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Clinicopathological, Cytological and Histopathological Studies on Liver and Kidney Affections in Camels

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Abstract: A total of 92 camels (*Camelus dromedaries*) were included in this study. Liver and kidney specimens as well as blood samples were collected from each camel during a period of January-August 2010 from two slaughter houses in Giza and Cairo governorates. Different liver and kidney affections were noticed and studied by cytological evaluation and histopathological examinations in correlation with hematological and serum biochemical tests. Results of liver and kidney specimens could be classified into two groups: Group A, (27.6%) showed no cytological or histopathological alterations. Group B, different liver affections (40.4%) and kidney affections (16.5%) were noticed. Concerning cytological and histopathological examinations of diseased livers, hepatic lipidosisbeside hepatitis, cirrhosis, choleostasis, hyperplasia of biliary epithelium and hepatic necrosis were observed. PCV %, Hb concentration and RBCs count showed significant decrease in cases of hepatic lipidosis and hepatic necrosis with insignificant decrease in cases of kidney affections. Activity of serum liver enzymes ALT, AST, ALP and GGTrevealed significant increase in cases of hepatic lipidosis, cholestasis and hepatic necrosis. BUN and serum creatinine showed significant increases withsignificant decrease of serum total proteins and serum albumin in cases of kidney affections.

Key words: Camel · Lipidosis · Nephrosis · Cirrhosis · Glomerulonephritis · Cytology

INTRODUCTION

Affections of liver in meat-producing animals constitute a major factor that reduce our national income, either directly through condemnation of the affected livers, or indirectly by their effect on the animal growth and so its meat production [1]. In camelids, liver disease has not been widely recognized. Although fatty liver disease is often observed during necropsy examination, primary liver diseases have been infrequently diagnosed [2]. Different types of degenerative changes and liver diseases of camels such as hepatic lipidosis, biliary hyperplasia, lymphocytic plasmacytic hepatitis, cholangitis, liver cirrhosis andcholangiohepatitis were demonstrated [3].

Concerning kidneys, the kidney of camel is characterized by long loop of henel, the ratio of medulla to cortex is 4:1 and the initial glomerular filtration rate is about one-half that of sheep and cattle which is responsible for production of highly concentrated urine [1]. Several factors as well as etiological agents are blamed in initiating renal affections, such factors are nutritional status and toxic agents either of exogenous or endogenous origin together with specific infectious agents. Environmental conditions are also considered as contributing factors in renal disease initiation.Diagnosis of hepatic and renal disorders can be made through the clinical history, hematological, biochemical, histopathological and recently cytological examinations.

Cytological examination of the tissue is being used with increasing frequency in animal medicine [4] as it describes the microscopical criteria of individual cells regardless the architectural pattern characterizing the tissue of origin. It can be performed quickly, easily and inexpensively with little or no risk in most cases [5,6].

Serum hematological and biochemical tests are needed to assess the severity and monitor the progress of the diseases. Tissue cytology, in correlation with histopathology; the gold standard technique, could determine the degree of liver or kidney dysfunction and finally to reach a definitive diagnosis [7]. The aim of the present work is to study the different liver and kidney

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affections in camels by cytological evaluation and histopathological examinations in correlation with hematological and serum biochemical tests.

MATERIALS AND METHODS

A total of 92 camels (*Camelus dromedaries*) were included in this study. From each camel, liver and kidney specimens as well as blood samples were collected during a period of January-August 2010 from two slaughter houses in Giza and Cairo governorates. Two blood samples (about 10 ml) were collected from each camel through jugular vein.

The first blood sample was anticoagulated by di-potassium salt of ethylene diamine tetra-acetic acid (EDTA) and was used for evaluating Hemogram [Total leukocyte (TLC) and erythrocyte counts (RBCs), Packed cell volume (PCV %), Hemoglobin concentration (Hb) and differential leukocytic count (DLC)] [8].

The second blood sample was collected in a clean centrifuge tube for serum separation. The clear non-hemolysed supernatant serum was harvested for biochemical studies {serum total proteins, serum albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate amino transferase (AST), gamma glutamyltransferase (GGT) activities [9], blood urea nitrogen (BUN) and serum creatinine [10]}. The mentioned serum biochemical parameters were assayed using reagent kits supplied by StanBio-Laboratories incorporation, USA.

For cytological examination, the impression smears from liver and kidney were taken after cutting the removed mass into two halves to obtain a freshly cut surface. The cut surface was blotted on clean paper towel. The surface was made small enough to make several rows of imprints, stained by field stain [11] thenexamined under the microscope [5]. For histopathological examination, tissue specimens including liver and kidney were collected and fixed in 10% neutral buffered formalin for preparing paraffin tissue sections at 4-6µ thickness. These sections stained with hematoxylin and eosin [12].

Statistical Analysis: Values were expressed as mean ± SD. Statistical comparisons between Group A (camels with no cytological or histopathological alterations) and Group B (camels with different liver affections) were made with completely randomized two ways ANOVA "Student-Newman-Keuls test" by COSTAT program version one. A probability "P" value of <0.05 was assumed for statistical significance. On the other hand, Student's T test was carried out to assess the significance of mean difference between Group A (camels with no cytological or histopathological alterations) and Group B (camels with kidney affections) by using SPSS ® program version sixteen.

RESULTS AND DISCUSSION

Results of liver and kidney specimens could be classified into two groups: Group A, 30 cases (27.6%) showed no cytological or histopathological alterations. Group B, 44 cases with different liver affections (40.4%) and 18 cases with kidney affections (16.5%) were noticed.

Concerning cytological and histopathological examination of diseased livers, 21cases (47.7%) suffered from hepatic lipidosisbeside12 cases (27.2%) showed hepatitis and cirrhosis, choleostasis with hyperplasia of biliary epithelium 3 cases (6.8%) and 8 cases of hepatic necrosis (18.1%). On the other hand, tubular disease was observed as kidney affections although the abnormalities of the glomeruli are not discernible cytologically and require histological analysis.

Clinicopathological Findings

Erythrogram: Mean values of the erythrogram [packed cell volume (PCV), hemoglobin concentration (HB), erythrocytes count (RBCs), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC)] are illustrated in Table 1 and 2. In comparison to the mean values of group A, PCV, Hb concentration and RBCs count showed significant decrease in cases of hepatic lipidosis and hepatic necrosis with insignificant decrease in cases of kidney affections. MCV and MCHC values revealed insignificant changes in all cases.

From the above mentioned data, there is normocytic normochromic nonregenerative anemia most likely due to anemia of chronic disease [13] as the chronicity of many hepatic diseases often leads to non-regenerative anemia in which bone marrow is not able to respond to the anemic state [14]. On the other hand, the insignificantdecrease in cases of kidney affections may be attributed to decrease erythropoietin production in addition to the circulating uremic inhibitors that may play a role in inhibiting erythropoiesis [8].

Leukogram: Mean values of the leukogram [total leukocyte count (TLC), neutrophil, lymphocyte and monocyte counts] are illustrated in Table 1 and 2.

Table 1: Hemogram of camels with no cytological or histopathological alterations (group A) and camels with different liver affections (group B) (Means ± SD)

	PCV (%)	HB (g/dl)	RBCs (×10⁶/ µl)	MCV (fl)	MCHC (g %)	TLC (×10³/µl)	Neut. (×10³/µl)	Lymp. (×10³/µl)	Mono. (×10³/µl)	Eosin. (×10³/µl)
Group A	25.00 ± 1a	12.57 ± 0.65 a	7.82 ± 0.60 a	31.17 ± 1.10a	49.80 ± 2.67a	10.43 ± 0.80 a	8.65 ± 0.34 a	1.56 ± 0.12 a	0.42 ± 0.03 a	0.21 ± 0.02 a
Hepatic lipidosis	20.00 ± 2 b	10.03 ± 0.68 b	6.47 ± 0.60 b	30.91 ± 0.38a	50.26 ± 2.11a	12.85 ± 0.35b	11.03 ± 0.30b	1.08 ± 0.04b	0.40 ± 0.03a	0.14 ± 0.02bc
Hepatitis	25.55 ± 1.53a	12.67 ± 0.80 a	7.87 ± 0.65 a	32.26 ± 1.44a	49.99 ± 0.41a	12.87 ± 0.50b	8.53 ± 0.34a	3.82 ± 0.15c	0.38 ± 0.02a	0.12 ± 0.01bc
Choleostasis	24.67±1.53ab	12.43 ± 0.86 a	7.81 ± 0.61 a	31.81 ± 0.89a	51.07 ± 1.51a	12.86 ± 0.67b	10.86 ± 0.57b	1.06 ± 0.07b	0.39 ± 0.04a	0.13 ± 0.01bc
Hepatic necrosis	20.00 ± 1 b	9.90 ± 0.26 b	6.27 ± 0.40 b	30.94 ± 0.45a	49.54 ± 1.33a	7.77 ± 0.67c	6.21 ± 0.53c	1.16 ± 0.10b	0.22 ± 0.01b	0.08 ± 0.01c
LSD	5.00	2.05	1.32	1.34	2.34	2.42	2.32	0.41	0.19	0.08

LSD represents least significant difference between different groups at probability P< 0.05.

Means with different superscripts (a,b,c) within a raw are significantly different at P< 0.05.

Table 2: Hemogram of camels with no cytological or histopathological alterations (group A) and camels with kidney affections (group B) (Means ± SD)

	PCV (%)	HB (g/dl)	RBCs(×10⁶/ µl)	MCV (fl)	MCHC (g %)	TLC (×10³/µl)	Neut. (×10³/µl)	Lymp. (×10³/µl)	Mono. (×10³/µl)	Eosin. (×10³/µl)
Group A	25.00 ± 1	12.57 ± 0.65	7.82 ± 0.60	31.17 ± 1.10	49.80 ± 2.67	10.43 ± 0.80	8.65 ± 0.34	1.56 ± 0.12	0.42 ± 0.03	0.21 ± 0.02
Kidney affections	23.00 ± 1	11.40 ± 0.56	7.11 ± 0.02	32.33 ± 1.34	49.56 ± 0.46	12.97 ± 0.57 *	11.34 ± 0.51 *	1.30 ± 0.06 *	0.40 ± 0.03	0.13 ± 0.01 *

* Significantly different from group A at P value <0.05

Table 3: Some biochemical parameters of camels with no cytological or histopathological alterations (group A) and camels with different liver affections (Means ± SD)

	T.protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	BUN (mg/dl)
Group A	7.80 ± 0.32 a	4.58 ± 0.64 a	3.33 ± 0.10 a	1.39 ± 0.2 a	12.33 ± 2.52 a	25.67 ± 5.13 a	75.67 ± 9.71 a	18.33 ± 7.64 a	25.13 ± 3.6 a
Hepatic lipidosis	6.76 ± 0.43b	2.98 ± 0.78b	3.78 ± 1.00b	0.91 ± 0.41b	46.67 ± 7.64b	62.33 ± 10.69b	128.33 ± 12.58b	48.33 ± 10.50b	25.40 ± 4.37a
Hepatitis	7.85 ± 0.30a	3.95 ± 0.37bc	3.91 ±0.29bc	1.01 ±0.16bc	55.67 ± 6.66bc	62.47 ± 11.68bc	63.67 ± 33.71a	19.33 ± 4.04a	25.00 ± 3.85a
Choleostasis	7.01 ± 0.72a	3.94 ± 0.77bc	3.97 ±0.25bc	1.02 ±0.32bc	43.33 ± 15.28bc	63.33 ± 5.86bc	145.00 ± 7.94bc	75.67 ± 6.51c	24.50 ± 4.29a
Hepatic necrosis	5.69 ± 0.46c	2.97 ± 0.04b	3.72 ±0.42bc	0.79 ±0.15bc	71.67 ± 7.64bc	34.67 ± 6.11abc	122.67 ± 7.23bc	68.00 ± 16.52bc	23.97 ± 5.40a
LSD	1.02	1.7	0.26	0.37	30.65	35.21	24.02	20.45	2.15

LSD represents least significant difference between different groups at probability P< 0.05.

Means with different superscripts (a,b,c) within a raw are significantly different at P< 0.05

In comparison to the mean values of group A, the leukogram showed significant leucocytosis in cases of hepatic lipidosis, choleostasis and hepatitis The DLC results, showed significant neutrophilia and lymphopenia in cases of hepatic lipidosis and choleostasis with significant lymphocytosis in cases of hepatitis. Significant neutropenia and lymphopenia were noticed in cases of hepatic necrosis. Cases of kidney affections revealed significant leucocytosis with significant neutrophilia and lymphopenia. From the above results, picture of stress was observed in cases of hepatic lipidosis, choleostasis and kidney affections in response to corticosteroid and epinephrine release, while in cases of hepatitis, lymphocytosis may be due to the antigenic stimulation of lymphoid tissues [15]. Hepatic necrosis showed significant neutropenia and lymphopenia which may be attributed to the excessive tissue demand occur in severe inflammation or endotoxemia causing an immediate accumulation, margination and activation of leukocytes in the microcirculation, especially in the alveolar capillaries [16].

Serum Biochemical Analysis: Statistical analysis of some biochemical parameters of camels with no cytological or histopathological alterations (group A) and camels with different liver affections are illustrated in Table 3. In comparison to the mean values of group A, the result of hepatic lipidosis, cholestasis and hepatic necrosis cases revealed significant increase in the activity of serum liver enzymes ALT, AST, ALP and GGT. In cases of hepatitis there is significant increase in the activity ALT and AST only. Significant decrease in A/G ratio due to decrease in serum albumin and increase in serum globulins concentrations with insignificant change in BUN were observed in all cases of hepatic affections.

In cases of hepatitis, an increased serum activity of ALT and AST is suggestive of hepatocellular membrane damage as ALT is a specific marker of hepatocellular injury in camel, although occasionally severe muscle damage may also increase serum ALT activity [17]. On the other hand,AST considers as a non-specific index for liver investigations as it elevated in skeletal or cardiac muscle diseases as well as liver diseases [18].

In cases of hepatic lipidosis, cholestasis and hepatic necrosis, increase serum activity of ALT, AST, ALP and GGT may suggest hepatobiliary diseases as hepatocytes injury or necrosis will result in extracellular leakage of ALT and AST which found in the cytoplasm of hepatocytes [17]. In cases of cholestasis, AST and ALT may be elevated due to liver damage as a secondary effect of cholestasis while in cases of hepatic lipidosis, GGT is only substantially increased when concurrent conditions such as pancreatitis or cholangiohepatitis are present [13].

Table 4: Some biochemical parameters of camels with no cytological or histopathological alterations (group A) and camels with kidney affections (Means ± SD)

	T.protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	BUN (mg/dl)	Creatinine (mg/dl)
Group A	7.80 ± 0.32	4.58 ± 0.64	3.33 ± 0.15	1.37 ± 0.25	25.13 ± 3.60	1.83 ± 0.22
Kidney affections	5.92 ± 0.55 *	3.07 ±0.13*	2.85 ± 0.43	1.08±0.13	49.56 ± 8.08 *	2.82±0.49*

* Significantly different from group A at P value <0.05

The changes observed in the concentrations of serum albumin and serum globulin content may be attributed to decreased protein production due to severe hepatic damageand increase in immunoglobulin in camel sera, respectively. BUN may be used to assess hepatic function. However, more than 80%o of the hepatic mass must be lost before any change in BUN is detected. Serum albuminand BUN concentrations are not very sensitive or specific indices for liver function as they can influenced by many other factors rather than liver affections [19].

Concerning cases of kidney affections, Statistical analysis of some biochemical of camels with no cytological or histopathological alterations (group A) and camels with kidney affections parameters are illustrated in Table 4. The results of BUN and serum creatinine showed significant increases while that of serum total proteins and serum albumin showed significant decreases. These changes may be attributed to the renal affection which confirmed cytologically by the presence of vacuolation and degeneration of renal tubular epithelial with necrotized cellular cast and histopathologically by tubular nephrosis and glomerulonephritis [20].

Cytological Findings

Hepatic Cytology: Camel's smears from Group A showed uniform large round to oval cells with abundant basophilic cytoplasm. Cells contain one or two round, centrally located nuclei with a single prominent pale blue nucleolus Fig. 1a. Normal hepatocytes often contain a small amount of dark brownish-green bile pigment Fig. 1b and occasionally small amount of dark brown-black pigmentthis pigment may be hemosidern Fig. 1c. Biliary epithelial cells; that are uniform in size with round nuclei and relatively small amount of pale blue cytoplasm may also see Fig. 1d. Other cells occasionally observed in small numbers in aspirates include macrophages (Kupffer cells), lymphocytes and neutrophils [4].

Hepatic Lipidosis: Most hepatocytes from hepatic lipidosis cases contain extensive, round, sharp delineated vacuoles Fig. 1e. The presence of clear round discrete vacuoles are usually suggestive of lipid; the vacuoles may vary in size from small to large ballooning vesicles that distend hepatocytes, pushing the nucleus to a side Fig. 1f.

From the above description, the hepatic smears revealed diffuse lipid accumulation in hepatocytes in response to the hepatocellular damage [21]. Excessive accumulation of fat in liver cells is a disease process termed hepatic lipidosis, fatty infiltration, or fatty liver. Hepatic lipidosis is increasingly recognized in cases of camelid illness and death after a period of inadequate energy intake. This disease may be explained as camels normally maintain higher blood glucose concentration (85-100 mg/dl), similar to that of non ruminant animals and greatly increased when stressed. Research suggests that animals become “insulin resistant” by aging, somewhat similar to becoming a diabetic. With insulin resistance, body cells will not utilize glucose efficiently and use the stored fat as an alternative energy source. This metabolic scenario would result in more rapid fat mobilization during periods of stress as in severe endoparasitism, inadequate dietary protein, extreme weather, interactions with herdmates, overcrowding and a variety of other stress factors leading to hepatic lipidosis [2, 22].

Hepatitis and Cirrhosis: Cytological smears from these cases showed two types of hepatitis; lymphocytic hepatitis and chronic active hepatitis (granulomatous inflammation). Lymphocytic hepatitis was characterized by increased numbers of small lymphocytes and plasma cells without increased numbers of macrophages Fig. 2a, while granulomatous inflammation was characterized by a mixed infiltrate of large numbers of macrophages beside lymphocytes, plasma cells with fibroblasts and occasionally eosinophils Fig. 2b. Hepatic cirrhosis was noticed with both types of hepatitis and may appear as a fibrocytic bundles Fig. 2 correactive fibroblasts between hepatic cells Fig. 2d.

From these cytological findings we can report that Inflammation was suspected in cytological specimens when the leukocytes were intimately associated with hepatic cells [4]. Inflammation of the liver may be neutrophilic (suppurative) or lymphocytic and It is usually due to cholangitis resulted from ascending bacterial infections [23]. Reactive fibrocytes were commonly seen a long with severe inflammation and careful should be taken not to over interpret this reactivity as a neoplastic activity. On the other hand, granulomatous inflammation of the liver is not uncommon as it also seen in liver of

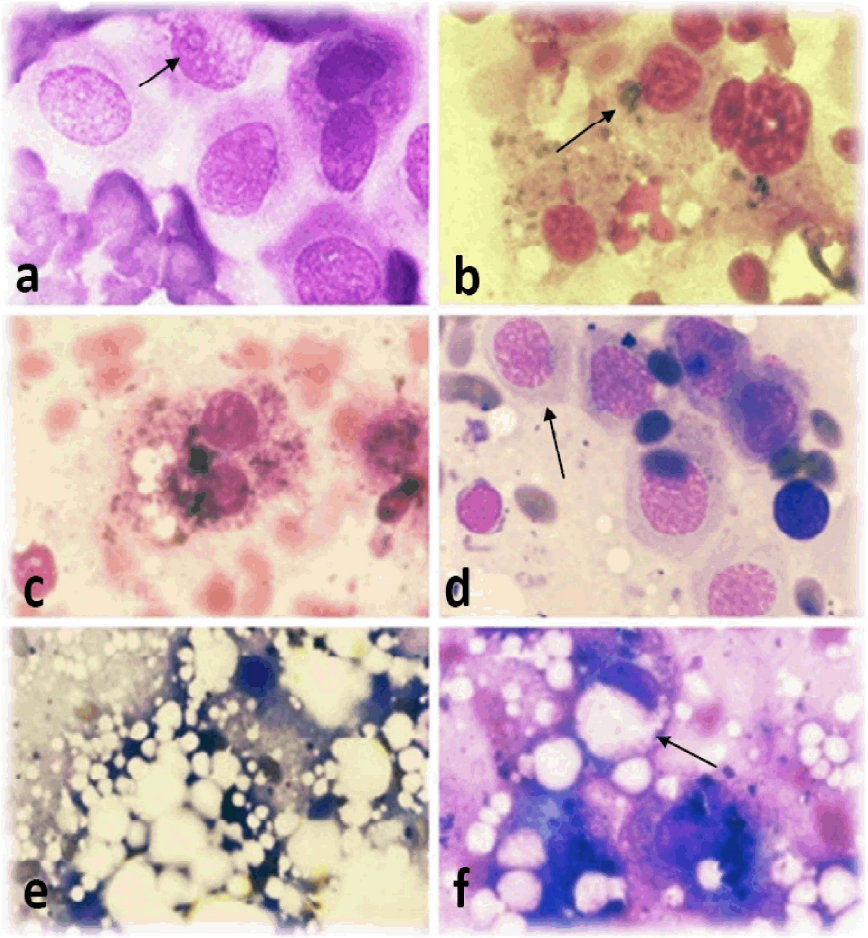

Fig. 1: a-Normal hepatocytes of group A. Cells contain one or two round, centrally located nuclei with a single prominent pale blue nucleolus (arrow) (Field stain x1000).
b- Normal hepatocytes of group A with a small amount of dark brownish-green bile pigment(arrow) (Field stain x1000).
c- Normal hepatocytes contain a small amount of hemosidern which is a more brown-black pigment than bile pigment (Field stain x1000).
d-Biliary epithelial cells of control group are small cells ,uniform in size with round nuclei and relatively small amount of pale blue cytoplasm(arrow) (Field stain x1000).
e- Liver smears from hepatic lipidosis cases revealed extensive and diffuse lipid accumulation in hepatocytes (Field stain x1000).
f-Hepatic lipidosis with ballooning vesicle that distend hepatocyte, pushing the nucleus to a side (arrow) (Field stain x1000).

Egyptian camels suffered from trypanosoma infection [24]. The differential diagnosis of granulomatous inflammation of the liver is broad and includes infectious etiologies as well as drug reactions.

Cholestasis: Hepatic smears from cholestasis appeared cytologically as accumulation of brownish-green pigment inside the individual hepatocytes Fig. 2e or between adjacent hepatocytes Fig. 2f. Moreover, there is hyperplasia of biliary epithelial cell with increases in cell and nuclear size Fig. 3a. Extracellular accumulation of bile, may be seen between hepatocytes.

From the above mentioned result we can conclude that bile formation is a secretory function of the liver which appears cytologically as intracellular or extracellular, brown-greenish pigment [25]. Its accumulation begins in bile canaliculi when the flow of bile is impaired at some point between the liver cells [4].

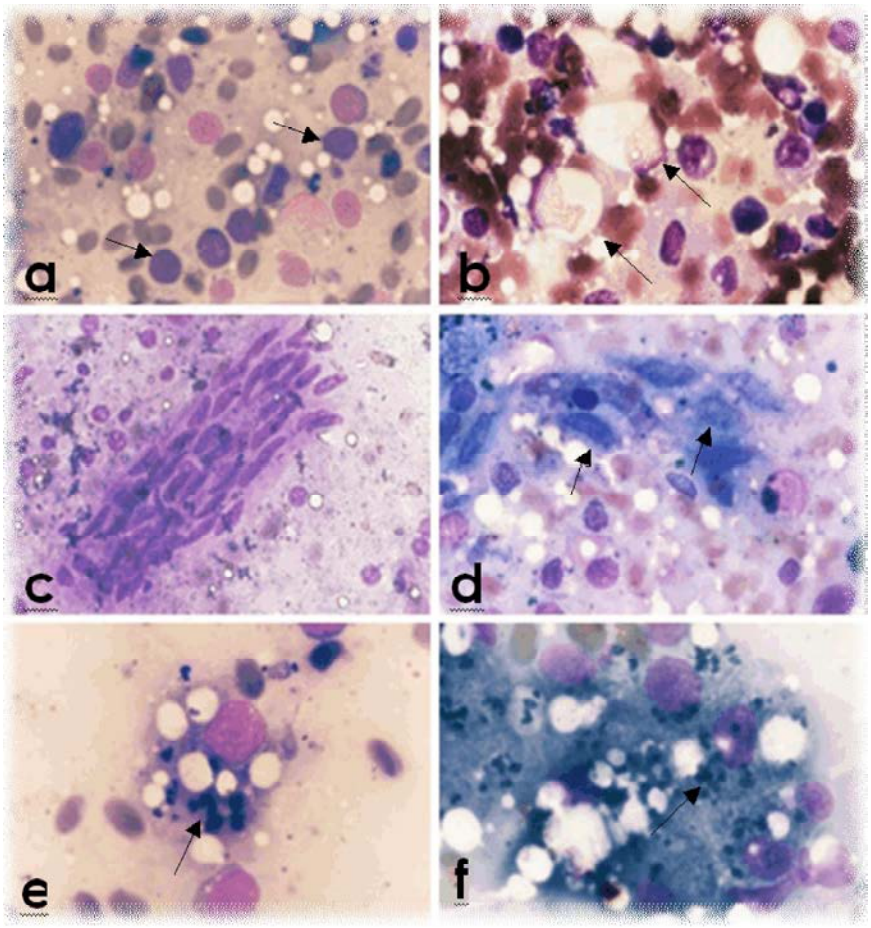

Fig. 2: a- Lymphocytic hepatitis showed increase numbers of reactive lymphocytes (arrow) without macrophages (Field stain x1000).
b-granulomatous inflammation with a mixed infiltrate of large numbers of macrophages(arrow) beside lymphocytes and plasma cells(arrow head) (Field stain x1000).
c- Fibrocytic bundles in cases of Hepatic cirrhosis (Field stain x1000).
d-Many reactive fibroblasts (arrow) were noticed in cases of Lymphocytic hepatitis(Field stain x1000).
e- Hepatic smears from cases of cholestasis with accumulation of brownish-green pigment(arrow) inside the individual hepatocyte with fatty infiltration (Field stain x1000).
f- Cluster of hepatocytes with accumulation of bile pigment (arrow) and fatty infiltration (Field stain x1000).

The types of cholestasis are divided into two types; an obstructive type of cholestasis where there is a mechanical blockage in the duct system such as can occur from a gallstoneor malignancy. The second type of cholestasis is the metabolic type which occurs because of cholangitis, intrahepatic cholestasis, primary biliary cirrhosis, genetic defects or acquired as a side effect of many medications [26].

Hepatic Necrosis: Hepatic smears from cases suffered from Hepatic necrosis showed completely necrotized hepatocytes with poorly delineated, light-colored, lacy appearing areas in the cytoplasm Fig. 3b. There was also mild infiltration of neutrophils, macrophages, lesser numbers of lymphocytes and plasma cells. karyolysis (complete dissolution of the chromatin) was pronounced in most hepatocytes Fig. 3c and a few numbers of hepatocytes were less severely affected.

From the above description, the hepatic smears revealed severe injury of hepatocytes resulted from liver cell necrosis. These findings appeared as a result of hepatopathy which may be resulted from endotoxin, Copper toxicity, Trypanosomes infection, Blue green algae toxicity or Infectious agents such as

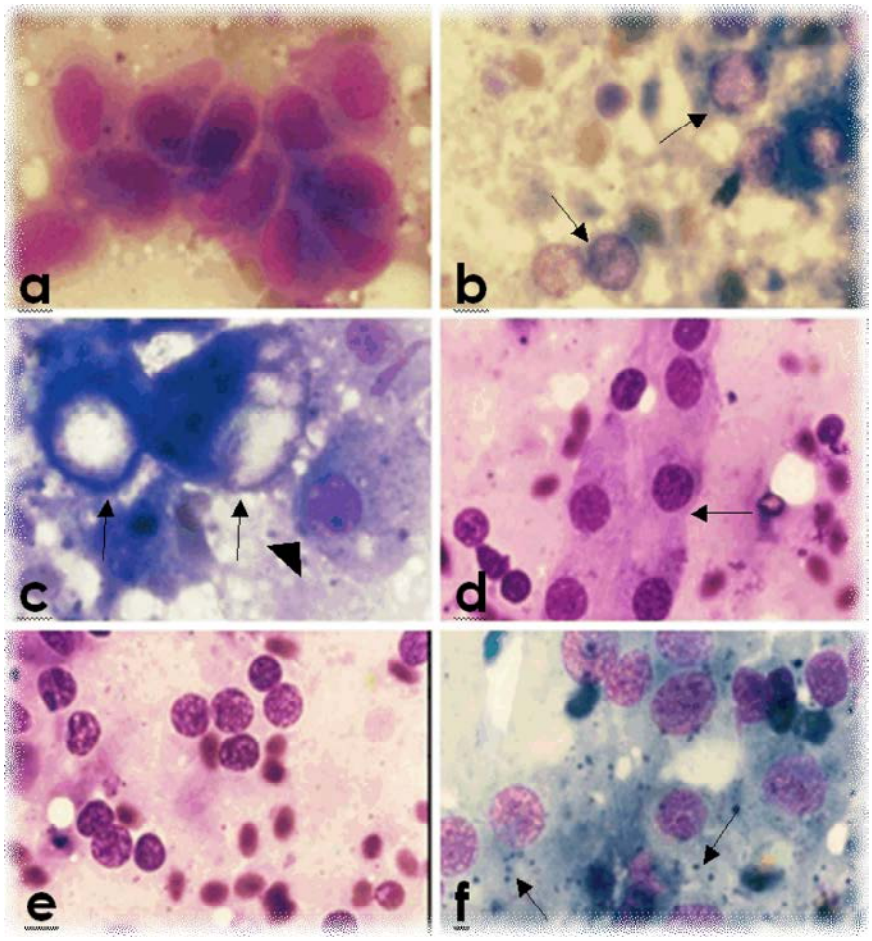

Fig. 3: a-Hyperplasia of biliary epithelial cells with increase in cell and nuclear size (Field stain x1000).
b- Hepatic smears from this group showed necrotized hepatocytes (arrow) with poorly delineated, light-colored, lacy appearing areas in the cytoplasm (Field stain x1000).
c- karyolysis (complete dissolution of the chromatin) was pronounced in most hepatocytes (arrow) and a few numbers of hepatocytes were less severely affected (arrow head) (Field stain x1000).
d-Renal tubular epithelial cells of group A often remain together as recognizable tubule fragment (arrow) (Field stain x1000).
e- Proximal convoluted tubule of group A; cluster of cells with light purple cytoplasm and indistinct cytoplasmic borders (Field stain x1000).
f- Distal convoluted tubule of group A; cell cluster seen with a blue-green granule (arrow) (Field stain x1000).

Salmonella spp., Listeria spp. or Chlamydia spp. The fore mentioned causes could induce hepatocellular necrosis through both direct and indirect effects on the liver. Indirectly by causing Kupffer's cells to release lysosomal enzymes, prostaglandins and collagenase that damage hepatocytes, or it may interact directly with the hepatocytes causing lysosomal damage, decreased mitochondrial function and necrosis. The hepatic necrosis may relate to the enteric infection which can invade the liver by way of septicemia or ascending cholangiobillary infection [27].

Renal Cytology: Renal impression smears from Group A showed renal tubular epithelial cells with abundant light blue cytoplasm and round nuclei. Renal tubular epithelial cells often remain together as recognizable tubule fragment of various sizes Fig. 3d. Cluster of cells with light purple cytoplasm and indistinct cytoplasmic borders are derived from proximal convoluted tubule Fig. 3e. The later cell cluster may see with a blue-green granule which is probably derived from the loop of henle or distal convoluted tubule Fig. 3f.

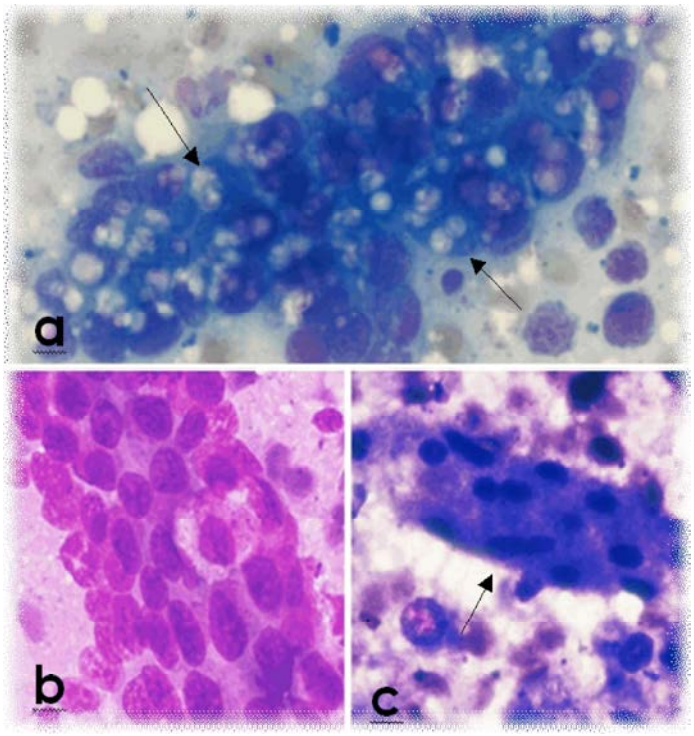

Fig. 4: a-Renal impression smears showed moderate degree of vacuolation and degeneration (arrow); reflect the tubular epithelium injury (Field stain x1000).
b- Renal impression smears showed cellular cast (Field stain x1000).
c- Renal impression smears showed necrotized cellular cast (arrow) (Field stain x1000).

Renal impression smears of group B showed moderate degree of vacuolation and degeneration on some areas; reflect the tubular epithelium injury Fig. 4a. Other smears appeared more affected in which two types of tubular casts were seen, cellular cast Fig.4b and necrotized cellular cast Fig. 4c. The later type was noticed with pyknotic nucleus and dark gray amorphous debris represents the necrotic material. Significance of tubular cast depends on the amount and type of cast present, similar to those in urine sediment. Tubular cast is a more advanced stage of tubular injury than cellular vacuolation and degeneration and reflect severe renal ischemia, infarction, prolonged exposure to toxic substance or administration of compounds that extremely irritating to the renal tubules [20].

Concerning Histopathological Alterations Observed in the Examined Livers: In this study, liver revealed various degenerative changes; the most demonstrated one was fatty change. The fatty change observed in this study either in the form of large focal area of hepatic steatosis with focal aggregation of mononuclear cells Fig. 5a or diffuse hepatic steatosis with diffuse fatty change of liver cells, mainly present as single large fat droplets (macrovesicular steatosis),which cause distortion of the cell Fig. 5b. Development of macrovesicular steatosis may have different causes such as diabetes, obesity or starvation. This result was supported by Tej Singh *et al*. [3] who mentioned that cloudy swelling, hydropicdegeneration, fatty change andamylodosis were the main degenerative changes recorded in the liver of camels. Cholangitis was also demonstrated in these cases which histologically characterized by hyperplasia of biliary epithelium. Formations of newly formed non functioning cholangioles as well as periductal infiltration with mononuclear cells Fig. 5c were demonstrated.

Karki [28] demonstrated severe biliary hyperplasia in adult Lama Alpaca in Nepal and attributed these lesions to the blockage of bile duct with liver fluke although no such lesion was identified at gross examination. One case of portal hepatitis associated with hepatic lipidosis was characterized by congestion of portal blood vessels with infiltration of portal area with mononuclear cells and portal fibroplasia Fig. 5d. Hepatic hemosiderosis was demonstrated in 3 cases Fig. 5e [3].

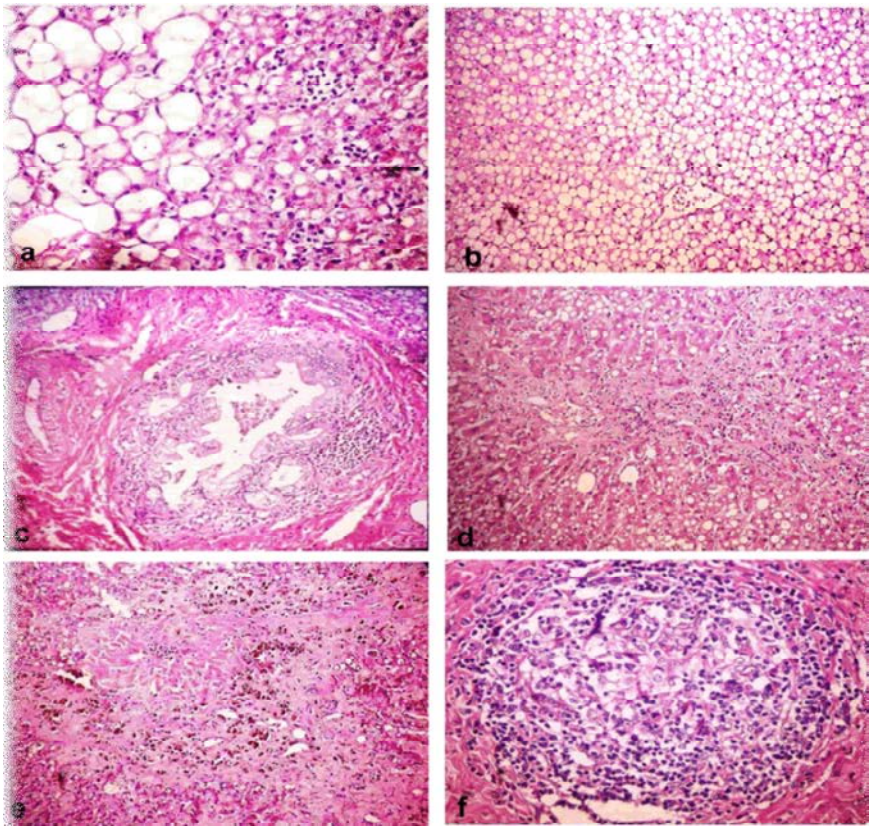

Fig. 5: a-Liver of camel showing large focal area of hepatic steatosis with focal aggregation of mononuclear cells (H&E X 400).
b-Liver of camel showing hepatic steatosis; diffuse fatty change of liver cells ,mainly present as single large fat droplets (macrovesicular steatosis) which cause distortion of the cell (H&E X200).
c-Liver of camel showing showed cholangitis; hyperplasia of biliary epathelium and formation of newly formed non functioning cholangioles as well as periductal infiltration with mononuclear cells (H&E X 20).
d-Liver of camel showing portal hepatitis; congestion of portal blood vessels with infiltration of portal area with mononuclear cells and portal fibroplasia(H&E X 200). e-Liver of camel showing hepatic hemosiderosis (H&E X 200).
e-Liver of camel showing hepatic hemosiderosis(H&E X 200).
f-Liver of camel showing granulomatous hepatitis; the granulome is consisted of circumscribed focal aggregation of epitheloid cells and mononuclear cells and encapsulated by delicate fibrous connective tissue capsule (H&E X 400).

Three cases showed granulomatous hepatitis; the granulome is consisted of circumscribed focal aggregation of epitheloid cells and mono nuclear cells and encapsulated by delicate fibrous connective tissue capsule Fig. 5 fsurrounding hepatocytes suffering from degenerative changes. These microscopic lesions were similar to those recorded by Abdelattif and Sakr *et al*. [16, 29] in the liver of Egyptian camels suffered from Trypanosoma infection.

Eight cases showed hepatocellular necrosis; five of them are associated with liver cirrhosis.The demonstrated liver necrosis in the form of minute to large focal area of hepatocellular necrosis infiltrated with mononuclear cell.

Different types of liver cirrhosis were diagnosed in this studys. Hepatic cirrhosis was in the form of portal, biliary, central,glissonian and pericellular cirrhosis. Massive cirrohosiswith atrophy of hepatic lobule Fig. 6a were demonstrated in 2 cases.Glissonian cirrhosis was characterized by marked thickening of the hepatic capsule due to fibrous connective tissue proliferation with vacuolar degeneration of the underlying hepatic cells Fig. 6b [29].

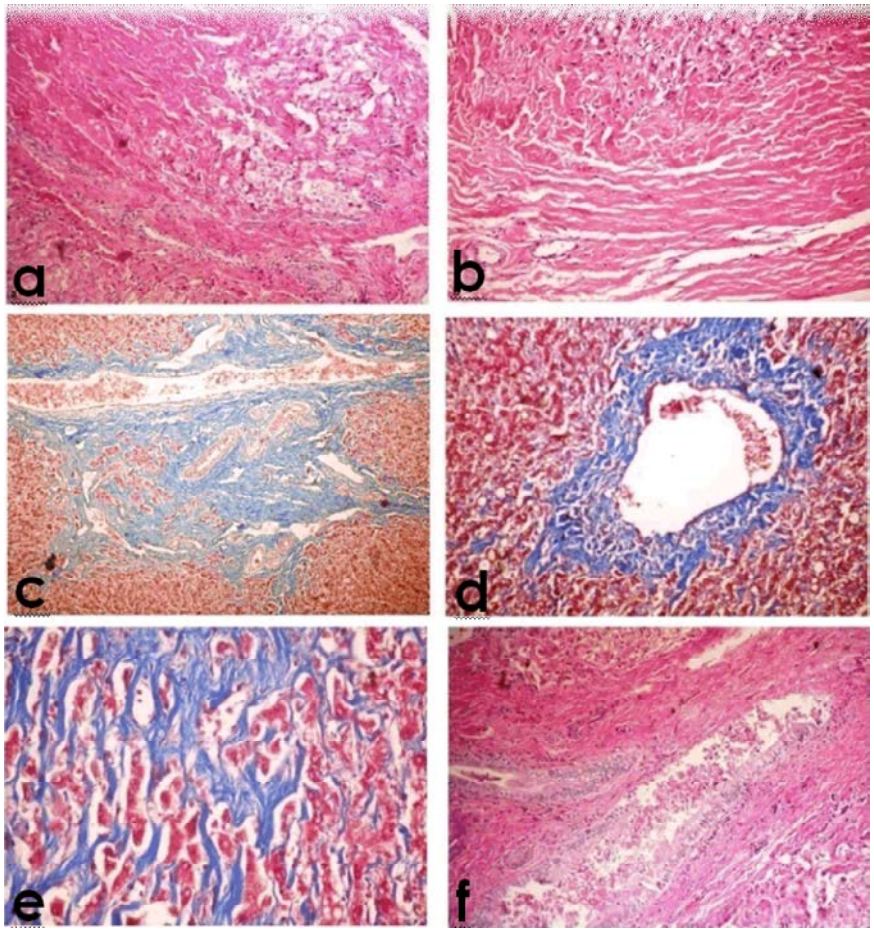

Fig. 6: a-Liver of camel showing massive cirrohosis with atrophy of hepatic lobule(H&E X 200)
b-Liver of camel showing Glissonian cirrhosis Marked thickening of the hepatic capsule(H&E X 400).
c-Liver of camel showing portal cirrhosis. Note; fibrous connective tissue proliferation enclosing the bile duc t(Masson´s Trichrome X 200).
d-Liver of camel showing central cirrhosis (Masson´s Trichrome X400).
e-Liver of camel showing pericellular cirrhosis (Masson´s Trichrome X400).
f- Liver of camel showing biliary hyperplasia and or severe destruction, desquamation of its lining epithelium with peribiliary fibrosis, mononuclear cell infiltration with formation of newly non functioning bile ductules (H&E X 200).

Portal cirrhosis showed fibrous connective tissue proliferation infiltrated by mononuclear cells and enclosed by degenerated hepatocytes Fig. 6c. Bile duct hyperplasia with thickening in the wall of portal blood vessels was noticed. Central cirrhosis were diagnosed histologically by fibrous connective tissue proliferation replaced the hepatic cells around central veins with degeneration of the adjacent hepatic cells Fig. 6d.

Pericellular cirrhosis Fig. 6e was reported associated with portal cirrhosis.Biliary hyperplasia and or severe destruction, desquamation of its lining epithelium with peribiliary fibrosis, mononuclear cell infiltration with formation of newly non functioning bile ductules Fig. 6fwere noticed. These microscopic pictures were also described by Gameel *et al.,* and Abdelattif [29, 30]. The Obstruction to intrahepatic bile flow leads to upstream bile ductular proliferation, inflammation and necrosis of adjacent periportal hepatic parenchyma, generalized choleostasis,portal tract scarring and bridging fibrosis. Cirrhotic liver is recognized as an end stage liver, which may be resulted from chronic toxicity due to ingestion of hepatotoxin, chronic extrahepatic and intrahepatic biliary obstruction,choleostasis together with chronic hepatitis [31].

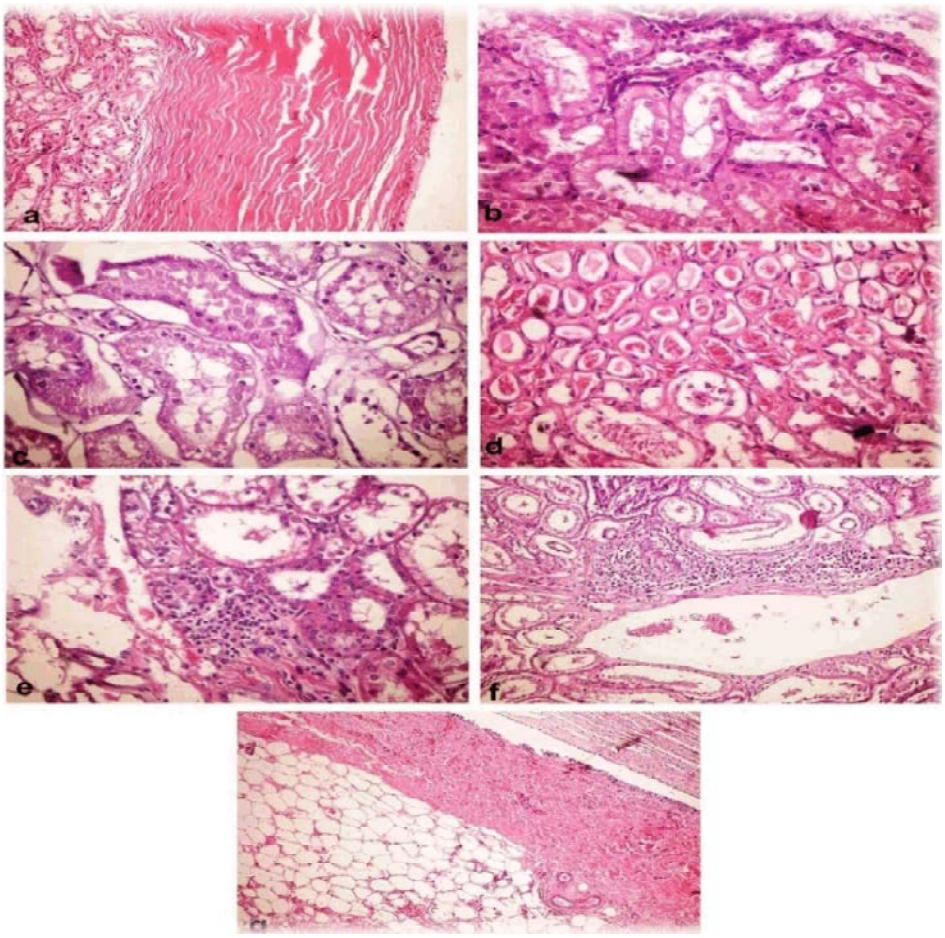

Fig. 7: a-Kidney of camel showing marked thickening of perirenal capsule and tubular nephrosis of the underlying renal tubules (H&E X 200).
b-Kidney of camel showing hyaline droplet nephrosis; note; presence of eosinophilic hyaline droplets in the cytoplasm of renal tubular epithelial cells (H&E X400).
c-Kidney of camel showing wrinkling of basement membrane of renal tubules together with degeneration of their epithelial lining and presence of eosinophilic hyaline droplets in the lumen of some renal tubules (H&E X 400).
d-Kidney of camel showing eosinophilic hyaline cast in the lumen of renal tubules (H&E X400).
e-Kidney of camel showing focal interstitial mononuclear cell aggregation (H&E X400).
f-Kidney of camel showing perivascular aggregation of mononuclear cells (H&E X200).
g-Kidney of camel showing fibroplasia of sub mucosa of renal pelvis and focal area of lipomatous metaplasia(H&E X200).

Concerning Histopathological Alteration Observed in the Examined Kidneys

Renal Capsule: Two cases showed marked thickening of perirenal capsule and tubular nephrosis of the underlying renal tubules Fig. 7a.

Tubulointerstitial Diseases: All examined kidneys showed tubular nephrosis as a response of prolonged ischemia or nephrotoxins. Hyaline droplet nephrosis with appearance of eosinophilic hyaline droplets in the cytoplasm ofrenal tubular epithelial cells Fig. 7b was also a common feature. These hyaline droplets represent accumulations of intracytoplasmic protein absorbed from the glomerular filtratedue to glomerulonephritis.Inter tubular edema with wrinkling of basement membrane of renal tubules associated with hyaline droplet nephrosis Fig.7c was also demonstrated. Almost all animals in this study had lesions of acute renal tubular necrosis. This lesion is most commonly caused by prolonged renal ischemia [32].

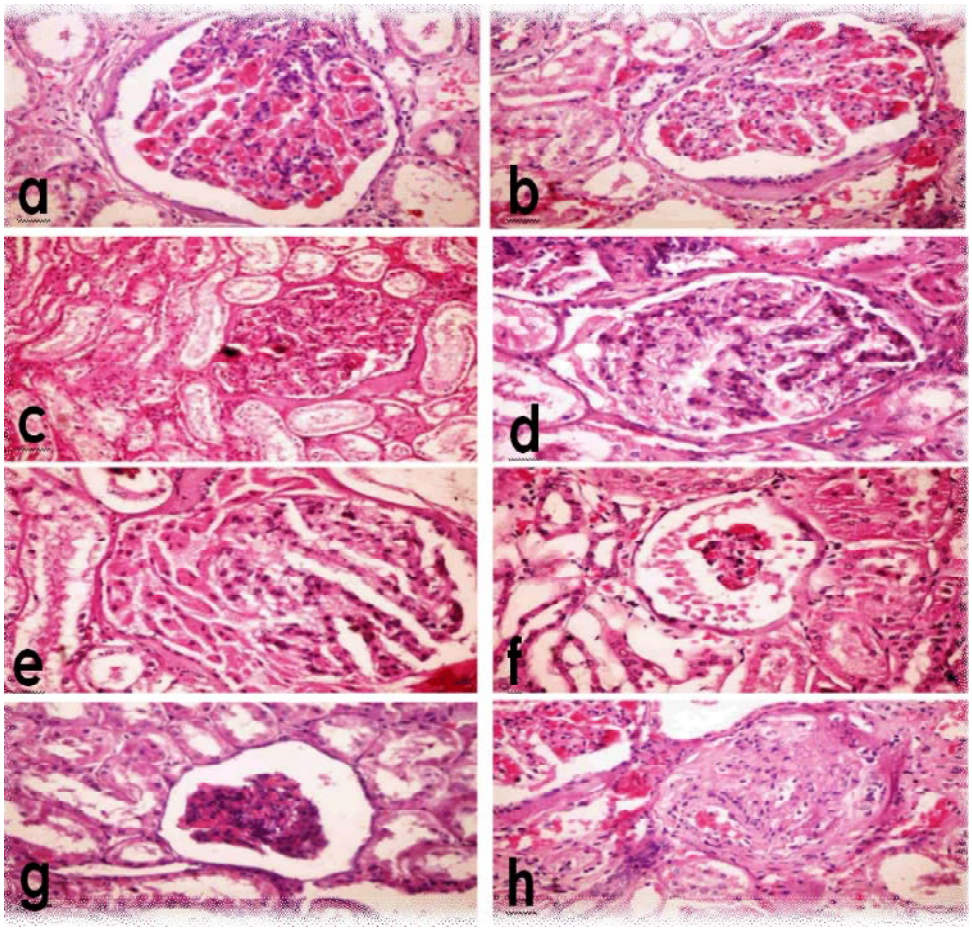

Fig. 8: a-Kidney of camel showing congestion of glomerular capillary tuffts with focal adhesion to pareital layer of Bowman´s capsule (H&E X 400).
b-Kidney of camel showing Focal thickening of pareital layer of Bowman;s capsule and Hypertrophy of its lining epithelial cells (H&E X 400).
c-Kidney of camel showing proliferative glomerulonephritis that was characterized by glomerular hypercellularity with adhesion to pareital layer of Bowman's capsule and tubular nephrosis of the surrounding renal tubules (H&E X200).
d-Kidney of camel showing membranous glomerulonephritis (H&E X 400).
e-Kidney of camel showing reflux of the detached proximal tubular epithelium into Bowman's space of the glomerulus(H&E X 400).
f-Kidney of camel showing glomerular atrophy with distension of Bowman's space which was filled with esinophilic hyaline materials(H&E X 400).
g-Kidney of camel showing glomerular hemorrhage and atrophy as well as distension of Bowman´s space (H&E X 400).
h-Kidney of camel showing global glomerulosclerosis (H&E X 400).

Toxic tubular nephrosis was also demonstrated in the present study. It characterized by extensive degeneration and necrosis of renal tubules especially at the proximal convoluted tubules, which are the most susceptible part to ischemia or toxic injury because of its high metabolic rate [33]. Nephrotoxins can directly damage the renal epithelial cells, particularly the proximal convoluted tubules either by alteration in ion transport at the luminal surfaceor indirectly by stimulation of rennin-angiotensin mechanism causing vasoconstriction and reduced blood flow that result in ischemia and tubular cell damage [34].

Intraluminal hemoglobin and hyaline cast Fig.7d were recorded in these examined cases. One case showed interstitial fibrosis with atrophy of renal tubules and thickening of their basement membrane. Renal fibrosis follows many renal lesions including primary inflammation of glomeruli, tubules, interstitial tissue,degeneration or necrosis of renal tubules. The mechanisms by which fibrosis is induced are related to destruction and loss of nephrons components by the inflammatory process and initiation of the healing process.

In renal tubular atrophy, recent experimental studies have demonstrated that after severe nephrotoxin-induced tubular epithelial cell injury, the remaining cells undergo accelerated apoptosis resulting in tubular atrophy.Interstitial fibrosis with focal interstitial mononuclear cell aggregation Fig. 7e were recorded in 2 cases. Three cases showed perivascular mononuclear cell aggregation Fig. 7f. Fibroplasia of sub mucosa of renal pelvis and focal area of lipomatous metaplasia Fig. 7g were demonstrated in only one case.

Glomerular Diseases: Four cases showed congestion of glomerular capillary tufts with focal adhesion to parietal layer of Bowman's capsule Fig. 8a. Focal thickening of parietal layer of Bowman's capsule and hypertrophy of its lining epithelial cells Fig. 8b accompany proliferative, membranous and membronoproliferative glomerulonephritis. Twocases showed proliferative glomerulonephritis that was characterized by glomerular hypercellularity with adhesion to parietal layer of Bowman's capsule and tubular nephrosis of the surrounding renal tubules Fig. 8c. The hypercellularity of glomerular tufts resulted from proliferation of endothelial and mesangial cells with influx of leukocytes leading to adhesion of tufts as well as degeneration and necrosis of renal tubules may be due to viral, bacterial or protozoal infections [35].

Four cases showed membranous glomerulonephritis Fig. 8d which characterized by thickening of the basement membrane of glomerular tuft with excessive mesangial matrix. Membranous glomerulonephritis was claimed to either excessive synthesis of glomerular basement membrane or trapping and deposition of macromolecules such as immune complexes which are selectively trapped in the glomerular filter.Renal tubules revealed necrobiotic changes with intraluminal accumulation of proteinous material. This was attributed to the glomerular damage with reduction of vascular perfusion and leakage of large quantities of glomerular filtrate [36].

One case showed reflux of the detached proximal tubular epithelium into Bowman's space of the glomerulus Fig. 8e. This lesion is associated with acute renal tubular necrosis; Infraglomerular tubular reflux is the movement or intrusion of detached proximal tubular epithelium into Bowman's space, around the renal glomerulus. This change is considered a very sensitive indicator of tubular epithelial damage and was found in relation to membranous glomerulonephritis [32].

Five cases showed glomerular atrophy with distension of Bowman's space which was filled with eosinophilic hyaline materials Fig. 8f were also demonstrated.Glomerular hemorrhage and atrophy as well as distension of Bowman's space Fig. 8g were demonstrated in one case. One case showed global glomerulosclerosis (the entire glomerular tuft is involved) which characterized by increase in the fibrous connective tissue, mesangial matrix and loss of glomerular capillary Fig. 8h. Glomerulosclerosis is considered not only the end stage of glomerulonephritis, but resulted from any chronic insults that leading to loss of nephrons function. Meanwhile glomerulosclerosis may result from increased mesangial matrix with deposition of immunoglobulin and complement on the glomerular basement membrane end by hyalinization of the glomerulus [37].

CONCLUSION

The present study concluded that hepatic lipidosis was the most hepatic affection noticed in the diseased livers. On the other hand, different glomerular and tubulointerstitial diseases were also seen in the examined camels. Although abnormal serum concentrations of hepatic enzymes have been found with hepatic necrosis, lipidosis, hepatitis and choleostasis, diagnosis of hepatic disease should not be dependent upon liver enzyme concentrations alone; as it cannot reach to a definitive diagnosis, cytology and histopathology must be considered in hepatic disease evaluation.

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