The Pharma Innovation Journal 2022; SP-11(11): 933-937

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(11): 933-937

www.thepharmajournal.com
Received: 22-08-2022
Accepted: 24-09-2022

PB Pandya
M. V. Sc. Scholar, Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

AA Vagh
Associate Professor and Head, Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

JP Joseph
Assistant Professor, Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

AK Bilwal
Assistant Professor, Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

VL Parmar
Assistant Professor, Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

Corresponding Author:
PB Pandya
M. V. Sc. Scholar, Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

Assessment of hepatobiliary ultrasound score with hemato-biochemical alterations between healthy dogs and dogs with hepatobiliary dysfunctions

PB Pandya, AA Vagh, JP Joseph, AK Bilwal and VL Parmar

Abstract
Objectives: The aim of study was to asses hepatobiliary ultrasound score between apparently healthy dogs and dogs with hepatobiliary dysfunctions diagnosed via clinical examination and hemato-biochemical analysis supported by ultrasonography.
Materials and Methods: A total of 22 dogs presented at Veterinary Clinical Complex, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Junagadh, showing clinical signs of hepatobiliary dysfunction were subjected to clinico-hemato biochemical and ultrasonographic (USG) examinations by standard methods. For assessment of ultrasound score Different ultrasonographic features were categorized on the basis of (1) liver surface, (2) parenchymal score (echogenicity of parenchyma and nodularity of parenchyma), and (3) biliary score (gallbladder wall thickness, amount of gall sludge, and visibility of bile duct). Six apparently healthy dogs served the control group.
Results: The dogs affected with hepatobiliary dysfunction showed clinical signs like lethargy, anorexia/hyporexia, diarrhoea, pale conjuctival mucous membrane, polyuria/polydipsia, ascites, fever, vomiting, jaundice, pain on abdominal palpation, emaciation, melena, constipation, limb/scrotal oedema and petechial haemorrhages. Significant decline in Hb, TEC, PCV and platelet counts and significant elevation in TLC and Neutrophils (%) was noted as compared to control values. Significant increase in ALT, ALP, GGT, total bilirubin, direct bilirubin and total bile acids; significant decline in total protein and albumin were also noted as compared to control values. Hepatosonography revealed different focal and diffuse lesions. Majority of the dogs affected with hepatobiliary dysfunctions showed moderate severity (14/22, 63.64 %).
Conclusions: When treating diseased animals, one must take into account the clinical and hemato-biochemical changes brought on by hepatobiliary dysfunction. Here, explained ultrasound score system can be used for screening of different hepatobiliary affections thus better diagnosis of the liver diseases in dogs can be given via visulizing liver damage.

Keywords: Dogs, hepatobiliary dysfunctions, hemato-biochemical, ultrasound score

Introduction
The biggest parenchymal organ in the body, the liver performs at least 1500 metabolic processes vital to the life of the host.[12] Drug metabolism, removal of exogenous and endogenous toxins, synthesis of vital substances such as albumin and blood clotting factors, protein, fat, and carbohydrate metabolism, vitamin storage and activation, glycogen, triglyceride, and mineral storage, activation, conversion, secretion, deactivation, and excretion of various hormones, bile salt synthesis, and bilirubin conjugation and excretion. Hepatobiliary dysfunction in dogs can be caused by drug-induced toxicity, viral infections, congenital or neoplastic diseases, metabolic abnormalities, degenerative processes, vascular injury, autoimmune diseases, and even severe trauma. Disorders of Biliary tract disorders include extrahepatic bile duct obstruction (EBDO) in dogs as well as diseases of the biliary tract itself, such as bacterial cholecystitis, cholangitis, cholangiohepatitis, and necrotizing cholecystitis. Cholangiohepatitis and cholecystitis are most frequently linked to Ascending infection.
Labrador Retrievers, Doberman Pinschers, English Cocker Spaniels, Bedlington Terriers, West Highland White Terriers, and many other breeds are the most susceptible breeds for occurrence of hepatitis [10].
The majority of dogs affected by hepatobiliary dysfunction over their lifetime are between the ages of 4 and 8 years old [8]. Females were more likely than males to suffer from liver disorders [16]. Jaundice, bleeding tendency, and ascites are typical clinical indicators of liver disease, while depression, lethargy, anorexia, vomiting, diarrhoea, and weight loss are non-specific symptoms [14].

The diagnosis of liver illnesses is difficult for a clinician since specific clinical indicators appear only when hepatocellular damage is severe. As a result, it necessitates a thorough diagnosis process that includes a clinical examination, ultrasound, laboratory tests, and cytology and/or histology [9].

Materials and Methods

Ethical approval: In this study the use of animals was approved by Institutional Animal Ethics Committee (IAEC) of College of Veterinary Science and Animal Husbandry, Kamdhenu University (KU), Junagadh, Gujarat, India (No. KU-JVC-IAEC-LA-90-22).

Study animals: This study included 6 apparently healthy dogs brought for regullar check up and vaccination at hospital and 22 dogs with hepatobiliary dysfunctions and it was carried out at Veterinary Clinical Complex, College of Veterinary Science & A. H., Kamdhenu University, Junagadh during 2021-2022.

Hemato-biochemical analysis: Two milliliters of whole blood was collected from the saphenous or cephalic vein of the dog using sterile disposable syringes into vacutainers with K3 EDTA for estimation of haematological parameters. Care was taken during collection and processing of blood sample to avoid haemolysis. Haematological parameters were performed by BC2800 Vet auto hematology analyzer. For separation of serum, 5 ml of blood was collected in clot accelerator vacutainer. Serum was separated immediately after clotting for estimation of biochemical parameters. All the parameters were performed using biochemical analyzing reagents kits (Randox laboratories, Northern Ireland) and with the use of Microlab 300 semi-automatic biochemical analyzer.

Ultrasonography: Hepatosonography was performed using EXAGO B-mode ultrasound machine. A convex probe of 3.5 MHz and 5 MHz frequencies were used for large and small dogs, respectively to perform hepatosonography. Hepatosonographic examination was performed by placing the animals in either lateral or dorsal recumbency. A liberal amount of coupling gel was applied to provide sufficient contact. Liver was scanned fully in both transverse and longitudinal sections by gently angling and moving the transducer from cranial to caudal and from right to left. The ultrasound parameters and their assigned scoring system, which were adopted from previously published literature, are depicted in Table-1. For assessment of ultrasound score Different ultrasonographic features were categorized on the basis of (1) liver surface, (2) parenchymal score (echogenicity of parenchyma and nodularity of parenchyma), and (3) biliary score (gallbladder wall thickness, amount of gall sludge, and visibility of bile duct). Each parameter was scored with a 0, 1, 2, or 3, and the ultrasound score was calculated by the sum of scores of all the six parameters for the individual dog. Further the dogs were categories into 3 different severity categories on the basis of total ultra sound score as mild (score 0-2), moderate (score 3-5), and severe (score 6-12).

Table 1: Calculation of hepatobiliary ultrasound score based on 3 score type ranging from 0-12

Score type	Clinical features	Scores			
		0	1	2	3
Surface score	Liver edge/border (0-2)	Sharp	Mildly blunt	Blunt	_
Parenchy-mal score	Parenchymal echogenicity (0-3)	Normal	Hypo/hyper-echogenicity	Inhomogeneous (mildly coarse)	Heterogeneous (coarse)
	Nodularity of parenchyma (0-2)	Smooth	Mildly irregular	Irregular	_
Biliary score	Gallbladder wall thickness (0-1)	<2 mm	>2 mm	_	_
	Gall sludge (0-3)	Normal	Increase	Stellate sludge	Stone
	Bile duct visibility (0-1)	No	Yes	_	_

Statistical analysis: Collected data were compiled, tabulated and analyzed using Microsoft Excel sheet and results were recorded as mean±standard error (SE). The changes in hemato-biochemical parameters and ultrasound score from diseased dogs with hepatobiliary dysfunctions were analyzed and compare with the healthy dogs data using Student t-test. $P<0.05$ was considered statistically significant.

Results and Discussion: A total 6 apparentely healthy dogs and 22 dogs with hepatobiliary dysfunctions were evaluated. In the present study majority of the dogs affected with hepatobiliary dysfunctions were Females (13/22, 59.09 %) and no. of affected male dogs were 9. In relation to total number of cases which had hepatobiliary dysfunctions the highest number of cases of hepatobiliary dysfunctions was found in dogs with 4-8 years age group (12/22, 54.55 %) followed by > 8 years (6/22, 27.28 %) and 1 – 4 years (4/22, 18.19%) age group in present study. highest occurance of hepatobiliary dysfunctions seen in Labrador Retriever (28.58 %) breed of dogs followed by Non Descript (25 %), German Shepherd (17.86 %), Pomeranian (10.71 %), Rottweiler (10.71 %), Saint Bernard (3.57 %) and Dalmatian (3.57 %). The findings are in accordance with Lakshi *et al.* (2017) who documented Labrador Retriever as mostly affected breed with hepatobiliary dysfunctions.

The total dogs affected with hepatobiliary dysfunctions showed different clinical symptoms like depression to lethargy, anorexia/hyporexia, diarrhoea, pale conjuctival mucous membrane, polyuria/polydipsia, ascites, fever, vomiting, jaundice, pain on abdominal palpation, emaciation, melena, constipation, limb/scrotal oedema and petechial haemorrhages.

Hemato-biochemical analysis: Hematological examination of the affected dogs revealed a highly significant ($P< 0.01$) decrease in the hemoglobin, PCV and TEC values as compared with healthy control dogs. In addition to impaired bone marrow responses, decreased erythrocyte survival time, decreased nutrient uptake due to inappetance or anorexia, and reduced availability of micronutrients from liver, the decrease in Hb in the current study can be attributed to increased degradation of erythrocytes due to increased transit time through spleen because of increased portal blood flow and/or increased fragility of erythrocytes due to high levels of bile acids [17]. The present finding is in similiar with Prebavathy *et al.* (2020) [13]. However, a significant ($P< 0.05$) increase in the

mean values of total leucocyte count was noticed in dogs with hepatobiliary dysfunctions as compared to healthy control dogs. The present finding was in similiar with Eman *et al.* (2018) [5] and Prebavathy *et al.* (2020) [13] who documented a TLC concentration of 15.55±2.84 x 10^3 /µl and 25.08±4.92 x 10^3 /µL in hepatic/ hepatobiliary dysfunction. Total leukocyte count increases may be caused by hepatocellular injury, infection, sepsis, or the ingestion of intestinal bacterial toxins [19]. The mean platelet counts were significantly (P< 0.05) decreased in the in dogs with hepatobiliary dysfunction as compared to healthy control dogs. These findings are in accordance with Elhiblu *et al.* (2015) [4], Eman *et al.* (2018) [5] and Prebavarthi *et al.* (2020) who reported mean values of platelet counts as 1.94±0.27, 1.31±0.1 and 0.88±0.29 (x 10^5/µl) in dogs with hepatic/ hepatobiliary dysfunctions.

Biochemical profile revealed a highly significant (p< 0.01) increase in the mean alanine amino transferase (ALT) activity in dogs with hepatobiliary dysfunction as compared with healthy control. Known as the most liver-specific enzyme, ALT is largely located in the cytoplasm of hepatocytes and is released into the serum when hepatocyte membrane permeability is enhanced or if there is hepatobiliary dysfunctions [9], Although acute hepatic damage is associated with the greatest aminotransferase levels, chronic liver diseases such as cirrhosis, chronic hepatocellular disease, parasite hepatopathy, and primary or metastatic neoplasia are all associated with more modest elevations in aminotransferase activity [1]. The mean values of ALP and GGT showed significant (P < 0.05) increase in dogs with hepatobiliary dysfunction when compared with healthy control. Similar findings were obserevd by Mircean *et al.* (2008) and Saravanan *et al.* (2014) but findings were in contrary with Assawarachan *et al.* (2019). Elevated ALP activity was regarded as a sign of either intrahepatic or extrahepatic biliary blockage because it was associated with cholestasis. The mean total bilirubin and direct bilirubin levels showed highly significant (P < 0.01) elevation in diseased dogs as compared with healthy control. Conjugated hyperbilirubinemia is usually secondary to hepatocellular diseases or cholestasis. Similar findings were recorded by Elhiblu *et al.* (2015) [4] and Lakshmi *et al.*, (2018) [7]. There was signficant (P < 0.05) decrease found in the mean value of TP in diseased dogs as compared with healthy control although mean values of Albumin showed highly significant (P< 0.01) drop in diseased dogs as compared with healthy control. Hypoalbuminemia in the present study might be due to the fact that liver being the main site of synthesis and degradation of most of the proteins by the hepatocytes and water and sodium retention that dilutes the content of albumin in the extracellular space in hepatic disorder is responsible for decrease in albumin concentration and it can be upto 60-80 % in cases of advance cirrhosis[18]. Findings of TP and Albumin were in accordance with Eman *et al.* (2018) [5] and Jana *et al.* (2019) [6]. The mean value of Total Bile Acids were significantly (P< 0.05) elevated in diseased dogs as compared with healthy control. Dirksen *et al.* (2017) [3] reported similar findings noted for TBA.

Table 2: Hematobiochemical parameters recorded in control group and dogs with hepatobiliary dysfunctions

Sr. No.	Parameters	Apparently healthy dogs (n=6)	Affected dogs with hepatobiliary dysfunctions (n=22)
1.	Haemoglobin (g/dl)	10.8±1.96	7.79±0.23**
2.	PCV %	40.2±0.428	25.31±0.58**
3.	TEC (x 106 / µl)	7.03±0.191	4.23±0.11**
4.	TLC (x 103 / µl)	11.7±0.783	16.4±0.79*
5.	**DLC (%)**		
	Neutrophils (%)	61±1.06	69.9±2.13
	Lymphocytes (%)	18.3±1.41	21.77±1.67
	Monocytes (%)	1.33±0.422	2.72±0.36
	Eosinophils (%)	0.5±0.224	0.91±0.1
	Basophils (%)	0.333±0.211	0.55±0.1
6.	Platelets	5.15±0.38	2.03±0.253*
7.	ALT (U/L)	72.3±1.6	145.88±14.4**
8.	ALP (U/L)	47.5±2.71	99.49±5.4*
9.	GGT (U/L)	9.28±0.73	20.36±1.7*
10.	Total Protein (mg/dL)	7.35±0.15	4.09±0.18*
11.	Albumin (mg/dL)	2.71±0.05	1.9±0.1**
12.	Total Bilirubin (mg/dL)	0.2±0.01	1.83±0.4**
13.	Direct Bilirubin (mg/dL)	0.09±0.01	1.29±0.3**
14.	TBA (mmol/L)	9.31±1.83	65.72±7.1*

*significantly at P < 0.05
**significantly at P < 0.01

Ultrasonography: Hepatosonography revealed Hyperechogenicity of liver parenchyma, normal liver size, normal thickness of gallbladder wall and anechoic free fluid in abdomen in 6 dogs; hyperechoic liver parenchyma with hyperechoic and thickened gall bladder wall and distended bile duct in 5 dogs; accentuated brightness of liver parenchyma with distinguish demonstartion of portal vasculature with overall reduced echogenicity in 5 dogs; increase in the size of the liver, rounding of caudal ventral margins projecting away from the costal arch, normal echogenicity and thickness of gallbladder wall without ascitic fluid in 2 dogs; diffusely hypoechoic liver with dilated caudal venacava, hepatic veins and enlarged liver in 1 dog; bright liver with shrunken irregular contour and massive anechoic free fluid in 2 dogs; hyperechoic gall bladder wall with normal hepatic parenchyma in 1 dog; hyperechoic sediment in gall bladder in 1 dog.

Dogs with hepatobiliary dysfunctions had significantly (P < 0.05) higher ultrasound scores than the apparently healthy dogs. Dogs with hepatobiliary dysfunctions had significant (P < 0.05) higher mean value of surface score (0.955±0.154) when compared to control animals (0.167±0.1). Dogs with

hepatobiliary dysfunctions showed highly significant ($P < 0.01$) elevation in mean value of parenchymal score (1.07±0.115) when compared to control animals. (0.0833±0.0833). The mean value of biliary score in dogs affected with hepatobiliary dysfunctions (0.575±0.0734) was significantly increased when compared to control group (0.11±0.0696). These findings are in accordance with Assawarachan *et al.*, (2019).

Majority of the dogs affected with hepatobiliary dysfunctions showed moderate severity (14/22, 63.64 %) followed by mild severity (7/22, 31.82 %) and sever severity (1/22, 4.55 %). In present study different hepatobiliary dysfunctions in dogs were found and out of the 28 dogs 50 % (11/22) had chronic hepatitis, 18.18 % (4/22) had cholengio hepatitis, 13.64 % (3/22) had acute hepatitis, 9.1 % (2/22) had steroid induced hepatopathy, 4.55 % (1/22) had hepatic congetion and 4.55 % (1/22) cholecystitis. About, 65% of the dogs were of moderate category and majority of them were affected with chronic hepatitis.

Conclusions: When treating diseased animals, one must take into account the clinical and hemato-biochemical changes brought on by hepatobiliary dysfunction. Here, explained ultrasound score system can be used for screening of different hepatobiliary affections thus better diagnosis of the liver diseases in dogs can be given via visulizing liver damage.

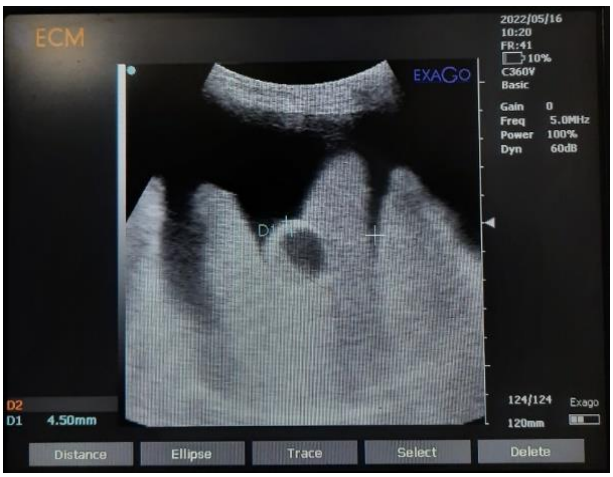

Fig 1: Chronic hepatitis

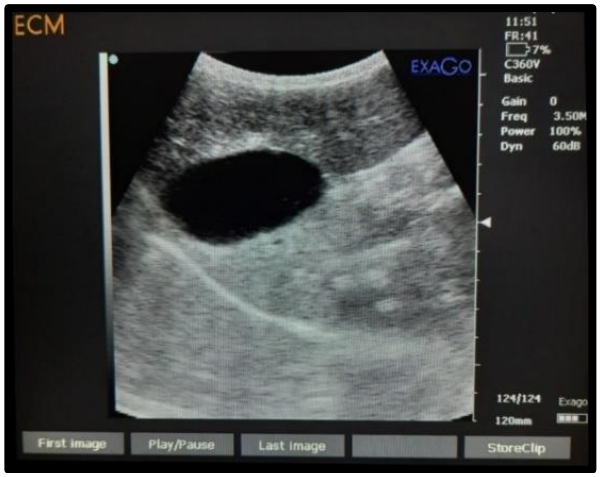

Fig 2: Normal liver parenchyma with gallbladder in apparently healthy dog

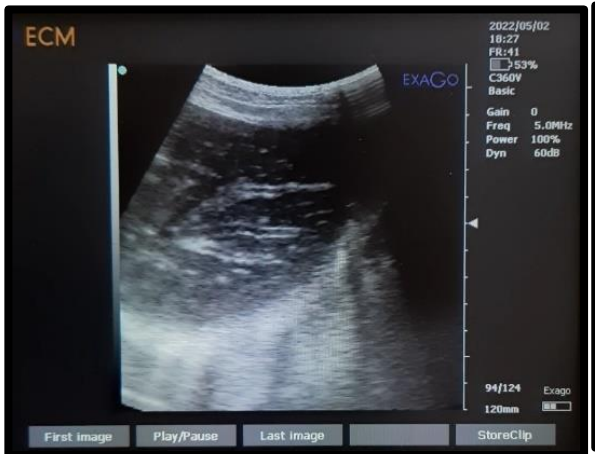

Fig 3: Cholengiohepatitis

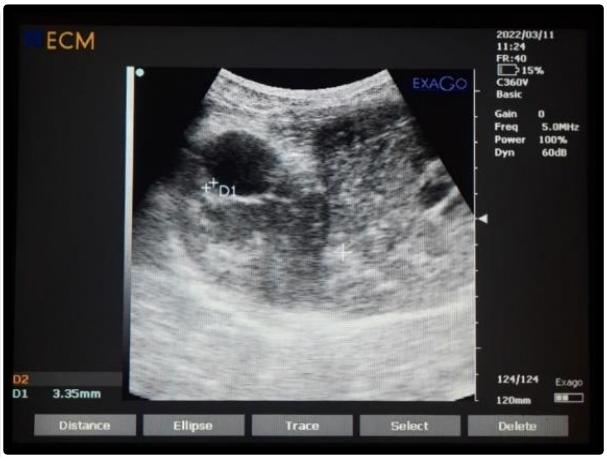

Fig 4: Acute hepatitis

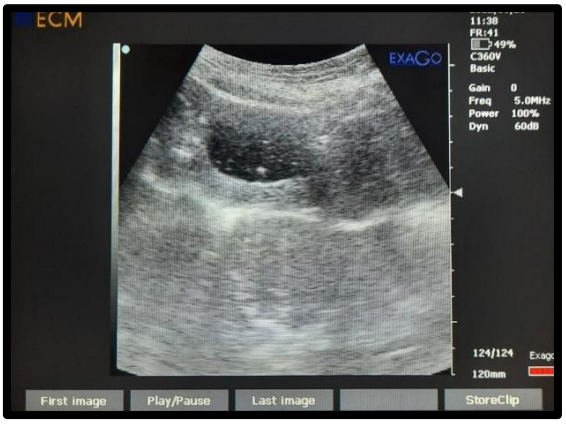

Fig 5: Gall stone

References

1. Abdallah AA, Nasr El-Deen NA, Neamat-Allah AN, El-Aziz A, Heba I. Evaluation of the hematoprotective and hepato-renal protective effects of Thymus vulgaris aqueous extract on thermally oxidized oil-induced hematotoxicity and hepato-renal toxicity. Comparative Clinical Pathology. 2020;29(2):451-461.
2. Assawarachan SN, Chuchalermporn P, Maneesaay P, Thengchaisri N. Evaluation of hepatobiliary ultrasound scores in healthy dogs and dogs with liver diseases. Veterinary World. 2019;12(8):1266-1272.
3. Dirksen K, Burgener IA, Rothuizen J, Van den Ingh TSGAM, Penning LC, Spee B, *et al*. Sensitivity and specificity of plasma ALT, ALP, and bile acids for hepatitis in Labrador Retrievers. Journal of Veterinary Internal Medicine. 2017;31(4):1017-1027.
4. Elhiblu MA, Dua K, Mohindroo J, Mahajan SK, Sood NK, Dhaliwal PS. Clinico-Hemato-Biochemical Profile of Dogs with Liver Cirrhosis. Veterinary World. 2015;8(4):487.
5. Eman SR, Kubesy AA, Baraka TA, Torad FA, Shaymaa IS, Mohammed FF. Evaluation of Hepatocyte-derived microRNA-122 for Diagnosis of Acute and Chronic Hepatitis of dogs. Veterinary World. 2018;11(5):667-673.
6. Jana S, Das PK, Banerjee D, Ghosh D, Mukherjee P, Mukherjee J. A case study on ascites of hepatic origin in a dog. Indian Journal of Animal Health. 2019;58(1):135-138.
7. Lakshmi K, Padmaja K, Nagaraj P, Gopala Reddy A, Gnana Prakash M. Hemato-Biochemical Studies of Hepatobiliary Disorders in Dogs. International Journal of Current Microbiology and Applied Sciences. 2018;7(01):1406-1411.
8. Lakshmi K, Padmaja K, Nagaraj P, Reddy AG, Prakash MG. Incidence of hepatobiliary disorders in dogs. The Pharma Innovation. 2017;6(5):70.
9. Lawrence YA, Steiner JM. Laboratory evaluation of the liver. Veterinary Clinics, Small Animal Practice. 2017;47(3):539-553.
10. Meyer HP, Rothuizen J. History and physical examination. In: Washabau, R.J. & Day, M. J., editors. Canine and Feline Gastroenterology. 1st ed. Ch. 61(liver). Saunders, St. Louis, MO, USA. 2013, 849-958
11. Mircean M, Giurgiu G, Scurtu I, Popovici C, Kiss T. Observations regarding the comparative value of ultrasonography and laboratory diagnosis of hepatobiliary diseases in dogs. Bulletin UASVM, Veterinary Medicine. 2008;65(2):20-25.
12. Mullakkalparambil Velayudhan J, Mondal D, Raja R, Kumar B, Mandal RSK, Bhatt S, *et al*. Hepatoprotectant potential of sodium alginate coated catechin nanoparticles (SACC-NPs) in rat model. Inorganic and Nano-Metal Chemistry. 2020;50(12):1334-1342.
13. Prebavathy T, Amaravathi P, Rajesh K, Vaikuntarao V, Bharathi S, Raghunath M. Haematobiochemical alterations in hepatic diseases in dogs. Journal of Entomology and Zoology Studies. 2020;8(5):1382-1384.
14. Richter KP. Diseases of the Liver and Hepatobiliary system. In: Handbook of Small Animal Gastroenterology. Todd R. Tams, (2nd ed.). W. B. Saunders and Co., Philadelphia. 2003, 286-352.
15. Saravanan M, Mondal DB, Sarma K, Mahendran K, Vijayakumar H, Sasikala V. Comprehensive study of haematobiochemical, ascitic fluid analysis and ultrasonography in the diagnosis of ascites due to hepatobiliary disorders in dog. Indian Journal of Animal Sciences. 2014;84(5):503-506.
16. Selgas AG, Bexfield N, Scase TJ, Holmes MA, Watson P. Total serum bilirubin as a negative prognostic factor in idiopathic canine chronic hepatitis. Journal of Veterinary Diagnostic Investigation. 2014;26(2):246-251.
17. Swanson KS, Kuzmuk KN, Schook LB, Fahey Jr GC. Diet affects nutrient digestibility, hematology, and serum chemistry of senior and weanling dogs. Journal of Animal Science. 2004;82(6):1713-1724.
18. Tantary HA, Soodan JS, Sahrish C, Ansari MM, Sandeep K, Taziyun I. Diagnostic studies in dogs with hepatic disorders. International Journal of Veterinary Science. 2014;3(4):210-215.
19. Twedt DC. Jaundice, hepatic trauma, and hepatic encephalopathy. The Veterinary Clinics of North America: Small animal practice. 1981;11(1):121-145.