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BACTERIOLOGICAL, PATHOLOGICAL AND BIOCHEMICAL STUDIES ON THE URINARY TRACT AFFECTIONS ON CATTLE AND BUFFALOES

(With 7 Tables and 7 Figures)

By

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دراسات بكتريولوجية وباثولوجية وبيوكيميائية على اصابات الجهاز البولى في الأبقار والجاموس

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هدفت هذه الدراسة إلى التعرف على التغيرات البكتريولوجية والباثولوجية والبيوكيميائية المصاحبة لإصابات الجهاز البولى في الأبقار والجاموس وأيضا تقديرأهمية إنزيمات الجهاز البولى فى تشخيص إصابات الجهاز البولى. تم إجراء الدراسة على عدد 75 حيوان منهم 45 من الأبقار و30 من الجاموس وكان هناك 15 من الأبقار وعدد 10 من الجاموس كمجموعة ضابطة لهذه الدراسة. تم تجميع عينات الدراسة بصورة عشوائية لإستخدامها فى إجراء الفحص الطبيعى والكيميائى والبكتيرى. وقد أظهر الفحص البكتريولوجى أن 80% من عينات البول و 77.3% من الاعضاء المفحوصة كانتٍ إيجابية للعزل البكتيرى. وقد استخدمت عينات (الكلى والحالب والمثانة) للفحص الهستوباثولوجى. تم فى هذه الدراسة مناقشة التغيرات الباثولوجية وعلاقتها بالتغيرات فى مكونات البول وزيادة انزيمات مصل الدم والبول في كلا من الأبقار والجاموس. وتعد زيادة انزيمات مصل الدم والبول مؤشر حقيقى للتغيرات الباثولوجية فى كلا من (الكلى والحالب والمثانة). كما تم التعرف على أنواع البكتريا الموجودة فى العينات التى تمت دراستها وقد كانت نسبة الإصابة بالميكروب القولونى هى 34.66% والبروتيس 32% والكلبسيلا 18.6% والميكروب العنقودى الذهبى 18.6% والميكروب السبحى20 % والسالمونيلا 2.6%. واثبت الفحص الباثولوجى ان الحيوانات كانت تعانى من الألتهاب الصديدى فى الكلى والحالب والمثانة.

SUMMARY

The goal of the present study was to screen the the bacteriological, pathological and biochemical changes associated with the urinary tract affections in cattle and buffaloes and also to evaluate the diagnostic importance of urinary enzymes in urinary tract affections. A total number 75animals, (45) cattle, and (30) buffaloes, were subjected to this study. Fifteen cattle and 10 buffaloes were considered the control groups. These animals were randomly selected. Bacteriological examinations reveald that 80% of examined urine samples and 77.3% of examined organs samples were positive for bacterial isolation. Samples from (kidney, ureter and urinary bladder) used for histopathological examination. This study, discussed pathological changes and relation to changes in urine constutants, with an increase in the urine and serum enzymes. Bacterial isiolated strains were, E coli resembled 34.66%, Proteous sp 32 %, Streptococcus feacalis 20 %, Staphylococcus aureus, 18.6%, Klebsilla 18.6%, and Salmonela sp.2.6%. According to the histopathological findings, the animals under investigation were suffered from pyelonephritis, uretritis and cystitis. Urine analysis is helpful in diagnosis of pyelonephritis, uretritis and cystitis. There were a significant increase in serum levels of AST, ALT, LDH and ALP enzymes activities. Significant increase in AST, and LDH enzymes activities in urine of cattle and buffaloes. There were also, a marked increases in both ALT and ALP enzymes activities levels in urine of diseased animals in comparing with the normal levels. Increased enzyme activities are considered reliable indicators of pathological changes in various tissues due to the realease of intracellular enzymes from damaged tissue. Increased activities of serum enzymes lead to increased activities of these enzymes in urine.

Key words: *Urinary tract, urinary enzymes, biochemical changes, serum, urine.*

INTRODUCTION

In Most African countries, cattle and buffalo are the most dominant source of animal protein (Adedeji *et al.,* 2006). These species of animals are the cheapest to produce owing to their feeding habit. Animal production can be significantly increased through improved breeding practices and efforts that will targeted at combating such constraints as diseases and poor nutrition.

Urinary tract affections consedered as a significant bacteriuria in the presence of clinical symptoms (Divers, 1996). Urinary tract infections may be limited to a single tissue or organ, or may spread upward to involve the entire system. Affection such as cystitis involves the bladder but may spread through the ureters to the kidneys. (Meyer and Harvey, 1998).

Bacterial infections of the kidney (pyelonephritis, renal abscesses) or the other parts of the urinary tract are often accompanied by the appearance of bacteria in practically all diseases of the kidney which will cause changes in urinary enzyme activities (Kramer *et al.*, 1997).

Urine analysis is one of the most important diagnostic tests that can help to localize disease, to determine causes of discolored urine and to identify inflammatory diseases of the urinary system (Pugh, 2002).

The activity of enzymes in plasma and other body fluids can be altered by many factors (their rate of release from organs, by the distribution of the enzyme in the extracellular compartment, rate and routes of enzyme elimination and inactivation. These factors are influenced by individual variability, disease, drugs, exercise, etc., which need to be considered to ensure a more efficient diagnostic use of enzymes in veterinary clinical practice (Braun *et al.*, 2008).

Reports which include studies of one or more urinary enzymes have related enzyme activity to disorders of the urinary tract and in this regard lactic acid dehydrogenase has been most studied. Alkaline phosphatase and transaminase have received appreciable investigation (Dawra *et al.*, 1991; Meyer and Harvey, 1998).

Urinary lactic acid dehydrogenase activity has been reported to be elevated in the kidney and bladder affections and alkaline phosphatase increase in cases of kidney diseases (Raab, 1972).

Serial measurements of urinary lactic acid dehydrogenase and urinary alkaline phosphatase have been considered particularly useful screening test of renal disease especially in the presence of normal urinary sediment (Price, 1982).

Increases in enzyme activities are considered reliable indicators of pathological changes in various tissues due to the release of intracellular enzymes from damaged tissue (Yokus, and Dilek-Cakir, 2006). Elevations of urinary enzyme activity have been reported in a variety of disease states (Meyer and Harvey, 1998) in which proteinurea is a frequent, if not constant, manifestation.

Kidney is one of the indispensable organs in the body, its function is to maintain constancy in the internal environment. Urine which is the byproduct of the regulatory activities of the kidneys is not only altered by diseases occurring in the organs but many extend extra renal conditions. The produced changes may be of diagnostic significance. Analysis of urine could therefore reveal alterations typical of diseases of theses organs in addition to providing valuable information concerning alterations in other physiologic processes in the body (Sirois, 1995).

The kidney tubular cells lie on a basal membrane with their apical membrane facing the tubule lumen. In tubular cell damage ،there is no increase in plasma enzyme activity, as enzymes are released immediately and completely into the urine, except in the case of very intense kidney damage (Kramer *et al.*, 1997). This allows early and sensitive detection of even moderate kidney damage. The amount of enzymes present in urine at any one time precisely reflects the damage, which has occurred very recently. This means that the progress of kidney damage can be monitored (Braun *et al.*, 2008).

The need for more sensitive renal monitoring techniques has increased interest in proximal tubular tests such as urinary enzymes (Cornelius, 1980; Radostits, 2003). Measurement of urinary cast excretion as an early marker of renal tubular damage, because the identification of a renal tubular marker with adequate sensitivity, low cost, and general availability can potentially decrease both the incidence and the severity of aminoglycoside-related renal damage.

A breakdown of renal tissue (renal infarction, acute tubular necrosis), leads to a rise in enzymatic activities of serum as lactate dehydrogenase, aminotransferase and alkaline phosphates (Braun *et al.*, 2008).

Renal tubular cells are rich in enzymes, with normal cellular turnover; cells desquamate and disintegrate in urine. Permeation of tubular membranes seems to contribute to urinary enzymatic activity, because enzymes from the luminal parts of the renal tubular cells are regularly found in high activities in urine, e.g. alkaline phosphates and amino peptidase (Raab, 1972).

Cellular turnover in various epithelia (Kidney-pelvis, ureter, and bladder) leads to the desquamation of epithelial cells, the enzymes of which may appear partially in urine after decomposition of the cells.

Direct and indirect methods of localization of the site of infection is a desirable and logical step to the proper management of cases with

urinary tract infections. Some methods of localization to the site of infection are based on isolation of the infecting organism from kidney biopsy material, ureteral urine or bladder urine. The indirect methods include assay of urinary enzymes and examination of the urine for leukocyte casts (Meyer and Harvey, 1998).

The assay for urinary lactate dehydrogenase enzyme represents a simple, inexpensive and reliable method to differentiate between kidney and bladder infections.

Various pathogenic bacteria produce lactate group of enzymes, one of them is lactate dehydrogenase. These pathogen including bacteroids fragilis, Escherichia coli, Klebsiella pneumonia and Staphylococcus aureus (Raab, 1972). This study aimed to screen the bacteriological, pathological and biochemical changes associated with the urinary tract affections and also to evaluate the diagnostic importance of urinary enzymes in urinary tract affections.

MATERIALS and METHODS

Materials:

Animals:

A total number of 75 cattle (45) and buffaloes (30) were included in the present study. These animals were selected randomly, also 15 cattle and buffaloes were subjected to careful clinical and laboratory examinations and used as control of the study.

The studied cases were of different sex and their ages between 5-7 years.

Sampling:

1- Blood sample:

To obtain clean non heamolyzed serum for analysis of enzyme activity (LDH, AP, GGT, ALT).

2- Urine samples:

Sterile urine samples were taken directly through urinary bladder puncture from each animal. Two ml were used for bacteriological examination, and about 5ml were used for physical, chemical and microscopic examination.

3- Tissue samples:

Urinary system samples (kidneys, ureters, and urinary bladder) were taken from slaughtered animals.

Collected urinary system samples (kidneys, ureters, and urinary bladder) were examined morphologically for any obvious macroscopic changes.

For pathological examination,specimens from the urinary system (kidneys, ureters, and urinary bladder) were cutted, trimed, labeled and fixed in 10% neutral buffer formalin for histopathological examination. The fixed tissues processed routeinly.Serial sections of 4 μm thickness were obtained and stained with heamotoxylin and Eiosin stain (Bancroft and Stevens, 1982).

Methods:

1- Slaughter house specimens:

Macroscopic inspection of both kidneys, to screen its texture for abscess, calculi, pus, hydronephrosis and any other lesions.

2- Urine analysis:

Routine urine analysis includes physical characters (color, odor, transparency, and specific gravity) while chemical examination included pH,nitrite, blood, protein, urobilinogen, glucose and ketone bodies was carried out.

Chemical examination of urine sample was done using test strips produced by Farma Laboratory. Egypt.

Microscopical examination of urine sediment was done after Kaneko *et al.* (1997). Urine samples were centrifuged at 2000 rpm for 10 minutes.

Bacteriological examination:

According to (Ayeni *et al.*, 2009; Raboisson *et al.*, 2010) urine samples were centrifuged at 2000 rpm. for 10 minutes to obtain sediment and subjected for bacteriological examination. The urine sediment was inoculated into enriched media (nutrient broth) for 24 hours then on nutrient agar, blood agar and MacConkey agar plates and incubated at 37◦c for 72 hours. Identification of bacterial isolates were carried out through direct smear stained by Gram'stain. Specimens were then cultured on the following media: Nutrient agar plates, Bloodagar (all isolates) and MacConkey agar plates(E-coli –Salmonalla sp.).

Statistical analysis: Recorded data were analyzed statistically using analysis of variance (ANOVA). The statistical differences between means were estimated by Duncons Multiple Range test. The computation was facilitated by statistical package SPSS (2000).

RESULTS

The total number of animals was 75animals, cattle (45), and buffaloes (30), where 15 cattle and 10 buffaloes were considered the control group. These animals were randomly chased as a screen study for the urinary affections which accompanied by bacteriological, pathological and biochemical changes. Their ages ranged between 3-6 years.

The most common bacterial urinary affections that present in this study were ilisturated in Table (1) and the prevalence of bacteria isolated from urine and organs samples of cattle and buffaloes were showed in Table (2). The results of the virological examinations were negative.

The gross picture and the histopathological results of the examined tissue were similar in both cattle and buffalo as the macroscopical examination showed inflamed pelvic and urteral mucous membrane as it was thick redden and coated with a thin exudate. The wall of the urinary bladder was thickened by edema and focally hemorrhagic. Microscopicall examination revealed:

A- Kidney

The glomeruli showed necrosis and diffuse cellular infiltration consisted mainly of endothelial cells, neutrophil cells and in some cases lymphocyte and macrophage cell (suppurative glomerulitis) were observed Fig. (1).

Severe inflammatory cellular infiltration, mainly neutrophil cells, were observed in the interstitial tissue between the tubules in the cortex and medulla (interstitial nephritis) Fig. (2). The renal tubules were dilated, contain some time proteineous debris, hyalinzed or granular casts and neutrophil cell Fig. (3). Their epithelium showed coagulative necrosis and in some cases were completely destructed Fig. (4). The blood vessels in the glomeruli were congested and the other renal blood vessels were congested and have thick walls of proliferating connective tissue Fig. (5,6).

B- Ureters and Urinary bladder:

Showed degeneration in their epithelium. The lamina propria was intenisly edematous and infiltrated with neutrophil cell. Hypremia and hemorrhage show also congestion in the blood vessels with prevascular cellular infiltration Fig. (7) that's indicating uretritis and cystitis.

Table 1: The common bacterial urinary affections in this study:

Type of isolates in urine and organs	Number	tnecreP(%)
E-coli	26	34.66
Proteus sp	24	32.0
Streptococcus feacalis	15	20
Staphylococcus aureus	14	18.6
Klebsilla sp.	14	18.6
ps allenomlaS	2	2.6

Table 2: Prevalence and percentage of bacteria isolated from urine and organs samples of cattle and buffaloes suffered from urinary tract infections:

samples / animal	Urine samples			Organs samples		
	No	+ve	%	No	+ve	%
Apparently healthy	25	10	40	25	8	32
Diseased	50	50	100	50	50	100
Total	75	60	80	75	58	77.32

Table 3: Results of physical examination of urine samples for cattle and buffaloes:

Disease	color	odor	foam	transparency	Sp. gravity
Control	yellow	urineferous	negative	clear	1.022-1.036
Pyelonephritis	Whitish to yellow	putrid	negative	turbid	1.040-1.050

Table 4: Results of chemical examination of urine samples for cattle and buffaloes:

Disease	pH	Nitrite	Protein	RBC	Glucose	ketones	urobilinogen
control	7-8	negative	negative	negative	negative	negative	normal
Pyelonephritis	8-9	negative	+30-++100	positive	negative	negative	normal

Table 5: Microscopic examination of urine sediment: for cattle and buffaloes:

Disease	RBC	WBC	Epith.cells	crystals	casts
Control	2-5	3-6	2-4	negative	negative
Pyelonephritis	32-40	+25-++75	7-11	Ca-carbonate Ca-oxalate	negative

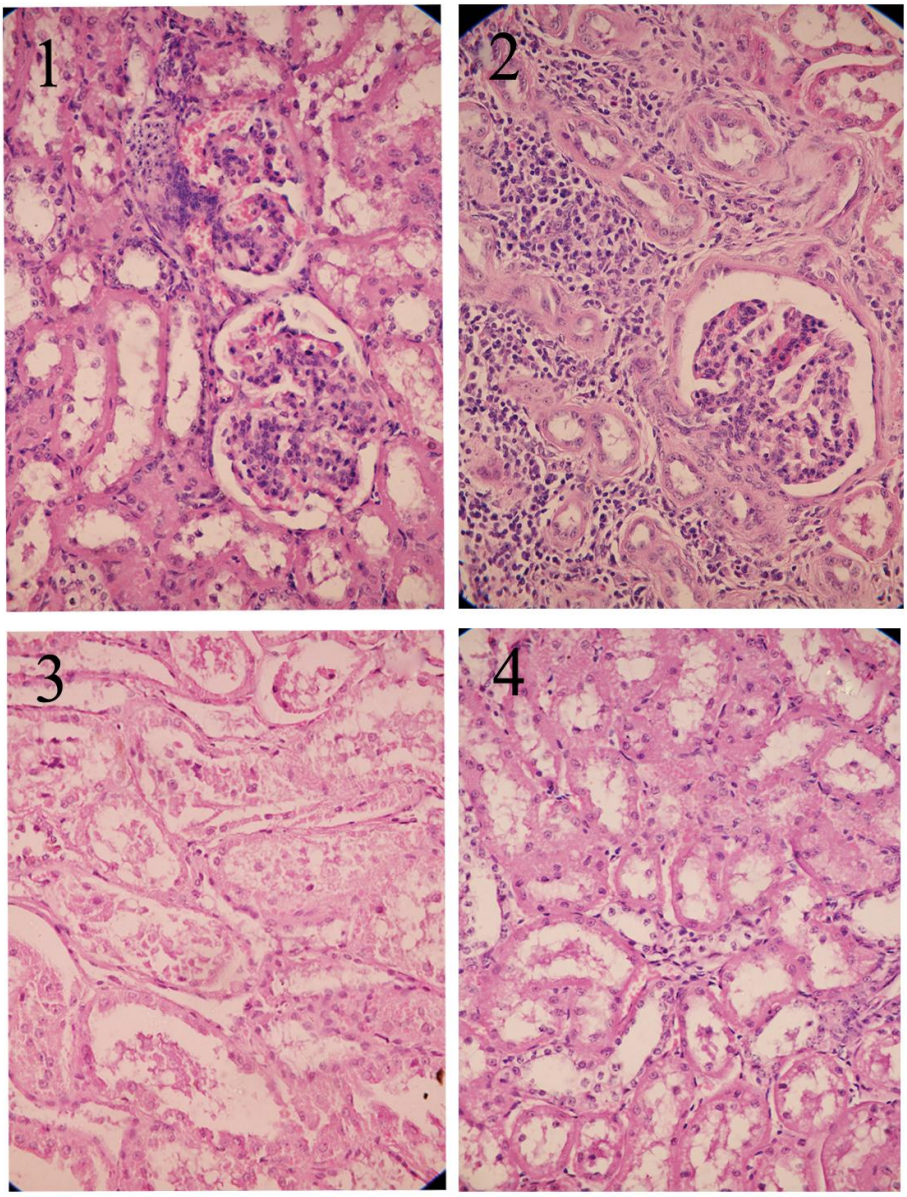
Table 6: Results of the biochemical (enzyme assay) of serum for cattle and buffaloes:

Disease	AST(U/L)		ALT(U/L)		LDH(U/L)		ALP(U/L)	
	cattle	Buffaloes	cattle	Buffaloes	cattle	Buffaloes	Cattle	Buffaloes
control	74.4±0.67	76.0±0.87	19.4±0.6	17.5±0.6	414±1.1	333±1.7	88.5±0.8	81.8±1.3
Pyelonephritis	101.0±1.09*	89.7±0.9*	28.8±0.6*	24.9±0.5	626 ±2.7*	518±1.1*	94.9±0.5*	90.5±0.6*

Table 7: Results of the biochemical (enzyme assay) urine of cattle and buffaloes:

Disease	AST(U/L)		ALT(U/L)		LDH(U/L)		ALP(U/L)	
	cattle	Buffaloes	cattle	Buffaloes	cattle	Buffaloes	cattle	Buffaloes
control	66.09±0.48	63.9±0.7	13.1±0.4	10.7±0.3	311±1.4	217±1.2	50 ±0.5	33.7±0.5
Pyelonephritis	72.3±0.53*	69.9±0.9*	14.7±0.5	12.5±0.4*	438±3.9*	248±4.7*	51.2±0.5	34.1±0.3

- = Significant ($p < 0.001$)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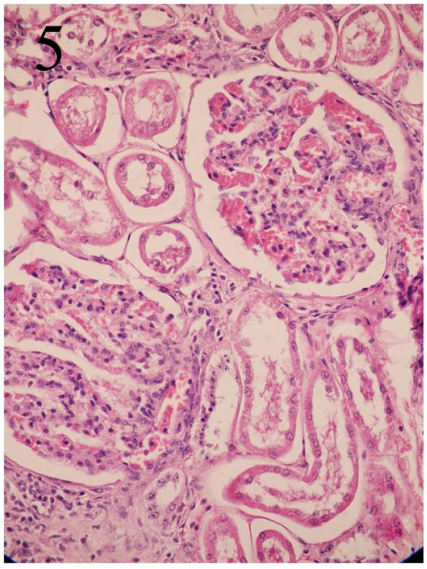
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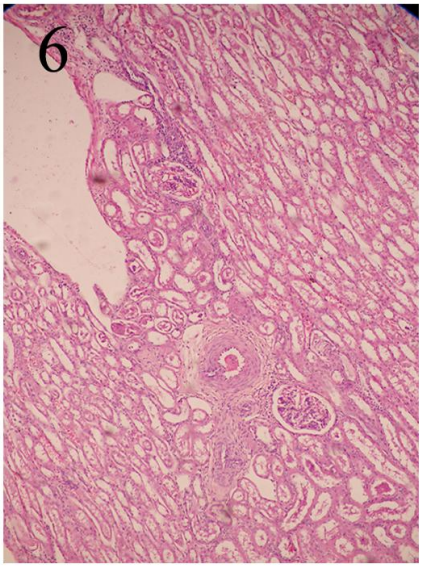
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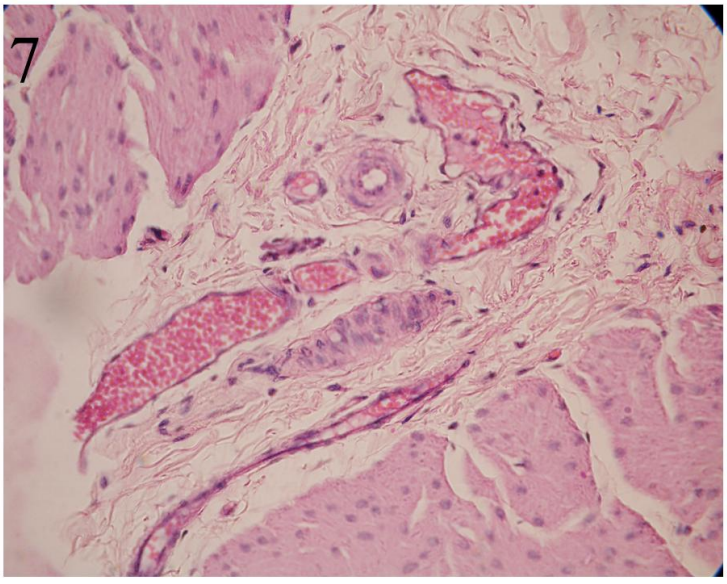
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DISCUSSION

Bacteriological examinations reveald that 80% of examined urine samples and 77.3% of examined organs samples were positive for bacterial isolation.

Bacterial isolated strains were tabulated in Table (1) where E coli resembled 34.66%, Proteous sp 32 %, Streptococcus feacalis 20 %, Staphylococcus aureus, 18.6%, Klebsilla 18.6%, and Salmonela sp.2.6%. These findings of bacteria isolets illustrate the causes of severe pyelonephritis which found through the pathological examinations.

Present results agreed with McGavin and Zachary (2007) as they stated that E coli especially the uropathogenic strains that produce virulence factors such as alpha hemolysin, adhesions and fimbria is one of the most common causes of lower urinary tract disease and pyelonephritis Also Proteus Sp., Kelebsiella Sp., Staphylococcus Sp. and Streptococcus Sp. are common causes of lower urinary tract infections and pyelonephritis in all species. The gross (inflammation and dilatation of the pelvic and ureters) and microscopic (suppurative glomerulitis, intratubular and interstitial inflammation) pictures appeared in present study resembles,in general, those described by (Rebhun *et al.,* 1989; Maxie, 1993) for the pyelonephritis.

The bacterial cystitis occurred when bacteria overcome normal defense mechanisms and attach to or invade the urinary bladder mucosa (Divers, 1996). In our cases, this purulent cystitis was characterized microscopically by congestion in the blood vessels with heavy infiltration of the urinary bladder mucosa with leucocytes and in sever conditions ulceration of the mucosa supervenes. These results were similar to those obtained by Sastry (1999).

All the abnormal changes occurred in the various parameters of the urine samples are good indicators of renal dysfunction or at least, presence of pathological conditions in the urinary examined tissues.

Presence of abnormal leukocyte counts, pyouria in the animals sampled suggests that there was an inflammation or tissue necrossis in the urinary examined tissues (Finco, 1980; Bush, 1993).

Detection of protein in urine is considered pathologic, and its presence attributed to either inability of the renal tubules to reabsorb the

protein as may occur in renal disorders or that there were some exudative inflammations of the urinary tract of animals showing this proteinuria (Meyer and Harvey, 1998).

The presence of RBC in the urine of animals sampled is of clinical significance since haematuria is encountered mostly in association with the urinary tract (Carnfield, 1986). Some of the diseases that result in haematuria include pyelonephritis, ureteritis and cystitis (Bush, 1993).

Increased in enzyme activities are considered reliable indicators of pathological changes in various tissues due to the realease of intracellular enzymes from damaged tissue.(Braun *et al.,* 2008). Increased activities of serum enzymes lead to increased activities of these enzymes in urine (Dawra *et al.,* 1991).

According to the biochemical results there were a significant ($p< 0.01$) increase in serum levels of AST, ALT, LDH and ALP enzymes activities of cattle and buffaloes. A significant ($p< 0.01$) *increase in AST, and LDH enzymes activities in urine of cattle and buffaloes was recorded. Also, there were a marked increase in both ALT and ALP enzymes activities levels in urine of diseased animals in comparing with the normal levels. These results agreed with, Yokus and Dilek-Cakir (2006); Braun *et al.* (2008), who proved that a breakdown of renal tissue (renal infarction, acute tubular necrosis), lead to a rise in enzymatic activities of serum as lactate dehydrogenase, aminotransferase and alkaline phosphates.

According to the pathological findings, various inflammatory processes of the kidney and of other parts of the urinary tract are accompanied by movement of cells that have a high enzyme content (leukocytes, lymphocytes). Such cells often enter urine, and their enzymes contribute to urinary enzymatic activity.

The present study reveled that, increased activities of serum enzymes lead to increased activities of these enzymes in urine and an increase in urinary enzymes levels is an indication of renal tubular damage.on the basis of this study, enzymeuria evaluation is easy to determine as a diagnostic test for urinary tract affections.

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