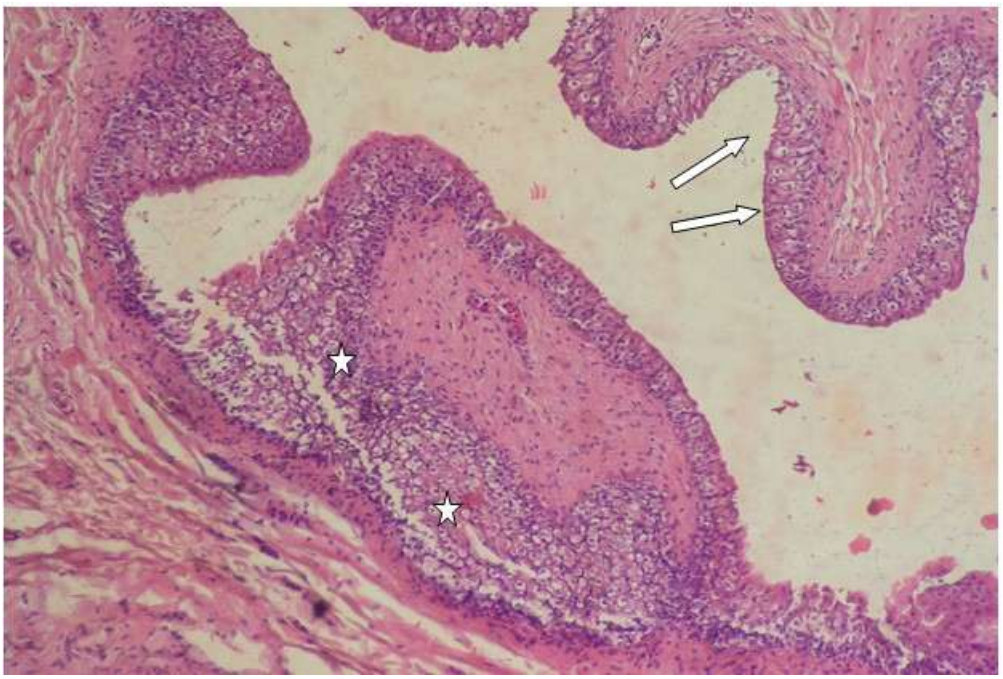

Fig. 45: Polypoid Cystitis in cattle with polypoid formation in the bladder wall (Arrows) associated with focal cellular reaction in the lamina propria (Stars). H&E. 10×10.

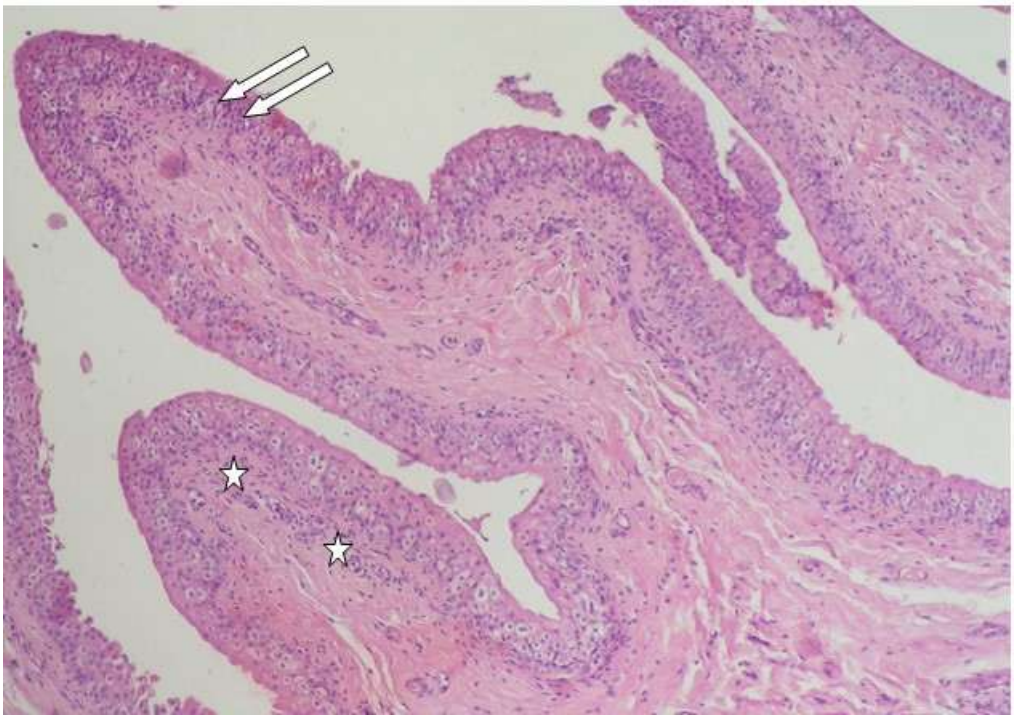

Fig. 46: Polypoid Cystitis in cattle showing hyperplasia of the bladder epithelium (Arrows) with diffuse monocytic and lymphocytic cell reaction in the subepithelial tissue in the bladder mucosa (Stars). H&E. 10×10.

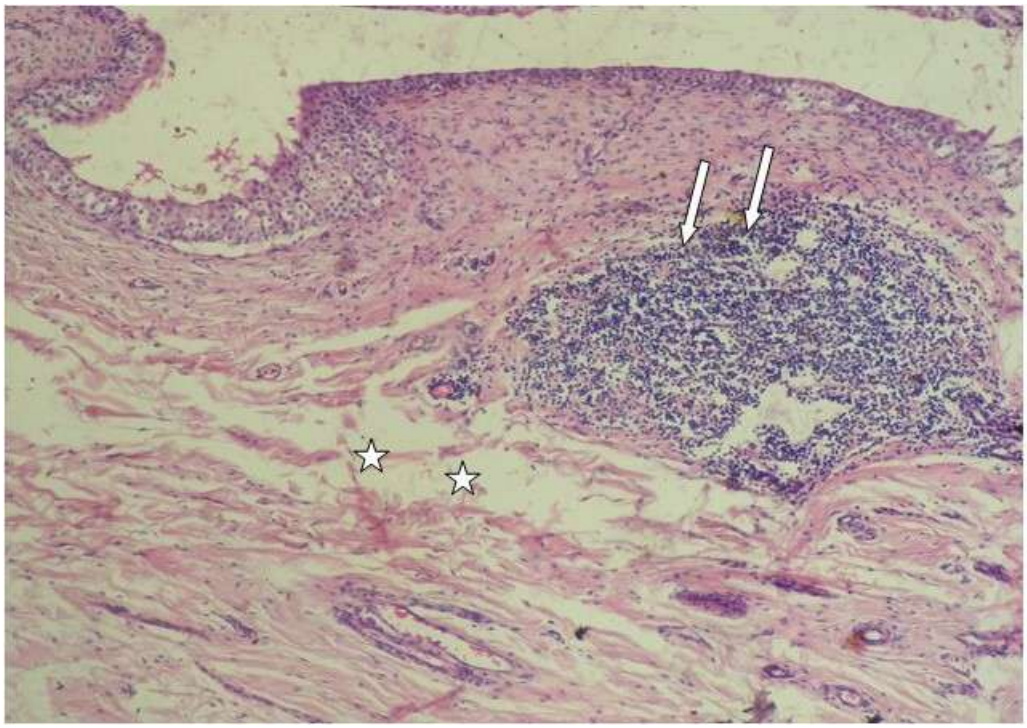

Fig. 47: Follicular cystitis in cattle showing focal cellular reaction in the lamina propria (Arrows) as well as congestion of the blood vessels and edema (Stars) in the bladder wall. H&E. 10×10.

DISCUSSION

DISCUSSION

No abnormal clinical signs were observed on any of the affected cases in the present study. Renal diseases are infrequently diagnosed in bovine practice, although kidney lesions are often detected in clinically healthy animals at slaughter. In practice, only advanced renal cases can be detected clinically because subtle clinical signs are usually found during the initial stages of disease (Radostits *et al*., 2006).

A complete diagnostic evaluation of renal conditions requires the determination of serum biochemical variables, such as creatinine, urea, and albumin, and urinalyses. However, urinalyses are rarely performed in bovine practice, and many early or mild renal symptoms/indicators are undiagnosed (Radostits *et al*., 2006).

Results from the present study showed that the blood serum biochemical analysis in cattle suffering from nephrosis had a significant increase in blood urea ($p = 0.03$) and creatinine ($P < 0.001$) levels, and non-significant changes in serum total protein, albumin, globulin, alkaline phosphatase and γGT. The mechanism responsible for glomerular injury with an ensuing nephrosis in cattle is probably multifactorial and is poorly understood (Johnson and Jamison, 1984). It can be difficult to establish renal disease by carrying out biochemistry. Normally, blood urea and serum creatinine are used to estimate the glomerular filtration rate. However, in ruminants, urea is recycled in a functional rumen, which can make it difficult to interpret blood urea (Beekhuis, 2022). Protein-losing nephropathy is a characteristic phenomenon in kidney diseases (Radostits *et al*., 2006). Yeruham *et al*. (2006) found differences in total protein and several protein fractions were found between affected and healthy animals. Plasma albumin is not normally lost in the urine to any appreciable extent, but certain diseases feature large

urinary losses of proteins with corresponding decreases in circulating levels (Kirschbaum, 2001; Peluso and Raguzzini, 2016). Kaneko *et al*. (2008) specified that kidney parenchymal damage might cause a change in urinary enzyme activity but not in serum activity that changes only when the lesion has already invaded most of the kidney's parenchyma. Aliyu *et al*. (2017) and Vysakh *et al*. (2017) found increased alkaline phosphatase activity and hepatic enzymes in cattle and experimental animals with kidney diseases.

In this study, blood urea and serum creatinine levels showed significant increases in cattle suffered from glomerulonephritis and associated with non-significant differences in serum total protein, albumin, and globulin levels, and in alkaline phosphatase and γGT activities. The elevation in blood urea and serum creatinine levels in case of nephritis agreed with several studies (Braun *et al*., 2008; Vysakh *et al*., 2017 and Ihedioha *et al*., 2019), and also come in agreement with those reported by Radostits *et al*., (2006), Niraj *et al*. (2009), Braun *et al*. (2008), Kaneko *et al*. (2008). Similar results were also obtained by Otter (2013), El-Deeb and Buczinski (2015), Vysakh *et al*. (2017), Aliyu *et al*. (2017), Gosselin *et al*. (2018), Ihedioha *et al*. (2019) and Elgioushy *et al*. (2020), Ruslie *et al*. (2021). However, Katsoulos *et al*. (2020) found that serum biochemical profiles did not show any suspicion of subclinical renal disease in cattle; all animals had serum albumin, BUN, and creatinine concentrations within the reference intervals.

Significant increase in serum creatinine level was the classical findings in case of cystitis and urolithiasis, measuring serum urea and creatinine is the first step for diagnosing the defect in excretion of metabolic wastes from the body and post-renal uremia might be sequelae to obstructive urolithiasis. Sharma *et al*. (2006) owed the increased levels of serum urea and creatinine in intact bladder group to decreased glomerular filtration rate because of back pressure on the kidneys.

Serum tests depend on either the accumulation in cases of renal insufficiency, of metabolites normally excreted by the kidney or the excretion of endogenous substances by the kidney. Determinations of blood urea and creatinine concentration are essential components of an evaluation of the urinary system. These serum indices of function are simple estimates of glomerular filtration because urea and creatinine are freely filtered by the glomerulus. Serum concentrations of urea and creatinine do not rise appreciably above the normal range until 60-75% of nephrons are destroyed. Blood urea and creatinine concentrations are influenced by blood flow to the kidneys. They also suffer from the disadvantage that their serum concentrations can vary with the rate of protein catabolism (Radostits *et al*., 2006).

Urine analysis is one of the most important diagnostic tests that can help localize disease, determine causes of discolored urine and identify inflammatory diseases of the urinary system (Constable *et al*., 2017). In contrast with plasma, the composition of urine is more unpredictable and reflects the continuously changing environment of the body, which is affected by diet, activity, and stress among other factors (Kirschbaum, 2001; Peluso and Raguzzini, 2016).

Results of physical examination of urine samples from examined cases showed that urine color was yellow in nephrosis, white to yellow in glomerulonephritis and pyelonephritis, white in cystitis, white to dark yellow in nephrolithiasis, and yellow in the control group. The abnormal change of urine color in the affected cases may be attributed to the presence of organized and none organized elements that are responsible also for turbidity of urine and absence of transparency, these results come in agreement with previous studies (Garry *et al*., 1990; Gaspari *et al*., 2006; Grauer, 2007;

Grauer. 2011, D'amico and Bazzi, 2003; Grauer, 2011, Isani *et al*., 2018 and Probo *et al*., 2022).

The presence of abnormal leukocyte count (pyuria) indicates inflammation or tissue necrosis of the urogenital tract. The presence of nitrite indicated bacterial infection which can also be accompanied by abnormal leukocyte content in urine. Proteinuria signifies inability of renal tubules to reabsorb the protein which can be seen in a wide range of renal dysfunctions (Parrah *et al*., 2013). Similarly, Krishnan *et al*. (2018) found that in chronic kidney diseases mostly characterized by proteinuria and inflammatory conditions, cytoskeleton (extracellular matrix) integrity is impaired, that leads to podocyte foot process effacement.

Proteinuria is an important marker of chronic kidney disease, it can result from damage of the glomerular barrier (glomerular proteinuria), damage to the proximal tubules (tubular proteinuria), and/or interstitial nephritis. Proteinuria should be interpreted along with other findings in the urinalysis. In the absence of active sediment or grossly visible hematuria, persistent and strongly positive reactions for protein suggest renal protein loss (Gaspari *et al*., 2006; Théron *et al*., 2017 and Toblli *et al*., 2012).

The urinary tract is constantly exposed to microorganisms that inhabit the gastrointestinal tract, but generally, the urinary tract resists infection by gut microorganisms. This resistance to infection is mainly ascribed to the versatility of the innate immune defenses in the urinary tract, as the adaptive immune responses are limited, particularly when only the lower urinary tract is infected. In recent years, as the strengths and weaknesses of the immune system of the urinary tract have emerged and as the virulence attributes of uropathogens are recognized, several potentially effective and

unconventional strategies to contain or prevent urinary tract infections have emerged (Abraham *et al*., 2015).

The obtained results showed that *E. coli* was found in 18 cases, *Corynebacterium renale* was found in 25 cases, *Staphylococcus* spp. was found in nine cases, *Streptococcus* spp. was found in 6 cases, and *Micrococcus* was found in 16 cases. The results showed also that *E. coli* was found in 5 cases of nephrosis, 4 cases of glomerulonephritis and interstitial nephritis, 7 cases of cystitis and 2 cases of nephrolithiasis. *Corynebacterium renale* was found in 7 cases of nephrosis, 8 cases of glomerulonephritis and interstitial nephritis, 8 cases of cystitis and 2 cases of nephrolithiasis. *Staphylococcus* spp. was found in 3 cases of nephrosis, 2 cases of glomerulonephritis and interstitial nephritis, 3 cases of cystitis and 1 case of nephrolithiasis. Streptococcus Sp. was found in 3 cases of nephrosis, 1 case of glomerulonephritis and interstitial nephritis, 2 cases of cystitis and no case of nephrolithiasis. *Micrococcus* was found in 5 cases of nephrosis, 8 cases of glomerulonephritis and interstitial nephritis, 3 cases of cystitis and no case of nephrolithiasis. Similar results were also obtained by Yanagawa and Honda (1978); Confer and Panciera (1995); Rebhun *et al*. (1989); Radostits *et al*. (2006); Yeruham *et al*. (2006) and El-Deeb and Elmoslemany (2016). Bacteria, frequently of mixed population, were isolated from 100 animals. Gram-positive bacteria prevailed, with *Staphylococcus* spp. and *Bacillus* spp. being the most common. *Escherichia coli* and *Acinetobacter* spp. were the most frequently recovered Gram-negative bacteria (Roperto *et al*., 2012). Similarly, Maxie and Prescott (1993) reported that infection is often mixed and, *C renale* may be present in cattle with nephritis, various enteric pathogens may be of more pathogenic importance.

According to Confer and Panciera (1995), a common bacterial cause of nephritis in cattle is an ascending infection, often associated with the reflux

of infected urine from the ureters and urinary bladder into the renal pelvis. It has also been reported that catheterization of the urinary bladder may initiate bacterial damage to the kidney (Van Metre and Divers, 2002).

The biological tissues contain a variety of antioxidants mechanisms that play a central role in the protection against ROS (Halliwell, 1991). Both the enzymatic and non-enzymatic antioxidants become overwhelmed during oxidative stress, due to excessive generation of ROS (Abd Ellah, 2010).

In the present study, cattle suffered from nephrosis and cystitis showed a significant increase in serum MDA, and significant decreases in serum TAC and NO. However, in case of glomerulonephritis, there were non-significant change in serum MDA, and significant decreases in serum TAC ($p = 0.05$) and NO. The overall findings of the study indicated increased oxidative stress in blood of all examined cases, which being more severe in case of nephrosis and cystitis. These results agree with previous studies done by Araújo *et al*. (2014); El-Deeb and Buczinski (2015) ; Soleimani *et al*. (2016) ; Vysakh *et al*. (2017) ; Rodrigues *et al*. (2018) ; Gumasta *et al*. (2019) ; Miyata *et al*. (2019) ; Elgioushy *et al*. (2020) and Hassan *et al*. (2021). Despite several experimental and clinical studies having explored the intricate mechanisms between urinary system diseases and oxidative imbalance, the pathophysiological mechanisms of organ damage have not been clarified (Pellegrino *et al*., 2019).

The common gross lesions observed in this study were hyperaemia and haemorrhage. At histopathological examination of the kidneys slight to severe mononuclear cell infiltrations were commonly observed. Haemorrhage and connective tissue proliferations were also seen. Neutrophil leucocyte infiltrations caused by pyelonephritis were observed in some cases and multiple stone formations were found in these cases. Widening of the

Bowman's space was a common histopathological finding especially when the stones were big, or the inflammatory reaction was severe. Calcium deposits and eosinophilic material were found in the medullary tubules and in lumens of the renal pelvis in some kidneys. Giant cell formations around the stone reactions were rarely observed. Hyperplasia of the pelvis renalis and tubulus epithelium was another finding which occurred seldom. Whereas sand-like material accumulation seen in urinary bladders in some cases. These results agree with those reported by El-Mashad *et al*. (2019) and Katsoulos *et al*. (2020).

The obtained findings agree also with those reported by Juránek and Bezek (2005). The authors reported that tissue injury and its healing are characterized by a sequence of various events influenced by the cause of the injury and other factors, such as the intensity of the damaging agent, the type of tissue, and the condition of the whole organism. The healing process is mediated by a variety of messengers released by the immune system; for example, phagocytes produce cytotoxic agents, which not only prevent the spread of infection but also remove host cellular particles that are damaged.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

A total number of 97 male cattle (18 - 20 months old) were selected form animals slaughtered in Mallawy slaughterhouse (El-Minia governorate, Egypt) and included in this study.

Blood samples were collected from each animal by jugular vein puncture before slaughtering. Two types of blood samples were collected:

a. Whole blood samples were collected in vacutainer tubes containing EDTA as anticoagulant for separation of plasma and used for measuring plasma Malondialdehyde (MDA) level.
b. Blood samples were collected in plain vacutainer tubes without anticoagulant for separation of serum and used for measuring serum biochemical parameters.

After slaughtering, animals were inspected carefully by the meat inspectors. Animals that showing postmortem pathological lesions in other organs rather than the urinary system were excluded from the study.

Urine samples were collected directly from the bladder using a disposable 10-ml syringe and preserved in ice bag until analysis. The kidneys and urinary bladder were collected from each slaughtered animal in sterile bags. They preserved in ice bags until the macroscopic investigations and the histopathological sampling in the laboratory. Tissue specimens from the kidney and bladder were preserved in formalin 10% for histopathological examination.

Cattle subjected to study (n.=97) were classified into different groups based on the gross and histopathological examination of the kidneys and urinary bladder. The detected urinary system diseases included: Nephrosis (n.=23), Glomerulonephritis and interstitial nephritis (n.= 23),

Nephrolithiasis (n.=23), and cystitis (n.=5). Animals that showed no abnormal clinical findings and no histopathological affections were considered as the control group (n.23).

Blood serum biochemical analysis in nephrosis are presented in Table 1 and Figs. 1-5. There were significant increases in serum urea ($p = 0.03$) and creatinine ($P < 0.001$) in diseased cattle when compared with the control. There were nonsignificant differences in total protein, albumin, globulin, alkaline phosphatase and γGT between the diseased cattle and the control. There was a significant increase in serum MDA ($p = 0.003$), and significant decreases in serum TAC ($p = 0.02$) and NO ($p = 0.01$) in diseased cattle when compared with the control.

Blood serum biochemical analysis in glumrulonephritis and interstitial nephritis are presented in Table 2 and Figs. 6-9. There was a significant increase in serum urea ($p = 0.01$) and creatinine ($P < 0.001$) in diseased cattle when compared with the control. There were nonsignificant differences in total protein, albumin, globulin, alkaline phosphatase and γGT between the diseased cattle when compared with the control. There was a nonsignificant change in serum MDA ($p = 0.07$), and significant decrease in serum TAC ($p = 0.05$) and NO ($P < 0.001$) in diseased cattle when compared with the control.

Blood serum biochemical analysis in cystitis are presented in Table 3 and Figs. 11-15. There was a non-significant change in blood urea ($p = 0.54$) but serum creatinine increased ($P < 0.001$) in diseased cattle when compared with the control. There were nonsignificant differences in total protein, alkaline phosphatase and γGT between the diseased cattle and the control, but serum albumin and globulins decreased ($p = 0.01$) in diseased cattle when compared with the control. There was a significant increase in serum

MDA ($p = 0.005$), significant decrease in serum TAC ($p = 0.02$) and significant increase in NO ($p = 0.02$) in diseased cattle when compared with the control.

Blood serum biochemical analysis in urolithiasis are presented in Table 4 and Figs. 16-18. There was a significant increase in creatinine ($P < 0.001$) in diseased cattle when compared with the control. There were nonsignificant differences in the mean values of total protein, albumin, globulin, alkaline phosphatase and γGT between the diseased cattle and the control.

Results of physical examination of urine samples are shown in Table 5. Urine color was yellow in nephrosis, white to yellow in glomerulonephritis and pyelonephritis, white in cystitis, white to dark yellow in nephrolithiasis, and yellow in the control group. Urine odor was uriniferous in cases of nephrosis, putrid and ammoniacal in some cases of glomerulonephritis and pyelonephritis, putrid in cases of cystitis, ammoniacal in cases of nephrolithiasis, and uriniferous in the control group. Foam was positive in all groups. Urine was cloudy to turbid in all groups.

Chemical examination of urine samples of examined cases is shown in Table 6. pH ranged from 7-8 for cases of nephrosis, glomerulonephritis and pyelonephritis, cystitis, and in the control group, whereas it recorded 6 in cases of nephrolithiasis. Urobilinogen was normal in all groups. Nitrite was positive in cases of nephrosis and cystitis, but it was negative in the other groups. Protein was high in positivity in cases of nephrosis and in other groups. Blood was positive in cases of nephrosis and cystitis, but it was negative in the other groups. Glucose and ketone bodies were negative in all the studied groups.

Microscopic examination of urine sediment is shown in Table 7. Affected cases showed increased numbers of WBC, RBCs (hematuria and pyuria) and epithelial cells than the control group. Crystals in the affected cases was amorphous phosphate and triple phosphate.

Types and number of bacterial isolates in urine are shown in Table 8. *E. coli* was found in 18 cases, *Corynebacterium renale* was found in 25 cases, *Staphylococcus* sp. was found in 9 cases, *Streptococcus* sp. was found in 6 cases, and *Micrococcus* was found in 16 cases.

The relation between the results of bacterial isolation and urinary disorders in diseased cattle cases is shown in Table 9. *E. coli* was found in 5 cases of nephrosis, 4 cases of glomerulonephritis and interstitial nephritis, 7 cases of cystitis and 2 cases of nephrolithiasis.

Corynebacterium renale was found in 7 cases of nephrosis, 8 cases of glomerulonephritis and interstitial nephritis, 8 cases of cystitis and 2 cases of nephrolithiasis. *Staphylococcus* sp. was found in 3 cases of nephrosis, 2 cases of glomerulonephritis and interstitial nephritis, 3 cases of cystitis and 1 case of nephrolithiasis. *Streptococcus* sp. was found in 3 cases of nephrosis, 1 case of glomerulonephritis and interstitial nephritis, 2 cases of cystitis and no case of nephrolithiasis. *Micrococcus* was found in 5 cases of nephrosis, 8 cases of glomerulonephritis and interstitial nephritis, 3 cases of cystitis and no case of nephrolithiasis.

CONCLUSION

- The most common renal affections in the studied areas are nephritis, nephrosis, renal calculi and cystitis.
- Blood oxidative stress increase in case of nephrosis, glomerulonephritis and cystitis.
- Lipid peroxidation increase in case of nephrosis, and cystitis.
- Total antioxidants capacity decrease in case of nephrosis, glomerulonephritis and cystitis.
- Blood serum creatinine level increase in cases of nephrosis, glomerulonephritis, cystitis and urolithiasis.
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REFERENCES

REFERENCES

Abd Ellah, M.R., 2010. Involvement of free radicals in animal diseases. Comparative Clinical Pathology 19, 615–619.

Abd Ellah, M.R., 2013a. Involvement of free radicals in diseases of the urinary system. Journal of Advanced Veterinary Research Volume 3, 52-56.

Abd Ellah, M.R., 2013b. Involvement of free radicals in parasitic infestations. Journal of Applied Animal Research 41, 69-76.

Abd Ellah, M.R., 2013c. Role of free radicals and antioxidants in mastitis. Journal of Advanced Veterinary Research 3, 1-7.

Abd Ellah, M.R., Khamis, G.F., Elnisr, N.A., 2012. Serum lipoproteins, antioxidants and urine biochemical constituents in camel cystitis. Comparative Clinical Pathology 21, 515-519.

Abraham, S.N., Miao, Y., 2015. The nature of immune responses to urinary tract infections. Nature Reviews Immunology 15, 655-663.

Adams, L., Franco, M.C., Estevez, A.G., 2015. Reactive nitrogen species in cellular signaling. Experimental Biology and Medicine 240 (6), 711-717.

Aliyu, A., Natala, A.J., Adamu, S., Igbokwe, I.O., Esievo, K.A.N., Useh, N.M., 2017. A comparative study on the haematology, biochemical alterations in serum composition and pathologic changes in the kidneys of cachectic and non-cachectic cattle in Zaria, Nigeria. Int. J. Biochem. Res. Rev. 19, 1-11.

Annas, S., Zamri-Saad, M., Jesse, F.F., Zunita, Z., 2015. Comparative clinicopathological changes in buffalo and cattle following infection by Pasteurella multocida B:2. Microb Pathog. 88, 94-102.

Araújo, A.M., Reis, E.A., Athanazio, D.A., Ribeiro, G.S., Hagan, J.E., Araujo, G.C., Damião, A.O., Couto, N.S., Ko, A.I., Noronha-Dutra, A., Reis, M.G., 2014. Oxidative stress markers correlate with renal dysfunction and thrombocytopenia in severe leptospirosis. The American Journal of Tropical Medicine and Hygiene 90 (4), p.719.

Bancroft, J.D., Stevens, A., Turner, R., 1982. Theory and practice of histological techniques. 2nd (ed.) churchill living stone. New York.

Bathla S, Rawat P, Baithalu R, et al., 2015. Profiling of urinary proteins in Karan Fries cows reveals more than 1550 proteins. J. Proteomics 127, 193-201.

Baude, L., Ardaillou, R., 1993. Involvement of reactive oxygen species in kidney damage. British Medical Bulletin 49, 621–629

Beekhuis, L., 2022. Pyelonephritis in adult cattle. Improve Veterinary Practice.https://www.veterinary-practice.com/article/pyleonephritis-cattle

Bernier, GV., Kim, DY., Nagy. DW., 2018. Immune complex glomerulonephritis of suspected iatrogenic origin in five Japanese Black calves. J. Vet. Med. Sci. 80, 823-828.

Braun, U., Nuss, K., Wehbrink, D., Rauch, S., Pospischil, A., 2008. Clinical and ultrasonographic findings, diagnosis and treatment of pyelonephritis in 17 cows. The Veterinary Journal 175 (2), 240-248.

Brenner, B.M., 2004. The Kidney; Saunders: Philadelphia, PA, USA.

Callens, Amanda J., and Joseph W.B., 2015. Urinalysis." Veterinary Clinics: Small Animal Practice 45, 621-637.

Che, R., Yuan, Y., Huang, S., Zhang, A., 2014. Mitochondrial dysfunction in the pathophysiology of renal diseases. American Journal of Physiology-Renal Physiology 306, F367-F378.

Coles, E.H., 1986. Collection of blood samples. In: Veterinary clinical pathology, 4th Ed., Coles, E.H. (Ed), Saunders, W.B. Company, Philadelphia, ISBN-13: 978-0721618289, pp. 46-47.

Confer, A.W., Panciera, R.J., 1995. The urinary system. In Thomson's Special Veterinary Pathology. 2nd edn. Eds W. W. Carlton, M. D. McGavin.St Louis, Mosby. pp 234-23

Constable, P. D., Hinchcliff, K. W., Done, S. H., Grünberg, W., 2017. Veterinary medicine -e-book: a textbook of the diseases of cattle, horses, sheep, pigs and goats. 11th ed. Elsevier Health Sciences.

Daenen, K., Andries, A., Mekahli, D., Van Schepdael, A., Jouret, F., Bammens, B., 2019. Oxidative stress in chronic kidney disease. Pediatric Nephrology 34 (6), 975-991.

D'amico, G., Bazzi, C., 2003. Pathophysiology of proteinuria. Kidney International, 63(3), 809-825.

Darenskaya, M.A., Kolesnikov, S.I., Rychkova, L.V., Grebenkina, L.A., Kolesnikova, L.I., 2018. Oxidative stress and antioxidant defense parameters in different diseases: ethnic aspects. Free Radical Biology and Medicine 120, S60.

Dijkstra, J., Oenema, O., Van Groenigen, J.W., Spek, J.W., Van Vuuren, A.M., Bannink, A., 2013. Diet effects on urine composition of cattle and N2O emissions. Animal 7(s2), 292-302.

Divers, TJ., 2008. Urinary tract diseases. In: Divers TJ, Peek SF, eds. Rebhun's Diseases of Dairy Cattle. St. Louis: Saunders, Elsevier, pp. 130-199.

Ejaz, A.A., Johnson, R.J., Shimada, M., Mohandas, R., Alquadan, K.F., Beaver, T.M., Lapsia, V., Dass, B., 2019. The role of uric acid in acute kidney injury. Nephron 142 (4), 275-283.

El-Deeb, W.M., Buczinski, S., 2015. The diagnostic and prognostic importance of oxidative stress biomarkers and acute phase proteins in urinary tract infection (UTI) in camels. PeerJ. 3, e1363.

El-Deeb, W.M., Elmoslemany, A.M., 2016. Acute phase proteins as biomarkers of Urinary Tract Infection in dairy cows: diagnostic and prognostic accuracy. Japanese Journal of Veterinary Research 64, 57-66.

Elgioushy, M.M., Elgaml, S.A., El-Adl, M.M., Hegazy, A.M., Hashish, E.A., 2020. Aflatoxicosis in cattle: Clinical findings and biochemical alterations. Environmental Science and Pollution Research 27, 35526-35534.

El-Mashad, A.B., Moustafa, S., Amin, A., 2019. Comparative pathological Studies on Kidneys affections in slaughtered farm animals. Benha Veterinary Medical Journal 36, 192-202.

Ferlizza, E., Fasoli, S., Cavallini, D., Bolcato, M., Andreani, G., Isani, G., 2020. Preliminary Study on Urine Chemistry and Protein Profile in Cows and Heifers. Pakistan Veterinary Journal 40 (4), 413-418.

Frijhoff, J., Winyard, P.G., Zarkovic, N., Davies, S.S., Stocker, R., Cheng, D., Knight, A.R., Taylor, E.L., Oettrich, J., Ruskovska, T., Gasparovic, A.C., 2015. Clinical relevance of biomarkers of oxidative stress. Antioxidants & Redox Signaling 23 (14), 1144-1170.

Galle, J., Seibold, S., Wanner, C., 2003. Inflammation in uremic patients: what is the link?. Kidney and Blood Pressure Research 26, 65-75.

Garry, F., Chew, D.J., Rings, D.M., Tarr, M.J., Hoffsis, G.F., 1990. Renal excretion of creatinine, electrolytes, protein, and enzymes in healthy sheep. American Journal of Veterinary Research 51(3), pp.414-419.

Gaspari, F., Perico, N., Remuzzi, G., 2006. Timed urine collections are not needed to measure urine protein excretion in clinical practice. American Journal of Kidney Diseases 47(1), 1-7.

Genestra, M., 2007. Oxyl radicals, redox-sensitive signalling cascades and antioxidants. Cellular Signalling, 19 (9), 1807-1819.

Gosselin, V.B., Kim, D.Y., Nagy, D.W., Shoemake, B.M., Shaw, D.P., Royal, A.B., Evans, T.J., Middleton, J.R., 2018. Immune complex glomerulonephritis of suspected iatrogenic origin in five Japanese Black calves. Journal of Veterinary Medical Science, 18, 823-828.

Grauer, G.F., 2007. Measurement, interpretation, and implications of proteinuria and albuminuria. Veterinary Clinics of North America: Small Animal Practice 37 (2), 283-295.

Grauer, G.F., 2011 Proteinuria: measurement and interpretation. Top Companion Anim Med. 26, 121-127.

Greene, E.L., Paller, M.S., 1991. Oxygen free radicals in acute renal failure. Mineral and Electrolyte Metabolism 17, 124- 132.

Gumasta, P., Dubey, A., Swamy, M., Sahni, Y.P., Anil, A., 2019. Immunohistochemical expression of oxidative stress markers in bovine tissues correlated to cadmium concentration. J. Anim. Res. 9, 721-726.

Gutteridge, J.M.C., Tickner, T.R., 1978. The characterisation of thiobarbituric acid reactivity in human plasma and urine. Analytical Biochemistry 91, 250-257.

Handelman, G.J., Walter, M.F., Adhikarla, R., Gross, J., Dallal, G.E., Levin, N. W., Blumberg, J.B., 2001. Elevated plasma F2-isoprostanes in patients on long-term hemodialysis. Kidney International 59, 1960–1966.

Hassan, F.M., El-Gazzar, B.A., El-Helbawy, M.G., Elhabashy, M.H., 2021. Evaluation of oxidant–antioxidant balance and total antioxidant capacity in urinary tract infection in children. Menoufia Medical Journal 34, 243.

Herman, N., Bourgès-Abella, N., Braun, J.P., 2019. Urinalysis and determination of the urine protein-to-creatinine ratio reference interval in healthy cows. J. Vet. Intern. Med. 33, 1-10.

Ihedioha, J.I., Ukachukwu, C.V., Ugochukwu, I.C.I., Anyogu, D.C., 2019. Evaluation of kidney function and urinary analytes in Nigerian trade cattle. Tropical Animal Health and Production 51, 1867-1875.

Isani, G., Ferlizza, E., Nally, J., 2018. Proteomic research in urine and other fluids. In: Proteomics in Domestic Animals: from Farm to Systems Biology, (De Almeida A, Eckersall D, Miller I, Eds), Springer, Cham, CH pp:121-47.

Jabarpour, M., Rashtchizadeh, N., Argani, H., Ghorbanihaghjo, A., Ranjbarzadhag, M., Sanajou, D., Panah, F., Alirezaei, A., 2019. The impact of dyslipidemia and oxidative stress on vasoactive mediators in patients with renal dysfunction. International Urology and Nephrology, 51, 2235-2242.

Johnson, R., Jamison, K., 1984. Amyloidosis in six dairy cows. J. Am. Vet. Med. Assoc. 185, 1538.

Juránek, I., Bezek, S., 2005. Controversy of free radical hypothesis: reactive oxygen species-cause or consequence of tissue injury?. General physiology and biophysics 24, 263.

Kaneko, Jiro Jerry., John W. Harvey., and Michael L. Bruss., 2008. Clinical biochemistry of domestic animals. Academic press.

Katsoulos, P.D., Athanasiou, L., Psalla, D., Marouda, C., Papchianou, M., Tsitsos, A., Boscos, C., Polizopoulou, Z.S., 2020. Urine protein-to-creatinine ratio in cattle with subclinical renal disease. Veterinary Clinical Pathology 49, 66-70.

Kirschbaum B., 2001. Total urine antioxidant capacity. Clinica Chimica Acta 305, 167–173.

Klahr, S., 2001. Urinary tract obstruction. Seminars in Nephrology 21, 133–145.

Krishnan, S.M., Kraehling, J.R., Eitner, F., Bénardeau, A., Sandner, P., 2018. The impact of the nitric oxide (NO)/soluble guanylyl cyclase (sGC) signaling cascade on kidney health and disease: a preclinical perspective. International Journal of Molecular Sciences, 19, 1712.

Kulcharyk, P.A., Heinecke, J.W., 2001. Hypochlorous acid produced by the myeloperoxidase system of human phagocytes induces covalent cross-links between DNA and protein. Biochemistry 40, 3648-3656.

Kurutas, E.B., Ciragil, P., Gul, M., Kilinc, M., 2005. The effects of oxidative stress in urinary tract infection during pregnancy. Mediators of Inflammation 4, 242–244.

Lobo, V., Patil, A., Phatak, A., Chandra, N., 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews 4, p.118.

Maciel, E.A., Athanazio, D.A., Reis, E.A., Cunha, F.Q., Queiroz, A., Almeida, D., McBride, A.J., Ko, A.I., Reis, M.G., 2006. High serum nitric oxide levels in patients with severe leptospirosis. Acta Trop 100, 256–260.

Maxie, M.G., Prescott, J.F., 1993. The urinary system. In Pathology of Domestic Animals. 4th edn. Vol 2. Eds K.V.F. Jubb, P.C. Kennedy, N. Palmer. Academic press.

Miyata, Y., Matsuo, T., Mitsunari, K., Asai, A., Ohba, K., Sakai, H., 2019. A review of oxidative stress and urinary dysfunction caused by bladder outlet obstruction and treatments using antioxidants. Antioxidants 8, 132.

Murray, GM., Sharpe, AE. 2009. Nephrotic syndrome due to glomerulopathy in an Irish dairy cow. Vet. Rec. 164,179-180.

Niraj, K.T., Christopher, R.G., Kenneth, S.L., 2009. Urinary system. In: K.S. Latimer (ed), Duncan and Prasse's Veterinary laboratory medicine: clinic pathology, (Wiley Blackwell, West Sussex, UK), pp. 253–282.

Nourmohammadzadeh, F., Haji Hajikolaei, MR., Sasani, F., Alidadi, N., 2010. Abattoir study of the prevalence of renal lesions in slaughtered cattle. Int. J. Vet. Res. 4, 173-175.

Otter, A., 2013, Diagnostic blood biochemistry and haematology in cattle, In Practice 35, 7–16.

Ozbek, E., 2012. Induction of oxidative stress in kidney. International Journal of Nephrology 2012, 465897–465899.

Parrah, J.D., Moulvi, B.A., Gazi, M.A., Makhdoomi, D.M., Athar, H., Din, M.U., Dar, S., Mir, A.Q., 2013. Importance of urinalysis in veterinary practice – a review, Vet World 6, 640–646.

Pellegrino, D., La Russa, D., Marrone, A., 2019. Oxidative imbalance and kidney damage: New study perspectives from animal models to hospitalized patients. Antioxidants 8, 594.

Peluso, I., Raguzzini, A., 2016. Salivary and urinary total antioxidant capacity as biomarkers of oxidative stress in humans. Pathology research international, 2016, Article ID 5480267.

Phaniendra, A., Jestadi, D.B., Periyasamy, L., 2015. Free radicals: properties, sources, targets, and their implication in various diseases. Indian journal of clinical biochemistry 30, 11-26.

Piech, T.L., Wycislo, K.L., 2019. Importance of urinalysis. Veterinary Clinics: Small Animal Practice 49, 233-245.

Powers, S.K., Ji, L.L., Kavazis, A.N., Jackson, M.J., 2011. Reactive oxygen species: impact on skeletal muscle. Comprehensive Physiology 1, 941.

Probo, M., Giordano, A., Rocca, V., Moretti, P., Paltrinieri, S., 2022. Preliminary study on the effect of season on urinary analytes in healthy Italian dairy cows. Veterinary Clinical Pathology 51, 408-413.

Radostits, O.M., Gay, C.C., Blood, D.C., Hinchcliff, K.W., 2006. Veterinary Medicine, 10th Ed. (Baillier Tindall, London, Philadelphia, New York).

Rawat, P., Bathla, S., Baithalu, R., 2016. Identification of potential protein biomarkers for early detection of pregnancy in cow urine using 2D DIGE and label free quantitation. Clin. Proteom. 13, 15.

Rebhun, W.C., Dill, S.G., Perdrizet, J.A., Hatfield, C.E., 1989. Pyelonephritis in cows: 15 cases (1982-1986). Journal of the American Veterinary Medical Association 194, 953-955.

Rodrigues, F.A.D.P., Santos, A.D.D.C., de Medeiros, P.H.Q.S., Prata, M.D.M.G., Santos, T.C.D.S., da Silva, J.A., Brito, G.A.D.C., Dos Santos, A.A., Silveira, E.R., Lima, A.Â.M., Havt, A., 2018. Gingerol suppresses sepsis-induced acute kidney injury by modulating methylsulfonylmethane and dimethylamine production. Scientific Reports 8, pp.1-10.

Roperto, S., Di Guardo, G., Leonardi, L., Pagnini, U., Manco, E., Paciello, O., Esposito, I., Borzacchiello, G., Russo, V., Maiolino, P., Roperto, F., 2012. Bacterial isolates from the urine of cattle affected by urothelial tumors of the urinary bladder. Research in Veterinary Science 93, 1361-1366.

Ruslie, R.H., Ramayani, O.R., Darmadi, D., Siregar, G.A., 2021. Oxidative stress markers in initial therapy and remission of nephrotic syndrome and serum malondialdehyde level predictor from routine laboratory test. Medicinski Glasnik 18, 90-95.

Salisbury, D., Bronas, U., 2015. Reactive oxygen and nitrogen species: impact on endothelial dysfunction. Nursing Research 64, 53-66.

Schnaper, H.W., 2017. The tubulointerstitial pathophysiology of progressive kidney disease. Advances in Chronic kidney Disease 24, 107-116.

Scholze, A., Jankowski, J., Pedraza-Chaverri, J., Evenepoel, P., 2016. Oxidative stress in chronic kidney disease. Oxidative Medicine and Cellular Longevity. Article ID 8375186, http://dx.doi.org/10.1155/2016/8375186

Shah, S.V., 2001. Role of iron in progressive renal disease. American Journal of Kidney Diseases 37 (Suppl. 2), S30–S33.

Sharma, P.D., Singh K., Singh, J., Kumar, A., 2006. Bacteriological, biochemical and histopathological studies in uroperitoneum in buffalo calves (Bubalus bubalis) Indian J. Anim. Sci. 76, 124–126.

Shirazi, M.K., Azarnezhad, A., Abazari, M.F., Poorebrahim, M., Ghoraeian, P., Sanadgol, N., Bokharaie, H., Heydari, S., Abbasi, A., Kabiri, S., Aleagha, M.N., 2019. The role of nitric oxide signaling in renoprotective effects of hydrogen sulfide against chronic kidney disease in rats: Involvement of oxidative stress, autophagy and apoptosis. Journal of Cellular Physiology 234, 11411-11423.

Snijder, P. M., van den Berg, E., Whiteman, M., Bakker, S. J. L., Leuvenink, H. G. D., van Goor, H., 2013. Emerging role of gasotransmitters in renal transplantation. American Journal of Transplantation, 13, 3067–3075.

Soleimani, G., Sadeghi-Bojd, S., Teimouri, A., Nakhaee, A., Sheikhhosseini, A. 2016. Evaluation of oxidant-antioxidant balance and total antioxidant

capacity of serum in children with urinary tract infection. Niger Med. J. 57, 114–118.

Somani, S.M., Husain, K., Whitworth, C., Trammell, G.L., Malafa, M., Rybak, L.P., 2000. Dose-dependent protection by lipoicacid against cisplatin-induced nephrotoxicity in rats: antioxidant defense system. Pharmacology and Toxicology 86, 234-241.

Svensson, L., Poljakovic, M., Demirel, I., Sahlberg, C., Persson, K., 2018. Host-derived nitric oxide and its antibacterial effects in the urinary tract. Advances in Microbial Physiology 73, 1-62.

Taccone-Gallucci, M., Lubrano, R., Meloni, C., Morosetti, M., Manca di Villahermosa, S., Scoppi, P., Palombo, G., Castello, M., Casciani, C., 1999. Red blood cell membrane lipid peroxidation and resistance to erythropoietin therapy in hemodialysis patients. Clinical Nephrology 52, 239-245.

Tanner, R.M., Brown, T.M., Muntner, P., 2012. Epidemiology of obesity, the metabolic syndrome, and chronic kidney disease. Current Hypertension Reports 14, 152-159.

Templar, J., Kon, S.P., Milligan, T.P., Newman, D.J., Raftery, M.J., 1999. Increased plasma malondialdehyde levels in glomerular disease as determined by a fully validated HPLC method. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association-European Renal Association 14, 946-951.

Théron, M.L., Piane, L., Lucarelli, L., Henrion, R., Layssol-Lamour, C., Palanché, F., Concordet, D., Braun, J.P.D., Trumel, C., Lavoué, R., 2017. Effects of storage conditions on results for quantitative and qualitative evaluation of proteins in canine urine. American Journal of Veterinary Research 78, 990-999.

Toblli, J.E., Bevione, P., Di Gennaro, F., Madalena, L., Cao, G., Angerosa, M., 2012. Understanding the mechanisms of proteinuria: therapeutic implications. International Journal of Nephrology, Article ID 546039 https://doi.org/10.1155/2012/546039.

Vaden, S.L., Pressler, B.M., Lappin, M.R., Jensen, W.A., 2004. Effects of urinary tract inflammation and sample blood contamination on urine albumin and total protein concentrations in canine urine samples. Veterinary Clinical Pathology 33, 14-19.

Vamvakas, S., Bahner, U., Heidland, A., 1998. Cancer in end-stage renaldisease: potential factors involved. American Journal of Nephrology 18, 89-95.

Van Metre, D.C., Divers, T.J., 2002. Urinary tract infection. In LargeAnimal Internal Medicine. 3rd edn. Ed B. P. Smith, St Louis, Mosby. pp 863-866

Venkataraman, K., Khurana, S., Tai, T.C., 2013. Oxidative stress in aging-matters of the heart and mind. International Journal of Molecular Sciences 14, 17897-17925.

Vientós-Plotts, A.I., Behrend, E.N., Welles, E.G., Chew, D.J., Gaillard, P.R., Busler, J.N., Lee, H.P., 2018. Effect of blood contamination on results of dipstick evaluation and urine protein-to-urine creatinine ratio for urine samples from dogs and cats. American Journal of Veterinary Research 79, 525-531.

Vysakh, A., Raji, N.R., Suma, D., Jayesh, K., Jyothis, M., Latha, M.S., 2017. Role of antioxidant defence, renal toxicity markers and inflammatory cascade in disease progression of acute pyelonephritis in experimental rat model. Microbial Pathogenesis 109, 189-194.

Whalan, J.E. 2015. A Toxicologist's Guide to Clinical Pathology in Animals. Springer International Publishing Switzerland. DOI 10.1007/978-3-319-15853-2_7

Wojcicka, G., Beltowski, J., 2001. Oxidative stress in glomerulonephri- tis. Postepy higieny medycyny doswiadczalnej 55, 855–869.

Yalcin, A.S., Yurtkuran, M., Dilek, K., Kilinc, A., Taga, Y., Emerk, K., 1989. The effect of vitamin E therapy on plasma and erythrocyte lipid peroxidation in chronic hemodialysis patients. Clinical Chimistry Acta 185,109–112.

Yanagawa, R., Honda, E., 1978. Corynebacterium pilosum and Corynebacterium cystitidis, two new species from cows. International Journal of Systematic and Evolutionary Microbiology 28, 209-216.

Yawata, Y., Jacob, H., 1975. Abnormal red cell metabolism in patients with chronic uremia: Nature of the defect and its persistence despite adequate hemodialysis. Blood 45, 231–239.

Yeruham, I., Elad, D., Avidar, Y., Goshen, T., 2006. A herd level analysis of urinary tract infection in dairy cattle. The Veterinary Journal 171, 172-176.

Zampelas, A., Micha R., 2015. Antioxidants in health and disease. Antioxid. Heal. Dis. 115,603-606.

الملخص

إشتملت الدراسة على عدد ٩٧ حيوان من الثيران تراوحت اعمارهم من ١٨ – ٢٠ شهرًا والتي تم ذبحها في مسلخ ملوي (محافظة المنيا ، مصر) وتم تضمينها في هذه الدراسة.

تم جمع عينات الدم من كل حيوان عن طريق ثقب الوريد الوداجي قبل الذبح. تم جمع نوعين من عينات الدم:

أ. عينات الدم الكامل في أنابيب مفرغة تحتوي على الإيثيلين داي أمين تترا أسيتيك أسيد (EDTA) كمضاد للتخثر لفصل البلازما واستخدمت لقياس مستوى المالون داي ألديهيد في البلازما.

ب. عينات الدم في أنابيب مفرغة بدون مادة مانعة للتخثر لفصل المصل واستخدمت لقياس المتغيرات البيوكيميائية في الدم.

بعد الذبح ، تم فحص الحيوانات بعناية. كما تم استبعاد الحيوانات التي تظهر آفات مرضية في أعضاء أخرى غير الجهاز البولي.

تم جمع عينات البول مباشرة من المثانة باستخدام محقنة سعة ١٠ مل وحفظها في الثلج حتى التحليل. تم جمع الكلى والمثانة البولية من كل حيوان مذبوح في أكياس معقمة. تم أخذ العينات النسيجية المرضية في المختبر. تم حفظ عينات من أنسجة الكلى والمثانة في الفورمالين بنسبة ١٠٪ للفحص التشريحي المرضي.

تم تصنيف الأبقار الخاضعة للدراسة (العدد = ٩٧) إلى مجموعات مختلفة بناءً على الفحص الشامل والمجهري لانسجة الكلى والمثانة البولية. اشتملت أمراض الجهاز البولي المكتشفة على: استحالات خلايا الكلية (عدد = ٢٣) ، التهاب كبيبات الكلى والتهاب الكلية الخلالي (عدد = ٢٣) ، حصوات الكلية (عدد = ٢٣) ، التهاب المثانة (عدد = ٥). تم اعتبار الحيوانات التي لم تظهر أي نتائج سريرية غير طبيعية ولم تكن هناك أعراض عينية وميكروسكوبية مرضية ضمن المجموعة الضابطة (عدد= ٢٣).

أظهرت نتائج التحليل الكيميائي الحيوي لمصل الدم في الماشية التي تعاني من التهاب الكلية زيادة معنوية في اليوريا في الدم ($p<0.05$) والكرياتينين ($P<0.001$) في الماشية المصابة بالمقارنة مع المجموعة الضابطة. كانت هناك فروق غير معنوية

في البروتين الكلي، الألبومين، الجلوبيولين، الفوسفاتيز القلوي و الجاما جلوتاميا ترانسفيريز بين الماشية المصابة و المجموعة الضابطة. كانت هناك زيادة معنوية في مصل المالون داي ألديهيد ($p<0.01$) ، وانخفاض معنوي في المقدرة الكلية لمضادات الاكسدة ($P<0.01$) و أكسيد النيتريك ($P<0.01$) في مصل الأبقار المريضة بالمقارنة مع المجموعة الضابطة.

أظهرت نتائج التحليل الكيميائي الحيوي لمصل الدم في الماشية التي تعاني من التهاب كبيبات الكلى والتهاب الكلية الخلالي زيادة معنوية في اليوريا في الدم ($P<0.01$) والكرياتينين ($P<0.001$) في الأبقار المصابة بالمقارنة مع المجموعة الضابطة. كانت هناك فروق غير معنوية في البروتين الكلي ، الألبومين ، الجلوبيولين ، الفوسفاتيز القلوي و الجاما جلوتاميا ترانسفيريز بين الماشية المصابة بالمقارنة مع المجموعة الضابطة. كان هناك تغيير غير معنوي في مستوى المالون داي ألديهيد ، وانخفاض معنوي في المقدرة الكلية لمضادات الاكسدة ($P<0.05$) و أكسيد النيتريك ($P<0.001$) في مصل الماشية المريضة بالمقارنة مع المجموعة الضابطة.

أظهرت نتائج التحليل الكيميائي الحيوي لمصل الدم في الماشية التي تعاني من التهاب المثانة تغير غير معنوي في اليوريا في الدم لكن الكرياتينين في الدم زاد ($P<0.001$) في الأبقار المصابة بالمقارنة مع المجموعة الضابطة. كانت هناك فروق غير معنوية في البروتين الكلي ، الفوسفاتيز القلوي و الجاما جلوتاميا ترانسفيريز بين الماشية المصابة و المجموعة الضابطة ، ولكن انخفض ألبومين الدم والجلوبيولين ($P<0.01$) في الأبقار المصابة بالمقارنة مع المجموعة الضابطة. كانت هناك زيادة معنوية في المالون داي ألديهيد في الدم ($P<0.01$) ، وانخفاض معنوي في المقدرة الكلية لمضادات الاكسدة في المصل ($P<0.05$) وزيادة معنوية في ($P<0.05$) NO في الأبقار المريضة بالمقارنة مع المجموعة الضابطة.

أظهرت نتائج التحليل الكيميائي الحيوي لمصل الدم في الماشية التي تعاني من تحص بولي انخفاض معنوي في اليوريا في الدم ($P<0.05$) وزيادة معنوية في الكرياتينين ($P<0.001$) في الأبقار المصابة بالمقارنة مع المجموعة الضابطة. كانت هناك اختلافات غير معنوية في متوسط قيم البروتين الكلي ، الألبومين ، الجلوبيولين ، الفوسفاتيز القلوي و الجاما جلوتاميا ترانسفيريز بين الماشية المصابة و المجموعة الضابطة.

اتضح من الفحص الظاهري لعينات البول: لون البول أصفر في الكلى ، أبيض إلى أصفر في التهاب كبيبات الكلى والتهاب الحويضة والكلية ، أبيض في التهاب المثانة ، أبيض

إلى أصفر داكن في إصابت حصوات الكلية ، وأصفر في المجموعة الضابطة. كانت رائحة البول بولية في حالات استحالات خلايا الكلية ورائحةالأمونيا في بعض حالات التهاب كبيبات الكلى. كانت الرغوة إيجابية في جميع المجموعات. كان البول غائما إلى عكر في جميع المجموعات.

الفحص الكيميائي لعينات البول للحالات التي تم فحصها: تراوح الأس الهيدروجيني بين ٧-٨ في حالات استحالات خلايا الكلية والتهاب كبيبات الكلى والتهاب المثانة. كان اليوروبيولوجين طبيعيًا في جميع المجموعات. كان النتريت إيجابياً في حالات التهاب الكلية والتهاب المثانة ولكنه كان سلبياً في المجموعات الأخرى. كان البروتين عالي الإيجابية في حالات استحالات خلايا الكلى. كان وجود الدم موجبًا في حالات استحالات خلايا الكلية والتهاب المثانة ، لكنه كان سالبًا في المجموعات الأخرى. كانت أجسام الجلوكوز والكيتون سلبية في جميع المجموعات الخاضعة للدراسة.

أظهر الفحص المجهري لرواسب البول وجود زيادة في أعداد خلايا الدم البيضاء وكرات الدم الحمراء والخلايا الظهارية بالمجموعات المرضية مقارنة بالمجموعة الضابطة. كانت البلورات في الحالات المصابة عبارة عن فوسفات غير متبلور وثلاثي فوسفات.

أنواع وعدد العزلات البكتيرية في البول: تم عزل بكتريا الإشريكية القولونية من ١٨ حالة ، و تم عزل بكتريا الوتدية الكلوية من ٢٥ حالة ، وتم عزل بكتريا *المكورات العنقودية* من ٩ حالات ، وتم عزل البكتريا السبحية من ٦ حالات ، كما تم عزل بكتريا *المكورات الدقيقة* من ١٦ حالة.

من هذه الدراسة تم استنتاج ما يلي:

- أكثر أمراض الكلى شيوعاً في المناطق المدروسة هي استحالات خلايا الكلية وحصوات الكلى والتهاب المثانة.
- زيادة الإجهاد التأكسدي للدم في حالة استحالات خلايا الكلى والتهاب كبيبات الكلى والتهاب المثانة.
- زيادة بيروكسيد الدهون في حالة استحالات خلايا الكلية والتهاب المثانة.
- انخفاض القدرة الكلية لمضادات الأكسدة في حالات استحالات خلايا الكلى والتهاب كبيبات الكلى والتهاب المثانة.

تأثير أمراض الجهاز البولي على بعض المكونات في الدم والبول في الأبقار

رسالة مقدمة من

ط . ب/ أسماء سعد عبد الله

(ماجستير العلوم الطبية البيطرية ٢٠١٥- جامعة أسيوط)

للحصول على دكتوراه الفلسفة في العلوم الطبية البيطرية

(التشخيص المعملي والإكلينيكي)

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