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Assessment of Doppler Ultrasonography and Hepatocyte MiRNA-122 for the Diagnosis of Acetaminophen-induced Acute Hepatic Injury in Dogs

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Abstract

THIS study aimed to evaluate the diagnostic value of Doppler ultrasound parameters of the hepatic artery, portal vein, and interlobar artery as well as the potential use of miRNA-122 for the diagnosis of acetaminophen-induced acute hepatic injury in dogs**.** Ten healthy mongrel dogs were orally administered 200 mg/kg body weight of acetaminophen daily for ten days. Clinical, Biochemical, Ultrasonographic, and Doppler ultrasonographic examinations were performed for all dogs on zero day before induction, 3rd day, and the 10th day of induction. The results of the current study showed that depression, dullness, and jaundice signs appeared in dogs on the 10th day of induction. Significant increase ($P<0.05$) in the level of ALT, AST, ALP, BUN, Creatinine Cholesterol, triglycerides, and total bilirubin coincident with a significant decrease in levels of total protein, and albumin at the same time. The measurement of miRNA-122 showed its elevation at 6.34-fold on the 3rd day, and 9.64-fold on the 10th day of induction. Ultrasonographic findings revealed increased echogenicity of liver parenchyma on the 3rd day and 10th day of induction. The resistive index value of the portal vein, hepatic artery, and renal interlobar artery showed a significant increase($P<0.05$) on the 3rd day, and 10th day of induction. The results of the current study revealed that Doppler ultrasonography is a useful diagnostic tool for early diagnosis of acute liver injury in dogs, and miRNA-122 could be used as a biomarker for early diagnosis of drug-induced acute liver injury in dogs.

Keywords: Acetaminophen, Dogs, Doppler ultrasonography, MiRNA-122, Ultrasound.

Introduction

Acute liver injury (ALI) is a life-threatening clinical case characterized by the sudden loss of hepatocyte function without a history of liver disease, many causes contribute to the occurrence of liver dysfunctions e.g. prolonged ischemia, toxicant exposure, dose-dependent drug reactions, and immune-mediated processes [1]. Although elevation of ALT is a sensitive and relatively specific biomarker for liver injury, current biochemical testing parameters do not always reflect dysfunction in the liver of humans [2]. The detection of drug-induced hepatotoxicity is still a critical health issue in small animals [3]. Acetaminophen is one of the most common drugs that can cause liver toxicity in dogs either by a single overdose or through repeated subtherapeutic doses above 100 mg/kg [4].

The majority of studies on the establishment of biomarkers for the diagnosis of liver damage caused by acetaminophen have concentrated on the possible application of novel molecular biomarkers like microRNA-122[5]. Small regulatory noncoding RNAs known as microRNAs (miRNAs) are utilized to monitor pathological changes resulting from drug-induced hepatic damage in dogs, like acetaminophen. MiRNAs can impact the stability of mRNA and/or the beginning and progression of protein translation[6]. About 70% of the total liver miRNAome is composed of miR-122, making it one of the most prevalent hepatic miRNAs [7]**.** Because miR-122 is abundant in the liver, it controls many important gene networks, including the hepatic circadian rhythm, lipid metabolism, and cell differentiation [8].

Doppler ultrasonography has been used to monitor hemodynamic changes in the liver, pancreas, kidney,

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and spleen, it is considered an advanced diagnostic technique for assessing hepatic vasculature [9]. Additionally, it is employed to determine the inner

diameters and hemodynamics of hepatic, portal, and splenic veins [10]. Therefore, to overcome the limitations of abdominal ultrasound which only detects changes in liver structure and parenchymal echogenicity, the combination of abdominal ultrasound and Doppler ultrasonography enhanced the diagnostic accuracy and was necessary for the identification of hepatic illnesses [9], and [10]. Due to severe intrarenal vasoconstriction which is linked to a decreased renal plasma flow and an increased renal arterial vascular resistance, renal hemodynamic changes start early in the course of the hepatic injury and may even occur before alterations in serum creatinine level are noticeable [11].

There were a few studies on the potential use of Doppler ultrasound for the early diagnosis of acetaminophen-induced acute liver injury in dogs. Previous studies such as [12], and [13] discussed the hepatotoxic effect of acetaminophen on the liver of animals, also few studies investigated the potential use of miRNA-122 for the diagnosis of drug-induced liver injury. Therefore, the current study was designed to assess the diagnostic value of Doppler ultrasound, and the potential use of miRNA-122 for the diagnosis of drug-induced acute liver injury in dogs compared to gold standard biochemical methods.

Material and Methods

Animals

Ten clinically healthy mongrel dogs' weights ranged from 10 to 18 Kg and their age ranged from 1 to 2 years were used in this study. Animals were housed in separate boxes with plenty of food and water. After two weeks of adaptation, the dogs were given orally 200 mg/kg of acetaminophen (Doliprane 1000mg, SANOFI company) daily for ten days for induction of acute liver injury in dogs according to the method described by [12]. Clinical, biochemical, abdominal ultrasonography, and Doppler Ultrasonographic examinations were performed for all dogs on zero day before induction, 3rd day, and the 10th day of induction.

Blood samples

Blood samples of 5 ml were collected in a sterile plain tube without an anticoagulant for serum separation for the analysis of biochemical parameters.

Biochemical analysis

The total protein (TP), albumin, total globulin, total bilirubin, direct bilirubin, blood urea nitrogen (BUN), creatinine, alanine amino transferees (ALT), alkaline Phosphatase (ALP), aspartate aminotransferase (AST), triglycerides, and cholesterol were analyzed using specific kits according to manufacturer instructions (Spectrum Diagnostic Kits, Egypt).

Hepatocyte-derived miRNA-122

Serum samples were stored at −20 till miRNAs-122 were measured [14]. Briefly, RNA from samples was extracted using RNeasy Mini Kit (Catalogue No. 74104, QIAGEN, Germany) according to manufacturer guidelines. Quantitative polymerase chain reaction (PCR) for the determination of miRNA-122 was done using Quantitect SYBR Green PCR Kit (QIAGEN, Germany, Cat. No. 204141), oligonucleotide primers and probes used in SYBR Green Real-time PCR are shown in Table(1) [14], and cycling conditions for SYBR Green Real-time PCR are shown in Table (2). Amplification curves and cycle threshold (CT) values were measured by the Stratagene MX3005P software, and the CT of each sample was compared with that of the control group to estimate the variation of gene expression on the RNA of different samples according to the method stated by [15] using the following ratio: ($2^{-\Delta\Delta Ct}$).

Whereas $\Delta\Delta Ct = \Delta Ct$ reference $- \Delta Ct$ target, ΔCt target = Ct control – Ct treatment, and ΔCt reference = Ct control- Ct treatment

Ultrasonographic examination

Using a 3.5–5 MHz convex probe, abdominal ultrasonography was carried out to assess the liver's parenchymal tissue, portal, and hepatic blood vessels, the biliary system, and the gallbladder according to the method mentioned by [16]. The region of increased echogenicity in the liver tissue was identified by analyzing the histogram. The full area of increased echogenicity in the region on a B-mode imaging grey scale was used to determine the region of interest (ROI). The total mean histogram of the region was calculated from each photo for all dogs [17].

Doppler Ultrasonography

Using Doppler ultrasound (duplex), (Sonoscape E2 portable color Doppler, with micro convex 5-8MHz transducer, China). The color assignment in all examinations was chosen so that flow toward the transducer appeared red while flowing away from the transducer appeared blue. Hepatic artery and portal vein imaging were included in the examination. To lower the measurement variability for the hepatic artery and portal vein resistive index, at least three measurements were taken and averaged [18]. For each kidney, the renal resistive index (RI) was calculated. The renal interlobar artery was first identified using the color Doppler, and it was subsequently moved over the selected artery to the pulsed wave by Doppler using a gate that was 1.5 mm wide, all animals were manually restrained and placed in either right or left lateral recumbency [11].

The resistive index of the hepatic artery, portal vein, and interlobar artery was measured by the ultrasound machine.

Histopathological examination

Dog liver and kidney autopsies were obtained, and the samples were preserved in 10% formol saline for a whole day. After washing with tap water, the specimens were dehydrated using methyl, ethyl, and 100% ethyl alcohol dilutions. Specimens were embedded in paraffin at 56 degrees in a hot air oven for 24 hours after being cleaned with xylene. Using a Leitz rotary microtome, paraffin wax beeswax tissue blocks were produced for sectioning at a thickness of 4 microns. Following deparaffinization and staining with hematoxylin and eosin, the resulting tissue sections were placed on glass slides for routine examination using a light electric microscope[19].

Statistical analysis

SPSS version 25 (SPSS, Chicago, IL, USA) was used to analyze the data. The findings were presented as mean ± SE. Acetaminophen's effects throughout the various time points were compared using a one-way ANOVA with repeated measures test. P-value < 0.05 was regarded as statistically significant, and the Tukey post-hock test was used to compare pairs of data and identify time points that differed from one another. A Pearson product-moment correlation was run to determine the relationship between biochemical parameters, doppler indices, and miRNA-122.

Results

Clinical findings

The dogs with induced acute liver injury showed signs of dullness and depression on the 3rd day after induction, off food, diarrhea, and jaundice appeared on the 10th day of induction Table (3).

Biochemical findings

There was a significant increase in the level of ALT, AST, and ALP on the 3rd day of induction, and on the 10th day of induction. The increase in ALT, AST, and ALP was 4,5,8 folds, respectively, on the 3rd day, and 5,5, 12 folds, respectively, on the 10th day of induction (Table 4).

The level of total protein, and albumin showed a significant decrease on both the 3rd and 10th day of induction (Table 4).

The level of blood urea nitrogen (BUN) showed a significant increase on the 3rd day and 10th day of induction. The increase in blood urea nitrogen level was more than 1-fold on the 3rd day of induction, and more than 4-fold on the 10th day of induction. There was a significant increase in the level of creatinine on the 10th day of induction as 2-fold (Table 4).

There was a significant increase in the level of cholesterol and triglycerides on the 3rd day of induction and showed higher significance on the 10th day of induction (Table 4).

The level of total bilirubin, and direct bilirubin showed a significant increase on the 3rd day of induction and showed higher significance on the 10th day of induction, but the level of indirect bilirubin showed a significant increase on the 10th day of induction (Table 4).

Hepatocyte-derived miRNA-122

The serum hepatocyte-derived miRNA-122 values showed a 6.3-fold increase on the 3rd day of induction, and a 9.6-fold increase on the 10th day of induction (Table 5).

Ultrasonographic findings

Before induction, the liver appeared with ultrasound homogenous in echogenicity with anechoic gall bladder and echogenic portal vein walls. Liver tissue was marginated by the hyperechoic diaphragm (Fig.1). there was a generalized increase in liver echogenicity after induction with a shift to hyperechogenic liver tissue on the 10th day of induction (Fig. 2).

Doppler ultrasonographic findings

A representative color Doppler flow images of the hepatic artery, portal vein, and interlobar artery were obtained in all animals on zero day before induction (Fig.3-5), on the 3rd day of induction, and the 10th day of induction (Fig.6-8). The resistive index (RI) of the hepatic artery, portal vein, and interlobar artery significantly increased ($P < 0.05$) on the 3rd day of induction and the 10th day of induction. End diastolic velocity (EDV) of the portal vein and hepatic artery significantly decreased ($P < 0.05$) on the 10th day of induction. EDV of the interlobar artery significantly decreased ($P < 0.05$) on the 3rd day of induction and the 10th day of induction (Table 6).

There was a positive correlation between levels of ALT, AST, creatinine, and BUN with the RI value of the portal vein, hepatic artery, interlobar artery, and miRNA-122 (Table 7).

Histopathological findings

Histopathological examination of the liver and kidney revealed that the hepatocytes in the parenchyma diffusely showed degenerative changes and necrobiosis (Fig. 9), swelling of the endothelial cells lining the glomerular tufts as well as swelling of the epithelial cells lining the tubules with stare shape lumen at the cortex (Fig. 10).

Discussion

Acute liver injury(ALI) is known as severe acute liver dysfunction that impairs liver function

(i.e. coagulopathy، jaundice), leading to hepatic encephalopathy [20]. One of the most widely prescribed medications for pain relief and fever reduction is acetaminophen (N-acetyl-p-aminophenol, or APAP). However, APAP hepatotoxicity is still one of the main causes of liver dysfunction [5]. The induction of ALI by APAP occurs due to the main proportion of acetaminophen being broken down in the liver by glucuronidation and sulphation and excreted through urine. A small portion (10–15%) is metabolized in the liver by cytochrome P450 isoforms into the alkylating and highly poisonous substance N-acetyl-p-benzoquinone imine (NAPQI), the glutathione (GSH) transforms NAPQI into a harmless form which excreted by bile [13]. When GSH levels are low due to an overdose of APAP that leads to the reduced ability for glucuronidation, and sulphation causes an increase in the quantity of processed APAP. This increased amount of NAPQI links to mitochondrial proteins to form cytotoxic protein adducts which cause hepatocyte necrosis [21]. The icterus or jaundice reported in the present study was attributed to the accumulation of bilirubin in the liver which is considered the major pigment in bile, during liver failure, the hepatocytes are unable to process bilirubin leading to the yellow discoloration of mucous membrane [22], these findings were reported by [23].

The current investigation showed that altered hepatic membrane permeability, hepatocellular necrosis, and inflammation to a degree corresponding with injured hepatocytes could be the cause of the increased levels of ALT and AST [24], and these findings agreed with [22]: [25] who all found that significant increase in liver enzymes in dogs with hepatic diseases. In dogs, these enzymes (ALT and AST) are released into the bloodstream when hepatocytes undergo damage from toxins (e.g., acetaminophen), inflammation, infections, or other liver diseases. The elevation of these enzymes correlates with the severity of hepatocyte injury, allowing veterinarians to assess liver function and damage levels based on blood test results.

Alkaline phosphatase (ALP) is an enzyme found in the biliary membrane of hepatocytes and serves as a marker for hepatobiliary damage [8]. In our study, the elevated ALP levels indicated injury to the biliary epithelial cells or canalicular membrane, likely caused by the hepatotoxic effects of acetaminophen, which promotes the formation of reactive oxygen species and inflammatory cytokines. [26]. These findings were similar to [12].

Concerning serum levels of BUN and creatinine there was a significant increase in the level of BUN and creatinine in the current study due to reduced kidney function associated with hepatocyte dysfunction due to the decreased ability of the liver to detoxify the toxic compounds [25], these findings were in agreement with [12] and [27].

In the current study, hypertriglyceridemia and hypercholesterolemia were linked to either decreased peripheral triglyceride catabolism because of a reduction of lipase activity or increased synthesis of triglyceride-rich lipoproteins [28]. Furthermore, impairment of the parenchymal cells of the liver lowered the excretion of extra cholesterol by biliary secretion and plasma LDL which could result in hypercholesterolemia [29]. These results were consistent with [30]. The liver is the main site for protein metabolism, where most plasma proteins are produced and broken down making them sensitive indicators for impaired liver function [24]. In addition, the liver is the main organ for the synthesis and degradation of albumin therefore liver injury leads to decreased levels of albumin [22]. Hyperbilirubinemia in the current study could be attributed to an imbalance in the rate of bilirubin generation, metabolism, and excretion. The increase in bilirubin level might be a result of diminished excretion due to severe hepatocyte damage or biliary obstruction [31]. These results agreed with [12], and [22].

Recently, several circulating microRNAs (miRNAs) have been known as sensitive and specific biomarkers for tissue injury [32]. For example, miR-122 has been demonstrated to elevate parallel with serum aminotransferase levels in case of drug-induced ALI, miR-122 is predominantly expressed in hepatocytes, and its levels increase in the bloodstream when liver cells are damaged. In dogs, elevated serum miR-122 can serve as a sensitive and early biomarker for liver injury, often rising before traditional liver enzymes like ALT or AST [6]. In the current study, miR-122 showed a significant increase on the 3rd day of induction and the 10th day of induction. These findings were consistent with previous studies which found that levels of serum miR122 were increased in case of acetaminophen-induced liver dysfunction [33]; [6]: [3]. Compared to traditional liver enzymes, miR-122 may be more specific to liver injury because it is expressed at very high levels in the liver, is stable in the blood, and accounts for 70% of all liver miRNA. This is because ALT and AST are present in skeletal muscle and were increased in the serum of patients with polymyositis and intense exercise [8].

A great noninvasive method for assessing liver parenchyma is ultrasonography. It is especially helpful in distinguishing between solid masses and cystic lesions, as well as between obstructive and nonobstructive icterus [34]. Ultrasonographic examination of the dogs in the current study showed increased echogenicity of liver parenchyma on the 3rd day and 10th day of induction that reflected hepatic damage, and these findings were similar to [12].

One of the most common causes of death in small animals is liver and renal dysfunction. The prognosis and treatment of renal or hepatic disease are significantly affected by early identification [35]. Understanding the Doppler indices of the liver portal vein and hepatic artery, such as maximum systolic blood flow (PSV), end-diastolic blood flow velocity (EDV), and resistive index (RI), is crucial for the early detection of liver diseases in dogs because changes in blood flow within the liver can be recognized as an early sign of acute liver injury. So, in the present study, we detected the hemodynamic changes in the portal vein and hepatic artery for early diagnosis of ALI in dogs, and there was a significant increase in RI of the portal vein, and hepatic artery on the 3rd day and 10th day of induction and EDV of portal vein and hepatic artery revealed significant decrease on 10th day of induction because of the perisinusoidal deposition of collagen، which hinders the blood flow in the sinusoids from the portal veins lead to increase RI [37], and these findings were consistent with [18] who reported that RI of hepatic artery was significantly increased in patients with acute hepatic failure, and the current study also revealed significant increase in RI of interlobar artery of the kidney on the 3rd day and 10th day of induction, and these findings were consistent with [11] because of renal RI increases as liver disease progresses [38].

RI is a vascular resistance indicator used in both human and veterinary medicine. Diastolic blood flow is decreased more than systolic blood flow when vascular resistance rises as a result of blockage or vasoconstriction, this fact is reflected in a higher decrease in end-diastolic velocity than in peak systolic velocity and, therefore, increased RI [11].

The histopathological examination of the liver and kidney in our study showed that hepatocytes in the parenchyma diffusely showed degenerative changes and necrobiosis, swelling of the endothelial cells lining the glomerular tufts as well as swelling of the epithelial cells lining the tubules with stare shape lumen at the cortex that might be attributed to hepatotoxic effect of APAP on liver tissue causing necrosis of hepatocyte. These findings were in accordance with [12], and [5].

Since ALT is found in the liver, skeletal and cardiac muscle, and kidneys, it is not currently thought to be liver-specific in the majority of commonly studied species, including humans [39]. Alternatively, Doppler ultrasound can be used for the early diagnosis of acute liver injury in dogs because of the positive correlation between the value of RI and the levels of ALT, AST, creatinine, and BUN.

There was a positive correlation between Doppler indices, and miRNA-122, both of them can overcome the lack of sensitivity of traditional liver enzymes for diagnosis of drug-induced acute liver injury in dogs.

Conclusion

The current study concluded that Doppler ultrasonography indices of portal vein, hepatic artery, and renal interlobar artery are useful noninvasive diagnostic tools for early detection of acute liver injury in dogs. Moreover, Hepatocyte-derived miRNA-122 is an easily measurable, and stable diagnostic blood biomarker for diagnosis of drug-induced ALI in dogs compared to routine biochemical hepatic markers.

Acknowledgments

Not applicable.

Funding statement

This study didn't receive any funding support.

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

All examinations were done after the approval of the Ethics Committee of the Faculty of Veterinary Medicine, Benha University, Egypt with the approval numberBUFVTM11-01-23).

TABLE 1. Oligonucleotide primers and probes used in SYBR Green real-time PCR.

Gene	Primer sequence (5'-3')	Reference
U6 (housekeeping)	GCTTCGGCAGCACATATACTAAAAT CGCTTCACGAATTTGCGTGTCAT	**[14]**
MiRNA-122	Gcgagcacagaattaatacgac **Tggagtgtgacaatggtgtttg**	**[14]**

TABLE 2.Cycling conditions for SYBR green real-time PCR according to Quantitect SYBR green PCR kit.

Gene	Reverse transcription	Primary Denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)		
			Secondary denaturation	Annealing (Optics on)	Extension	Secondary denaturation	Annealing	Final denaturation
U6 (housekeeping)	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	60˚C 30 sec.	72˚C 30 sec.	94˚C 1 min.	60˚C 1 min.	94˚C 1 min.
MiRNA-122	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	55˚C 30 sec.	72˚C 30 sec.	94˚C 1 min.	55˚C 1 min.	94˚C 1 min.

TABLE 3. Frequency of clinical signs recorded in dogs with induced acute liver injury.

Clinical signs	Number of cases	Percentage
Dullness	**8/10**	**80%**
Depression	**8/10**	**80%**
Jaundice	**3/10**	**30%**
Off food	**7/10**	**70%**
Diarrhea	**4/10**	**40%**

TABLE 4. Serum biochemical alterations in dogs with induced- acute liver injury.

Biochemical parameters	Zero-day before induction(n=10)	3rd day after induction(n=10)	10th day after induction(n=10)	P-value
ALT(u/L)	17.9^{c}±0.3	60.06^{b}±1.4	71.99^{a}±1.7	0.000
AST(u/L)	31.21^{c}±0.7	57.74^{b}±1.4	84.71^{a}±5.2	0.000
Urea (mg/dl)	27.2^{c}±0.7	43.03^{b}±1.07	75.99^{a}±1.13	0.000
Creatinine (mg/dl)	0.71^{b}±0.03	0.81^{b}±0.01	2.15^{a}±0.3	0.000
ALP(u/L)	425.68^{c}±10.62	1286^{b}±32.1	1756^{a}±43.84	0.000
Cholesterol(mg/dl)	186.75^{c}±4.66	261.87^{b}±6.5	299.43^{a}±7.4	0.000
Triglycerides (mg/dl)	47.87^{c}±1.2	115.81^{b}±2.8	132.5^{a}±3.3	0.000
Total protein(g/dl)	8.2^{a}±0.11	6.07^{b}±0.2	5.99^{b}±0.3	0.000
Albumin (g/dl)	4.2^{a}±0.06	2.11^{b}±0.3	2.15^{b}±0.3	0.000
Total globulin (g/dl)	3.98^{a}±0.05	2.96^{a}±0.4	2.84^{a}±0.6	0.227
Total bilirubin (mg/dl)	0.7^{c}±0.02	3.13^{b}±0.3	5.52^{a}±0.4	0.000
Direct bilirubin(mg/dl)	0.29^{c}±0.04	1.06^{b}±0.03	2.61^{a}±0.25	0.000
Indirect bilirubin (mg/dl)	0.41^{b}±0.03	2.07^{b}±0.2	2.88^{a}±0.3	0.001

Data are presented as (Mean ± SE). S.E = Standard error. Values with different superscript letters in the same row are significantly different at ($P<0.001$)

TABLE 5. Serum level of miRNA-122 in dogs with induced-acute liver injury.

Group	U6 CT	MiRNA-122	Δ CT	Δ Δ CT	Fold change
Zero-day	20.15	23.47	0	0	0
3rd day	20.43	21.08	-0.28	2.39	6.34
10th day	20.39	20.44	-0.24	3.03	9.64

miRNA = microRNAs, U6 = spliceosomal, CT = Cycle threshold

TABLE 6. Value of RI, PSV, and EDV of the portal vein, hepatic artery, and interlobar artery in dogs with induced-acute liver injury

	Zero-day before induction			3rd day of induction			10th day of induction			*p-value*
	Portal vein	Hepatic artery	Interlobar artery	Portal vein	Hepatic artery	Interlobar artery	Portal vein	Hepatic artery	Interlobar artery	0.000***
RI	0.44^{c}±0.04	0.52^{c}±0.04	0.5^{c}±0.02	0.8^{b}±0.03	0.71^{b}±0.09	0.63^{b}±0.03	0.97^{a}±0.01	0.86^{a}±0.04	0.92^{a}±0.03	0.000***
PSV (cm/sec.)	17.79^{a}±1.9	31.29^{a}±4.03	23.51^{a}±1.6	27.21^{a}±4.3	33.35^{a}±0.89	25.05^{a}±6.1	30.09^{a}±5.1	38.15^{a}±6.43	34.4^{a}±2.9	0.000***
EDV (cm/sec.)	12.15^{a}±2.7	14.31^{a}±1.44	16.87^{a}±1.1	9.86^{a}±1.3	9.6^{ab}±0.62	8.69^{b}±1.1	6.34^{b}±0.2	5.72^{b}±2.76	2.72^{c}±1.5	0.000***

Data are presented as (Mean ± SE). S.E = Standard error. Values with different. superscript letters in the same row are significantly different at (P<0.000). RI: Resistive index, PSV: Peak systolic velocity, EDV: End diastolic velocity.

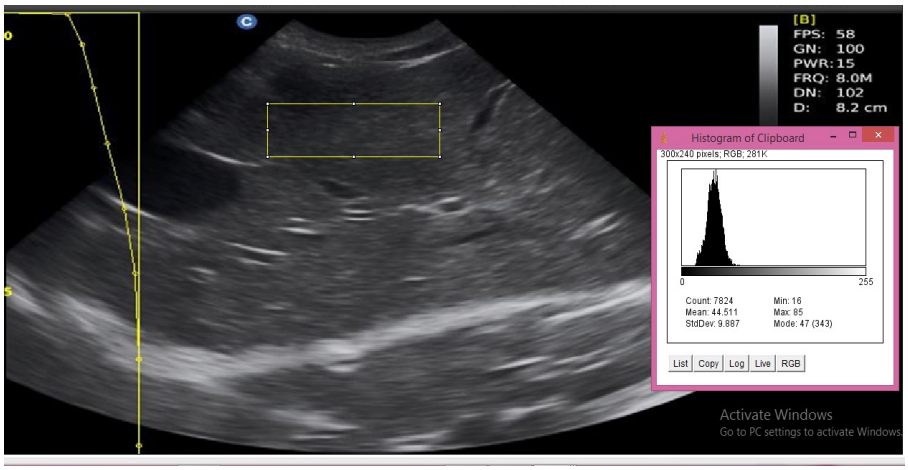

Fig. 1. Ultrasonographic image of the liver, and respective histogram on zero day before induction showed that liver is homogenous in echogenicity with anechoic gall bladder and echogenic portal vein walls. Liver tissue was marginated by hyperechoic diaphragm.

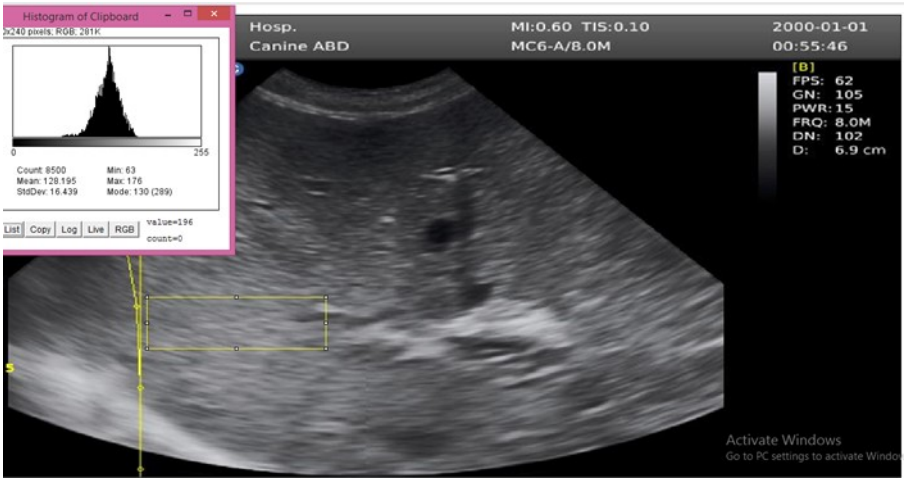

Fig. 2. Ultrasonographic image of the liver, and respective histogram on 10th day of induction showed diffuse hyperechoic liver parenchyma.

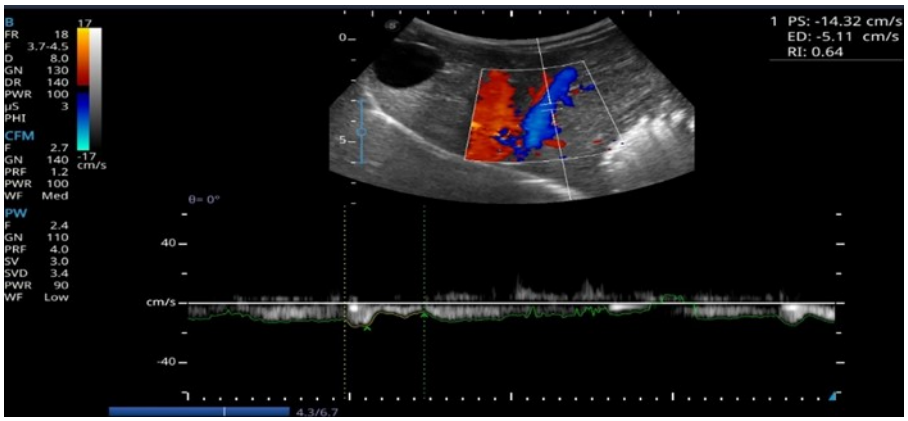

Fig. 3. Pulsed wave Doppler ultrasound image of portal vein on zero day before induction showing RI equal 0.64.

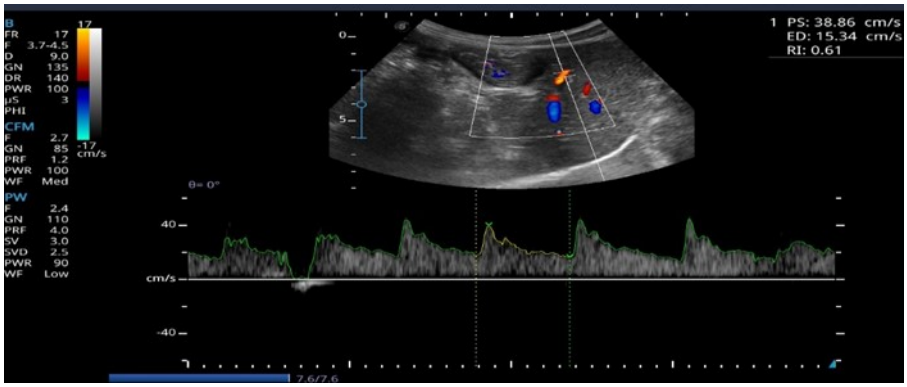

Fig. 4. Pulsed wave Doppler ultrasound image of hepatic artery on zero day before induction showing RI equal 0.61.

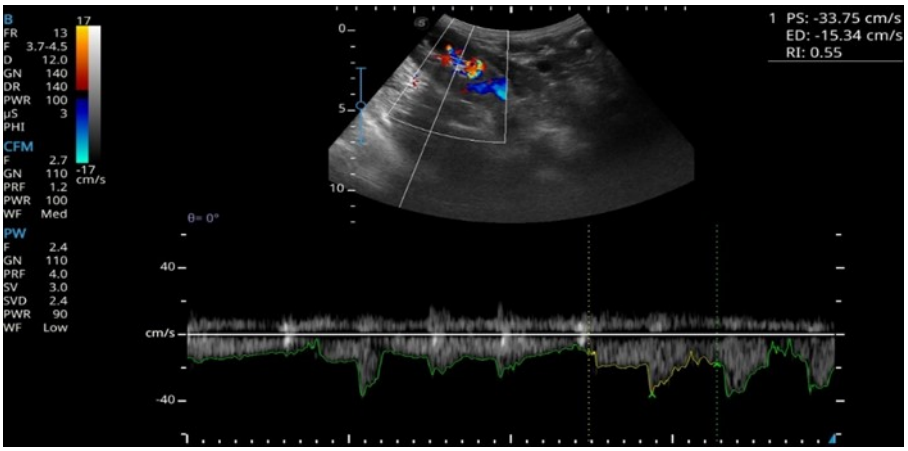

Fig. 5. Pulsed wave Doppler ultrasound image of interlobar artery on zero day before induction showing RI equal 0.55.

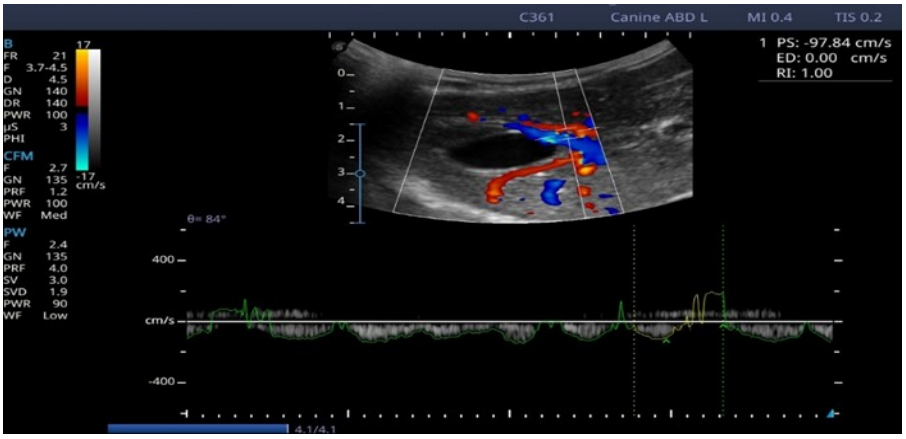

Fig. 6. Pulsed wave Doppler ultrasound image of portal vein on 10th day of induction showing RI equal 1.

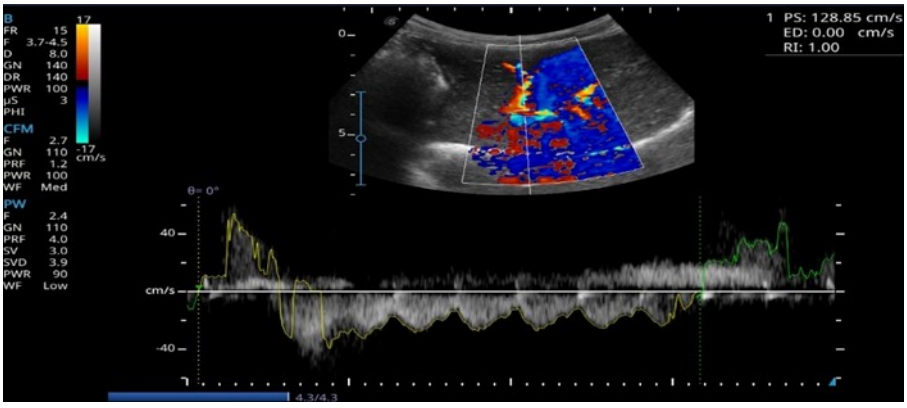

Fig. 7. Pulsed wave Doppler ultrasound image of hepatic artery on 10th day of induction showing RI equal 1.

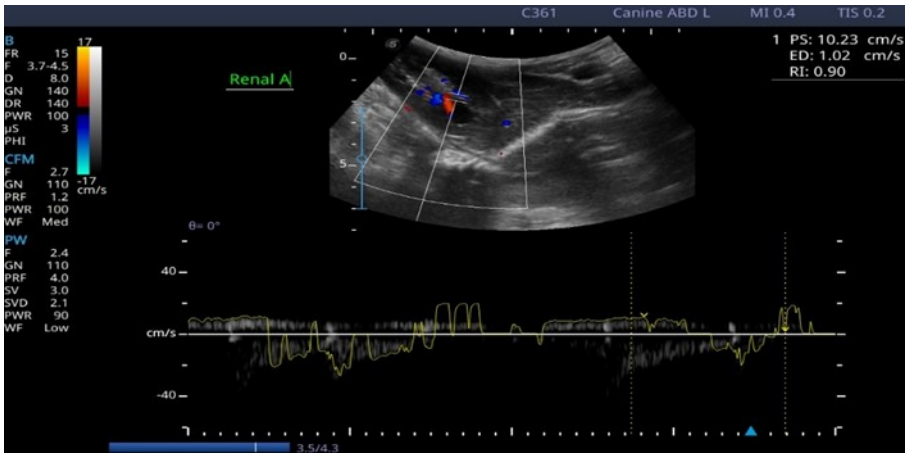

Fig. 8. Pulsed wave Doppler ultrasound image of inter lobar artery on 10th day of induction showing RI equal 0.9.

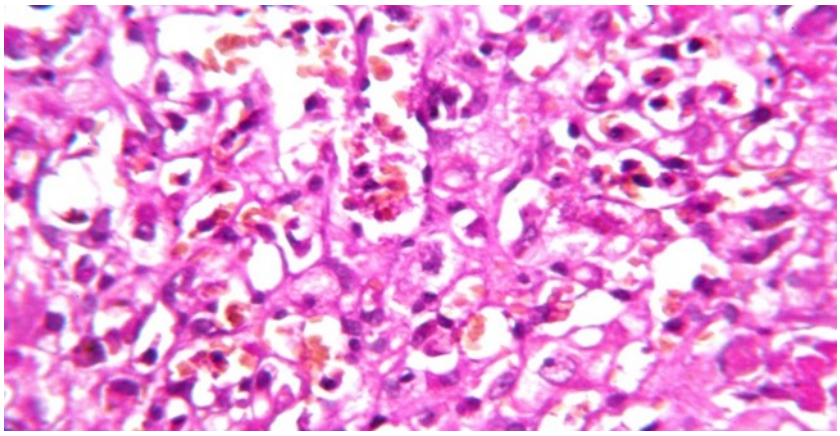

Fig. 9. The histopathological examination of the liver of dogs showed that the hepatocytes in the parenchyma showed degenerative changes and necrobiosis in diffuse manner (H&E 80).

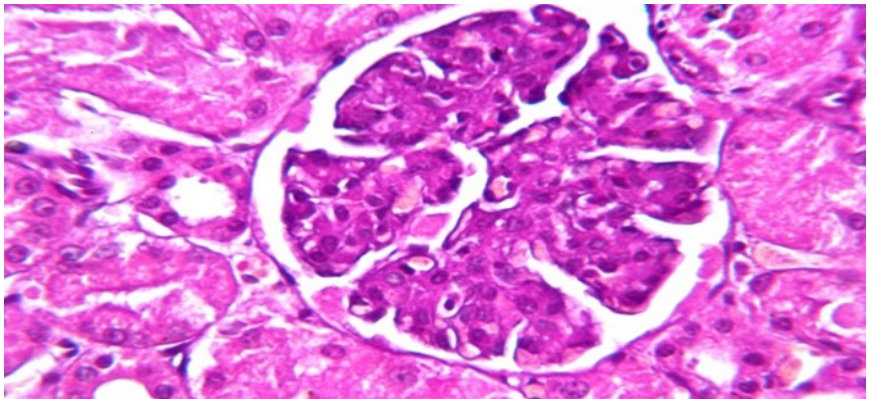

Fig. 10. The histopathological examination of the kidney of dogs showed Swelling of the endothelial cells lining the glomerular tufts as well as swelling of the epithelial cells lining the tubules with stare shape lumen at the cortex (H&E 80).

	ALT	AST	BUN	Creatinine	ALP	Cholesterol	triglycerides	Total protein	albumin	Total globulin	Total bilirubin	Direct bilirubin	Indirect bilirubin	RI of hepatic artery	RI of portal vein	RI of interlobar artery	miRNA-122
ALT	1	.949**	.853**	.648**	.990**	.986**	.922**	-.921**	-.765**	-.577**	.921**	.839**	.940**	.923**	.894**	.844**	.791**
AST		1	.953**	.818**	.981**	.980**	.766**	-.791**	-.681**	-.476**	.932**	.900**	.902**	.895**	.895**	.882**	.915**
BUN			1	.901**	.918**	.900**	.587**	-.717**	-.526**	-.507**	.944**	.975**	.850**	.813**	.797**	.916**	.978**
Creatinine				1	.733**	.715**	.333*	-.499**	-.206	-.489**	.729**	.840**	.570**	.617**	.618**	.718**	.886**
ALP					1	.994**	.877**	-.730**	-.560**	.954**	.898**	.946**	.923**	.897**	.890**	-.730**	.868**
Cholesterol						1	.866**	-.872**	-.766**	-.511**	.935**	.866**	.941**	.927**	.929**	.856**	.834**
triglycerides							1	-.914**	-.785**	-.550**	.733**	.589**	.827**	.832**	.801**	.626**	.498**
Total protein								1	.657**	.773**	-.866**	-.775**	-.891**	-.832**	-.776**	-.736**	-0.629**
Albumin									1	.029	-.653**	-.484**	-.778**	-.610**	-.772**	-.501**	-0.447**
Total globulin										1	-.599**	-.619**	-.527**	-.589**	-.379**	-.554**	-0.457**
Total bilirubin											1	.965**	.965**	.880**	.822**	.941**	0.904**
Direct bilirubin												1	.863**	.802**	.747**	.940**	0.952**
Indirect bilirubin													1	.903**	.838**	.883**	0.798**
RI of hepatic artery														1	.842**	.885**	0.758**
RI of portal vein															1	.709**	0.705**
RI of interlobar artery																1	0.924**
miRNA-122																	1

ficient between biochemical parameters, Doppler indices, and miRNA-122.

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تقييم التصوير بالموجات فوق الصوتية الدوبلر وخلايا الكبد MiRNA-122 لتشخيص الإصابة الكبدية الحادة الناجمة عن الأسيتامينوفين في الكلاب

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الملخص

تهدف هذه الدراسة إلى تقييم القيمة التشخيصية الموجات فوق الصوتية دوبلر للشريان الكبدي والوريد البابي والشريان بين الفصوص بالإضافة إلى الاستخدام المحتمل لـ miRNA-122 لتشخيص الإصابة الكبدية الحادة الناجمة عن عقار الاسيتامينوفين في الكلاب. تم إعطاء عشرة كلاب هجينة صحية عن طريق الفم 200 ملغم / كغم من وزن الجسم من عقار الاسيتامينوفين يوميًا لمدة عشرة أيام. تم إجراء الفحوصات السريرية والكيميائية الحيوية والموجات فوق الصوتية والدوبلر لجميع الكلاب في يوم الصفر قبل الاصابة واليوم الثالث واليوم العاشر من الاصابة. وأظهرت نتائج الدراسة الحالية ظهور علامات الاكتئاب والبلادة واصفرار الجسم في الكلاب في اليوم العاشر من الاصابة. ارتفاع معنوي ($P<0.05$) في مستوى ALT، AST، ALP، BUN، الكوليسترول الكرياتينين، الدهون الثلاثية والبيليروبين الكلي تزامن مع انخفاض معنوي في مستويات البروتين الكلي والألبومين في نفس الوقت. أظهر قياس miRNA-122 ارتفاعه عند 6.34 ضعفًا في اليوم الثالث، و9.64 ضعفًا في اليوم العاشر من الاصابة. كشفت نتائج التصوير بالموجات فوق الصوتية عن زيادة صدى حمة الكبد في اليوم الثالث واليوم العاشر من الاصابة . أظهرت قيمة RI للوريد البابي والشريان الكبدي والشريان الكلوي بين الفصوص زيادة كبيرة ($P <0.05$) في اليوم الثالث واليوم العاشر من التحريض. كشفت نتائج الدراسة الحالية أن التصوير بالموجات فوق الصوتية دوبلر هو أداة تشخيصية مفيدة للتشخيص المبكر لإصابة الكبد الحادة في الكلاب، ويمكن استخدام miRNA-122 كمؤشر حيوي للتشخيص المبكر لإصابة الكبد الحادة الناجمة عن استخدام الادوية في الكلاب.

الكلمات الدالة: الاسيتامينوفين، الكلاب، موجات فوق صوتيه دوبلر، MiRNA-122 المشتق من خلايا الكبد، الموجات فوق الصوتية .