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Ultrasonography guided diagnosis of canine hepatopathy ascites and its therapeutic management

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Abstract
The present study was aimed at ultrasonographic changes and laboratory investigations for the diagnosis and therapeutic management in 30 ascitic cases of canines. The study comprised of Group I with 10 number, were apparently healthy on clinical and USG examination. Routine clinical examination was recorded on 0,15,30, and 60 days. Patients in group II and III showed marked like inappetence, abdominal distension with fluid thrill, respiratory distress, lethargy, melena. Radiography revealed ground glass appearance of abdomen, hepatomegaly and other hepatopathy disorders in these two groups. Anechoic abdominal free fluid was found in all 20 dogs, hyperechoic liver parenchyma in 14 dogs, hypoechoic liver in 4 dogs and mixed echotexture in 2 dogs. Marked hepatomegaly were found in 4 dogs, shrunken liver in 3 dogs and apparently normal liver size in 14 ascitic dogs. The resultant diagnosis was chronic hepatitis, hepatomegaly, nodular hyperplasia, liver cirrhosis, cholecystitis. The group II animals (10 no.) with mild and moderate ascites detected through USG were treated with silybin @ 1mg/kg b.wt OD orally, and common treatment regimen (parenteral essential amino acid + oral amino acid and multivitamin, ceftriaxone-tazobactum @ 20mg/kgbw I/M OD, parenteral furosemide followed by oral furosemide +spiranolactone and other supportive treatment). The group III animals (10 no.) with severe ascites were treated with combination of silybin @1 mg/kg bwt and SAMe @ 18mg/kg b wt OD orally, along with common treatment regimen. With selected therapeutic management in group II and III, abdominal fluid volume was cleared by one month. The treatment with the combination therapy of silybin and SAMe in therapeutic regimen-B was found to be better efficacious than silybin alone in therapeutic regimen-A.

Keywords: Cholecystitis, echogenicity, hepatomegaly, Silybin, S-Adenosyl methionine

Introduction
Ascites in canines is a very common condition with 68% occurrence [1] and mainly observed as accumulation of clear serous fluid in the peritoneal cavity. Chronic liver failure, congestive heart failure, kidney disorder, malnutrition, severe endoparasite infestation, protein losing enteropathy and abdominal neoplasia of different origin [2, 3] are the leading cause of ascites and the most common among them is cirrhosis of liver accounting for 75% [4]. Common clinical signs of ascites in dogs were in appetence, halitosis, vomiting, respiratory distress, lethargy, persistent distended abdomen, pedal oedema, melena with icterus mucus membrane and in few cases distended jugular vein, cough, cyanotic tongue, seizers and syncope [5-8]. Diagnostically ascites is observed as ground glass appearance of abdomen with diffuse fluid opacity [9] in radiography, mixed echogenicity with visceral organ such as liver (hyper echoic) floating in anechoic abdominal fluid in ultrasonography [10] and in electrocardiogram the common findings are the low voltage QRS complexes, deep Q wave with tachy- arrhythmia[5]. Chronic inflammation of liver or chronic hepatitis and liver cirrhosis is clinically manifested as ascites, as a sequalae to idiopathic origin, drug toxicity [11], portal hypertension, neurohormonal activation and hypoalbuminemia [12, 13]. Clinical signs resulting from liver disorder are vogue and non-specific with ascites being one of them, hence a battery of tests such as ultrasonography, radiography, serum biochemistry (AST, ALT, ALP) and biomarker such as micro-RNA-122, are being carried out to assess the hepatopathy origin [6, 14] and ultrasound guided liver biopsy is indicated for confirmation of liver cirrhosis [15, 16, 17]. Ascites developed due to increase in hydrostatic pressure (cirrhosis and congestive heart failure), reduced oncotic pressure (nephrotic syndrome) and increase in peritoneal fluid production. Chronic hepatitis mostly treated to eliminate the causative agent such as drug toxicity (D-penicillamine (D-Pen) in case of copper toxicity), necro inflammatory hepatic disease

(hepatoprotective and antioxidant agent such as SAMe, Vitamin E, Ursodeoxycholic acid, Silymarin) and ascites (diuretic such as spironolactone and abdominal paracentesis [11].

The aim of the present study was to detect changes in hemogram and serum biomarker, ultrasonographic findings in ascites of hepatic origin and to improve the condition of patient through restoration of altered hepatic functions without any side effect on other vital organs as well as preventing their recurrence through highly proven hepatoprotective drugs, silybin and S-adenosyl-L-methionine (SAMe).

2. Material and methods

The present work consists of thirty clinical cases presented to Teaching Veterinary Clinical Complex and Department of Veterinary Clinical Medicine, Odisha University of Agriculture and Technology, Bhubaneswar during 2019-2020. Twenty animals affected with ascites of hepatic origin were divided into 2 groups of 10 animals each and age, sex, breed, age chief complaint, patient's regular diet, duration of the disease, history of other illness and post-treatment were recorded. Detailed clinical examination, haemato-biochemical alteration (DC, TLC, Hb, AST, ALT, GGT, TSP, Albumin, Globulin, A: G ratio, BUN, urea, Creatinine), radiography (lateral and ventrodorsal orthogonal view), ultrasonography and electrocardiogram examination for accurate diagnosis were carried out along with ultrasonographic guided biopsy of liver with Trucut biopsy needle (Fig 1). B-mode ultrasonography was conducted by using GE Logiq F8 ultrasound machine (microconvex probe of 4.2-10 MHz) to evaluate the altered function of hepatic parenchyma, gallbladder, biliary system and portal vasculature. Ultrasound guided abdominocentesis was done for collection of peritoneal fluid [18]. 5ml ascetic fluid was collected by abdominocentesis, inserting 18 gauze needle 2-3 cm caudal and towards left from the midline of umbilicus at 30 40-degree angle (Fig 2). Fluid then dispensed in EDTA vial, clots activator vial and sterile tube as per the requirement. The treatment protocols were set as follows:

Groups	Drug, Dose rate and route of treatment	Separate liver protectant and antioxidant therapy
Group-I	No medication	
Group-II	Common regimen (1. Essential amino acid (Astymin-3) @ 5ml /kg bw I/V for 5 days 2. Furosemide (Lasix) @ 2mg/kgbw BID I/M for 3-5 days 3. Furosemide +spironolactone @ 2mg/kgbw +1mg/kgbw BID orally followed by parenteral diuretics for suitable period 4. Antibiotic (Ceftriaxone +tazobactum) @20mg/kgbw OD IM for 5 days 5.Ranitin @2mg/kg bw OD I/M for 5 days 6. Multi vitamin and Essential amino acid (Verol) @0.5ml/kgbw BID orally for 30 days	Regimen A (Silybin @1mg/kg/day orally for 30 days)
Group-III		Regimen B (Silybin +SAMe @1mg/kg+18mg/kg/day orally for 30 days)

3. Statistical analysis

All the data recorded in the above experiments were statistically analysed using SPSS-22 computer package employing one-way ANOVA, repeated measure anova and post-hoc analysis conducted by Duncan multiple range test.

Fig 1: Core needle biopsy of liver with Trucut biopsy needle

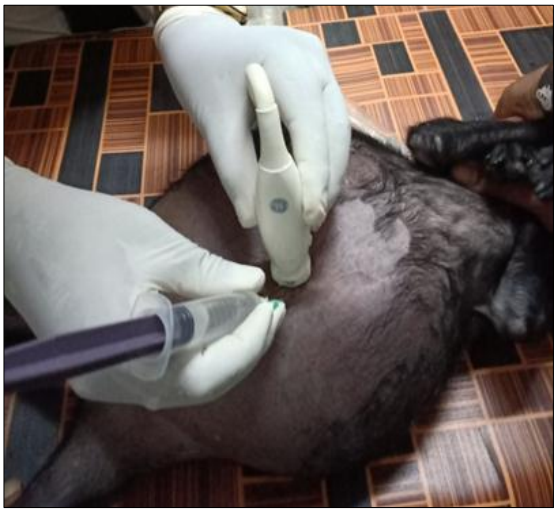

Fig 2: Ultrasound guided abdominal paracentesis for collection of ascitic fluid

4. Result and Discussion

The different clinical signs like abdominal distension, edema of dependant part, respiratory distress, paleness of mucous membrane, lethargy, anorexia, melena in the animals of Group I, II and III were documented on day 0, 15, 30 and day 60 in Table-1. The ultrasonographic findings were recorded in figure-3, 4, 5, 6 and table-2 and radiographic views in figure-7, 8. The diagnosis of twenty clinical cases were made as in table-3. The cytology of the sample collected through core needle biopsy shows presence of giant multinucleated hepatocytes with stacking on each other indicating liver cirrhosis and carcinoma with ultimate failure of hepatocytes and deposition of fluid in peritoneum leading to ascites (Fig 16).

Table 1: Alleviation of clinical sign in 3 groups

Clinical signs	Day-0			Day- 15			Day- 30			Day- 60		
	GR-I	GR-II	GR-III	GR-I	GR-II	GR-III	GR-I	GR-II	GR-III	GR-I	GR-II	GR-III
Reduced appetite to Anorexia	-	+++	+++	-	++	++	-	+	-	-	-	-
Accumulation of fluid in peritoneal cavity with abdominal fluid thrill	-	+++	+++	-	+	-	-	-	-	-	+	-
Paleness of mucous membrane	-	++	++	-	+	-	-	-	-	-	-	-
Edema of dependent part	-	+++	+++	-	-	-	-	-	-	-	-	-
Respiratory distress	-	++	++	-	+	-	-	-	-	-	-	-
Lethargy	-	++	++	-	+	-	-	-	-	-	+	-
Melena	-	+	+	-	+	-	-	-	-	-	-	-
	+: mild, ++: moderate, +++: severe, -: absent											

Table 2: Ultra sonographic findings in ascitic dogs affected with hepatobiliary disorders

Sl. No.	USG parameter	Features	No. of animals
1.	Echogenicity	Hypoechoic	4
		Hyperechoic	14
		Mixed echotexture	2
2.	Liver size	Normal liver size	13
		Hepatomegaly	4
		Reduced	3
4.	Liver margins	Sharpe	13
		Rounded	7
5.	Ascites	Mild	3
		Moderate	4
		Severe	13
6	Gall bladder	Thickened wall	2
		Normal	18

Table 4: Ultrasonographic evaluation of liver in different Groups

USG parameter	Day-0			Day- 15			Day- 30			Day- 60		
	GR-I	GR-II	GR-III	GR-I	GR-II	GR-III	GR-I	GR-II	GR-III	GR-I	GR-II	GR-III
Liver size	-	+	+	-	+	+	-	+	+	-	+	+
Echogenecity	-	+++	+++	-	+++	++	-	++	+		++	+
Irregular Liver margins	-	+++	++	-	+++	+++	-	+++	+++	-	+++	+++
Echotexture	-	+++	+++	-	+++	+++	-	+++	++	-	+++	++
Presence of peritonial fluid	-	+++	+++	-	+	+	+	-	-	-	+	-
Vascularity (portal and hepatic vein)	+++	+	+	+++	+	+	+++	++	++	+++	+	++
	+: mild, ++: moderate, +++: severe, -: absent											

Table 4: Diagnosis of 20 clinical cases

Sl. No.	USG diagnosis	No. of animals
1.	Chronic Hepatitis	8
2.	Hepatomegaly	4
3.	Nodular hyperplasia	3
4.	Liver cirrhosis	3
5.	Cholecystitis	2

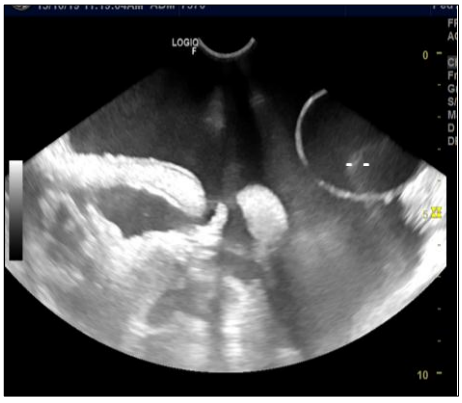

Fig 3: Abdominal USG showing ascitic fluid (A) and floating intestinal loops inside the ascitic fluid (B), Urinary bladder (UB)

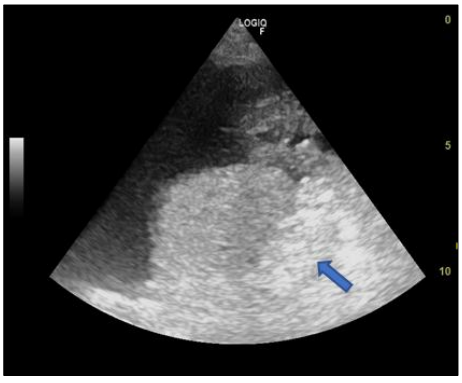

Fig 4: Irregular liver margin with multiple hyperechoic nodular structure on liver parenchyma

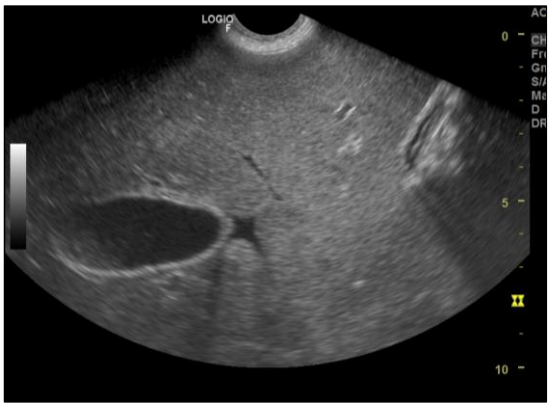

Fig 5: Cholecystitis with thickened gall bladder

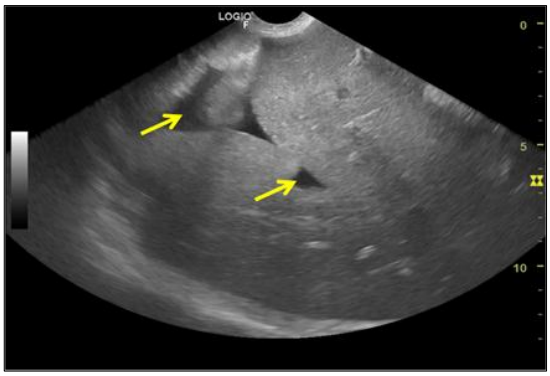

Fig 6: Ascetic fluid was decreased, but still present in-between the hepatic lobes (Gr-II, Day 15)

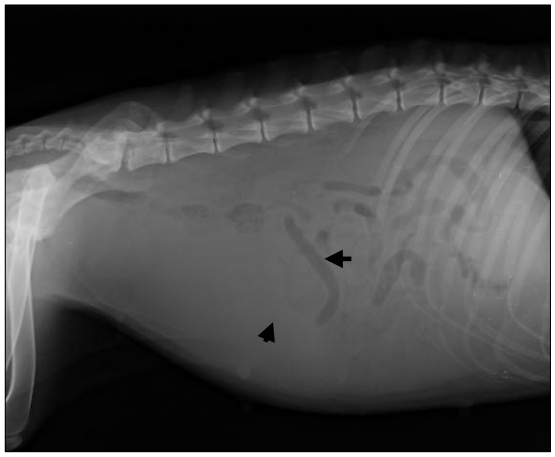

Fig 7: Lateral abdominal radiograph showing ground glass appearance of abdomen

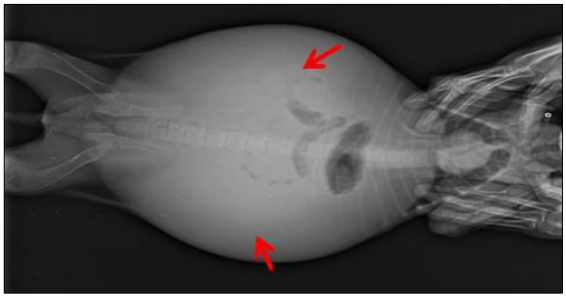

Fig 8: Ventrodorsal view of abdomen with ground glass appearance

The mean total haemoglobin, total leucocyte count, protein and A/G ratio, AST, ALT are presented in histogram as follow (Fig 9, 10, 11, 12, 13 and 14).

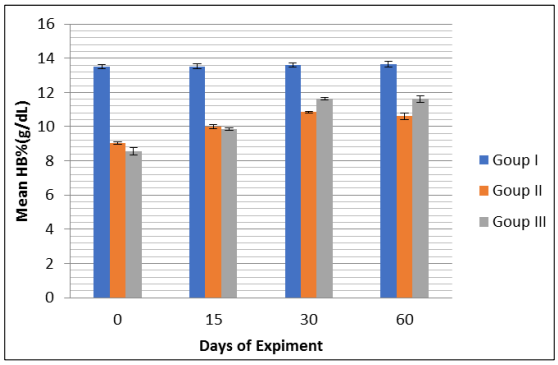

Fig 9: Mean ± S.E, Haemoglobin concentration (g/dL) in different Groups

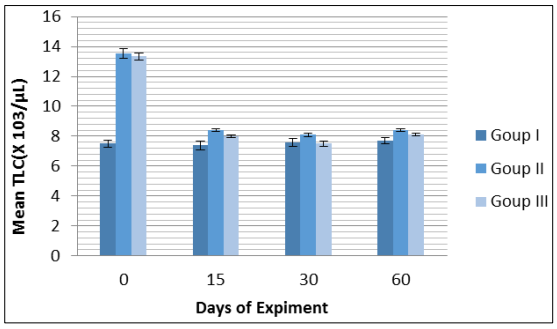

Fig 10: Mean ± S.E, total leucocyte count (X 10^3/mm^3) in different Groups

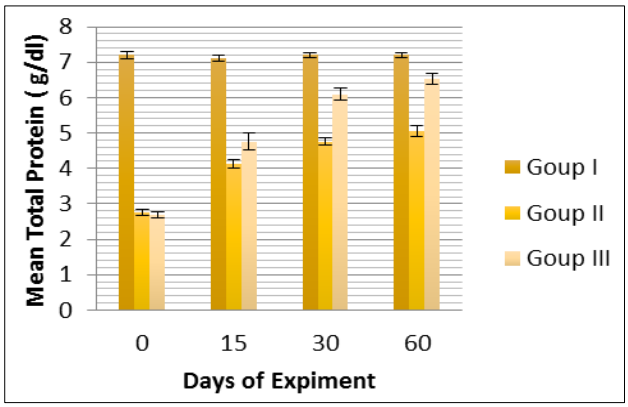

Fig 11: Mean ± S.E, protein concentration (g/dL) in different Groups

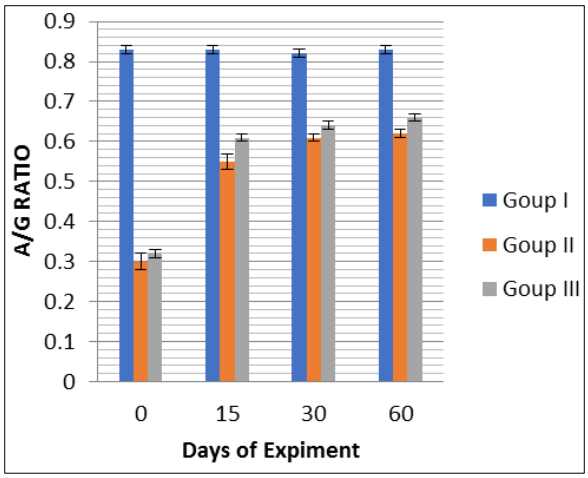

Fig 12: Mean ± S.E, Albumin and globulin ratio in different Groups

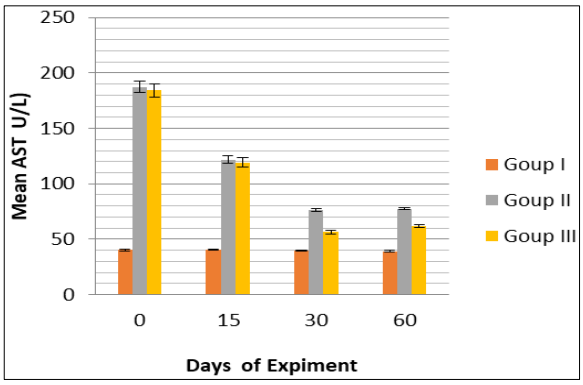

Fig 13: Mean ± S.E, AST (units/L) in different Groups

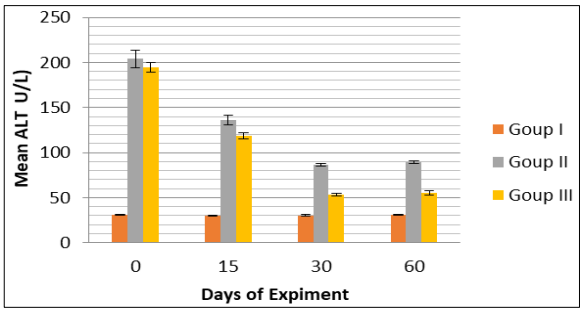

Fig 14: Mean ± S.E, ALT (untis/L) in different Groups

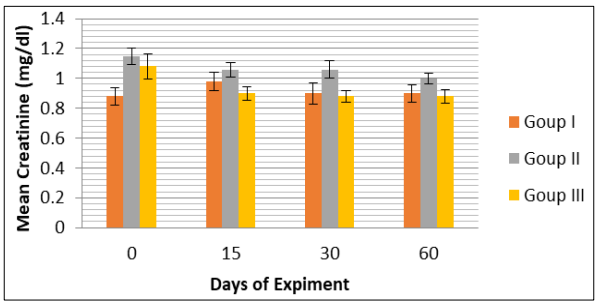

Fig 15: Mean ± S.E, creatinine (units/L) in different Groups

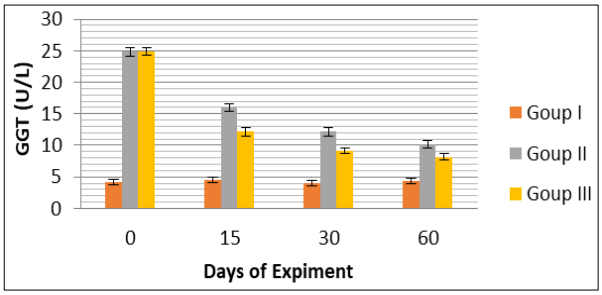

Fig 16: Mean ± S.E, GGT (units/L) in different Groups

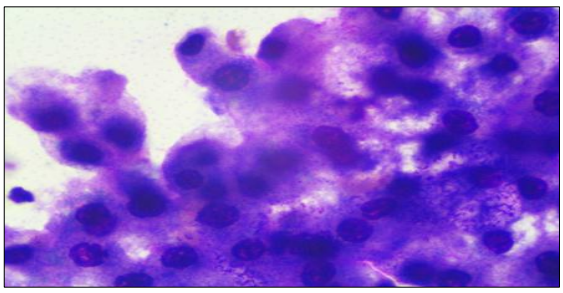

Fig 16: Multinucleated hepatocyte with stacking on each other (conglomeration) indicating carcinoma

Normal healthy dog's hepatic parenchyma appears coarse in echo texture, hypo echoic than spleen and hyper echoic or iso echoic than right kidney having pear shaped gall bladder. Hepatic veins do not have echogenic walls; however, that is present in portal veins. The normal hepatic parenchyma has uniform level of echogenicity with evidence of portal and hepatic veins. These findings are in agreement with Lamb, 1995 [19] and Chaudhary *et al.* 2008 [20], who sated that liver was less echogenic than spleen, hyper echoic or iso echoic than right kidney with anechoic gall bladder and portal veins with well-defined echogenic wall. In our study, 15 cases were found to be diffuse parenchymal disorders of the liver comprising of liver cirrhosis (3), hepatomegaly (4), chronic Hepatitis (8). And out of rest 5 cases, 3 cases were of focal parenchymal disorders of the liver (nodular hyperplasia liver) and 2 as biliary tract disorders. Diffuse hepatic disease indicates an extensive disease process which can be defined poorly through anatomical alterations. Hyperechogenicity of liver parenchyma with anechoic free fluid in abdomen separating the intra-abdominal organs giving an appearance of floating intestines was detected in 8 dogs suggested ascites due to hepatitis in agreement with Voros *et al.*,1991 [21] and Nyland and Mattoon, 1995 [22]. Focal hyperechoic parenchymal changes in the liver were noticed in three dogs suggestive of nodular hyperplasia. The findings are in agreement with Voros *et al.* 1991[21] and Mannion, 2006 [23]. Ultrasonographic changes in two dogs with cholecystitis revealed normal echogenicity of hepatic parenchyma, thickened gallbladder wall and sludge. The findings are in agreement with Nyland and Mattoon, 1995 [22] and Kumar *et al.* 2022 [1].

In present study SAMe is used along with silybin as hepatoprotective drug in group III ascitic dogs. It is in concurrence with Shih *et al.*, 2007 [24]. Methionine gets actively transported into the liver and in presence of the enzyme methionine adenosyl transferase it gets converted to S-adenosyl methionine (SAMe) but in case of liver injury SAMe could not be synthesize at the desired level for which it becomes essential to supplement SAMe in the food. Administration of SAMe in cirrhosis and intrahepatic cholestasis replenishes hepatic GSH reserves, thereby improving their tolerance for free radical and re-perfusion type cell damage (Centre, 2000 [25]). Liver support in the form of supplementation with antioxidants like S-adenosyl methionine, milk thistle and vitamin E in dogs had a protective role particularly in inflammatory hepatopathies (Marks, 2012) [26]. Silybin exerts its antioxidant property through inactivation of cytokines and other inflammatory transcription factors along with inhibition of lipid peroxidation. Silymarin plays the pivotal role in the synthesis of DNA and other proteins, restoration of glutathione level as well as inhibition of collagen synthesis (Webster *et al.*, 2019 [11]).

Conclusion

The phytosomal phospholipid complex silybin is the form of silybin having greater absorption rate and lesser dose rate i.e. @ 3-5 mg/kg body weight. The treatment with the combine therapy of silybin @1mg/kgbw and SAMe @18 mg /kgbw daily orally for 30 days was found to be better efficacious than silybin @1mg /kgbw in respect to alleviation of clinical sign, reduction in accumulated peritoneal fluid, restoration of hepatic dysfunction and normalization of haemato

biochemical alteration.

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