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# Diagnostic Studies on Acetaminophen Toxicosis in Dogs

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**Abstract:** Seventeen apparently healthy mongrel dogs aged from 1-2 years were used in this study. Ultrasonographic, clinicopathological, cytological and histopathological changes associated with the uses of acetaminophen as maximum therapeutic and toxic doses were studied. The dogs were divided into three groups as follow; first group without receiving any treatment as a control group, second and third group was orally received 128 and 200 mg kg-1 B.W as a maximum therapeutic and toxic dose of acetaminophen, respectively. The experiment continued for 10 days, through which collection of samples from all groups of dogs was performed on the 3rd and the 10th day post treatment (p.t). Ultrasonographic examination revealed hyperechodensity of liver parenchyma at 3rd day in some dogs. The ultrasonographic changes became more pronounced in the third group at the 10th day (p.t) in liver parenchyma and renal cortex expressed by hyperechogenicity and disappearance of echogenic portal vein walls, especially in toxic dose of acetaminophen. The clinicopathological changes revealed the presence of hemolytic anemia. Increases activities of liver enzymes as well as serum bilirubin, blood glucose, blood urea nitrogen (BUN) and serum creatinine concentrations in all experimental dogs were observed. The cytological examination showed the presence of hepatic toxicity, hyperplasia of splenic tissue, vacuolation and degeneration of renal tubular epithelial cells and different degrees of bone marrow (B.M) hypocellularity. All the above mentioned changes were more pronounced in the toxic dosed group than in the maximum therapeutic dosed group in comparison to the control one. These results were correlated with ultrasonographic and histopathological findings.It was concluded that, acetaminophen should be used only in limited amounts for limited periods as its signs of toxicity appeared even with its therapeutic dose.

**Key words:** Acetaminophen · Ultrasonographic · Clinicopathological · Cytological · Histopathological

## INTRODUCTION

Companion animals are at risk for developing toxicosis to prescription drugs as well as over-the-counter (OTC) medications, either by deliberate administration of the medication by owners, or by accidental consumption of improperly stored drugs. Dogs are more likely to eat the pills or ointment when the container finally breaks open. On the other hand, cats are generally more discrete about what they put in their mouths and are less likely to voluntarily ingest medications. Veterinarians occasionally use OTC drugs to treat a variety of their patients' maladies. The most common of these are probably the non-steroidal anti-inflammatory drugs (NSAIDs), acetaminophen is one of the top two most common household medications and it is not surprise that acetaminophen toxicity is commonly reported. In fact, between January 1998 and March 2000, veterinarians at the Animal Poison Control Center (ASPCA), USA, consulted on over 1050 cases of accidental exposure to acetaminophen in dogs and cats [1]. We prescribe NSAIDs to treat musculoskeletal inflammation, influenza, cold and control fevers [2, 3]. Acetaminophen toxicity produces the most common form of acute hepatic failure in the United States and United Kingdom, accounting for 39% of cases in a recent report [4]. In dogs the toxic effects of acetaminophen include hepatic damage, kidney failure and serious hematologic disorders as Heinz bodies

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formation and hemoglobin damage (non functioning hemoglobin) [5-9]. The clinical signs of acetaminophen toxicity are related to hemoglobin damage and hepatotoxicity, these signs include tachypnea, dyspnea, cyanosis, icterus, vomiting, hypothermia and facial or paw edema [10-12]. In our study, we didn't find any Egyptian paper deal with the cytological findings associated with acetaminophen toxicity. In addition to the present study aimed to determine which of the following tools; ultrasonographic, clinicopathological, cytological and histopathological examinations, were more rapid and accurate for the diagnosis of acetaminophen toxicity in its maximum therapeutic and toxic dose.

## MATERIALS AND METHODS

**Animals and Experimental Design:** Seventeen apparently healthy mongrel dogs aged from 1-2 years were used in this study. The dogs were divided into three groups as follow; first group composed of 5 dogs left without receiving any treatment as a control group, second and third group was composed of 6 dogs for each and orally received 128 and 200 mg $kg^{-1}$ B.W as a maximum therapeutic and toxic dose of acetaminophen according to Schlesinger [2] and Paget and Barnes [11], respectively. The experiment continued for 10 days, through which clinical signs, clinical examination and collection of samples from all groups of dogs were performed at the 3rd and the 10th days post treatment (p.t). All groups were subjected to ultrasonographic, clinicopathological, cytological and histopathological examinations.

**Clinical Examination:** Each dog was clinically examined for respiratory and pulse rates, rectal temperature, lymph nodes palpation, tactile percussion and abdominal palpation [12].

**Ultrasonographic Examination:** Ultrasonography for each fasted dog (24 hrs fasting) was performed by positioning it in a dorsal recumbency. Cranial ventral abdomen was clipped and sheaved, then covered with coupling gel. Transverse and longitudinal scans were taken using Pie-Medical Scanner (Maastricht, Netherlands) and sector transducer with alternating frequency of 5.0-7.5 MHz according to the method described by Nyland *et al*. [13].

**Collection and Preparation of Samples**

**Blood Samples for Clinicopathological Examination:** Two blood samples were taken from each dog (anterior median vein). The first blood sample was anticoagulated by di-potassium salt of ethylene diamine tetra-acetic acid (EDTA) and was used for evaluating hemogram. The second blood sample was collected in a clean centrifuge tube and was allowed to clot, then centrifuged at 3000 rpm for 10 minutes for serum separation. The clear non-hemolysed supernatant serum was harvested for biochemical studies.

**Tissue Specimens for Cytological Examination:** After necropsy, the impression smears from liver, spleen and kidney at 3rd and 10th days (p.t) were taken after cutting the removed mass into two halves to obtain a freshly cutted surface. The cutted surface was blotted on clean paper towel. The surface was made small enough to make several rows of imprints, then stained by field stain [14], washed and examined under the microscope [15]. Bone Marrow (B.M) aspiration was collected from all groups, stained by field stain and evaluated [16].

**Tissue Specimens for Histopathological Examination:** Tissue specimens including liver, spleen and kidney were collected at 3rd and 10th days (p.t) and fixed in 10% neutral buffered formalin for preparing paraffin tissue sections at 4-6μ thickness. These sections were stained with hematoxylin and eosin [17].

**Hematological and Serum Biochemical Studies**

**Hematological Studies:** Total erythrocyte leukocyte and counts were recorded using an improved Neubauer hemocytometer. Packed cell volume (PCV%) was estimated by the microhematocrit technique. Hemoglobin concentration was colorimetrically determined using the cyanmethemoglobin method. Differential leukocytic count was performed on Giemsa stained blood smears [18].

**Serum Biochemical Studies:** Serum samples were prepared to assay the following biochemical studies: serum total proteins was determined by the Biuret reaction according to Weichselbaun [19], serum albumin was determined according to Dumas and Biggs [20] and serum globulins were determined by subtracting value of serum albumin from the value of serum total proteins. A/G ratio was obtained by subdividing values of serum albumin by those of serum globulins. Colorimetric determination of alanine amino transferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) activities was performed according to Dumas and Biggs [20], Reitman and Frankel [21] and Tietz [22], respectively. Blood glucose level was determined as described by Trinder [23]. Blood urea nitrogen (BUN) was determined

using enzymatic method according to Tabacco *et al*. [24]. Serum creatinine was assayed using the method described by Fabiny and Ertingshausen [25]. Assay of bilirubin was carried out by a test kit according to the Jendrassik-Grof method described by Doumas *et al*. [26]. The above mentioned serum biochemical parameters were assayed using reagent kits supplied by StanBio Laboratories incorporation, USA.

**Statistical Analysis:** Values were expressed as mean ± SD. Statistical comparisons among the means of different experimental groups 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.

## RESULTS AND DISCUSSION

**Clinical Signs:** The observed clinical signs included anorexia, weight loss, weakness, tachypnea, dyspnea, icterus, vomiting, hypothermia, lethargy and apathy, these signs were similar to those observed by Aaronson [10] and Wongnawa *etal*. [27]. These findings appeared at the 3rd day (p.t) of the third group and became more pronounced at the 10th day (p.t) of both second and third experimental groups. These clinical signs may be attributed to the present hemolytic anemia,especially in the third group.

**Clinical Examination:** The clinical examination revealed significant increases in both respiratory and pulse rates (Table 1) which may be attributed to the presence of hemolytic anemia. This hemolytic anemia resulted in stimulation of chemoreceptor trigger zone (CRT) leading to the increases of both respiratory and pulse rates, especially in third group. The examined rectal temperature was normal to subnormal. The superficial lymph nodes were free in all experimental groups and the mucous membranes were pale. The physical examination revealed presence of anterior abdominal pain with moderate hepatomegally which was supported by liver histopathological findings [28].

**Ultrasonographic Examination:** Sonographic examination of the control group showed homogenous hypoechoic liver parenchyma interrupted by anechoic gall bladder and echogenic portal vein walls. Liver parenchyma is margined by hyperechoic diaphragm. The hepatic parenchyma appeared more echoic than renal cortex and lesser echoic than spleen (Fig. 1a and b). At the 3rd day (p.t), sonographic examination of the second group (only three of the six dogs) revealed slight increase in echodensity of liver parenchyma, which considered the early detectable sonographic findings (Fig. 1c). At the 3rd day (p.t) the third group (five of six dogs) showed markedly increased in the echogenicity of the liver parenchyma was with lesser clearance of portal vein wall echogenicity (Fig. 1d). The increased echogenicity reflected the hepatic damage and its replacement by fibrous connective tissue [13]. These findings were supported by the histopathological findings of the liver. At the 10th day (p.t), both experimental groups showed marked increase in echogenicity of liver parenchyma with disappearance of echogenic portal vein wall. These changes were more pronounced in the third group than those of the second group. At the 10th day (p.t), both experimental groups showed marked diffuse increase in echodensity of liver parenchyma (Fig. 1e and f). These changes revealed the aggressive increases in echogenicity of liver parenchyma with disappearance of echogenic portal vein wall, this observation was more pronounced in the third group than those in the second group. The renal cortex showed an increased echodensity than spleen, especially in the third group. These increased renal cortex density matched with renal damage that was observed by histopathological examination.

Table 1: Effect of Acetaminophen Toxicosis on general clinical examination of dogs in different experimental groups (means ± SD)

| | | 3rd day (p.t) | | | 10th day (p.t) | | |
|---|---|---|---|---|---|---|---|
| | Unit | First gp | Second gp | Third gp | First gp | Second gp | Third gp |
| Respiratory rate | rate/min | 25±3.0 | 27±2.2 | 29±3.0 | 25±3.0 | 31±1.6 | 37±1.8 |
| Pulse rate | rate/min | 62±1.7 | 69±1.9 | 74±2.8 | 62±1.7 | 73±2.1 | 88±2.2 |
| Rectal temp. | °C | 38.1±0.3 | 38.4±0.2 | 38.3±0.3 | 38.1±0.3 | 38.3±0.4 | 38.1±0.3 |

First group: represents the control group.

Second group: represents the maximum therapeutic dosed group.

Third group: represents the toxic dosed group.

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.

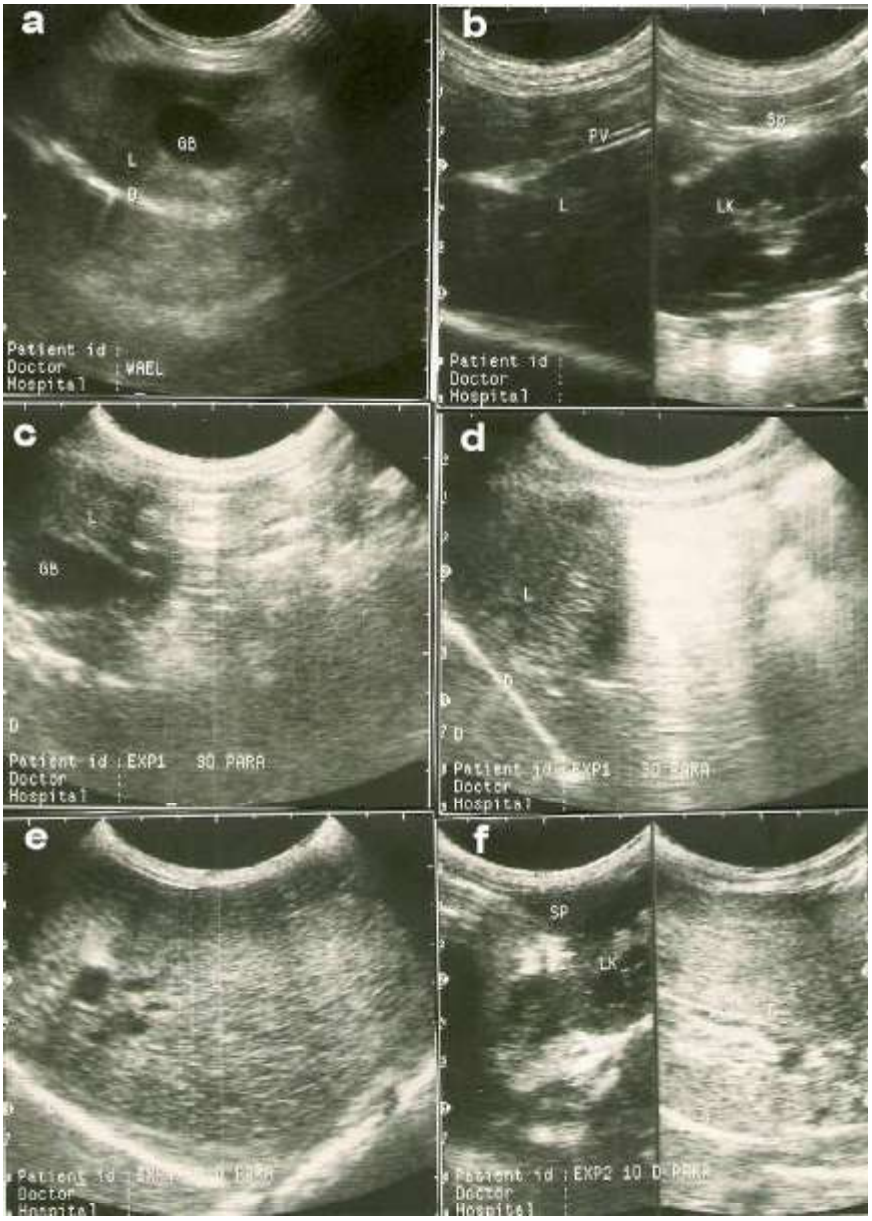


Fig. (1)Scan-A: Normal sagittal scan of the liver (L) parenchyma appeared homogenous moderately hypoechoic interrupted by echolucent gall bladder and margined by hyperechoic diaphragm (D).Scan-B: Double B-scan displaying that echogenicity of the spleen was nearly isoechoic with or more echoic than liver parenchyma. Liver parenchyma was little bit more echoic than renal cortex of left kidney (L.K.) with prominence of echogenic portal vein walls at the same depth and gain settings (control dog). Scan-C: Hepatic scan of second group at the 3$^{rd}$ day (p.t) revealed moderate echoic liver parenchyma interrupted with anechoic gall bladder.Scan-D: Hepatic scan of third group at the 3$^{rd}$ day (p.t) showed moderate increase in echogenicity and less clearance of echogenic portal vein walls.Scan-E: Hepatic scan of second group at the 10$^{th}$ day (p.t) displayed marked diffuse hyper-echodensity and less clearance of echogenic portal vein wall.Scan-F: Sagittal double B-scan of liver, spleen and kidney of third group at the 10$^{th}$ day (p.t) revealed markedly diffuse hyper-echogenicity of hepatic parenchyma and disappearance of echogenic portal vein wall. Hepatic parenchyma showed marked increases in the echodensity than the spleen and renal cortex. Renal cortex displayed increased echodensity than spleen.

Table 2: Effect of Acetaminophen Toxicosis on hemogram and M/E of dogs in different experimental groups (means ± SD)

| | | 3rd day (p.t) | | | 10th day (p.t) | | | |
|---|---|---|---|---|---|---|---|---|
| | Unit | First gp | Second gp | Third gp | First gp | Second gp | Third gp | LSD |
| PCV | % | 32.00±1.00$^{a}$ | 29.00±1.00$^{b}$ | 26.00±2.00$^{c}$ | 32.00±1.00$^{a}$ | 27.00±1.00$^{b}$ | 24.00±2.00$^{c}$ | 1.78 |
| Hb | g/dl | 11.20±0.20$^{a}$ | 11.00±0.85$^{a}$ | 11.83±1.10$^{a}$ | 11.10±0.20$^{a}$ | 11.80±1.10a$^{b}$ | 12.57±0.50$^{b}$ | 0.96 |
| RBCs | $\times10^{6}/\mu l$ | 5.30±0.11$^{a}$ | 4.98±0.56$^{a}$ | 4.10±0.90$^{b}$ | 5.24±0.05$^{a}$ | 4.30±0.98$^{b}$ | 3.97±0.49$^{b}$ | 0.79 |
| MCV | fl | 60.37±0.68$^{a}$ | 58.57±5.16$^{a}$ | 64.80±9.58$^{a}$ | 60.97±1.30$^{a}$ | 64.93±14.21$^{a}$ | 60.73±3.81$^{a}$ | 9.43 |
| MCHC | g% | 34.97±0.45$^{a}$ | 37.90±1.67$^{b}$ | 45.59±4.26$^{c}$ | 34.63±0.45$^{a}$ | 43.60±2.45$^{b}$ | 52.46±2.37$^{c}$ | 2.95 |
| TLC | $\times10^{3}/\mu l$ | 7.58±2.00$^{a}$ | 9.01±2.00$^{a}$ | 11.89±2.00$^{b}$ | 7.40±0.33$^{a}$ | 11.28±0.25$^{b}$ | 6.92±0.34$^{b}$ | 1.80 |
| Neut. | $\times10^{3}/\mu l$ | 5.23±2.00$^{a}$ | 6.75±2.00$^{a}$ | 9.39±2.00$^{b}$ | 5.16±0.11$^{a}$ | 8.88±0.29$^{b}$ | 4.71±0.20$^{ac}$ | 1.79 |
| Lym. | $\times10^{3}/\mu l$ | 2.16±0.65$^{a}$ | 1.71±0.20$^{b}$ | 1.54±0.20$^{b}$ | 1.60±0.28$^{a}$ | 1.58±0.20$^{a}$ | 1.70±0.20$^{a}$ | 0.42 |
| Mono. | $\times10^{3}/\mu l$ | 0.15±0.02$^{a}$ | 0.36±0.20$^{b}$ | 0.80±0.15$^{c}$ | 0.14±0.01$^{a}$ | 0.67±0.20$^{b}$ | 0.27±0.02$^{ac}$ | 0.17 |
| Eosin. | $\times10^{3}/\mu l$ | 0.30±0.02$^{a}$ | 0.18±0.02$^{ab}$ | 0.12±0.02b$^{c}$ | 0.59±0.28$^{a}$ | 0.22±0.02$^{b}$ | 0.13±0.02$^{bc}$ | 0.14 |
| M/E | - | 0.91±0.20$^{a}$ | 0.91±0.19$^{a}$ | 0.88±0.11$^{a}$ | 0.93±0.25$^{a}$ | 0.89±0.11$^{a}$ | 0.91±0.21$^{a}$ | 0.23 |

First group: represents the control group.

Second group: represents the maximum therapeutic dosed group.

Third group: represents the toxic dosed group.

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.

**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)] of different experimental groups are illustrated in Table 2.

In comparison to the mean values of the control group, PCV% of both experimental groups showed significant ($P < 0.05$) decreases throughout the experiment. Hb concentration values showed significant ($P < 0.05$) increases in the third group on 10th day (p.t). RBCs values revealed significant ( $P < 0.05$) decreases in all experimental groups except on the 3rd day (p.t) in the second group. All the before mentioned changes were more pronounced in the third group than in the second group. MCV values revealed insignificant changes in all experimental groups, while those of MCHC showed significant ($P < 0.05$) increases. On microscopical examination of the stained blood film, poikilocytosis, anisocytosis, Hienz bodies, eccentrocyte and target cells were observed,especially in the third group (Fig. 4a and b).

From the above mentioned data the hemolytic anemia was determined by the increases of Hb concentration and MCHC value. This anemia may be attributed to the toxic effect of acetaminophen metabolites on hemoglobin [29]. These metabolites began to bind iron and cellular material resulting in the presence of non function hemoglobin [30], membrane oxidative injury (eccentrocytes) and Heinz body formation which were permanent injuries leading to shortened erythrocyte survival [16, 31].

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

Compared to the control group, the results of the second and third groups showed significant ($P < 0.05$) leukocytosis throughout the experiment except on the 3rd day (p.t) of the second group and on the 10th day (p.t) in the third group. The DLC results showed significant ($P < 0.05$) neutrophilia on the 3rd and the 10th day (p.t) of the third and the second group, respectively. Significant ($P < 0.05$) lymphopenia was observed in all experimental groups on the 3rd day (p.t). Monocytosis was seen except on the 10th day (p.t) of the third group. Significant ($P < 0.05$) eosinopenia was reported in all groups except on the 3rd day (p.t) of the second group.

From the above results typical picture of stress was observed in all experimental groups in response to damaged red cells, corticosteroid and epinephrine release [2]. Except on the 10th day (p.t) in the third group, insignificant leukopenia was observed. This leukopenia may be attributed to the hypocellularity of B.M, which was supported by our cytological examination of B.M smears.

Table 3: Effect of Acetaminophen Toxicosis on serum biochemical parameters of dogs in different experimental groups (means ± SD)

| | | 3rd day (p.t) | | | 10th day (p.t) | | | |
|---|---|---|---|---|---|---|---|---|
| | Unit | First gp | Second gp | Third gp | First gp | Second gp | Third gp | LSD |
| ALT | U/L | 41.90±2.70 | 53.80±5.20$^{b}$ | 88.20±2.00$^{c}$ | 41.80±2.75$^{a}$ | 64.90±1.30$^{b}$ | 91.00±4.10$^{c}$ | 4.12 |
| ALP | U/L | 17.50±0.61 | 21.40±1.17$^{b}$ | 32.10±1.18$^{c}$ | 17.40±0.66$^{a}$ | 29.10±2.10$^{b}$ | 42.10±2.00$^{c}$ | 1.78 |
| GGT | U/L | 21.80±3.50 | 27.50±2.70$^{b}$ | 41.20±2.40$^{c}$ | 21.63±3.65$^{a}$ | 31.20±1.60$^{b}$ | 39.10±2.00$^{c}$ | 3.45 |
| T.bili. | mg/dl | 0.28±0.02 | 0.53±0.06$^{b}$ | 0.92±0.02$^{c}$ | 0.28±0.02$^{a}$ | 0.72±0.09$^{b}$ | 1.03±0.14$^{c}$ | 0.09 |
| D.bili. | mg/dl | 0.10±0.01 | 0.23±0.04$^{b}$ | 0.43±0.01$^{c}$ | 0.10±0.01$^{a}$ | 0.35±0.01$^{b}$ | 0.42±0.03$^{c}$ | 0.03 |
| I.bili. | mg/dl | 0.18±0.01 | 0.29±0.02$^{b}$ | 0.49±0.01$^{c}$ | 0.18±0.01$^{a}$ | 0.37±0.07$^{b}$ | 0.62±0.12$^{c}$ | 0.07 |
| T.prot. | g/dl | 8.07±0.08 | 8.50±0.16$^{a}$ | 8.67±0.99$^{a}$ | 8.02±0.09$^{a}$ | 8.40±0.19$^{a}$ | 8.60±2.00$^{a}$ | 1.15 |
| Alb. | g/dl | 3.37±0.60 | 4.20±0.31$^{a}$ | 3.40±0.25$^{a}$ | 3.65±0.20$^{a}$ | 3.60±0.17$^{a}$ | 3.70±2.00$^{a}$ | 1.10 |
| Glob. | g/dl | 4.70±0.61 | 4.30±0.15$^{a}$ | 5.18±0.74$^{a}$ | 4.37±0.11$^{a}$ | 4.80±0.02$^{a}$ | 4.85±0.00$^{a}$ | 0.50 |
| A-G | - | 0.73±0.21 | 0.98±0.11$^{b}$ | 0.65±0.04$^{a}$ | 0.84±0.07$^{a}$ | 0.75±0.03$^{a}$ | 0.76±0.41$^{a}$ | 0.25 |
| Gluc. | mg/dl | 93.20±2.00 | 110.20±2.00$^{b}$ | 111.60±2.00$^{b}$ | 93.90±1.68$^{a}$ | 86.40±2.00$^{b}$ | 70.80±2.00$^{c}$ | 2.45 |
| BUN | mg/dl | 19.10±2.00 | 26.37±4.16$^{b}$ | 45.20±2.00$^{c}$ | 19.10±1.90$^{a}$ | 41.70±2.00$^{b}$ | 49.80±2.00$^{c}$ | 3.12 |
| Creat. | mg/dl | 0.51±0.20 | 0.93±0.20$^{b}$ | 1.55±0.20$^{c}$ | 0.51±0.20$^{a}$ | 1.07±0.20$^{b}$ | 1.49±0.20$^{c}$ | 0.25 |

First group: represents the control group.

Second group: represents the maximum therapeutic dosed group.

Third group: represents the toxic dosed group.

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.

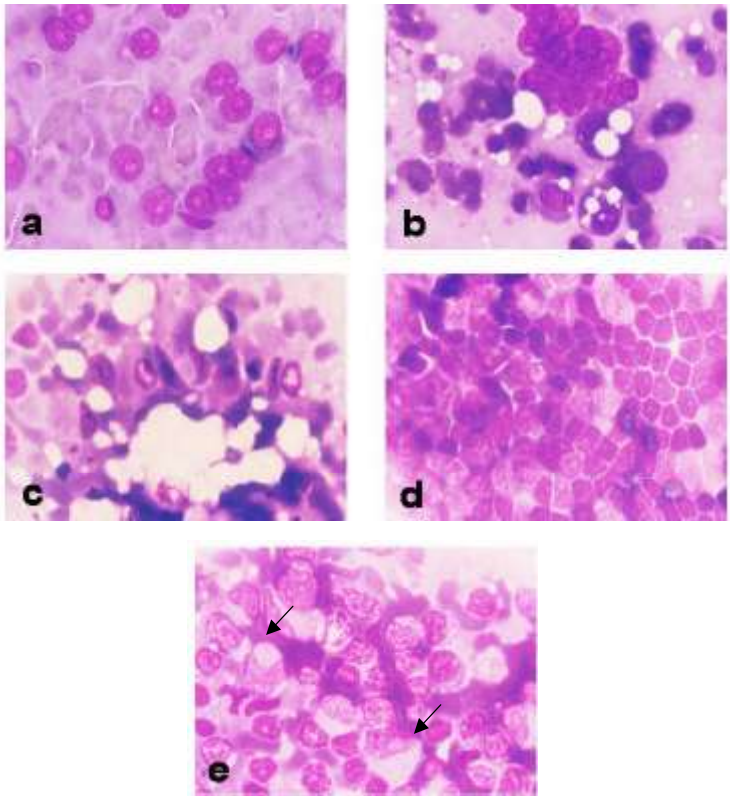


Fig. 2: a-Hepatic tissue showed normal hepatocyte (polyhedral cell with round hucleus and prominent single nucleoll). (Field stain x 1000) b-Hepatocyte showed vacuolar changes. (Field stain x 1000) c-Hepatocytes showed sever hepatocyte degeneration. (Field stain x 1000) d- Normal spleen tissue cytology (high number of lymphocyte). (Field stain x 1000) e- Spleen tissue showed hyperpiasia (increase number of macrophages and plasma cell (arrow). (Field stain x 1000)

**Serum Biochemical Evaluation:** Statistical analysis of different serum biochemical parameters is illustrated in Table 3.

Compared the result of both experimental groups to that of the control one, the hepatic enzymes (ALT, ALP and GGT) were significantly ($P < 0.05$) increased throughout the experiment. These elevations were probably as a result of hepatic damage which supported cytologically by the presence of intracellular hepatic vacuoles and histopathologically by the presence of centrolobular necrosis with massive degenerative changes in the hepatocytes. This hepatic damage may be caused directly by the acetaminophen metabolites or by hypoxia from the present anemia [2, 32]. The increased bilirubin concentration (especially unconjugated one) was likely a result of accelerated RBCs destruction while, the elevated blood glucose level was a stress response [2]. The increased concentrations of BUN and serum creatinine were attributed to the renal affection which supported sonographically by increased renal cortex echodensity, cytologically and histopathologically by the presence of vacuolation and degeneration of renal tubular epithelial. The changes observed in the concentrations of serum total proteins, serum albumin, serum globulins and A/G ratio were insignificant throughout the experiment which may be attributed to the equilibrium between the increased protein concentration, decreased protein production and increased protein losses due to the present hemoglobinemia, hepatic damage and renal affections, respectively.

**Cytological Findings**

**Liver Cytology:** Impression smears from the control group showed normal hepatocyte, which appeared as large, round to polyhedral cell with basophilic cytoplasm. The nucleus is round, centrally located with a distinctive large, single nucleolus (Fig. 2a). Hepatic smears of the second group on the 3$^{rd}$ and 10 $^{th}$ day (p.t) revealed mild to moderate vacuolar changes in hepatocytes that are recognized as discrete, round intracellular vacuoles which scattered throughout the back ground of the slides (Fig. 2b). The hepatic smears of the third group on the same experimental days showed that nearly all the hepatocytes are distended by multiple vacuoles of different size with little visible cytoplasm and the nuclei are pushed to the periphery (Fig. 2c).

From the above description, the hepatic smears revealed that the injury of hepatocytes resulted from the lipid accumulation in response to the hepatocellular damage [33]. The third group appeared the highly affected group in which the hepatocytes were difficult to recognize as the result of extensive and diffuse lipid accumulation [34]. All these signs of hepatic toxicity may be resulted from the toxic effect of acetaminophen or its metabolites after the depletion of glutathione stores [35].

**Spleen Cytology:** Impression smears of normal splenic tissue revealed the same microscopic features of normal lymph nodes. These smears are usually highly cellular results from the presence of large number of small lymphocytes, moderate number of intermediate lymphocytes and low number of plasma cells and lymphoblasts (Fig. 2d). Cytological evaluation of splenic smears showed splenic hyperplasia (Fig. 2e) on the 10$^{th}$ day (p.t) of both experimental groups as a result of increased macrophage, plasma cell and lymphoblast numbers with the predominance of small lymphocytes [36]. This hyperplasia of splenic tissue may be attributed to the presences of Heinz bodies, which is primarily filtered through the spleen [37].

**Renal Cytology:** Smears obtained from control group (normal kidney) are usually highly cellular and contain primarily tubular epithelial cells. These cells have abundant, slightly basophilic cytoplasm with round, slightly eccentric nucleus (Fig. 3a). Renal tubular cells are often remaining together as recognizable tubule fragments of various sizes (Fig. 3c). Glomeruli appeared as lobulated clusters of slender, splindloid cells (Fig. 3b).

Renal smears of the second group showed moderate to severe degree of vacuolation and degeneration (Fig. 3d). Smears of the third group appeared more affected, whereas the tubular cells degenerated into dark gray amorphous debris represent the necrotic material (Fig. 3e). These findings appeared as a result of nephrotoxicity which may be attributed to the insufficient glutathione in the renal parenchyma [3, 38].

**Bone. Marrow Cytology:** Results of cytological examination of B.M smear on the 3$^{rd}$ day (p.t) revealed normal cellularity (more than 75% cells) in all experimental groups (Fig. 4c), while those at the 10$^{th}$ day (p.t) showed different degrees of hypocellularity (less than 75% cells). This hypocellularity is more pronounced in the third group than the second group in comparison to the control one (Fig. 4d and e). The myeliod / erythoid ratio in all experimental groups showed insignificant changes.

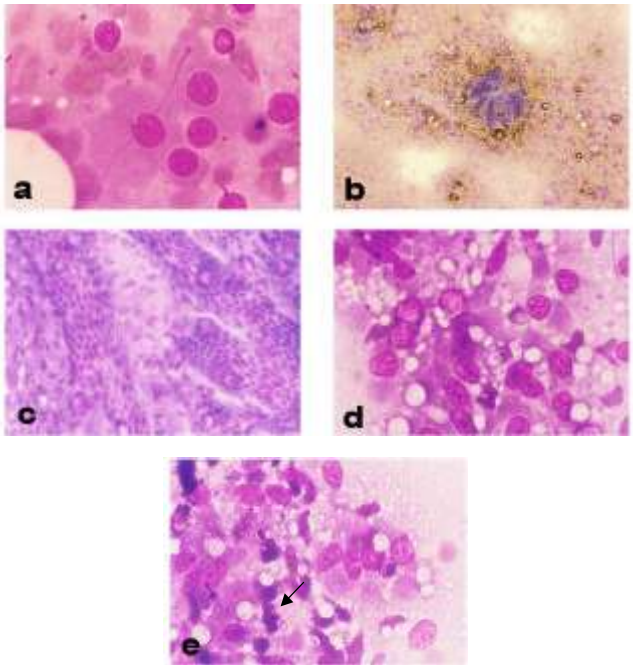


Fig. 3: a-Renal tissue showed normal renal epithellal cells (round cell with slightly eccentric nucleus). (Field stain x 1000) b- Normal glomerulus (clusters of splindlbold cells). (Field stain x 1000) c-Normal renal tubular epithellal cells (Field stain x 400) d-Renal tissue showed vacculation and degeneration of tubular epithellum. (Field stain x 1000) e-Renal tissue showed sever tubular degeneration with dark blue amorphous debris (arrow). (Field stain x 1000)

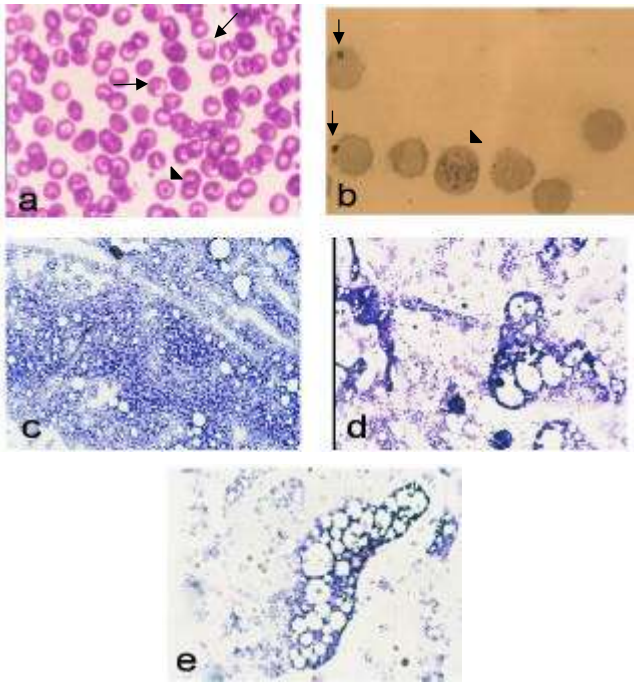


Fig. 4: a-Blood film showed eccentrocyte (arrow) and target cell (head arrow). (Gimsa stain x 1000). b- Blood film showed heinz bodles (arrow) and reticulocyte (head arrow). (NMB stain x 1000) c-Bone marrow smear showed normal cellularity. (Field stain x 100) d-Bone marrow smear showed moderate degree of hypocellularity. (Field stain x 100). e-Bone marrow smear showed sever degree hypocellularity. (Field stain x 100).

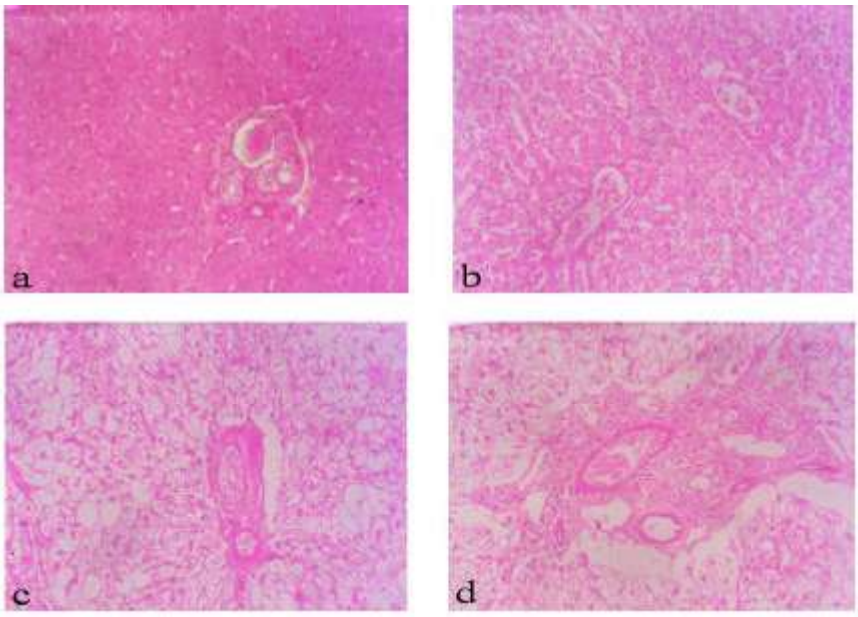


Fig. 5: (a): Liver of dogs at the 3rd day (p.t) of the second group showing swelling of hepatocytes together with portal tract congestion. (HandE×200).(b): Liver of dogs at the 10th day (p.t) of the second group showing congestion with dissociation of the hepatic.parenchyma.(HandE×100).(c): Liver of dogs at the 3rd day (p.t) of the third group showing congestion of portal blood vessels and bile duct hyperplasia. (HandE×200).(d): Liver of dogs at the 10th day (p.t) of the third group showing portal tract congestion. (HandE×200)

This B.M hypocellularity may be observed due to renal insufficiency resulting in decrease of erythropoietin production [18]. This renal insufficiency was supported by increases of BUN and serum creatinine concentrations.

**Histopathological Findings**

**Liver Histopathology:** Histopathological examination of hepatic tissue on the 3rd day (p.t) in the second group showed swelling of hepatocytes with eosinophillic granular cytoplasm and congestion of the portal area (Fig. 5a). On the 10th day (p.t) severe congestion with disorganization of the hepatic cords was seen (Fig. 5b). On the 3rd day (p.t) in the third group, liver appeared markedly vacuolated together with centrolobular necrosis, while bile duct hyperplasia and congestion were the predominant changes in the portal tract (Fig. 5c). On the 10th day (p.t) centrolobular necrosis with massive degenerative changes in the hepatocytes was observed. Portal area was also affected with congested blood vessels (Fig. 5d).

From the above histopathological findings, acute liver damage was observed as a result of acetaminophen toxicity. This hepatotoxicity may be attributed to the formation of highly reactive metabolite N-acetyl-P-benzoquinoneimine (NAPQI) by the hepatic cytochrome P-450. NAPQI is initially detoxified by conjugation with reduced glutathione (GSH), when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules [39, 40].

**Spleen Histopathology:** The second group on the 3rd day (p.t) showed normal splenic lymphoid follicles (Fig. 6a), while the 10th day (p.t) thickened splenic septae were appeared (Fig. 6b). Moreover, on the 3rd and 10th day (p.t) in the third group, severe congestion with vasculitis (Fig. 6c) and thickened fibrosal septae (Fig. 6d) were observed, respectively.

These splenic congestion and vasculitis may be resulted from the hyperplasia of monocyte-phagocytic system of the spleen and erythrophagocytosis.

**Renal Histopathology:** Histopathological alterations in renal tissue on the 3rd day (p.t) in the second group showed congestion of the interstitial blood vessel with vasculitits, represented by thickening of muscular wall and perivascular edema (Fig. 7a). Thickened capsule was an evident on the 10th day (p.t) (Fig. 7b). On the other hand, kidneys at the 3rd day (p.t) of the third group revealed vaculation of the glomerular epithelium with congestion and vasculitis (Fig. 7c). On the 10th day, (p.t) there were degeneration with vacuolation of some renal tubular epithelial and necrosis of others (Fig. 7d).

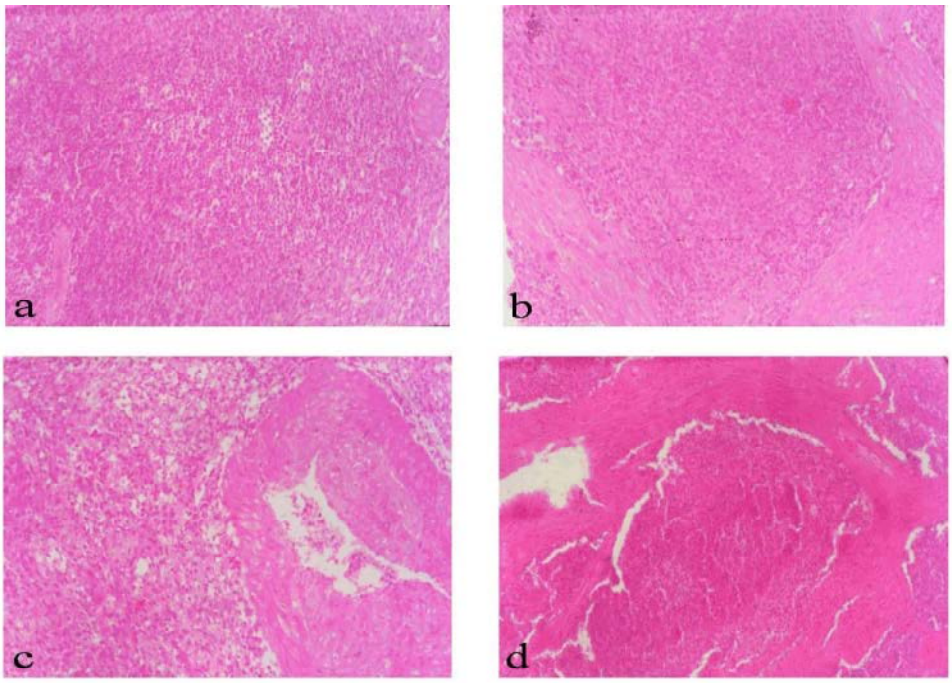


Fig. 6: (a): Spleen of dogs at the 3rd day (p.t) of the second group showing normal lymphoid follicles. (HandE×200). (b): Spleen of dogs at the 10th day (p.t) of the second group showing thickened splenic septae. (HandE×200). (c): Spleen of dogs at the 3rd day (p.t) of the third group showing severe congestion with vasculitis. (HandE×200). (d): Spleen of dogs at the 10th day (p.t) of the third group showing thickened fibrosal septae. (HandE×200)

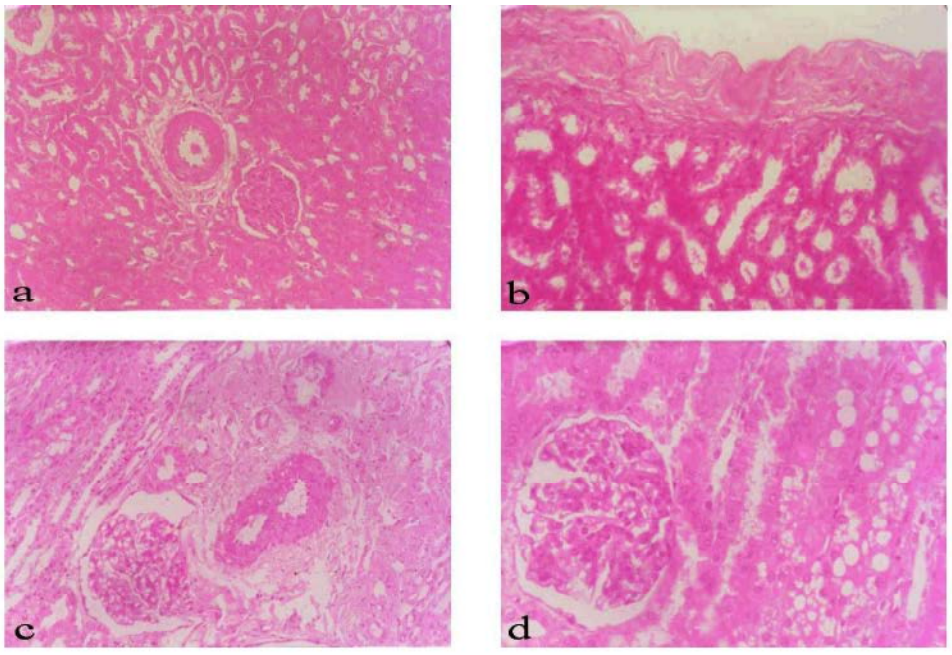


Fig. 7: (a): Kidneys of dogs at the 3rd day (p.t) of the second group showing congestion with vasculitis. (HandE×100).(b): Kidneys of dogs at the 10th day (p.t) of the second group showing thickened renal capsule (perinephritis). (HandE×200).(c): Kidneys of dogs at the 3rd day (p.t) of the third group showing vacuolation of the glomerular epithelium, congestion and perivascular fibrosis. (HandE×200) (d): Kidneys of at the 10th day (p.t) of the third group showing degeneration with vacuolation of some tubular epithelial and necrosis of others. (HandE×200)

Kidney failure may occur as a result of severe hepatic failure. In the presence of hepatotoxicity, the hepatic metabolism of acetaminophen may be "spill over" to the kidney which resulted in its nephrotoxicity as a result of insufficient glutathione in the renal parenchyma [41].

In conclusion, the present study recommended that, acetaminophen should be used only in limited amounts for limited periods as its signs of toxicity appeared even with its therapeutic dose. Ultrasonographic examination was a sensitive tool in defining the nature of lesions in most cases of mild acetaminophen toxicity. On the other hand, cytological findings should be correlated with hematological and serum biochemical examinations to avoid unrealistic expectations and over interpretation.

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