EFFICACY OF GLUTATHIONE S-TRANSFERASE (GST) FOR EARLY DIAGNOSIS OF HEPATIC DISORDERS IN DOGS

ANJU A.D.
(16-MVP-32)

THESIS

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF VETERINARY SCIENCE

(Veterinary Clinical Medicine, Ethics and Jurisprudence)

2018

Faculty of Veterinary and Animal Sciences
Kerala Veterinary and Animal Sciences University

DEPARTMENT OF VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
POOKODE, WAYANAD-673 576
KERALA, INDIA

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DECLARATION

I hereby declare that this thesis, entitled **"EFFICACY OF GLUTATHIONE S-TRANSFERASE (GST) FOR EARLY DIAGNOSIS OF HEPATIC DISORDERS IN DOGS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or society.

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CERTIFICATE

Certified that this thesis, entitled **"EFFICACY OF GLUTATHIONE S-TRANSFERASE (GST) FOR EARLY DIAGNOSIS OF HEPATIC DISORDERS IN DOGS"** is a record of research work done independently by **Anju A.D.** (16-MVP-32) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Date:

Dr. Vinu David.P.
Chairman
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We, the undersigned members of the advisory committee of **Anju A.D. (16-MVP-32)**, a candidates for the degree of master of Veterinary Science in Veterinary Clinical Medicine, Ethics and Jurisprudence, agree that the thesis entitled **"EFFICACY OF GLUTATHIONE S-TRANSFERASE (GST) FOR EARLY DIAGNOSIS OF HEPATIC DISORDERS IN**

DOGS" may be submitted by **Anju A.D. (16-MVP-32)** in partial fulfilment of the requirement for the degree.

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ACKNOWLEDGEMENTS

I feel great sense of satisfaction and fulfillment in submitting this tiny piece of research work. Completion of this thesis would not have been possible without the support and guidance of so many people. I would like to take this opportunity to extend my deepest sense of gratitude and words of appreciation towards those, who dedicated their valuable time and energy for me.

The guidance of one's teachers and superiors is of paramount importance in his/her academic life. In this regard it is a privilege for me to register my profound regards and

deep sence of gratitude towards the chairman of my advisory committee ***Dr.Vinu David P,*** *Assistant Professor, Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, for his benevolent guidance, critical appreciation, whole hearted and sustained encouragement, liberal attitude and univocal help extended during the entire period of my master studies as well as excellent supervision in execution and conducting research work and completion of manuscript.*

I am at loss of words to express my heartfelt gratitude to ***Dr. Biju P Habeeb****, Assistant Professor, Department of Veterinary Clinical Medicine, Ethics and Jurisprudence and member of advisory committee, for the inspiration conferred by him throughout my degree program. He taught me that a research should always be seen and applied in the context of a needy society as a whole. He provided the vision and advice necessary for me to proceed through hard times in my research work*

I owe my sincere gratitude to ***Dr. N. Madhavan Unny****, Assistant Professor, Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Mannuthy and member of advisory committee for his valuable suggestions, timely help and moral support rendered during entire period of research work.*

I was indeed fortunate to have ***Dr. Dinesh P.T****, Assistant Professor, Department of Veterinary Surgery and Radiology as a member of my advisory committee. His valuable comments and indispensable suggestions helped to improve my work.*

I treasure the clinical expertise, moral encouragement and great affection shown by ***Dr. Umesh C.G****, Assistant Professor, Department of Veterinary Clinical Medicine.*

My deep sense of gratitude to ***Dr. Manju K Mathew****, Assistant Professor, Department of Veterinary Clinical Medicine, for her expert suggestions during my research period and her constant encouragement to complete my work.*

I am extremely grateful to ***Dr. Sindhu O.K*** *Assistant Professor, Department of Veterinary Clinical Medicine for her encouragement, technical help and constant advice.*

I am immensely grateful to ***Dr. Anju Vargheese*** *and* ***Dr. Raghu Raveendran*** *fo their valuable suggestions, intellectual advices and helps for performing ELISA*

Words are not enough to express my deepest sense of gratitude and indebtedness to my teachers ***Dr. K.D. John Martin, Dr. Bibin, Dr. Sooryadas, Dr. Hiran, Dr. Azeez Zareena, Dr. Anoop Raj, Dr. Naicy Thomas, Dr. Elizabeth, Dr. Dinesh, Dr. Lijo John, Dr. Naheef, Dr. Janus, Dr. Leeba, Dr. E.M Muhammed, Dr. Vinod Kumar, Dr. Deepa Chirayath and Dr. Deepa P.M,*** *for their crucial contributions throughout this study and providing me with all the encouragement and help.*

I would also like to acknowledge the ***Dean, College of Veterinary and Animal Sciences, Pookode*** *for providing necessary facilities to carry out my research work.*

I sincerely thank ***Dr. Sunanda C****,* ***Department of Statistics*** *for helping me in the statistical analysis of data.*

No amount of words could express my gratitude for the support and encouragement from my seniors, colleagues and juniors especially ***Anjali Ramesh, Swathy Krishna, Honey Shaju, Aswathy S, , Amithy S Nair, Amrutha B.M, Amrutha C.N, Parvathy G Nair, Sumanth, Annie, Helna, Christina, Rasanath, Megha, Rani, Sruthi Chandramohan.***

I thank all my 2015, 2016 and 2017 M.V.Sc batchmates individually for being of great support to me during the various stage of my research work. I acknowledge with the 2012 and 2013 batch UG students for their timely help shown throughout the research work.

Co operations rendered by ***Dr. Jayesh****, Veterinary Surgeon, Veterinary Poly Clinic, Sulthan Bathery* ***Dr. Leena*** *Veterinary Surgeon, District Veterinary Centre Kozhikode.*

I treasure the unconditional love, encouragement and moral support given by my family - ***Achan, Amma, Anila and Anusree*** *who enriched my soul and taught me to survive in this world.*

Your arrival into my life was a landmark, remembering all the love and strength your hands gave me these days ***Shijuchetta****. The impossible was made possible with your presence on the other side, till this moment and always.*

Above all, I bow my head before God for his abundant grace and unfathomable love showered on me.

ANJU A.D.

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1. INTRODUCTION

There is nothing truer in this world than the love of a dog. The unconditional love and companionship that it gifts us make the dog one of the most loyal pet in the planet. The strong bond which develop between the owner and his pet is as emotional and intense as human bond, sometimes even more so. The nickname "Man's best friend" was thus one well earned. The number of canine patients in veterinary clinics increase day by day owing to increased attachment and concern of owners for their beloved pets. Liver disease is one of the common cause of death in dogs as it is rarely detected during early stages.

The largest parenchymal organ of the body, the liver, carries out a diverse number of processes essential to maintain the normal body homeostasis. Some of the pivotal processes carried out by liver include synthesis of fats, plasma proteins and various clotting factors; metabolism of amino acids, fats and carbohydrates, storage of glycogen as an energy source, secretion of bile for digestion and detoxification or excretion of toxins and drugs.

Hepatic disorders are an important cause of morbidity and mortality in dogs. Damage of hepatocytes occur due to several infectious agents, toxins, neoplasms, and immune mediated reactions. Various laboratory diagnostic procedures and imaging techniques are available for the diagnosis of liver disorders. However many of these procedures/techniques present their own set of challenges. Certain procedures/tests are more confirmatory in specific liver diseases. Most of the clinical signs in hepatic disorders will be exhibited only when 75-80% of the liver is damaged and the conventional liver function tests will yield a good result only during that period.

Glutathione S-transferases (GST) are a family of enzymes that catalyze conjugation of reduced glutathione to a wide range of substrates, usually resulting in detoxification. They also function as transport proteins. The Alpha GST isoenzyme is located mainly in the centrilobular hepatocytes and thus is a sensitive and relatively specific liver biomarker. So novel biomarkers like GST are helpful in the early detection of hepatocellular injury in dogs. The present study aims to compare the estimation of GST with other diagnostic protocols for hepatic disorders.

In most cases veterinarians have to utilize appropriate diagnostic tests to arrive at a proper conclusion. Only a few cases of hepatic disorders can be diagnosed by physical examination and history. Other diagnostic imaging technique like radiology/ultrasonography will aid in the conformatory diagnosis in hepatic disorders. Choosing the most appropriate diagnostic

test which helps to arrive at an accurate diagnosis is one of the toughest challenges faced by the veterinarian. Proper understanding of the pathophysiology of liver disorders and knowledge of the characteristic abnormalities that they may cause in clinical and laboratory findings can provide a basis for test selection, interpretation and early diagnosis. A study entitled "Study of Efficacy of Glutathione S-transferase for early diagnosis of hepatic disorders in dogs." was hence planned and undertaken with the following objectives:

1. To study the incidence of hepatic disorders in dogs in Wayanad district.

2. Efficacy of GST (Glutathione S-transferase) as biomarker for early diagnosis of liver diseases in dogs

3. To compare GST as biomarker with various diagnostic protocols in hepatic disease diagnosis.

4. Therapeutic management of liver diseases.

2. REVIEW OF LITERATURE

2.1 CANINE LIVER DISEASES

Canine liver has high regenerative capability and functional reserve capacity which will cause an obscurity in diagnosing functional injuries by conventional means. These diagnostic techniques were only feasible when more than 55 per cent of hepatic impairment occur (Kumar *et al*., 2012).

An assortment of insults cause development of hepatic disease in canines. Mild damage of the liver may renovate itself due to its high regenerative power. Severe or constant hepatic impairment cause progressive and self-perpetuating chronic hepatic dysfunction (Rutgers, 1996).

Precise and judicious diagnosis of hepatic diseases is a challenging duty to the veterinarian, since diseased animals may show unfocused clinical signs. More over some of the pretentious animals are asymptomatic at the time of presentation. Exact clinical signs may arise in animals only with advanced hepatic disease (Lawrence and steiner 2015).

2.2 SIGNALMENT

2.2.1.**Breed**

Rutgers (1996), Center (1999), Johnson (2000) and Boisclair *et al.* (2001) described that certain breeds such as Dobermann pinschers, Bedlington terriers, Sky terriers and West highland terriers show more incidence of chronic hepatitis when compared to other breeds. These breeds had an inherited defect in the copper excretion and transport which will lead to excess copper accumulation.

Breeds such as Labrador retrievers and Cocker spaniels had an high incidence of cirrhosis and chronic hepatic disease (Andersson and Sevelius 1991).

According to Patnaik *et al*. (1981) primary hepatic neoplasms were observed in 18 different breeds and no breed predominance was noticed.

Harkin (2008) reported breed predisposition for portosystemic shunts and copper storage hepatopathies.

Pooja *et al.* (2010) opined that Pomeranians were more predisposed to primary or secondary hepatopathies.

2.2.2 Age

Dogs above five years of age were more prone to cirrhosis and chronic hepatitis (Fuentealba *et al*., 1997).

Rutgers and Haywood (1998) reviewed that idiopathic chronic active hepatitis was mainly observed in dog with an average age of four to seven years.

Patnaik *et al*. (1981) reported that hepatocellular carcinomas were mainly observed in dogs with an average age of 11 years.

Dixit *et al*. (2010) and Holt *et al.* (1995) found that intrahepatic portosystemic shunts (PSS) were more commonly seen in younger dogs than in older ones and average age of occurrence was found to be 17 months

Farrar *et al.* (1996) reported that hepatic abscesses were seen in dogs with average age ranging from 8-15 years (mean of 10.6 years) where as Schwarz *et al.* (1998) reported that the incidence was more common between 4-16 years (mean of 11.5 years) .

2.2.3 Sex

According to Andersson and Sevelius (1991) male Cocker spaniels and Labrador Retriever females were more prone to hepatic diseases.

Incidence of hepatocellular carcinomas was more predominantly observed in males than in females and the male female ratio is l.7:1 (Patnaik *et al*., 1980).

Rutgers and Haywood (1988) observed that when compared to males, females were more predisposed to liver diseases especially the occurrence of chronic active hepatitis.

Dixit *et al.* (2010) reported that intrahepatic PSS was more frequently observed in male dogs while cirrhosis and hepatitis were higher in females.

2.3 GENERAL CLINICAL EXAMINATION

Abnormal physical findings in early phase of Infectious canine hepatitis (ICH) include increased rectal temperature, transient or biphasic fever, tonsillar enlargement, subcutaneous edema of head, neck and dependant parts, hepatomegaly and petechial and ecchymotic haemorrhage. Icterus was uncommon in acute ICH (Greene, 1990).

Rothuizen and Meyer (2000) observed that physical examination was informative in only a minority of dogs with liver disease. Possible clinical findings in dogs with liver diseases include icterus, hepatomegaly, splenomegaly, ascites and pale mucus membrane, with an occassional petechiation of skin or mucous membrane.

Hepatomegaly and cranial abdominal mass were the most common finding on physical examination of the abdomen in dogs with hepatic impairment (Patnaik *et al*., 1980).

Sherding (2013) observed that icterus was generally visible when serum bilirubin concentration exceeded 2-3 mg/dL or when it was greater than five to ten fold above the

reference range. Jaundice which is a highly specific clinical sign is always correlated with hyperbilirubinemia.

Icterus, hepatomegaly, palpable abdominal mass and peritoneal effusions constitute the prominent clinical alterations observed in primary and secondary hepatic diseases (Voros *et al*., 1991).

Boothe *et al.* (1992) reported that icterus, fever, weight loss, ascites, anaemia, pale mucous membrane and gastro-intestinal bleeding were the main physical examination findings of dogs with experimentally induced hepatotoxicosis.

On abdominal palpation of healthy animals, liver edges was found to be sharp. In animals with hepatic disease hepatomegaly, microhepatica, hepatodynia and ascites were the important findings observed during palpation of abdomen. The ascitic abdomen had a characteristic pear shape and a sharp fluid rebound can be felt on the opposite side during abdominal percussion. (Peden *et al.,* 1982).

2.4 CLINICAL SIGNS

Kumar and Varshney (2006) observed clinical manifestations like anorexia, vomiting, pyrexia, melena, weight loss, arrhythmia, ascites, peripheral nerve deficits, and lymphadenopathy suggestive of hepatic involvement in dogs with ehrlichiosis. Pale mucosa, ascites and hepatomegaly were marked in dogs with infection of *Babesia gibsoni* and *Ehrlichia canis*.

Varshney and Hoque (2002) studied 24 clinical cases of canine hepatopathy and reported different clinical signs like nausea/vomiting, jaundice, mild anaemia, abdominal distension, constipation, diarrhoea, head pressing, convulsion, hypersalivation, muscle tremor and melaena. Chronic anorexia, weakness, emaciation and depression were the non specific signs seen in most of the affected dogs.

Clinical signs associated with liver disease in canines include depression, weakness, anorexia, polydypsia, polyuria, jaundice, vomiting, ascites, weight loss, hepatomegaly and hepatic coma. A two year old bitch had anoestrus for 18 months after contracting liver disease (Strombeck and Gribble, 1978).

Canine superficial necrolytic dermatitis or hepatocutaneous syndrome was manifested as ulcerative, erythematous lesions in footpads, pressure points and mucocutaneous junctions in dogs (Nyland and Park, 1996).

Sevelius (1995) observed that ascites was one of the most common clinical finding in chronic hepatitis predominantly associated with cirrhosis and chronic active hepatitis.

In early stages of chronic hepatitis clinical signs were vague like depression, anorexia, weight loss, vomiting and diarrhoea where as in advanced stages major clinical manifestations include icterus, ascites, polyuria, polydypsia, and neurological signs of hepatic encephalopathy (Rutgers and Haywood, 1988 and Johnson, 2000).

Jaundice and melaena were observed in chronic active hepatitis in canines (Center, 1995 and Varshney and Hoque, 2002).

Sevelius (1995) reported cirrhosis as an end stage of chronic hepatitis, leading to liver failure with jaundice, ascites and hepatic encephalopathy.

Ascites developed due to portal hypertension, hypoalbuminemia or hormonal changes associated with hepatic dysfunction cause excessive sodium retention (Hunt *et al*., 1993).

Carmichael *et al*. (1996) reported cases of hepato-cerebellar degeneration in Bermese mountain dogs with clinical signs varying from mild ataxia, stumbling, incoordination, head tremors, nystagmus and falling sideways or backwards. Neurological examination revealed slight proprioceptive deficits in limbs with normal cranial and spinal reflexes. Patellar reflex was exaggerated with hypermetria and hypotonia of hind limbs.

Clinical signs of hepatic encephalopathy include mild signs like lethargy, inappetance, vomiting, behavioural changes, ataxia and weakness to severe signs like amaurosis, head pressing, pacing, seizures and coma (Rutgers, 1996).

Clinical signs associated with hepatic abscess include lethargy, vomiting, anorexia, diarrhoea, trembling, polyuria, polydypsia (Schwarz et *al*., 1998).

Farrar *et al*. (1996) observed that clinical signs such as fever, dehydration, abdominal pain, hepatomegaly and signs of bleeding like epistaxis, ecchymoses or haemotochezia were observed in 14 dogs having hepatic abscess. They also reported clinical signs like stunted

growth, weight loss, polyuria and polydypsia, vomiting, inappetance and neurologic signs like bizarre behaviour, ataxia, seizures and signs of depression in dogs with PSS.

According to Nyland (1984), fever, severe depression, vomiting, anorexia, weight loss and hepatomegaly were the clinical signs associated with canine hepatic lymphosarcoma. Lamb *et al*. (1990) reported that dogs with histologically confirmed lymphosarcoma had a variety of non specific clinical signs like weight loss, anorexia, lethargy or depression, hepatomegaly and lymphadenopathy.

Boothe *et al.* (1992) reported clinical signs indicative of liver disease in experimentally induced hepatotoxicosis in relative order of appearance as fever, weight loss, ascites, icterus, vomiting, gastro-intestinal hemorrhage and hepatic encephalopathy.

2.5 DIAGNOSTIC IMAGING TECHNIQUES

2.5.1 Radiography

According to Kealy and McAllister (2000), generalized enlargement of the liver was associated with rounding of the caudoventral edge on a lateral radiographic view. The caudal liver edge projected beyond the costal arch with displacement of the stomach caudally and dorsally on lateral view and caudally and to the left on ventrodorsal view.

Partington and Biller (1995) opined that focal hepatic enlargement could be detected by alteration in the hepatic margins with localized displacement of fundus, gastric body, pylorus, right kidney, cranial duodenal flexure, transverse colon and head of the spleen or diaphragm. Liver lobe enlargement displacing the body and pyloric region of the stomach dorsally and to the left has been reported by Pechman (1998).

According to Neer (1992), radiopaque choleliths could be identified on survey abdominal radiographs whereas radiolucent stones require advanced diagnostic procedures such as cholecystography and ultrasonography.

Abdominal radiography revealed hepatomegaly in cases of hepatic amyloidosis (Loeven, 1994).

Radiographic evaluation of alteration in liver size was subjective and insensitive to subtle changes (Godshalk *et al*. 1988 and Barr, 1992).

O'Brien (1978) suggested that the radiographic appearance of cirrhosis varied with the stage and severity of the disease. He reported that a small, dense liver with irregular nodular surface with or without pneumoperitoneograph as the most common radiographic appearance in cirrhosed liver.

Suter (1982) expressed opinion that radiographic appreciation of reduced liver size (microhepatica) was more difficult than hepatomegaly.

Capnoperitoneography, the special contrast radiographic procedure, enhanced the visceral visualization of abdominal organs in general and was very useful in the evaluation of liver lobes and its borders, especially the diaphragmatic border (Kumar *et al*., 2012).

2.5.2 Ultrasonography

Hepatic ultrasonography was indicated in liver disease with biochemical changes or clinical symptoms such as jaundice, vomiting, weight loss, hypersalivation, lethargy and also in cases with vague malaise, ascites or pyrexia of unknown origin (Godshalk *et al.*, Johnson, 1994 and Mannion, 2006).

Vijayanand *et al.* (2006) stated that ultrasonographic examination of liver was helpful for visualising the echogenecity and size of the liver and this technique was very useful for early diagnosis of hepatitis in dogs.

Nyland and Park (1983) reported that hepatic ultrasonography played an important role in detecting wide variety of liver diseases non-invasively. Hepatic mass lesions, vascular abnormalities, gall bladder/biliary disease and parenchymal pathology can be easily diagnosed by this technique.

Thushara *et al*. (2006) explained the ultrasonographic findings of ascites due to liver cirrhosis. They observed that in these cases liver was hyperechoic with irregular borders had abnormal architecture and was uniformly granular. Free peritoneal fluid present in the abdomen was seen as anechoic areas.

Tripathi (2008) observed that in case of hepatitis important ultrasound findings observed were hypoechoic liver parenchyma, distended gallbladder and hepatomegaly whereas in hepatic tumours mixed echogenecity (hypoechoic and hyperechoic areas) of liver was seen. Apart from

this multiple hyperechoic nodules present through out the hepatic parenchyma, hepatomegaly and distended gall bladder were also detected in hepatic tumour case.

Sarma *et al.* (2009) stated that hepatomegaly was the main ultrasound finding observed in all dogs suffering from ascites. Hepatic cyst was also observed in a single case.

Johnson (1994) reported ultrasound as an effective tool for the evaluation of portal hypertension and its effects. But Lamb and Mahoney (1994) reported difficulty in diagnosing portal hypertension with standard ultrasound imaging. The observations noted by them were enlarged main portal and extra hepatic portal veins, multiple portosystemic collateral vessels, ascites, splenomegaly and abnormal liver echogenicity in dogs with portal hypertension.

Godshalk *et al*. (1988) found that qualitative ultrasonic assessment of liver size can be done using static B- mode sonography.

2.5.2.1 Normal ultrasonographic pattern of liver

Nyland *et al*. (2002) observed that the normal hepatic parenchyma had uniform medium level of echogenicity, with normal interruption caused by the hepatic and portal veins. The echotexture of liver was coarser and more hypoechoic compared to spleen. Where as upon comparison to the renal cortex, the liver was found to be more hyperechoic.

Kealy and McAllister (2000) noted that the hepatic tissue was loosely granular with even echotexture and echogenicity. The portal veins were identified by their bright hyperechoic walls. Hepatic vessels were seen as anechoic linear and circular areas. Hepatic arteries and bile ducts were not usually identified. The gall bladder was seen as a large pear shaped anechoic structure on the right side of the liver. The gall bladder size on ultrasonography was variable depending on whether or not the animal had any food recently.

2.5.2.2 Abnormal ultrasonographic patterns of liver

Barr (1988) observed a mixed pattern of increased and decreased echogenicity in cirrhosis. A similar pattern of solitary echogenic patch was also seen in fatty infiltration of liver in dogs.

Partington and Biller (1995) reported that diffuse liver diseases like steroid hepatopathy, cirrhosis and fatty liver produced hyperechoic changes wheras hepatic congestion, hepatitis, and lymphoma caused hypoechoic changes.

Cartee *et al.* (1993) observed that the ultrasound examination could not established normal liver size but usually it will not extend caudally beyond xiphoid and hypochondriac regions and cranially from seventh rib to diaphragm.

In intra-hepatic portosystemic shunts, the liver echotexture was diffuse or patchy with hyperechoic areas. Moreover an overall reduction in liver area with distended and tortuous shunting vessel could be observed (Voros *et al*., 1991).

Nyland (1984) and Lamb (1990) reported that lymphosarcoma produced hypoechoic foci in the liver.

Farrar *et al.* (1996) found that ultrasonographic appearance of hepatic abscess in eight cases were hypoechoic or anechoic while one case was heteroechoic. In four cases, the hepatic lesions became more echogenic from hypoechoic or anechoic to heteroechoic in follow up abdominal ultrasonography.

Szatmari *et al.* (2004) concluded that ultrasonography was a reliable diagnostic tool to non invasively characterize the underlying disease process in hepatic vascular abnormalities. The authors also added that a dilated left testicular or ovarian vein was found to be an important indicator of acquired portosystemic shunt.

2.5.3 Ultrasound guided liver biopsy

Center (2007) explained different methods of liver biopsy techniques such as ultrasound guided procedures, laparoscopy, percutaneous procedure and keyhole approach. He stated that liver biopsy will be helpful for differentiating acute and chronic disorders, stage of carcinoma and assessment of response to therapy.

According to Rockey *et al.* (2009) liver biopsy was helpful for accurate diagnosis of hepatic diseases and to predict its prognosis.

In percutaneous hepatic biopsy, ultrasound could be used to guide the biopsy needle (Nyland and Park, 1983 and Rutgers, 1996).

Hoppe *et al.* (1986) compared manual ultrasound guided biopsy technique with an automated method and reported that the automatic method yielded better quality samples.

Lamb (1990) reported that ultrasonography provided an accurate, relatively non-invasive means of guiding liver biopsy and he described different procedures with Tru-Cut biopsy needle, fine needle aspiration and automated biopsy devices.

2.6 LABORATORY EXAMINATION

2.6.1 Haematology

Mild regenerative anemia produced as a result of gastro intestinal bleeding or rarely due to spontaneous bleeding associated with coagulopathy and normocytic normochromic non regenerative anaemia were the main haemogram changes observed in dogs with hepatic insufficiency. In liver diseases leucocytosis with neutrophilia was one of the importantant non-specific finding in haematological examination (Voros *et al*., 1991).

Loeven (1994) reported that neutrophilic leucocytosis with shift to left observed in hepatic amyloidosis cases.

Liptak *et al.* (2004) reported that mild regenerative anaemia, thrombocytosis, elevation of cytosolic enzyme ALT and leucocytosis were observed in canines with hepatocellular carcinoma.

2.6.2 Serum Biochemistry

Increase in the conventional liver enzymes in serum was found to be relatively higher than the incidence of liver disease due to a wide number of nonhepatic problems influencing the hepatic enzyme activity (Center, 2007).

Higher levels of Alanine amino transferase (ALT), Alkaline phosphatise (ALP), Aspartate amino transferase (AST) and bilirubin were observed in hepatic impairement caued by leptospirosis (Greene, 1990).

2.6.2.1. Alanine aminotransferase (ALT)

Kaneko *et al*. (1997) reported that the mean normal level of ALT in dogs was 47 ± 26 IU/L. ALT values correlated with the conventional microscopic examination of liver at autopsy.

A value between 40-400 units indicated minor to moderate necrosis while a value above 400 indicated severe liver necrosis.

Increase in serum ALT activity was considered to be liver-specific in dogs. Alanine aminotransferase activity can increase with severe muscle necrosis, but simultaneous evaluation of serum creatine kinase activity can rule out a muscle source (Valentine *et al*., 1990 and Center, 1995).

Increase in serum ALT activity had highest sensitivity (80-100%) for hepatic inflammation and necrosis, vacuolar hepatopathy, and primary neoplasia (hepatocellular carcinoma, cholangiocarcinoma). ALT had less sensitivity (50-60%) in hepatic congestion, metastatic neoplasia, and portosystemic vascular anomalies (Center, 1995).

Dossin *et al*. (2005) reported that the half life of serum ALT was 2.5 days. ALT activity was found to be low or within the normal range at end stage liver disease as there was no active damage during that period (Hall and German, 2005).

2.6.2.2 Alkaline phosphatase (ALP)

Alkaline phosphatase and alanine aminotransferase were the liver enzymes most often seen increased in hepatocellular carcinomas (Kosovsky *et al*., 1989).

The mean ALP level in serum of dogs was found to be 66 ± 36 IU/L (Kaneko *et al*., 1997).

The increase in ALP with hepatic necrosis and chronic hepatitis was usually found to be more than three to six times the upper normal limit (Center, 1996).

Following an acute, severe hepatic insult there is only a minimal increase in ALP in contrast to ALT and AST (Meyer and Harvey, 1998 and Meyer and Twedt, 2000).

Tissues that had the highest levels of ALP activity in descending order were the intestinal mucosa, kidney (cortex), placenta, liver, and bone in dogs (Center, 1995).

2.6.2.3 Bilirubin

Kaneko *et al.* (1997) reported that the normal level of total serum bilirubin in canines was 0.1 to 0.5 mg/dl while that of conjugated and unconjugated bilirubin were 0.06 - 0.12mg/dl and 0.01 – 0.49 mg/dl respectively.

Twedt (1985) found that 75 per cent of the cases with chronic hepatitis had abnormal high bilirubin concentrations.

Increased bilirubin alone is of little diagnostic significance in canine hepatobiliary disease (Rothuizen and Van- Den- Brom, 1987).

Elevation of bilirubin was reported in canine hepatocellular carcinoma, canine biliary carcinoma and canine neuroendocrine epithelial tumors (Patnaik et al., 1981).

Center (2007) reported that endogenous organic anion cholephils like bilirubin and bile acids were the proven markers of hepatic disease, cholestasis and portal perfusion adequacy.

2.6.2.4 Total protein

Kaneko *et al*. (1997) reported mean level of serum total protein, albumin, globulin and A:G ratio in canines as 6.1 ± 5.2 g/l, 2.91 ± 1.9 g/l, 3.40 ± 5.1 g/l and 0.83 ± 0.16 respectively.

Low serum protein and low albumin concentration were observed by Boothe *et al.* (1992) following a chronic hepatic insult.

In a retrospective study on hepatic abcess in dogs, hypoalbuminemia and hypoprotenemia was detected (Farrar *et al*., 1996).

Liver was the source of all albumin and most globulins, except gamma globulin , and so serum total protein and especially albumin could be considered as markers of hepatic function (Hall and German, 2005).

2.6.2.5. Albumin

Kaneko *et al.* (1997) reported normal mean value of serum albumin in canines 2.91 ± 1.9 g/l.

Richter (2003) opined that hypoalbuminemia resulting from hepatic disease suggest chronic dysfunction. As the serum half-life of albumin was 7-21 days (depending on the disease

state and the serum concentration), there should be a prolonged hepatic disease before serum albumin concentration decreases.

Hypoalbuminemia and reduced total serum protein were observed in primary hepatitis in dogs (Poldervaart, 2009).

2.6.2.6 *Globulin*

The mean level of serum globulin in dogs was reported as 3.40 ± 5.1 g/l by Kaneko *et al.* (1997)

Strombeck and Gribble (1978) reported slight hypoalbuminemia and hyper gammaglobulinemia in dogs with chronic active hepatitis.

Misra *et al.* (2001) believed that the decrease in albumin and increase in globulin levels in hepatobiliary disorders led to decrease in Albumin:Globuin ratio.

2.6.3 Serum alpha glutathione S transferase (GST) level

According to Lidbury and Suchodolski (2016) GST is a potential biomarker of liver injury in dogs and cats and Alpha GST is one among the four isoenzymes produced by hepatocytes.

In animal and human models GST has a significant role in early diagnosis of organ damage (Kilty *et al*., 1998). GST is an ideal biomarkers of organ damage because they exhibit many of the required characteristics such as high cytosolic concentration, relatively short half-life and specific localisation.

Ozer *et al*. (2008) reported that glutathione-S-transferases (GSTs) were examples of important inducible phase II detoxification enzymes. It plays an important role as potential markers of liver injury in dogs and cats.

Among the four GST isoenzymes produced by hepatocytes Alpha Glutathione-S-transferase (α GST) is one of the major isoenzymes (Coles *et al*., 2001).

Giffen *et al*. (2002) viewed that α GST concentration in serum was found to be higher than AST and ALT activities in an experiment conducted in rat by inducing acute hepatic injury.

Knapen *et al*. (2000) reported that in humans half life of α GST was much lesser when compared to ALT and AST.

Kilty *et al*. (1998) observed that in case of hepatocellular injury the serum αGST concentration showed a rapid increase initially and subsequent return to lower limit after a total hepatic vascular exclusion. Solid phase immunoassays (EIAs) were used for the detection of αGST level in serum samples. Here standard/sample GST was captured by means of a specific anti-GST antibody coated onto the solid phase.

Maina *et al.* (2016) conducted a comparative study in human patients with liver failure and found that α GST was a more responsible marker of hepatic injury/recovery with real-time assessment of hepatic disease.

2.7 HISTOPATHOLOGY

Histopathological examination was essential to determine the presence of hepatic damage and to identify any possible underlying cause. It can help in establishing the chronicity, activity and prognosis of a disease (Sterczer *et al*., 2001).

Lorenzi (2010) found that liver cytology was only useful for initial evaluation of liver diseases. He stated that it cannot distinguish benign focal inflammatory disease from progressive chronic disease, and was unable to establish the extent of lesion.

Andersson and Sevelius (1991) reported that chronic hepatitis refer to the later stage of chronic active or chronic progressive hepatitis. Chronic active hepatitis was characterised by portal inflammation with mononuclear or mixed inflammatory cells along with piecemeal necrosis, bridging necrosis and periportal fibrosis extending from the portal triads into the hepatic parenchyma. Chronic progressive hepatitis had a similar pattern, but piecemeal and bridging necrosis were absent.

Cirrhosis refer to end stage liver disease with necrosis, fibrosis, hepatocyte degeneration and marked architectural distortion (Sevelius, 1995)

Non-specific vacuolar hepatopathy was the histopathological finding in dogs with secondary liver disease subsequent to diseases of other systems like acute pancreatitis, acute or chronic small intestinal disease, extrahepatic bacterial infection, shock, anaemia and congestive heart failure (Rutgers, 1996).

2.8 URINE ANALYSIS

Tripathi (2008) reported that the colour of urine was yellowish brown to greenish yellow in canine hepatic diseases.

Krimizigul *et al*. (2005) observed that the specific gravity of urine ranged between 1.005-1.030 in dogs.

Santilli and Gerboni (2003) discussed that many patients with hepatobiliary disease had PU and PD and with low urine specific gravity. Some dogs with portosystemic vascular anomalies (PSVA) have detectable ammonium biurate crystalluria due to concurrent hyperuricaemia and hyperammonemia. They also observed that it was normal for some dogs (particularly male dogs) to have some conjugated bilirubin in their urine, but presence of hyperbilirubinuria is an indicative finding of excessive extravascular haemolysis or hepatobiliary disease.

Loeven (1994) observed proteinuria, bilirubinuria and positive reaction to urobilinoingen in hepatic amyloidosis.

2.9 TREATMENT

2.9.1 Antibiotics

Antibiotics were widely used in dogs with chronic hepatitis, non specifically to modify enteric flora and to reduce the incidence of secondary infections (Sevelius, 1995 and Center, 1999).

Ampicillin, amoxycillin, cephalexin and enrofloxacin were the preferred antibiotics for the treatment of hepatitis, cholangiohepatitis, cholecystitis and hepatic abscess (Rutgers, 1996).

Honeckman (2003) reported usage of various commonly used antibiotics in case of liver disorders like fluroquinolone, metronidazole, ampicillin, amoxicillin and clavulanic acid.

Antibiotics that rely on hepatic clearance or those which are potentially hepatotoxic like tetracycline, sulphonamide, chloramphenicol and erythromycin should be avoided (Bexfield and Watson 2009).

2.9.2 Corticosteroids

Prednisolone might be given at a dose rate of 1-2mg/Kg. body weight once daily for two to four weeks, followed by tapering dose or in combination with Azathioprine at a dose rate of 1-2 mg/Kg body weight (Rutgers, 1996).

2.9.3 Ursodeoxycholic acid

Ursodeoxycholic acid prevented reduction in levels of hepatic cytochrome p450 isoenzyme following hepatocyte damage due to hydrophobic deoxycholic acid especially during cholestasis (Tomida *et al.,* 1999) at a dose rate of 10-15 mg/Kg orally (Rutgers, 1996, Johnson, 2000 and Leveille- Webster, 2000).

Udayasree *et al.* (2006) reported successful management of cholestatic hepatitis in a Labrador dog with ursodeoxycholic acid.

2.9.3 Copper Chelation Therapy

Rutgers (1996), Johnson (2000) and Varshney (2002) opined D-penicillamine as a common copper chelating agent that can be used at a dose rate of 10-15 mg/Kg. Trientine or 2,3,2 tetramine can also be used as a decoppering agent at the dose rate of 10-15 mg/Kg. orally atleast one hour before meals.

2.9.4 Silybin

According to Sario *et al* (2005) new silibin-phosphatidylcholine-antioxidant complex could be an interesting drug to be tested in patients with chronic liver diseases.

Martin *et al.* (1984) reported that silibin alone or in combination with choline exhibit a good regenerative effect in hepatic tissue, reducing the extend of lesion and time needed for recovery. Silibin was usually given at the dose rate of 10 mg/Kg body weight.

Tiwari *et al*. (2001) evaluated the efficacy of silymarin in the management of clinical cases of hepatitis/hepatic congestion in dogs. The drug showed clinical improvement and reduction in activity of various enzymes like alanine aminotransferase, gama glutamyl transferase and serum alkaline phosphatase in the treated group.

2.9.5 Antioxidants

Vitamin E, Zinc and S-adenosyl methionine could be used as an antioxidant in treating canine liver diseases (Honeckman, 2003).

Center *et al*. (2007) stated that SAM-e increased hepatic and red blood cell glutathione levels. He also suggested that SAM-e was helpful for treating toxic hepatopathies in humans, particularly phenobarbital-induced hepatopathy. Recent work suggest that it might be helpful for curing steroid hepatopathy in dogs.

Vitamin E had an effective antioxidant activity in dogs with liver disease and can be used at a dose rate of 400 - 600 U/day in medium-sized dogs. It was effective for treating dogs suffering from copper storage disease (Twedt, 1985).

Zinc can be supplemented as zinc sulphate at a dose rate of 2 mg/Kg. or as zinc gluconate at a dose rate of 3mg/Kg/day for 30 days followed by a total dose of 50 mg orally at 12 hour interval in canines with liver diseases (Johnson, 2000).

2.9.6 Fluid therapy

Rutgers (1996) observed that 2.5-5 per cent dextrose or 0.9 per cent saline can be given to animals with liver damage depending upon the degree of dehydration.

Hypokalemia associated with hepatic failure was treated by adding 20-30 meq of potassium chloride per litre of fluid administered (Bunch, 2000).

2.9.7 Control of coagulopathies

Treatment of coagulopathies in dogs with hepatic failure included administration of plasma to replenish the coagulation factors along with heparin to prevent disseminated intravascular coagulation. Subcutaneous or intramuscular administration of Vitamin K at the dose rate of 2 mg/Kg was also recommended (Rutgers, 1996).

2.9.8 Control of Ascites

Ascites in chronic hepatic failure could be managed with restriction of salt intake (Rutgers and Haywood, 1988). Furosemide could be given at a dose rate of 1-2 mg/Kg as diuretic, but has to be supplemented with potassium chloride in case of prolonged treatment

(Varshney, 2002) or should be combined with Spiranolactone (Rutgers, 1996 and Johnson, 2000).

2.9.9 Indigenous medicines

Zafar and Ali (1998) reported that *Circhorium intybus* had a potent anti hepatotoxic activity and is a major component in trade preparations like like Liv-52, Geriforte, Acilvan and Livex.

Gadgoli and Mishra (1999) reviewed that p-Methoxy benzoic acid which is an active ingredient of *Capparis spinosa* had antihepatoxic activity.

3. MATERIALS AND METHODS

The study was conducted in the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, Pookode during the period from April 2017 to June 2018.

A total of 40 dogs brought to the Teaching Veterinary Clinical Complex, Pookode and other Veterinary hospitals of Kerala Veterinary and Animal Sciences University with clinical signs suggestive of hepatic diseases were selected for this study.

Dogs showing specific signs of liver involvement such as jaundice, ascites, hepatomegaly, hepatic encephalopathy (Rutgers, 1996 and Rothuizen and Meyer, 2000) and non specific signs of liver disease such as vomiting, diarrhoea, polyuria, polydypsia, anorexia, weight loss, depression (Rutgers, 1996) were selected for this study. For comparison purpose, a control reference population of ten apparently healthy dogs were randomly selected.

3.1 SIGNALMENT

Signalment of the animals presented (age, breed, sex, body weight, vaccination and deworming status) were noted and detailed history of illness and the treatment given if any was collected from the owner.

3.2 GENERAL CLINICAL EXAMINATION

A thorough clinical examination of the selected patients were conducted as proposed by Nelson and Couto (2009). Different aspects of the clinical examination includes recording the respiration, heart rate, pulse rate, rectal temperature and colour of the mucous membrane. The findings of auscultation of the heart and respiratory area were recorded. Patients were also examined for superficial lymph node enlargement and for the presence of skin bruises, petechiation, ecchymoses and hepatocutaneous syndrome. Abdominal palpation and ballottement were carried out to check for the presence of pain (hepatodynia), fluid accumulation, organomegaly or abnormal mass. Oral cavity was also examined for ulcerations and abnormal odours. Clinical signs of hepatic encephalopathy (dementia, seizures, changes in personality and motor disturbances) as well as those of reactive hepatopathy were thoroughly investigated.

3.3 CLINICAL SIGNS

Presence of various clinical signs like loss of appetite, vomiting, skin lesions, diarrhoea, icterus, abdominal pain, ascites, hepatomegaly, hepatic encephalopathy, pigmented urine, polyuria, polydypsia and depression were assessed.

3.4 COLLECTION OF CLINICAL MATERIALS

Various clinical materials like blood and urine were collected at the time of admission. In appropriate cases skin and liver biopsy samples were also collected. A total of six millilitres of whole blood was collected aseptically by puncturing the saphenous or cephalic vein. Two millilitres blood was collected in an EDTA coated vial for haematology (Benjamin, 2005) and remaining four milliliters of whole blood was collected in serum vial for the estimation of various biochemical parameters. Serum was also stored at -20ºC for performing ELISA to detect Alpha GST enzyme level. Thin peripheral blood smears were prepared, air dried, fixed and stained with Giemsa stain (1:10) dilution. These smears were then examined under oil immersion objective of the microscope to find out the presence of any haemoprotozoan infection and to evaluate RBC morphology (PLATE 1.d). Ten apparently healthy animals were selected and utilized for obtaining the normal haemato-biochemical parameters.

3.5 DIAGNOSTIC IMAGING TECHNIQUES

3.5.1 Radiography

Radiography was carried out using 500 mAs, 125 kVp x-ray machine (Siemens conventional X-ray Machine) (PLATE 1.f). The position, field of view and the exposure factors were dictated by the size of the subject. The animal was positioned in right lateral recumbency for a better visualization of the liver. Various radiographic exposure factors were selected as per the case, ranging from 12 to 24 mAs and 58 to 84 kVp at a constant focal film distance (ffd) of 90 cm.

3.5.2 Ultrasonography

3.5.2.1 Principle

Rantanen and Ewing (1981) and Barr (1988) described various display modes, equipments and principles of image interpretation in ultrasonography.

Cartee and Robert (1981); Lamb (1990) and Cartee *et al*. (1993) explained the principles of ultrasound production and transmission through tissues.

Kealy and McAllister (2000) observed that ultrasound waves were generated when an electrical impulse applied to a lead zirconate crystal produce piezo-electric effect. Lower frequency (2.0-3.5 MHz) ultrasound waves travel farther into the tissue, but the image they produce will be relatively poor. Higher frequency (7.5-10 MHz) sound waves gets attenuated in tissues more quickly, but the resolution of resulting image will be better.

Nyland *et al.* (2002) stated that areas of high echo intensity were referred to as echogenic, hyperechoic, or echo rich whereas areas of low echo intensity were termed as echo poor or hypoechoic. Areas with no echoes were said to be echo free or anechoic.

3.5.2.2 Equipment

Hepatic ultrasonography was carried out by using Aloka IPF-1503 ultrasound machine with 5.0 and 7.5 MHz transducer (PLATE 1.e).

3.5.2.3 Ultrasound scanning procedure

Animal was placed in dorsal recumbancy. Hair in the abdominal portion was removed and acoustic coupling gel was applied to the skin. Liver scanning was accomplished by positioning the transducer directly under the sternum at the xiphoid angle directed slightly cranially, attempting to avoid the air in the stomach. Sagittal and transverse scans of the liver was made by sweeping the sound beam back and forth from side-to-side and cranial to caudal in an attempt to visualize the entire liver (Nyland and park, 1983; Nyland *et al.* 1995; Varshiney and Hoque, 2002).

The ultrasonograms were reviewed for alterations in the echogenicity of liver parenchyma, contour, hepatic vasculature and liver size. The parenchymal lesions were classified into focal and diffuse. The echogenicity of liver parenchyma was described as normal, hypoechoic, hyperechoic or mixed echogenicity. Liver size was assessed subjectively by comparing the caudal position of liver to the costal arch (Nyland *et al*, 1995).

3.5.3. Ultrasound guided liver biopsy

Ultrasound guided liver biopsy was performed in appropriate cases and it was carried with a BARD biopsy gun. Haemostatic profiles include assessment of whole blood clotting time by capillary tube method direct evaluation of platelet count using Rees-Ecker solution (Benjamin, 1998) check before liver biopsy to rule out coagulopathies due to liver damage.

For liver biopsy, scan head was placed on anterior abdomen, just caudal to the xiphoid and abdomen was scanned sagitally. The area to be biopsied was identified avoiding major hepatic vessels. The biopsy site was prepared in a sterile manner. Local anaesthesia was attained through ring block using 2 per cent Lignocaine solution. Patient was placed on dorsal recumbancy. A small skin incision was made with No 11 BP blade. The needle of the biopsy gun was advanced through this skin incision towards the left side to avoid accidental puncturing of gall bladder, in a free hand approach. The needle was introduced at an angle of 15º or 30º to the transducer. Biopsy was then made when the needle tip and the target organ/lesion was clearly seen (Barr, 1995; Hager *et al.,* 1995; de Rocke *et al*., 1999) (PLATE 2). Tissue was immediately transferred to 10 per cent formalin solution and labelled.

3.6 LABORATORY EXAMINATION

3.6.1 Haematology

Haematological evaluation was performed for all the cases as per the method described by Schalm *et al*. (1975). Mindray BC-2800Vet Machine was used for the analysis (PLATE 1.a). The following parameters were observed.

1. Haemoglobin (Hb) [g/dL]
2. Volume of packed red cells (VPRC) [%]
3. Total erythrocyte count [10^6 / µL]
4. Total leucocyte count (TLC) [10^3/ µL]
5. Differential leucocyte count (DLC) [%]
6. Platelet count [x 10^5/ cmm]

3.6.2 Serum Biochemistry

Various biochemical parameters like total protein, albumin, globulin, A:G ratio, total bilirubin and liver enzymes like Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) were estimated by using semiautomatic serum biochemistry analyser (MISPAVIVA 2578-10/17) machine (PLATE 1.b).

Serum total protein was estimated by modified Biuret method described by Weichselbaum (1946) while albumin was estimated by bromo cresol green dye binding method as described by Doumas *et al*. (1971). Total bilirubin was estimated using the method of Jendrassik and Groff (1938) and direct bilirubin was estimated as described by Schellong and Wende (1960).

Alanine aminotransferase was measured based on the standard reference method of the International Federation of Clinical Chemistry (IFCC). Alkaline phosphatase was measured following the recommendations of Deutsche Gesellschaft fur Klinischechemie by Anon (1970).

Standard diagnostic kits from Agappe diagnostics Pvt Ltd, were used for the estimation of above mentioned biochemical parameters.

3.6.3. Serum alpha glutathione-S-transferase (α-GST) level by ELISA

3.6.3.1 Detection principe of α-GST ELISA kit

Alpha GST ELISA kit is based on double-sandwich ELISA technique. Here the pre-coated antibody is canine α-GST monoclonal antibody and the detecting antibody is biotin labelled polyclonal antibody (PLATE 3).

3.6.3.2 Detection procedure of α-GST ELISA kit

1. Added samples and different concentrations of canine Alpha GST standard samples to corresponding wells (100µl for each well), 0 ng/ml well should be filled with standard diluents. Sealed the reaction wells with adhesive tapes, hatching in incubator at 37ºC for 90 min.

2. ELISA plate was washed twice.

3. Biotinylated canine Alpha GST antibody liquid was prepared 30 min in advance.

4. One hundred microlitres of biotinylated Canine Alpha GST antibody liquid was added into each well. Sealed reaction wells with adhesive tapes, hatching in incubator at 37ºC for 60 min.

5. ELISA plate was washed twice.

6. Enzyme conjugate liquid was prepared 30 min in advance.

7. Enzyme-conjugate liquid to each well (100µl for each). Seal reaction wells with adhesive tapes, hatching in incubator at 37ºC for 30 min.

8. ELISA plate was washed 5 times.

9. Added 100µl colour reagent liquid to individual well (also into blank well), hatching in dark incubator at 37ºC. When colour for high concentration of standard curve become darker and colour gradient appear, the hatching can be stopped. The chromogenic reaction should be controlled within 30 min.

10. Added 100µl colour reagent C to individual well (also into blank well). Mix well. Read OD (450nm) within 10 min.

3.7 HISTOPATHOLOGY

Formalin prepared liver tissue samples collected were send to the Department of Veterinary Pathology for histopathological examination.

3.7 URINALYSIS

Urine sample was collected from all animals. The physical, chemical and microscopical examination of the collected samples were carried out using Uristick (DIRUI Industrial Co.,Ltd.) (PLATE 1.c).

3.8 TREATMENT

Specific treatment was given to the affected animal based on the disease diagnosed and these animals were randomly divided into two treatment groups of 20 animal each. One group was administered with the liver supplement containing silibin-phosphatidylcholine antioxidant complex and the other group was treated with a herbal liver supplement containing *Circhorium intybus* and *Capparis spinosa* for one month.

3.9 STATISTICAL ANALYSIS

The data obtained was subjected to statistical analysis as per Snedecor and Cochran (1994). SPSS 24.0 statistical software package was utilized for the analysis of results.

4. RESULTS

Forty animals with clinical signs suggestive of hepatic disease presented to the Teaching Veterinary Clinical Complex, Pookode and other Veterinary hospitals of Kerala Veterinary and Animal Sciences University were selected for the research work. Various data with respect to signalment, general clinical examination, clinical signs, diagnostic imaging techniques (radiography, ultrasonography and ultrasound guided liver biopsy), laboratory examinations (hematology, serum biochemistry and Alpha GST estimation), histopathology, urine analysis and therapeutic response were recorded from the selected cases. Out of 40 cases studied, 20 were from clinically suspected cases of leptospirosis, six animals were with cirrhotic liver, five had

babesiosis, three had hepatic tumour and two each had hepatocutaneous syndrome, drug intoxication, and pyometra respectively (Fig.1). Out of 7822 canine cases reported in Wayanad district 60 (0.77%) animal had hepatic disorders during the period from April 2017 to June 2018.

4.1 SIGNALMENT

4.1.1 Breed

The highest incidence of liver disease was found in Labrador (32.5 per cent) followed by Rottweiler (25 per cent), German Shepherd (15 per cent), Pug (7.5 per cent), Non-descript (7.5 per cent), Spitz (5per cent), Dachshund (2.5 per cent), Great dane (2.5 per cent) and Pekingese (2.5 per cent) breeds (Table 1 and Fig.2)

Table 1- Breed wise occurrence of hepatic diseases in dogs (n=40).

Sl. No	Breed	No. of cases	Per cent (Occurrence)
1	Labrador	13	32.5
2	Rottweiler	10	25
3	German Shepherd	6	15
4	Pug	3	7.5
5	Non-descript	3	7.5
6	Spitz	2	5
7	Dachshund	1	2.5
8	Great dane	1	2.5
9	Pekingese	1	2.5

4.1.2 Age

Age wise incidence among dogs showed that 45 per cent of cases was observed in young age (1month to 4 years), followed by 40 per cent in middle age (4 to 8 years) and 15 per cent during geriatric age (>8 years). The age of the affected animals ranged from 2 months to 10 years (Table 2 and Fig.3).

Table 2- Age wise occurrence of hepatic diseases in dogs (n=40).

Sl. No.	Age range	No. of cases	Per cent (Occurrence)
1	1 month to 4 years	18	45
2	4 to 8 years	16	40
3	8 to 12 years	6	15

4.1.3 Sex

Out of the 40 liver affected cases 24 were males (60 per cent) and 16 were females (40 per cent).

4.2 GENERAL CLINICAL EXAMINATION

Upon general inspection 26 dogs (65 per cent) exhibited varying degree of depression, dullness and lethargy and 14 (35 per cent) dogs were found to be alert. Nervous signs exhibited by these dogs include tremors, aggression and head pressing, dementia, stupor and collapse.

Upon examination of the body condition in affected dogs, it was found that 36 (90 per cent) animals exhibited with loss of weak condition where as the body condition of 4 (10 per cent) animals were good.

4.2.1 Respiration rate

The mean ± SE value of respiratory rate in dogs with liver disease was 50.89 ± 1.78 per minute.

4.2.2 Heart rate

The mean ± SE value of heart rate in dogs with liver disease was found to be 125.123 ± 1.73 per minute. Tachycardia was detected in 12 (30 per cent) cases, bradycardia in 2 (5 per cent) cases and the remaining 26 (65 per cent) cases had normal heart rate.

4.2.3 Pulse rate

The mean ± SE value of pulse rate in dogs with liver disease was noted as 123.39 ± 2.99 per minute.

4.2.4 Rectal temperature

The mean ± SE values of rectal temperature in dogs with liver diseases was found to be 101.9 ± 0.17 ºF. Twenty four (60 per cent) animals had normal body temperature, 12 had pyrexia (30 per cent) and the rest 4 (10 per cent) had subnormal body temperature.

4.2.6 Examination of mucous membrane

In this study, mucus membrane of 18 (45 per cent) dogs was pale, icteric in 15 (37.5 per cent), congested in 4 (10 per cent) and 3 (7.5 per cent) dogs had normal (pale roseate) mucus membrane.

4.2.7 Abdominal palpation

Upon abdominal palpation 6 (15 per cent) had peritoneal effusion (ascites or haemoperitoneum), two cases (5 per cent) each had hepatomegaly and abdominal mass respectively which was later confirmed with plain radiography and/or ultrasonography and/or exploratory laparotomy.

Table 3: Vital body parameters of dogs in various hepatic diseases (n=40)

Vital body parameters	Rectal temperature (ºF)	Heart rate (per minute)	Respiration rate (per minute)	Pulse rate (per minute)
Normal range	101-103	70-120	18-34	70-120
Diseased animals	101.9	125.123	50.89	123.39

4.1 CLINICAL SIGNS

The main clinical signs exhibited in dogs affected with liver disease were lethargy (90 per cent), anorexia (85 per cent), vomiting (55 per cent), diarrhoea (45 per cent), pyrexia (40 per cent), ascites (15 per cent), haematuria (10 per cent), crusty ulcerative skin lesions (5 per cent) and hepatic encephalopathy (2.5 per cent) (Table. 4 and Fig.4).

Table 4- Clinical signs observed in dogs with hepatic diseases (n=40).

Sl No.	Clinical sign	Number of cases
1	Vomiting	22
2	Anorexia	34
3	Lethargy	36
4	Pyrexia	16
5	Diarrhoea	18
6	Hematuria	4
7	Ascites	6

8	Skin lesions	2
9	Hepatic encephalopathy	1

4.3 DIAGNOSTIC IMAGING TECHNIQUES

4.3.1 Radiography

In the present study when 40 dogs with clinical signs suggestive of liver diseases were subjected to plain abdominal radiograph 13 (32.5 per cent) had radiographic changes. Hepatomegaly was observed in 4 (10 per cent) cases, with the liver found extended beyond the rib cage. In 3 cases neoplasms mass were observed adjacent to liver. Ascites was noticed in 6 (15 per cent) cases on lateral abdominal radiograph (PLATE 4).

4.3.2 Ultrasonography

Utrasonography showed an overall reduction in echogenicity of liver parenchyma in both transverse and sagittal scan. Portal vessel wall appeared as white specks in the hypoechoic liver parenchyma. Liver borders were found to be rounded in 8 out of 40 (20 per cent) cases indicating hepatomegaly. In two cases hypoechoic nodules were seen surrounded by hyperechoic parenchyma suggestive of hepatocutaneous syndrome. The gall bladder was seen as anechoic ovoid shaped structure with a tapering neck. The gall bladder was found to be distended in all anorectic animals. No ultrasonographic abnormalities were observed upon scanning the gall bladder. Gall bladder sludge was observed in two cases. In ascites liver lobes were found floating in the abdominal fluid. Upon ultrasound examination in clinically suspected cases of leptospirosis, renomegaly, loss of corticomedullary distinction, increased cortical echogenicity and a hyperechoic medullary band were observed (PLATE 4 & 5).

4.4 LABORATORY EXAMINATION

4.4.1 Haematology

The haematological values of diseased and healthy animals were presented in Table. 5. The mean haemoglobin level in diseased animals was 9.944 ± 0.596 g/dL. The mean total erythrocyte count (TEC) and VPRC were 4.743 ± 0.342 $\times 10^6$ cells/cmm and 38.97 ± 1.43 per

cent respectively. The mean platelet count of diseased animals was 3.033 ± 0.257 $\times 10^5$ cells/cmm.

The mean total leucocyte count in diseased animals was 34.897 ± 3.364 10^3cells/cmm with 87.217±1.242 per cent granulocytes, 9.669±1.136 per cent lymphocytes and 2.779±0.171 per cent monocytes.

Statistical analysis of the haematological parameters revealed a significant elevation ($p \leq 0.01$) in the level of TLC and a significant reduction ($p \leq 0.01$) in the values of TEC, haemoglobin and monocytes when compared to healthy controls. A significant reduction ($p \leq 0.05$) was observed in the levels of lymphocyte and platelet count where as a significant elevation ($p \leq 0.05$) was observed in the granulocyte count when compared to healthy animals (Table. 5)

Table-5. Mean haematological values of animals with hepatic diseases in comparison to healthy animals (n=40).

Parameters	Diseased animals (n=40)	Healthy animals (n=10)	t- value	p- value
Hb (g/dL)	9.944 ± 0.596	14.072 ± 0.469	5.435**	<0.001
VPRC (%)	38.97 ± 1.43	33.75 ± 2.87	1.627^{ns}	0.126
TEC ($\times 10^6$/ cmm)	4.743 ± 0.342	6.704 ± 0.360	3.940**	0.001
TLC($\times 10^3$/cmm)	34.897 ± 3.364	11.869 ± 0.942	6.590**	<0.001
Lymphocyte (%)	9.669 ± 1.136	15.531 ± 1.428	2.476*	0.017
Monocyte (%)	2.779 ± 0.171	4.830 ± 0.609	3.236**	0.008
Granulocytes (%)	87.217 ± 1.242	80.899 ± 2.042	2.369*	0.022
Platelet ($\times 10^5$/cmm)	3.033 ± 0.257	4.164 ± 0.357	2.069*	0.044

-* represent significant difference between the means at $p \leq 0.05$

-** represent significant difference between the means at $p \leq 0.01$

-^{ns} represent no significant difference between the means of diseased and apparently healthy dogs.

4.4.2 Serum Biochemistry

The serum biochemical values of diseased and healthy animals were presented in Table. 6. Mean serum ALT and ALP values of diseased animals were 114.044±29.69 IU/L and 206.013±37.18 IU/L respectively.

The mean serum total protein of diseased animals was 7.932±0.0364 g/dL. Albumin, globulin and A/G ratio were 2.744±0.113 g/dL, 5.249±0.3004 g/dL and 0.569±0.027 respectively.

The mean serum total bilirubin of diseased animals was 1.168±0.241 mg/dl.

Statistical analysis of the above serum biochemical parameters revealed a significant increase in total bilirubin and globulin ($p \leq 0.01$) values when compared to healthy animals. The total protein was also significantly elevated ($p \leq 0.05$) and A/G ratio was found to be significantly lowered ($p \leq 0.05$).

Table-6. Mean serum biochemical values of animals affected with hepatic diseases in comparison to healthy animals (n=40).

Parameters	Diseased animals (n=40)	Healthy animals (n=10)	t- value	p-value
ALT (IU/L)	114.044 ± 29.69	76.956 ± 6.310	0.619^{ns}	0.539
ALP (IU/L)	206.013 ± 37.18	86.2 ± 5.152	1.618^{ns}	0.112
Total Protein (g/dL)	7.932 ± 0.0364	6.007 ± 0.114	2.645*	0.011
Albumin (g/dL)	2.744 ± 0.113	2.542 ± 0.074	1.490^{ns}	0.144
Globulin (g/dL)	5.249 ± 0.3004	3.465 ± 0.098	3.012**	0.004
A/G (Ratio)	0.569 ± 0.027	0.739 ± 0.031	2.381*	0.021
Bilirubin (mg/dL)	1.168 ± 0.241	0.234 ± 0.018	3.856**	<0.001

-* represent significant difference between the means at $p \leq 0.05$

-** represent significant difference between the means at $p \leq 0.01$

-^{ns} represent no significant difference between the means of diseased and apparently healthy dogs.

4.4.3. Serum alpha glutathione-S-transferase (α-GST) level

The serum Alpha GST values of diseased and healthy animals were presented in Table. 7. The mean serum Alpha GST of diseased animals was found to be 4.376 ± 0.436 ng/ml and that of control group was 2.101 ± 0.199 ng/ml. A two fold increase was observed in the mean value of the diseased group when compared to healthy control group indicating significant hepatic damage (PLATE 3).

Statistical analysis of the serum Alpha GST revealed a significant increase in value ($p \leq 0.01$) when compared to healthy controls.

Table-7. Serum Alpha GST values of animals affected with hepatic diseases compared with that of healthy animals

Parameter	Diseased animals (n=40)	Healthy animals (n=8)	t- value	p-value
Alpha GST (ng/ml)	4.376 ± 0.436	2.101 ± 0.199	4.745**	<0.001

-** represent significant difference between the means at $p \leq 0.01$

4.5 HISTOPATHOLOGY

Histopathological examination of the liver samples revealed two cases of hepatocellular carcinoma, one case each of hepatic lymphoma and liver cirrhosis. H&E staining technique was used for the histopathological examination (PLATE 7).

4.6 URINALYSIS

4.6.1 Gross examination of urine in dogs with hepatic insufficiency

The urinary pH of 40 dogs in the present study was within the range of 5.5 to 8.3. Thirty three dogs (82.5 per cent) had acidic urinary pH of 5.5-7 and 7 dogs (17.5 per cent) had pH of 7-8.3 as shown in Table 8.

The colour of the urine was normal (straw coloured) in 19 (47.5 per cent) dogs, dark yellowish in 12 (30 per cent) dogs and light yellowish in 9 (22.5 per cent) dogs.

Urine odour was normal (ferrous) in 37 (92.5 per cent) dogs ammoniacal in two (5 per cent) dogs and sweet fruity in a single case which was due to the presence of ketone bodies. Turbidity was observed on the urine of 4 (10 per cent) dogs (Table 10).

Urine specific gravity of healthy dogs in control group varied from 1.015-1.030. In the present study, the urine specific gravity of diseased animals was found to be less than 1.015 in 4 (10 per cent) dogs, more than 1.030 in 2 (5 per cent) dogs and within the normal range in remaining 34 (85%) dogs (Table 8).

Table 8: Gross examination of urine in dogs with liver diseases (n=40)

Gross examination of urine (n=40)		**Total**
pH	5.5-7	33
	7-8.3	7
Colour	Straw coloured	19
	Dark yellow	12
	Light yellow	9
Odour	Ammoniac	2
	Fruity sweet	1
	Nil	37
Turbidity	Present	4
	Absent	36
Specific gravity	<1.015	4
	1.015-1.030	34
	>1.030	2

4.6.2 Chemical analysis of urine in dogs with hepatic dysfunction

Fresh urine samples of all dogs were subjected to complete chemical analysis. Varying degree of proteinuria was observed as an important finding in majority of cases. The degree of proteinuria was trace in 6 (15 per cent) cases, + in 10 (25 per cent) cases, ++ in 4 (10 per cent) cases and +++ in 3 (7.5 per cent) dogs (Table. 9).

In the present study, glucose level was found in traces in 5 (12.5 per cent) dogs, + in 2 (5 per cent) dogs, ++ in 2 (5 per cent) dogs and +++ in 3 (7.5 per cent) animals. Presence of blood was found in a total of 10 cases. Six (15 per cent) animals had + level, ++ in 3 (7.5 per cent) cases, +++ level was observed in 1 (2.5 per cent) dogs. Bilirubin level was found to be + in 15 (37.5 per cent) dogs, ++ in 3 (7.5 per cent) and +++ in 4 (10 per cent) dogs. Urine sample of 14 animals (35 per cent) revealed leucocytosis, urobilinogen was present in 11 (27.5 per cent) cases and two had ketone bodies (5 per cent). Nitrite was absent in all samples.

Table 9: Chemical analysis of urine in dogs with liver diseases (n=40)

Parameter	Result	Total
Glucose (mg/dL)	Negative	28 (70 per cent)
	Trace	5 (12.5 per cent)
	+	2 (5 per cent)
	++	2 (5 per cent)
	+++	3 (7.5 per cent)
Blood	Negative	30 (75 per cent)
	+	6 (15 per cent)
	++	3 (7.5 per cent)
	+++	1 (2.5 per cent)
Protein (g/dL)	Negative	17 (42.5 per cent)
	Trace	6 (15 per cent)
	+	10 (25 per cent)
	++	4 (10 per cent)
	+++	3 (7.5 per cent)
Bilirubin (mg/dL)	Negative	18 (45 per cent)

	+	15 (37.5 per cent)
	++	3(7.5 per cent)
	+++	4 (10 per cent)
Leucocytes	Present	14 (35 per cent)
	Negative	26 (65 per cent)
Ketone bodies (mg/dL)	Present	2 (5 per cent)
	Absent	38 (95 per cent)
Nitrites (mg/dL)	Absent	40 (100 per cent)
Urobilinogen (mg/dL)	Present	11(27.5 per cent)
	Absent	29 (72.5 per cent)

4.2.1.4 Treatment and therapeutic Response

All the affected animals were treated for with liver supplement containing the acive principle silibin-phosphatidylcholine antioxidant (Zn and Vit. E) shows better improvement compared to other treatment group. Eighty five per cent of the animals treated with silibin-phosphatidylcholine showed good therapeutic response While 62 per cent of the animals treated with liver supplement containing *Circhorium intybus* and *Capparis spinosa* showed clinical improvement.

5. DISCUSSION

Liver disease in dogs can develop as a result of many different insults. Mild damage may repair itself since the liver has tremendous regenerative capabilities. Severe and/or chronic damage, however, may lead to progressive and self-perpetuating chronic liver disease. Incidence of liver disorder in Wayanad district was found to be 0.77%. The clinical signs of liver diseases were usually non-specific and laboratory testing was essential for its recognition and evaluation. A confirmatory diagnosis was usually based on a combination of laboratory tests, radiography, ultrasonography on histopathological examination of liver biopsy. Conventional liver function test will give good results only when more than 55 per cent hepatic damage occur. One of the

toughest challenges faced by the veterinarian is to choose an appropriate test for accurate diagnosis. An early reliable diagnostic test was of atmost important in the treatment of hepatic disease. Estimation of novel biomarkers like Alpha GST plays an important role in diagnosing hepatic diseases. The results of this study are discussed below in detail. This study was carried out to find the efficacy of glutathione S- transferase (GST) in the early diagnosis of hepatic disorders in dogs.

5.1 SIGNALMENT

5.1.1Breed

In this study the highest incidence of liver disease was found in Labrador Retrievers followed by Rottweilers, German Shepherd, Spitz, Pug, Nondescript, Dachshund, Great Dane and Pekingese. This finding is in accordance with the studies conducted by different workers like Andersson and Sevelius (1991), Tiwari (2002), Hoffman *et al.* (2006) and Bexfield *et al.* (2012) where they stated that Labrador Retriever was at an increased risk for developing chronic hepatitis along with other breeds like Doberman Pinscher, Rottweiler, Dalmatian, Cocker Spaniel, Sky Terrier, Standard Poodle, German Shepherd, and Beagles. Higher population of Labrador Retriever breed in and around Wayanad district may be one of the important reason for the higher incidence.

5.1.2 Age

The mean age of dogs affected with hepatic disease was found to be 4.5 years. Tiwari (2002) in his study reported that the mean age of dogs with hepatic disease was 4.3 years, which is almost similar to the mean age reported reported in this study. In another study, Strombeck and Gribble (1978) reported 5.3 years as the mean age which was also not significantly different from our study.

5.1.3 Sex

In the present study, out of the 40 liver affected cases 24 were males and 16 were females. According to Rutgers and Haywood, (1988) and Andersson and Sevelius, (1991) male dogs were at high risk of liver diseases since they wander more and get exposed to infections or environmental/toxic hazards. This observation concur with the findings of our study.

5.2 GENERAL CLINICAL EXAMINATION

In the present study the behavioural changes exhibited by 65 per cent animals varied from depression, dullness and lethargy. Dementia, tremors, aggression, head pressing, stupor, circling, convulsions, hepatic encephalopathy and collapse were the nervous signs exhibited by the diseased animals. These clinical signs were found similar to the study conducted by Conn and Bircher (1988) where they stated that hepatic motor disturbances like depression, seizures, dementia, behavioural changes and hypersalivation was seen in hepatic encephalopathy. The findings of physical examination in this study was also found to be in agreement with the observations of includes Barrett *et al.* (1976) Taboada (1991), Rothuizen and Van Den Ingh (1998) and Javier *et al.* (2003).

5.2.1 Respiration

The mean ± SE values of respiratory rate in animals with liver disease was 50.89 ± 1.78 per minute. Than (2017) stated that the increase in respiratory rate was a compensation to meet the oxygen demand in anemic/icteric animals.

5.2.3 Heart rate

The mean ± SE value of heart rate in animals with liver disease was found to be 125.123±1.73 per minute. Mean value of heart rate was within the normal limits (Kaneco *et al.,* 2016)

5.2.4 Pulse rate

The mean ± SE value of pulse rate in animals with liver diseases was 123.39 ± 2.99 per minute (Kaneco *et al.,* 2016).

5.2.5 Rectal temperature

The mean ± SE value of rectal temperature in animals with liver disease was found to be 101.9 ± 0.17 ºF. Pyrexia was observed in 12 cases, subnormal body temperature was seen in 4 animals and remaining 24 animals had normal body temperature. According to Twedt (1981), pyrexia could be a consequence of hepatocellular damage, infection, sepsis or absorption of intestinal bacterial toxins. Subnormal body temperature was often observed at the end stage of any disease.

5.2.6 Examination of mucus membrane

In this study, mucus membrane of 18 (45 per cent) dogs was pale, icteric in 15 (37.5 per cent), congested in 4 (10 per cent) and 3 (7.5 per cent) dogs had normal (pale roseate) mucus membrane. The high incidence of pale mucus in the study was in agreement with the findings of Johnson (2000) who reported that pale mucous membrane may be due to anaemia associated

with chronic liver disease/gastrointestinal haemorrhage/excessive haemorrhage from neoplasm. Cotter (2000) stated that anaemia due to hepatic diseases occur as a result of shortened red cell life span due to decreased ATP.

5.2.7 Abdominal palpation

Upon abdominal palpation 6 (15 per cent) had peritoneal effusion (ascites or haemoperitoneum), two cases (5 per cent) each had hepatomegaly and abdominal mass respectively which was later confirmed with plain radiography and/or ultrasonography and/or exploratory laparotomy. These observations were in agreement with the reports of Rothuizen and Mayer (2000) who noted that physical examination was informative only in a few dogs suffers from liver diseases.

5.3 CLINICAL SIGNS

The main clinical signs exhibited in liver affected cases were lethargy in 36 animals, anorexia in 34 animals, vomiting in 22 cases, diarrhoea in 18 cases, pyrexia in 16 cases, ascites in 6 cases, haematuria in 4 animals, crusty ulcerative skin lesions in 2 animals and hepatic encephalopathy in a single case. Holt *et al* (1995) and Varshney and Hoque (2002) observed that clinical signs associated with liver dysfunctions are related to neurological and gastrointestinal signs but they are very variable, non-specific and vague. Skin bruises and crusty ulcerative skin lesion were observed due to the ruptur of underlying blood vessels, decreased synthesis of blood coagulation proteins and blood coagulation inhibitors (Feldman 1980).

5.4 DIAGNOSTIC IMAGING TECHNIQUES

5.4.1 Radiography

In the present study when 40 dogs with clinical signs suggestive of liver diseases were subjected to plain abdominal radiograph 13 (32.5 per cent) had radiographic changes. Hepatomegaly was observed in 4 (10 per cent) cases, with the liver found extended beyond the rib cage. In 3 cases neoplasms mass were observed adjacent to liver. Ascites was noticed in 6 (15 per cent) cases on lateral abdominal radiograph. These observations were in accordance with the findings of Kealy and McAllister (2000) were they reported an increased proportion of caudoventral liver margins beyond the costal arch in dogs with hepatomegaly. Radiographic evaluation of alteration in liver size was subjective and insensitive to subtle changes and hence accurate evaluation of mild generalized changes in liver size was difficult to diagnose as reported by Godshalk *et al*. (1988) and Barr (1992).

5.4.2 Ultrasonography

Utrasonography showed an overall reduction in echogenicity of liver parenchyma in both transverse and sagittal scan. Portal vessel wall appeared as white specks in the hypoechoic liver parenchyma. Liver borders were found to be rounded in 8 out of 40 (20 per cent) cases indicating hepatomegaly. In two cases hypoechoic nodules were seen surrounded by hyperechoic parenchyma suggestive of hepatocutaneous syndrome. The gall bladder was seen as anechoic ovoid shaped structure with a tapering neck. The gall bladder was found to be distended in all anorectic animals. No ultrasonographic abnormalities were observed upon scanning the gall bladder. Gall bladder sludge was observed in two cases. In ascites liver lobes were found floating in the abdominal fluid. Upon ultrasound examination in clinically suspected cases of leptospirosis, renomegaly, loss of corticomedullary distinction, increased cortical echogenicity and a hyperechoic medullary band were observed. These observations were in agreement with Root (1974) and O‟Brien (1978) who reported that diffuse hepatomegaly cause a substantial portion of caudoventral liver margins to get projected beyond the costal arch with rounding of caudal liver edges. Undulating and frilled smooth liver margin were reported by Partington and Biller (1995) in ascites.

5.4.3 Ultrasound guided liver biopsy

BARD biopsy gun was used for ultrasound guided liver biopsy for histopathological examination. Hoppe *et al.* (1986) compared manual, ultrasound guided biopsy technique with an automated method and he reported that the automatic method yielded better quality samples.

5.4 LABORATORY EXAMINATION

5.4.1 Haematology

In haematological examination significant granulocytic leukocytosis (34.897 ± 3.364 x 10^3 cells/µL) and anaemic changes like reduction in haemoglobin (9.944 ± 0.596 g/dL), platelet (3.033 ± 0.257 $\times 10^5$/ cmm) and total erythrocyte count (4.743 ± 0.342 $\times 10^6$/cmm) were observed. An elevated leucocyte count, decreased platelet, RBC and haemoglobin were also reported by Birnbaum *et al.* (1998), Greenlee *et al.* (2004) and Geisen *et al.* (2007) which was in agreement with the findings of our study. Thrombocytopenia observed in the present study might

be due to vasculitis, increased platelet consumption due to excessive intravascular coagulation and a reduced rate of platelet production in the bone marrow as reported by Greene *et al*. (2012).

5.4.2 Serum Biochemistry

The conventional markers associated with hepatocellular injury and biliary tract disorders include ALT and ALP. Serum biochemical values of ALT and ALP of diseased animals were 114.044 ± 29.69 IU/L and 206.013 ± 37.18 IU/L respectively. In this study it was observed that ALT values were within normal range in animals with hepatic damage. This finding was in accordance with a study carried out by Lidbury and Steiner, (2013) where they stated that established laboratory tests used to assess the liver function had important limitations. They also stated that abnormalities in serum aminotransferase concentrations often lag behind the changes in hepatocellular integrity. In general, an increase in the serum aminotransferase reflect the relative extent of active hepatocellular damage, but not necessarily its aggregate severity. An additional limitation of using aminotransferases as markers for hepatocellular injury is their relatively long plasma half-lives (47 h for ALT) (Beckett and Hayes 1993, Loguercio *et al.* 1998).

Another work which substantiate our study was ALP elevation reported by by Center, (2007) in which he reported that serum ALT and ALP were increased, at least to some extent in most of the liver disorders. He reported that measurement of serum alkaline phosphatase (ALP) activity alone was not liver specific as the enzyme could be elevated in a number of non-hepatic diseases. In our study also it was found that mean values of ALP was found to be higher when compared to the healthy group.

In this study the mean serum total protein and albumin level of diseased animals were 7.932 ± 0.0364 g/dL and 2.744 ± 0.113 g/dL respectively. These results were in accordance with the reports of Suleyman and Ehsan (2017) where they stated that hypoalbuminemia is a relatively insensitive marker for hepatic insufficiency and is only likely to be seen in patients with advanced chronic liver disease or portosystemic shunts (PSSs).

Globulin and A/G ratio were 5.249 ± 0.3004 g/dL and 0.569 ± 0.027 respectively. Hyperglobulinemia and decreased A/G ratio observed in this study comparison to healthy group. Hall (1985) opined that hyperglobulinemia was frequently encountered in liver disease. Anderson and Sevelius (1991) and Ward, (2006) stated that hyperglobulinemia and decreased

A:G ratio in chronic liver disease were due to increased level of gamma globulin fractions which may be associated with enhanced systemic immunoreactivity.

The mean serum total bilirubin was found to be 1.168 ± 0.241 mg/dL. Hyperbilirubinemia observed in cases of hepatitis could be attributed to hepatocyte damage or biliary obstruction associated with hepatic inflammation (Bush 2002) which was found similar to the findings of our study.

5.4.3. Serum alpha glutathione-S-transferase (α-GST) level

The mean serum Alpha GST of diseased animals was 4.376±0.436 ng/ml and that of control group was 2.101±0.199 ng/ml. Two fold increase was observed in the mean value of the diseased group when compared to healthy control group. Serum Alpha GST levels showed a significant elevation ($p \leq 0.01$) in diseased group when compared to controls group. Ozer et al. (2008) reported that the inducible phase II detoxification enzyme Alpha glutathione-S-transferase (αGST) is a potential marker of liver injury in dogs. Alpha Glutathione-S-transferase alpha (GSTα) was one of four GST isoenzymes produced by hepatocytes (Coles *et al*., 2001). Giffen *et al.* 2002 found that when acute hepatic injury was induced in rats, the resulting increase in serum α GST concentration was of greater magnitude than ALT and AST activities. In dogs, the potential of α GST as a marker of hepatocellular injury was demonstrated by Kilty *et al.* (1998) and they observed a rapid increase in serum αGST concentration and followed by subsequent return to baseline in hepatic diseases.

In a study conducted by Sidlova *et al*. (2003) in humans estimation of serum α-glutathione S-transferase (s-GSTA) activity in patients with cystic fibrosis (CF) was more confirmatory when compared with conventional liver function tests or hepatic ultrasound scan (US). They reported a significantly higher level of s-GSTA (2.05 µg/l) in affected patients in comparison to control group (1.55 µg/l) and no significant correlation was observed in the CF group between s-GSTA and conventional liver function tests (ALT, AST, ALP and GMT). They found that a raised s-GSTA level was a marker for hepatobiliary damage in CF patients. Thus from the above studies it can be concluded that serum Alpha GST is a more sensitive marker than transaminases for monitoring hepatocellular integrity. It can be used as early predictor of hepatic damage in CF patients. The above findings were in concurrence with the results obtained in our present study.

5.5 HISTOPATHOLOGY

Histopathological examination of the liver samples revealed two cases of hepatocellular carcinoma, one case each of hepatic lymphoma and liver cirrhosis. H&E staining technique was used for the histopathological examination. These findings were in accordance with Anderson and Sevelius (1991) where they stated that cirrhosis cause necrosis, fibrosis, hepatocyte degeneration and marked architectural distortion.

In hepatocellular carcinoma liver had a normal lobular pattern with anisocytosis where as in hepatic lymphoma infiltrating lymphocytes were present in hepatocyte lobules and few hepatocyte showed fatty change. These findings were in concurrance with the reports of Cullen *et al*. (2006), Van den Ingh *et al.* (2006) and Winkle *et al.* (2006).

5.**6** URINALYSIS

5.6.1 Gross examination of urine in dogs with hepatic insufficiency

In this study 82.5 per cent animals had acidic urinary pH (5.5-7) and 17.5 per cent animals had alkaline pH (7-8.3). In our study colour of the urine was normal (straw coloured) in 47.5 per cent dogs, dark yellowish in 30 per cent dogs and light yellowish in 22.5 per cent dogs. Ammoniacal smell was present in 5 per cent dogs due to renal impairement and sweet fruity odour was detected in 2.5 per cent cases due to the presence of ketone bodies (DKA). Ten per cent of animals had turbidity in urine whereas turbidity was absent in 90 per cent cases. Turbidity mainly due to the presence of crystals, casts or proteinuria. The above gross changes observed in urine were in accordance with the findings of Forrester and Brandt (1994) in which they stated that in case of hepatic diseases pH, colour, odour and tubidity changes will be seen.

Specific gravity of urine was within the normal range (1.015-1.030) in 85 per cent dogs, less than 1.015 in 10 per cent dogs, more than 1.030 in 5 per cent dogs. A low urine specific gravity is common in patients with liver disease due to polyuria and polydipsia. Renal impairment associated with liver dysfunction also can contribute to the low urine specific gravity (Bexfield and Watson 2006 and Chapman and Hostutler 2013).

5.6.2 Chemical analysis of urine in dogs with hepatic dysfunction

Varying degree of proteinuria was observed as an important finding in majority of cases. The degree of proteinuria was trace in 6 (15 per cent) cases, + in 10 (25 per cent) cases, ++ in 4 (10 per cent) cases and +++ in 3 (7.5 per cent) dogs. In the present study, glucose level was

found in traces in 5 (12.5 per cent) dogs, + in 2 (5 per cent) dogs, ++ in 2 (5 per cent) dogs and +++ in 3 (7.5 per cent) animals. Presence of blood was found in a total of 10 cases. Six (15 per cent) animals had + level, ++ in 3 (7.5 per cent) cases, +++ level was observed in 1 (2.5 per cent) dogs. Bilirubin level was found to be + in 15 (37.5 per cent) dogs, ++ in 3 (7.5 per cent) and +++ in 4 (10 per cent) dogs. Urine sample of 14 animals (35 per cent) revealed leucocytosis, urobilinogen was present in 11 (27.5 per cent) cases and two had ketone bodies (5 per cent). Nitrite was absent in all samples. Forrester (1997) reported that proteinuria was observed in CHF/ liver diseases/ genital diseases, lower urinary tract infections (UTIs) / renal diseases and Diabetes mellitus contribute to the presence of glucose in urine. He also stated that the presence of blood in urine was mainly due to haematuria or haemoglobinuria and findings were in agreement with the present study. Nelson and Couto (1998) and Leib and Monroe (1997) reported that the common finding in urinalysis consistent with hepatobiliary diseases include bilirubinuria. Urobilinogen was increased in the urine following haemolysis and the dogs suffering from hepatitis can develop clinical and laboratory evidence of renal tubular dysfunction as reported by Langlois *et al.* (2013).

4.2.1.4 Treatment and therapeutic Response

In this study eighty five per cent of the animals treated with silibin-phosphatidylcholine complex and 62 per cent of the animals treated with liver supplement containing *Circhorium intybus* and *Capparis spinosa* showed good therapeutic response 30 days after the initiation of treatment. These findings were in accordance with Sario *et al.* (2005) who stated that silibin-phosphatidylcholine-antioxidant complex can be used for treating patients with chronic liver disease. Martin *et al.* (1984) reported that silibin alone or in combination with choline exhibited a good regenerative effect in hepatic tissue, reducing extend of lesion and time needed for recovery.

6. SUMMARY

The present study entitled "Efficacy of glutathione S- transferase (GST) for early diagnosis of hepatic disorders in dogs" was conducted in the Department of Veterinary Clinical Medicine, College of Veterinary Animal Sciences, Pookode during the period from April 2017 to June 2018. Liver is a multi-purpose organ having important role in blood detoxification, break down of drugs, energy metabolism, storage of vitamins and glycogen, production of bile acids and manufacturing of important proteins for blood clotting. Because of its central role in many of the bodily functions, any damage to liver will lead to manifestation of variety of symptoms. In this study forty animals clinical signs suggestive of hepatic dysfunction were subjected to detailed signalment, general clinical examination, diagnostic imaging techniques (radiography and ultrasonography), laboratory examinations (hematology, serum biochemistry and Alpha GST estimation by ELISA technique), histopathology and urine analysis for confirmatory diagnosis. The data obtained were analysed statistically by comparing with the healthy group.

Incidence of liver disorders in Wayanad district was 0.77% during the period from April 2017 to June 2018. Labrador Retriever breed had highest susceptibility followed by Rottweiler. Among the 40 cases studied, age wise occurrence of hepatic disorders was higher (45 per cent) in young animals (1month to 4 years) followed by 40 per cent in middle aged dogs (4 to 8 years) and 15 per cent in geriatric patients (>8 years). Out of 40 liver affected cases 24 were males (60 per cent) and 16 were females (40 per cent).

Upon general clinical examination the mean value of respiration in animals with liver disease was 50.89 ± 1.78 per minute, heart rate was 125.123+1.73 per minute, pulse rate was found to be

123.39 ± 2.99 per minute and rectal temperature was found to be 101.9+0.17 ºF. In this study, mucus membrane of 18 (45 per cent) dogs was pale, icteric in 15 (37.5 per cent), congested in 4 (10 per cent) and 3 (7.5 per cent) dogs had normal (pale roseate) mucus membrane. Upon abdominal palpation 6 (15 per cent) had peritoneal effusion (ascites or haemoperitoneum), two cases (5 per cent) each had hepatomegaly and abdominal mass respectively. The main clinical signs exhibited by diseased animals were lethargy in 36 animals, anorexia in 34 animals, vomiting in 22 cases, diarrhoea in 18 cases, pyrexia in 16 cases, ascites in 6 cases, haematuria in 4 animals, crusty ulcerative skin lesions in 2 animals and hepatic encephalopathy in a single case.

Among the diagnostic imaging techniques, radiographic examination revealed ascetic changes in 6 cases, hepatomegaly in 4 animals, neoplasms in 3 cases and remaining had no abnormalities in hepatic size, contour and architecture. On ultrasonographic evaluation hepatomegaly was seen in 8 cases with rounded liver borders. Two cases had hypoechoic nodules surrounded by hyperechoic parenchyma suggestive of hepatocutaneous syndrome. Liver lobes and abdominal organs were seen floated over the ascitic fluid present in the abdominal cavity in ascites cases. Renomegaly, loss of corticomedullary distinction, increased cortical echogenicity and hyperechoic medullary bands were observed in clinically suspected leptospirosis cases.

In haematological examination significant granulocytic leukocytosis (34.897±3.364 x 10^3 cells/µL) and mild anaemia were observed. Serum ALT and ALP values of diseased animals were 114.044 ± 29.69 and 206.013 ± 37.18 IU/L respectively. Mean total serum bilirubin of diseased animals was 1.168 ± 0.241 mg/dl. The mean total serum protein, albumin, globulin and A/G ratio were 7.932±0.0364, 2.744±0.113, 5.249±0.3004 g/dl and 0.569±0.027 respectively.

Serum Alpha GST Showed a significant elevation ($p \leq 0.01$) when compared to healthy controls. The mean serum Alpha GST of diseased animals was 4.376±0.436 ng/ml and that of control group was 2.101±0.199 ng/ml. Two fold increase was observed in the mean value of the diseased group compared to healthy control group which indicates its significance for diagnosing hepatic disorders in dogs.

Gross changes in Color, pH, odour, turbidity and specific gravity were recorded in urine samples. Chemical analysis of urine was performed for estimating glucose, protein, bilirubin,

leukocytes, ketone bodies, blood, nitrites and urobilinogen level in the urine in which varying degrees of proteinuria, glucosuria, haemoglobinuria, ketone bodies were reported.

The hepatic diseases observed were classified as clinically suspected leptospirosis cases (50 per cent), liver cirrhosis (15 per cent), babesiosis (12.5 per cent), hepatic tumour (7.5 per cent) and hepatocutaneous syndrome, drug intoxication and pyometra (5 per cent each).

All the affected animals were treated for with liver supplement containing the acive principle silibin-phosphatidylcholine antioxidant (Zn and Vit. E) shows better improvement compared to other treatment group. Eighty five per cent of the animals treated with silibin-phosphatidylcholine showed good therapeutic response While 62 per cent of the animals treated with liver supplement containing *Circhorium intybus* and *Capparis spinosa* showed clinical improvement.

The following conclusions were drawn from the study:

1) Incidence of liver disorders in wayanad district was 0.77% during the period from April 2017 to June 2018.
2) Eighty two per cent of cases with hepatic imparement showed an elevation of Alpha GST activity where as ALP and ALT were elevated in 65 and 15 per cent cases respectively.
3) Estimation of Alpha GST was more efficacious for diagnosing liver diseases when compared to other diagnostic tests.
4) Liver supplement containing silibin-phosphatidylcholine antioxidant had good therapeutic response compared to herbal preparation containing *Circhorium intybus* and *Capparis spinosa*.

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ABSTRACT

The present study on "Efficacy of glutathione S- transferase (GST) for early diagnosis of hepatic disorders in dogs" was carried out in the Department of Clinical Veterinary Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, Pookode during the period from April 2017 to June 2018. The aim of the study was to study the incidence of hepatic disorders in dogs in Wayanad district, efficacy of GST as biomarker for early diagnosis in liver diseases in dogs, to compare GST as biomarker with various diagnostic protocoal in hepatic diseases diagnosis and therapeutic management of liver diseases. Forty dogs clinical signs suggestive for hepatic dysfunction were subjected to diagnostic imaging techniques (radiography and ultrasonography), laboratory examinations (haematology, serum biochemistry and Alpha GST estimation by ELISA technique), histopathology and urine analysis. Incidence of liver diseases in Wayanad district was 0.7 per cent. Highest incidence was observed in male, Labrador breed with young dogs (1 month to 4 yrs). Haematology revealed reduced Hb, TEC and monocyte and elevated TLC. Serum biochemistry revealed increase in total protein, total bilirubin and globulin levels and reduced Albumin/Globulin ratio. Dogs with hepatic disorders, the mean Alpha GST level was found to be 4.376±0.436 mg/dl. Urinanalysis revealed varying degrees proteinuria, glucosuria, haemoglobinuria and ketonuria. The main clinical signs exhibited in hepatic disorder dogs were vomiting, anorexia, lethargy, pyrexia, diarrhoea, haematuria, skin lesions, ascites and hepatic encephalopathy. 25 per cent of total affected case had ultrasonographic changes were as radiographic changes was observed in 32.5 per cent cases. Conditions like hepatocellular carcinoma, hepatic lymphoma and cirrhosis were diagnosed by liver biopsy. Affected dogs were randomly divided in to two treatment groups. One group was treated with silibin-phosphatidylcholine antioxidant (Zn and Vit. E) and other group was treated with a herbal liver supplement containing *Circhorium intybus* and *Capparis spinosa.* Eighty five per cent of dogs treated with silibin-phosphatidylcholine showed good therapeutic response while 62 per cent of the doga treated with liver supplement containing *Circhorium intybus* and *Capparis spinosa* showed clinical improvement. A comparative study carried out among different diagnostic procedures found that 82.6 per cent of cases with hepatic impairement showed an elevation of Alpha GST activity whereas ALP was elevated in 65 per cent cases and ALT elevated in 15 per cent cases. This study conclude that the estimation of Alpha GST was more efficatious in diagnosing liver diseases.

Academic form no.19

KERALA VETERINARY AND ANIMAL SCIENCES UNIVERSITY
FACULTY OF VETERINARY AND ANIMAL SCIENCES
PROGRAMME OF RESEARCH WORK FOR THESIS FOR MASTERS DEGREE

1. Title of thesis:

Efficacy of Glutathione S-transferase (GST) for early diagnosis of hepatic disorders in dogs.

2(a). Title of the departmental / KVASU research project approved: NA

(b). Code no. If any, and order by which the departmental / KVASU research project is approved: NA

3(a). Name of the student:

Anju A.D.

(b). Admission no:

16-MVP-032

4(a). Name of the major advisor (Guide):

Dr. Vinu David. P

(b). Designation:

Assistant Professor
Department of Veterinary Clinical Medicine Ethics & Jurisprudence
College of Veterinary and Animal Sciences,
Pookode,Wayanad-673576.

5. Objectives of the study:

1. Incidence of hepatic disorders in dogs in Wayanad district.
2. Efficacy of GST as biomarker for early diagnosis of liver diseases in dogs
3. Compare GST as biomarker with various diagnostic protocols in hepatic disease diagnosis.
4. Therapeutic management of liver diseases.

6. Practical/Scientific utility:

Hepatic disorders are important cause of morbidity and mortality in dogs. Damage of hepatocytes occurs due to several infectious agents, toxins, neoplasms, and immune mediated reactions. Various laboratory diagnostic procedures and imaging techniques are available for diagnosis of liver disorders. However many of these procedures/techniques present their own set of challenges. Certain procedures/tests are more confirmatory in specific liver diseases. Most of the clinical signs in hepatic disorders will be exhibited only when 75-80% of the liver is damaged. Novel biomarkers like GST are helpful in the

early detection of hepatocellular injury in dogs. The present study aims to compare GST with other diagnostic protocols for hepatic disorders.

7. Important publications on which the study is based:

Rutgers and Haywood (1988) reviewed that jaundice, ascites, hepatic encephalopathy, hepatomegaly or micro hepatica, drug intolerance and coagulopathy as specific clinical signs of hepatic disorders and nonspecific clinical signs include depression, anorexia, weight loss, diarrhoea or vomiting, polyuria or polydypsia and pigmenturia.

According to Biller *et al*. (1992) ultrasonography is an accurate non-invasive method for detection and monitoring of hepatic disorders.

According to Partington and Biller (1995) the quality of radiographic examination of liver could be maximized if the animal was fasted for 18 to 24 hours. An enema given four hours before the examination increased the quality of radiograph by decreasing the overlying bowel opacities and organ compression.

Kilty *et al.* (1998) stated that GST enzyme serve as an ideal biomarker of liver damage.

Zafar and Ali (1998) reported that *Circhorium intybus* is a potent anti-hepatotoxic plant and major component in indigenous drugs like Liv-52, Geriforte, Acilvan and Livex.

Gadgoli and Mishra (1999) reviewed that p-Methoxy benzoic acid active ingredient from the plant *Capparis spinosa* had antihepatotoxic activity.

Stockhaus *et al.* (2004) reported that histopathologic and cytologic examination of liver biopsy samples were the gold standard test for the diagnosis of liver diseases.

According to Sario *et al* (2005) new silybin-phosphatidylcholine-antioxidant complex could be an interesting drug to be tested in in patients with chronic liver disease

Tantary *et al.* (2014) reported that mean values of haemoglobin, packed cell volume (PCV), total erythrocyte count (TEC) and platelet count were significantly decreased and biochemical mean values of alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (ALP) were significantly increased in hepatic patients.

According to Lidbury and Suchodolski (2016) GST is a potential biomarker of liver injury in dogs and cats and α-glutathione-S-transferase (αGST) is one among the four isoenzymes produced by hepatocytes.

8. Outline of technical programme:

Dogs presented to the Teaching Veterinary Clinical Complex, Pookode, and other Veterinary hospitals of Kerala Veterinary and Animal Sciences University will be screened for the study. A minimum of 30 animals with the signs of anorexia, polydypsia, polyuria, jaundice, vomiting, ascites, weight loss etc will be subjected to detailed study using the proforma attached(Appendix-III). General clinical examination and system wise examination will be carried out in selected animals. Blood samples will be collected and subjected to haemato-biochemical studies. Plasma GST levels will be estimated by ELISA method (Kilty *et al*.,1998). Diagnostic imaging procedures like radiography and ultrasonography will be carried out to detect structural deformities of the liver. Appropriate clinical cases will be subjected to liver biopsy. Total animals will be divided into two groups. One group will be treated with herbal preparation containing extracts of hepatoprotective plants *Circhorium intybus* and *Capparis spinosa* and second group will be treated with preparation containing silybin-phosphatidylcholine-antioxidant (Zn and Vit E) complex. Antibiotics, fluids and other supportive treatments will be given as per the clinical condition of the animal and response will be evaluated. The results will be statistically analysed (Snedecor and Cochran, 1994).

9. Main items of observation to be made:

1. Signalment, Anamnesis, and Clinical signs.

2. Clinical and physical examination findings.

3. Haemato-biochemical parameters
   a) Haemoglobin (g/dL)
   b) VPRC (%)
   c) Total erythrocyte count ($10^6/\mu L$)
   d) Total leukocyte count ($10^3/\mu L$)
   e) Platelet count ($10^3/\mu L$)
   f) ALT (u/L)
   g) ALP (u/L)
   h) Total bilirubin (mg/dL)
   i) Total protein (g/dL)
   j) Albumin (g/dL)
   k) Globulin (g/dL)
   l) A/G ratio

4. GST level (U/ml)

5. Urine analysis.

6. Diagnostic imaging techniques.

a) Radiographic findings

b) Ultrasonographic findings

7. Histopathology (appropriate cases only)

10. Facilities:

a. Existing facilities in the Teaching Veterinary Clinical Complex will be utilized.

b. Additional facilities required: Chemicals and biologicals.

11. Duration of the study:

Four semesters

12. Financial estimate:

a. Chemicals and biologicals: Rs. 20000/-

b. Contingencies: Rs. 5000/-

Total : Rs. 25000/-

13. Signature of the Student

1**4. Signature of the Major Advisor:**

Place: Pookode

Date: 22-03-2017

Name and signature of the members of the advisory committee:

Chair person

Dr.Vinu David.P

Assistant Professor,
Department of Veterinary Clinical Medicine, Ethics and Jurisprudence,
College of Veterinary and Animal Sciences, Pookode, Wayanad-673576.

Members

1. **Dr.Biju P. Habeeb**
   Assistant Professor and Head i/c,
   Department of Veterinary Clinical Medicine, Ethics and Jurisprudence,
   College of Veterinary and Animal Sciences, Pookode, Wayanad-673576.

2. **Dr. N. Madhavan Unny**
   Assistant Professor,
   Department of Veterinary Clinical Medicine, Ethics and Jurisprudence,
   College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651.

3. **Dr. Dinesh P.T**
   Assistant Professor,
   Department of Veterinary Surgery and Radiology.
   College of Veterinary and Animal Sciences, Pookode, Wayanad-673576.

APPENDIX-I

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acid from *Capparis spinosa*. *J. Ethnopharmacol.* **66**: 187-192.

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APPENDIX-II

Time frame of work:

Semester -1

1. Collection of literature

2. Preparation of project proposal

Semester-2

1. Collection of literature

2. Procurement of chemicals and diagnostic kits.

Semester -3

Detailed evaluation of clinical cases of dog with hepatic disorders

Semester -4

1. Compilation of clinical and laboratory data evaluation and interpretation of results

2. Thesis writing and submission

CERTIFICATE

Certified that the research project has been formulated observing the stipulations laid down under the Prevention of Cruelty to Animal Act (Amendment, 1998).

Pookode, Dr. Vinu David P.

22/03/2017 (Major Advisor)

APPENDIX-III

Sl.No :

Case Number :

Owner details,

Name :

Address :

Phone No. :

1. **Signalment**

Animal	Species	Breed	Age	Sex	Colour	Body wt

2. **Anamnesis**

Present :
Past :

Deworming history :

Vaccination history :

Dietary Management :

Previous treatment history if any:

3. General examination

1. Respiration :

2. Pulse:

3. Temperature :

4. Mucous membrane :(pale/congested/icteric)

5. Popliteal lymph node :

4. Clinical Signs:

5. Physical examination of abdomen and liver

6. Diagnostic imaging techniques:

a) Radiography

b) Ultrasonography

7.Laboratory examination

Hemato-biochemical parameters

Sl No:	Parameters	Result
	Hemoglobin(g/dL) Red blood cells(10^6/µL) PCV(%) TLC(10^3/µL) Platelet count(10^3/µL) DLC (% and absolute count) a) Neutrophils b) Lymphocytes c) Monocyte d) Eosinophis e) Basophils ALT(u/L) ALP(u/L) Total bilirubin(mg/dL) Total protein(g/dL) Albumin(g/dL)	

	Globulin(g/dL) A/G ratio Plasma GST (U/ml)	

9. Urine analysis:

a. Physical properties
b. Chemical properties
c. Microscopical examination

10. Liver biopsy

11. Diagnosis

12. Treatment regime adopted:

13. Result:

CURRICULUM VITAE

1. Name of the candidate: Anju A.D.
2. Date of birth: 11-02-1992
3. Place of birth: Sulthan Bathery
4. Marital status:Married
5. Permanent address: Anju Nivas
   Muthanga P.O
   Sulthan Bathery
   Wayanad-673592

6. Major field of specilisation: Veterinary Clinical Medicine Ethics and Jurisprudence

7. Educational status:BVSc and AH

8. Professional experience: Nil

9. Membership in professional societies: IVA

Plate 1. Instrumentation

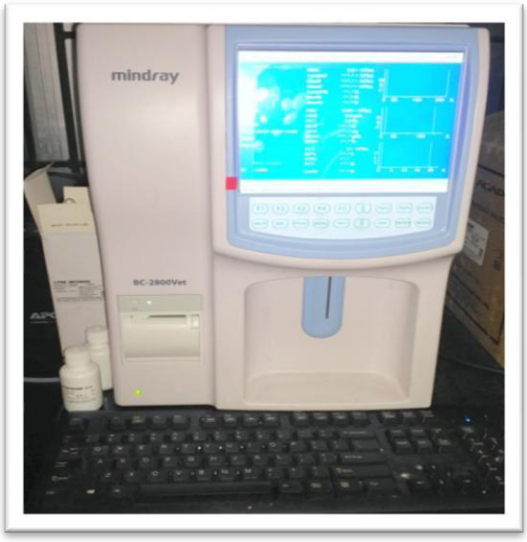

Plate 1. a

Plate 1. b

Plate 1. c

Plate 1. d

Plate 1.a : Haematological Analyser

Plate 1.b : Biochemical Analyser

Plate 1.c : Urine Analyser

Plate 1.d : Microscope

Plate 1.e. Ultrasound Unit

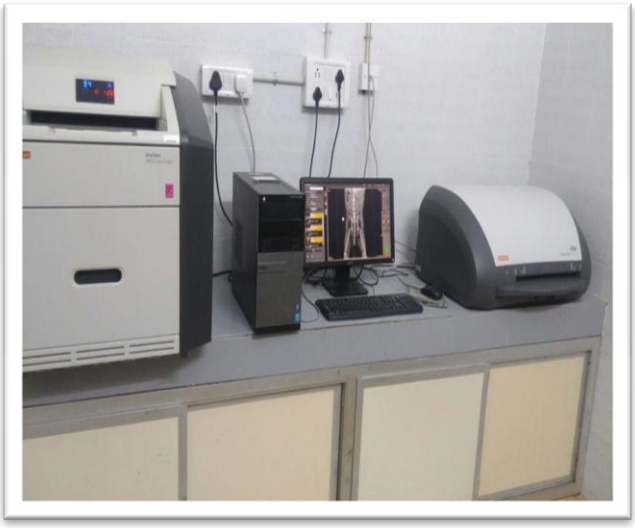

Plate 1.f. Digital Radiography unit

Plate 3. ELISA

Plate 3.a

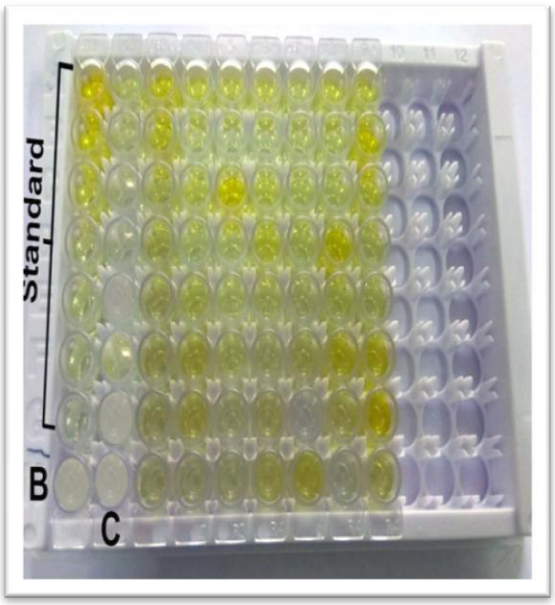

Plate 3.b

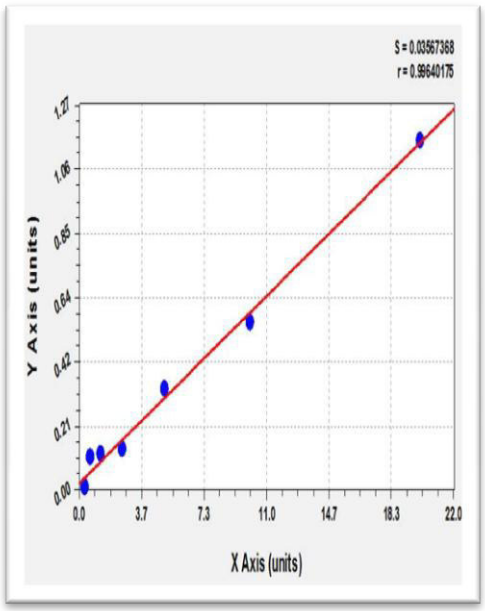

Plate 3.c

Plate 3.a : MY BioSource.com Canine α-GST ELISA Kit

Plate 3.b : ELISA result

Plate 3.c : Standard curve from serial dilutions of standard

Plate 7. Histopathology

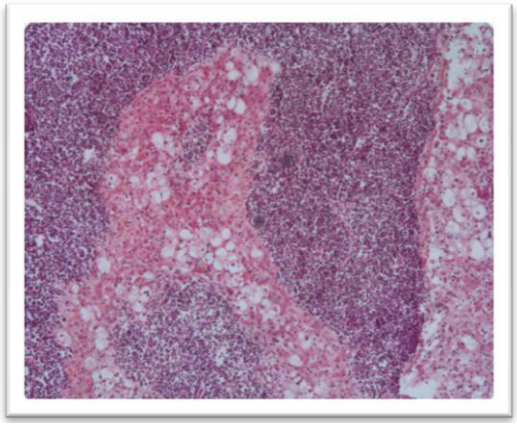

Plate 7.a

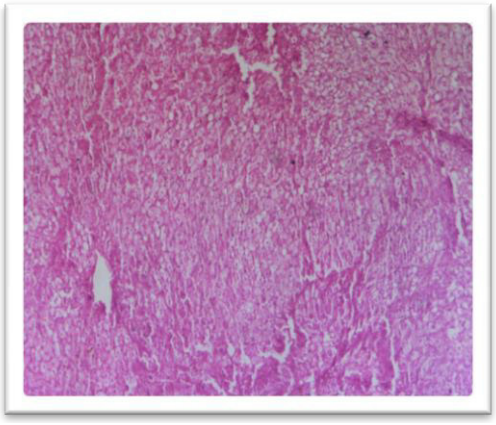

Plate 7.b

Plate 7.a: Hepatic lymphoma: Infiltrating lymphocytes in the hepatocyte lobule with fatty changes.

Plate 7.b: Hepatocellular carcinoma: Normal liver lobule pattern is lost with hepatocytes showing anisocytosis, with clear cytoplasm and centrally placed nucleus.

Plate 2. Liver biopsy

Ultrasound guided liver biopsy technique

BARD biopsy gun

Plate. 5. Ultrasonographic abnormalities in liver disorders

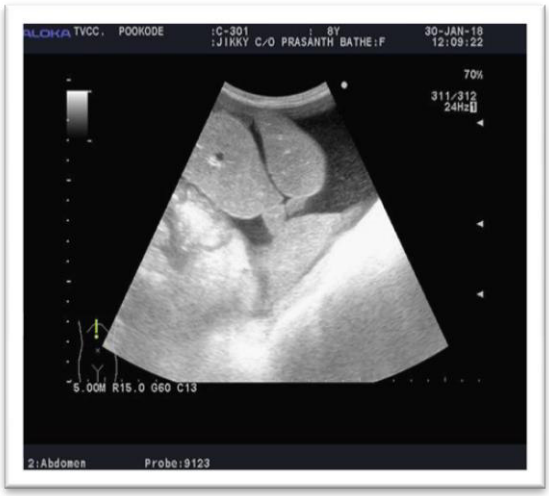

Plate 5.a

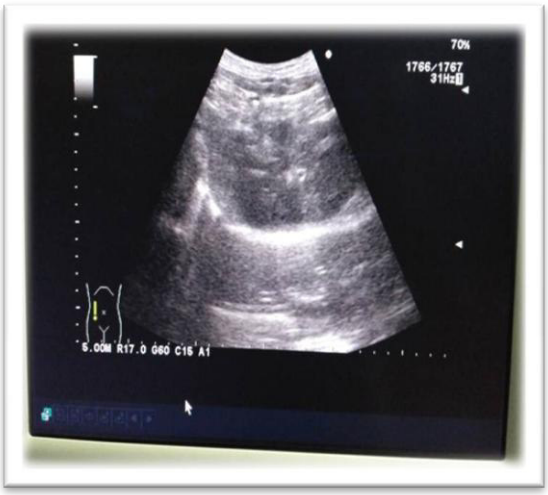

Plate 5.b

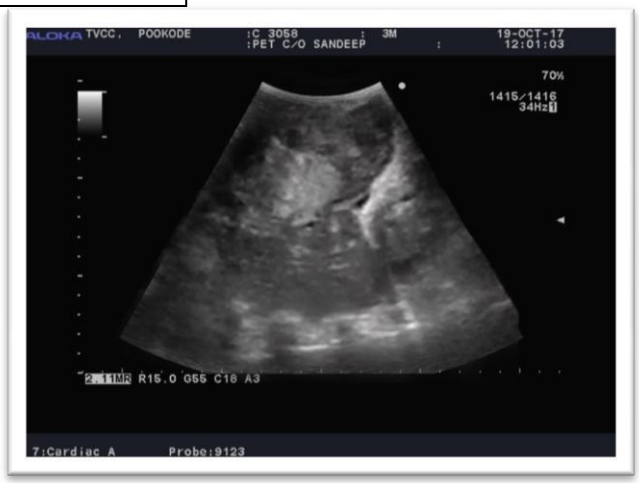

Plate 5.c

Plate 5.a : Ascitic fluid with floating liver lobes

Plate 5.b : Hyoechoic nodules surrounded by hyperehoic parenchyma suggestive of hepatocutaneous syndrome.

Plate 5.c : Irregular echotexture of parenchyma and multifocal areas of hyperechogenicity indicative of hepatic neoplasm.

Plate.6 . Ultrasonographic changes of liver and kidney in Leptospirosis

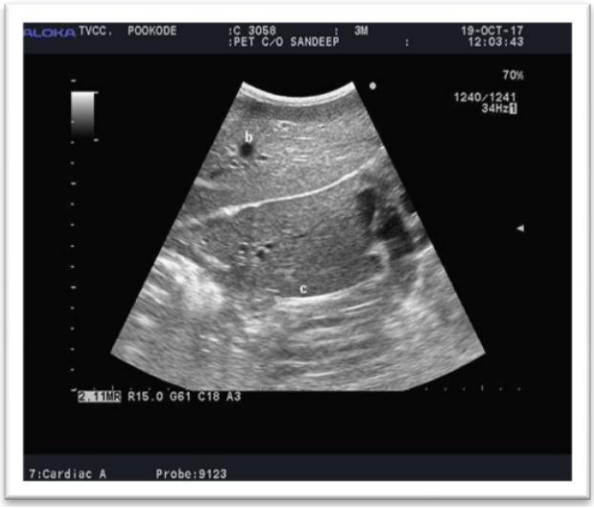

Plate 6.a

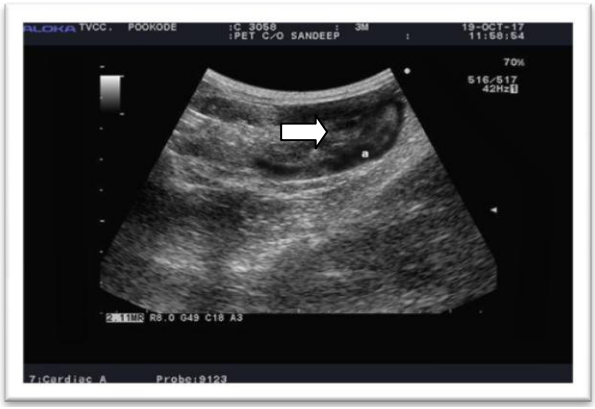

Plate 6.b

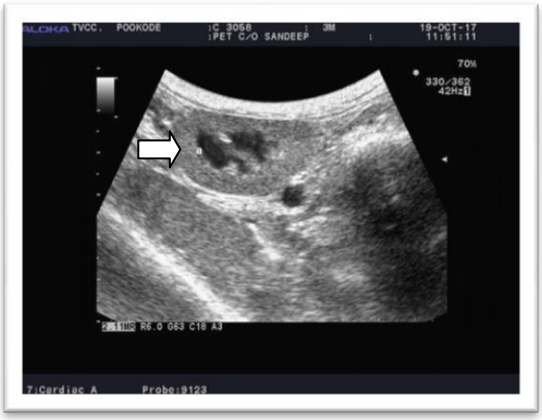

Plate 6.c

Plate 6.a : Decreased hepatic echogenicity (hypoechoic parenchyma) indicating hepatitis and congestion.

Plate 6.b : Thickened medullary band observed as increased echogenicity.

Plate 6.c : Loss of corticomedullary distinction.

Plate 4. Radiographic changes in hepatic diseases

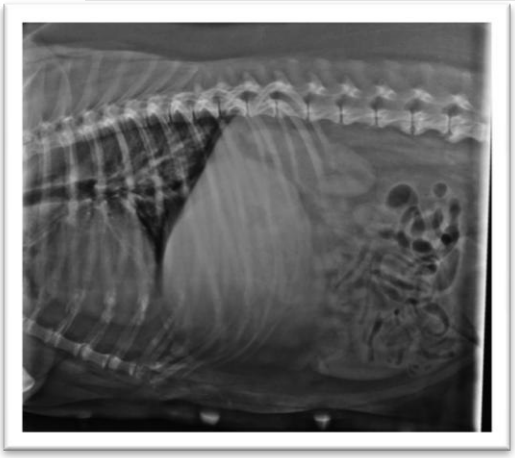

Plate 4.a

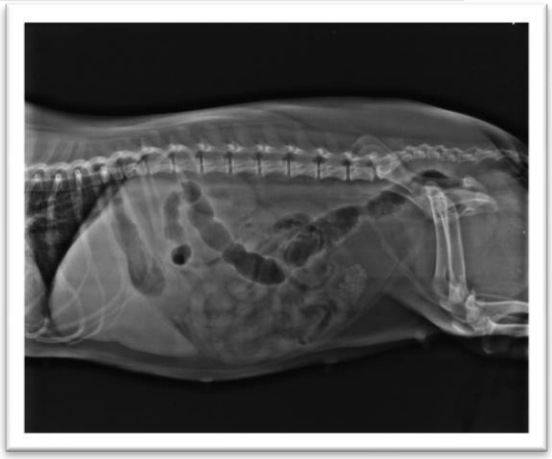

Plate 4.b

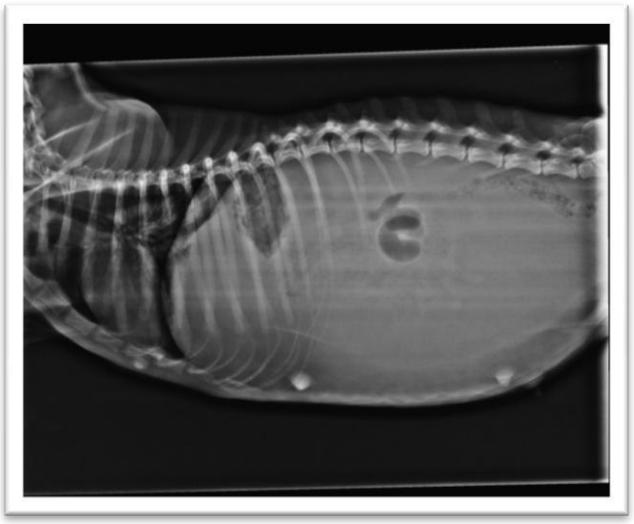

Plate 4.a : Severe hepatomegaly with caudodorsal displacement of stomach.

Plate 4.b : Hepatomegaly with projection of caudal edge of liver outside the rib cage.

Plate 4.c : Asctic abdomen

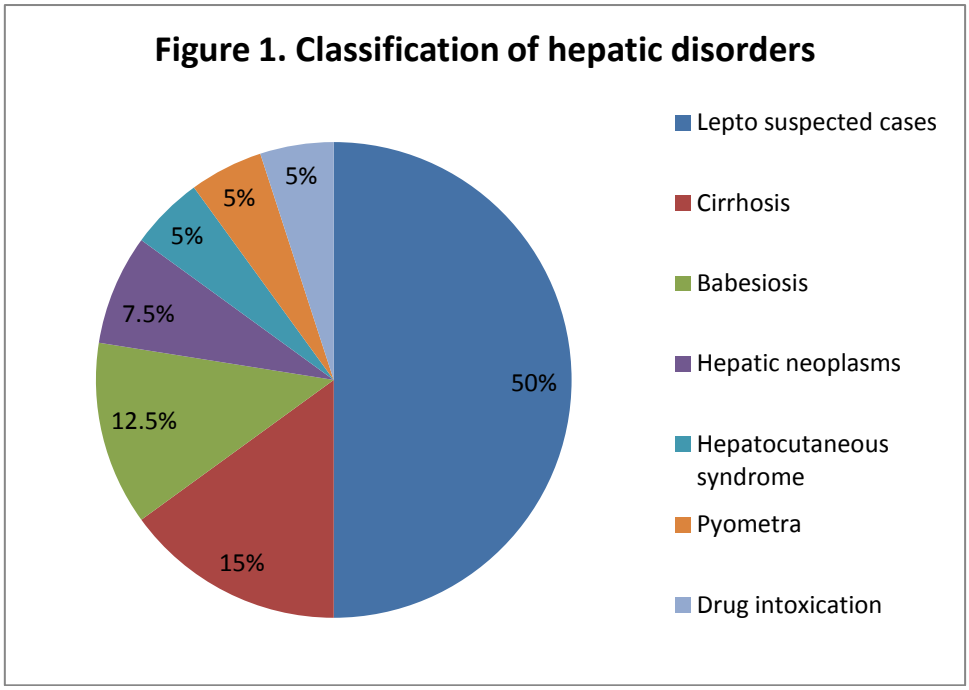
Figure 1. Classification of hepatic disorders
50%
15%
12.5%
7.5%
5%
5%
5%
Lepto suspected cases
Cirrhosis
Babesiosis
Hepatic neoplasms
Hepatocutaneous syndrome
Pyometra
Drug intoxication

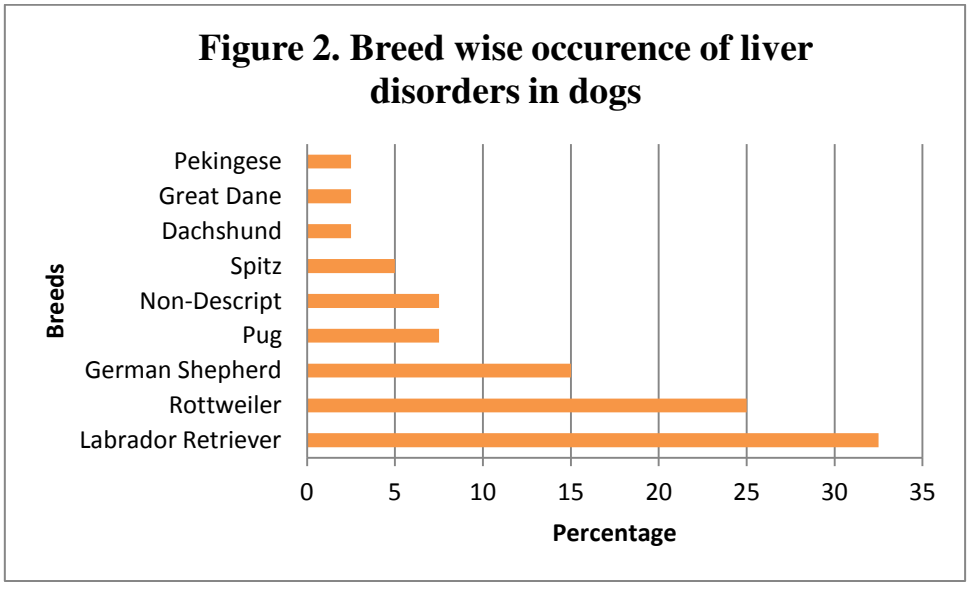
Figure 2. Breed wise occurence of liver disorders in dogs
Breeds
Pekingese
Great Dane
Dachshund
Spitz
Non-Descript
Pug
German Shepherd
Rottweiler
Labrador Retriever
0
5
10
15
20
25
30
35
Percentage

Figure 3. Age wise occurrence of hepatic diseases in dogs

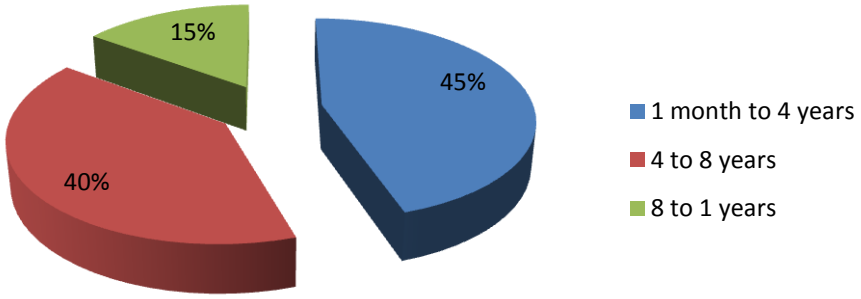

Figure 4. Clinical signs observed in dogs with hepatic diseases