

Evaluating the Effectiveness of Ursodeoxycholic Acid in Various Treatment Approaches for Canine Liver Dysfunction: A Comparative Study

Abstract

Liver dysfunction in dogs presents significant challenges to veterinary practice, with clinical symptoms ranging from modest to severe. For the development of optimised treatment strategies, this study was conducted at the College of Veterinary and Animal Sciences, G.B.P.U.A. & T. Pantnagar, U.S. Nagar (Uttarakhand) from September 2021 to April 2022 in dogs diagnosed with hepatic dysfunction. The study involved a therapeutic trial on dogs with liver impairment. 18 dogs diagnosed with hepatic dysfunction were divided into three groups, i.e., B, C, and D. Each group received different therapeutic protocols, and changes in hematobiochemical profiles were observed and compared before and after treatment. Dogs in Group B received ursodeoxycholic acid, Group C received ursodeoxycholic acid plus silymarin, and Group D received ursodeoxycholic acid and L-ornithine Ornithine L-aspartate. Haematological and biochemical parameters were evaluated at presentation and after 21 days of treatment. Ursodeoxycholic acid demonstrated efficacy in improving biochemical parameters, with additional benefits observed when combined with L-Ornithine, L-Aspartate, or Silymarin. Group D exhibited the most significant improvement, suggesting the effectiveness of combination therapy. These findings underline the importance of regular monitoring and appropriate therapeutics in managing hepatic dysfunction in dogs, with combination therapies offering enhanced recovery through hepatoprotective, antioxidant, and anti-inflammatory mechanisms.

1. Introduction

The liver is a prime organ in the body that is involved in several functions, including metabolism, purification, vitamin and trace mineral storage, and immunogenic regulation. But because of its dual blood supply and high blood flow, the liver is more susceptible to disease than other systems and organs [1] [2]. Hepatic dysfunction was first defined in humans as a potentially reversible disorder resulting from severe liver injury and including signs of encephalopathy shortly after the onset of symptoms in patients with no prior history of liver disease [3]. It was then further defined as an altered alanine aminotransferase (ALT) plasma concentration and a progressively increasing serum bilirubin concentration [3] [4]. Hepatic

disorders include hepatocellular, reversible and irreversible injury (necrosis), portosystemic shunt, neoplasia (primary hepatic and secondary), and hepatic fibrosis or cirrhosis [5]. Endocrine diseases such as diabetes mellitus, hyperadrenocorticism (Cushing's disease), and hyperthyroidism can all cause impaired liver function because of their effects on the organ [6]. Hepatic dysfunction often results from various etiological agents such as bacteria, viruses, fungi, toxins, and drugs [7]. It was estimated that disease of the liver had a prevalence of about 1.06% among all diseases of dogs presented to veterinary clinics [8]. The clinical manifestations of hepatic disorders are frequently nonspecific. Dogs with hepatic dysfunction may be present for a number of clinical signs, including anxious symptoms such as polydipsia, polyuria, lethargy, ascites, jaundice, and nervous disorders. Thus, hepatic illness is always doubtful until a blood biochemical test is performed [9]. Extensive hepatobiliary screening comprises a hematobiochemical profile, analysis of urine, measurement of clotting time, liver function tests, ultrasonography, radiography, bile cytology, and histopathology [10]. It has been reported that after a 70% partial hepatectomy, the liver returns to its normal size and function in about two weeks due to hepatocyte and cholangiocyte replication [11] [12]. Hence, due to the regenerating capacity of the liver, hepatic dysfunction can be managed with a good therapeutic approach. Treatment for a variety of hepatobiliary disorders usually emphasises removing predisposing factors, reducing their impact, regenerating damaged hepatocytes, and restoring hepatic dysfunctions [13]. Various therapies are available for the proper management of liver dysfunction, which include steroids, diuretics, antioxidants, a diet with appropriate protein, fluid therapies, antibiotics, and hepatic protectants [14]. Medicines that are commonly used as therapy in canine hepatic dysfunction aim at reversal of inflammation, reducing advancement of fibrosis, shielding against hydrophobic bile acid damage, and defending from oxidative damage [15]. Among the variety of drugs exhibiting these properties, some are silymarin, ursodeoxycholic acid, L-Ornithine, L-Aspartate and Ursodeoxycholic acid which were studied in the therapeutic management of hepatic dysfunction in dogs by various researchers [16] [17]. The present research aims to find the therapeutic efficacy of different drugs in combination with ursodeoxycholic acid to find an effective therapeutic protocol for canine hepatic dysfunction.

2. Material and Methods

In this study, a therapeutic trial was conducted in clinical cases of dogs of both sexes of different age groups (4 months to 15 years) and breeds, including Mongrel, Labrador retriever,

German shepherd, Doberman Pinscher, Pomeranian, Rottweiler, Pug, Pitbull, and Himalayan sheep dog exhibiting clinical signs of liver impairment presented to Veterinary Clinical Complex College of Veterinary and Animal Sciences, G.B.P.U.A. & T, Pantnagar U.S. Nagar (Uttarakhand). Dogs suspected of having hepatic dysfunction were subjected to hematobiochemical examination, radiography, and ultrasonography. 18 dogs that were diagnosed with hepatic dysfunction were randomly divided into 3 groups, irrespective of their age, breed and sex with 6 animals in each group, namely Group B, Group C, Group D. The dogs in each group were subjected to different therapeutic protocols, and changes in their hematobiochemical profiles were noticed. Dogs in group B were given Ursodeoxycholic acid @15mg/kg b.w./day orally, and dogs in group B were given Ursodeoxycholic acid (15 mg/kg b.w./day orally) and Silymarin (Sylibon syrup, 2 tsp BID orally), while dogs in group D were given Ursodeoxycholic acid and L-Ornithine L-Aspartate (Hepamerz syrup, 2 tsp TID orally) as a therapeutic protocol. Dogs in group A were selected as healthy controls. The changes in hematobiochemical parameters were compared on the day of presentation and at the end of the trail, i.e., day 21, to assess the efficacy of various therapeutic protocols.

Haematological parameter

Approximately 2 mL of blood was taken from a cephalic or saphenous vein using dry disposable syringe vials containing EDTA (ethylene diamine tetraacetic acid) and antiseptic procedures with appropriate safety protocols. The sample so acquired was labelled, and the blood parameters of freshly collected samples were analysed for evaluation of various blood parameters manually as per the standard protocol mentioned by Jain, 1986.

Biochemical parameter

A sterile syringe was used to collect 3 ml of blood, which was then immediately transferred to a test tube without any anticoagulant. The blood was then allowed to coagulate for roughly 1 hour in a slant posture before being centrifuged for 10 minutes at 2,000 to 3,000 rpm to get the serum. The separated serum was then placed into a dry Eppendorf tube with a micropipette for measurement of different serum parameters on a spectrophotometer using an Erba diagnostics kit. Blood biochemical parameters were estimated on the UV double beam spectrometer LI-2700 (Lassnay International Limited). The values of several serum parameters were calculated manually.

2.3. Statistical Analysis

The data was expressed as Mean±SE. Standard error of mean and p-values were used to determine whether there was any significant difference among different treatment groups using unpaired t test with the help of SPSS software version 22.0

3. Result and discussion

Dogs affected by liver ailment usually display a spectrum of clinical signs, which includes depression, weakness, nervous signs, jaundice, anorexia, vomiting, change in spleen size, diarrhoea, emaciation, dark brown urine, pyrexia, polydipsia, polyuria, epigastric pain, ascites, coma, change in liver size, dark or light-coloured stools, haemorrhage, and urticaria [19]. Similar clinical manifestations were documented in the present study, with the predominant signs being dullness, inappetence, vomiting, polydipsia, fever, and emaciation observed in 74.46%, 57.44%, 53.01%, 51.06%, 46.80%, 31.91%, and 42.56% of cases, respectively. Other signs such as icterus, ascites, epigastric pain, and neurological disorders were seen as sporadic in occurrence. Dogs with hepatic dysfunction exhibit major alterations in haematological and biochemical parameters [20]. Similar findings were observed in this study. Hepatic dysfunction has a predictable prognosis with a good therapeutic approach. Meyer et al. (1997) studied the effect of ursodeoxycholic acid on chronic hepatitis in dogs and found a decrease in biochemical parameters such as ALT, AST, ALP, cholesterol, and bilirubin, thereby improving hepatic ailment. Ursodeoxycholic acid has a number of different methods of action, one of which is increasing the quantity of hydrophilic bile acid while decreasing the harmful endogenous hydrophobic bile acids in cholestatic liver disease by a dilutional action; moreover, it also protects hepatocytes from bile acid-induced apoptosis and increases hepatobiliary secretion [21].

In this study, recovery was evaluated on the basis of resolution of clinical signs and improvement in hematobiochemical parameters. The average duration of disappearance of clinical signs was least in group D (6–14 days), followed by group C (8–16 days), and group B (6–19 days). In the haematological study, it was observed that there was a significant increase in haemoglobin, packed cell volume, and total erythrocyte count in groups C and D and significant changes in neutrophils and lymphocytes in all therapeutic groups post-treatment as compared to the day of presentation, while total leucocyte count and platelets

improved non-significantly in all treatment groups post-treatment (**Table 1**). There was improvement in all treatment groups, but better values were seen in group D, followed by group C and group B, respectively. Improvement in mean values of haematological parameters during therapeutic study in all treatment groups was due to improved liver function due to the effect of hepatoprotectives on impaired liver. This improvement might be due to the effect of improved liver function due to the effect of hepatoprotectives in impaired livers and the use of ancillary treatments including antipyretics, antacids, antibiotics, and other supportive and hematinic treatments, which were similarly observed by Singh et al., 2019.

Findings of biochemical parameters revealed a significant decrease in total bilirubin (ALT, AST, GGT, ALP, and blood glucose) and a significant increase in total protein (albumin, while parameters such as globulin, A:G ratio, blood urea nitrogen, serum creatinine, and cholesterol differed non-significantly in all treatment groups post-treatment as compared to the day of presentation (**Table 2**). Improvement was seen in all therapeutic groups due to hepatoprotective drugs, but there was more improvement in group D, followed by group C, where urosdeoxycholic acid was used in combination with L-ornithine, L-aspartate, and silymarin, respectively. L-ornithine L-aspartate dissociates into its constituents, ornithine and aspartate, which are readily absorbed by active transport. L-ornithine serves as an intermediary in the urea cycle in periportal hepatocytes in the liver and as an activator of carbamoyl phosphate synthetase and L-aspartate by transamination to glutamate via glutamine synthetase in perivenous hepatocytes, as well as by skeletal muscle and the brain, by which ammonia is detoxified [23]. L-ornithine L-aspartate was also found effective in canine hepatic dysfunction by Hudyma and Slivinska (2015) when used in combination with phospholipids. Silymarin has a hepatoprotective nature and manifests anti-fibrotic, anti-inflammatory, immunomodulating, hepatocyte-regenerating quality, anti-oxidant, and anti-lipid peroxidative properties [25]. The hepatoprotective nature of silymarin was also observed by Kumar et al. (2013) in dogs with hepatic dysfunction. Various treatments, including S-adenosylmethionine, zinc, and D-penicillamine, have demonstrated efficacy in addressing hepatic dysfunction in canines across diverse research studies [27] [28]. In this study, a combination of urosdeoxycholic acid and L-ornithine L-aspartate was found to be more effective as a therapeutic agent in canine hepatic dysfunction, followed by a combination of urosdeoxycholic acid and silymarin and urosdeoxycholic acid as an individual therapeutic agent.

Conclusion

From the present research work, it is concluded that regular monitoring with proper therapeutics in dogs affected by hepatic dysfunction can contribute to a good prognosis. It was established better improvement in clinical signs and hematobiochemical in group D is combination of urosdeoxycholic acid + L-Ornithine L-Aspartate had better improvements in dogs with hepatic dysfunction. These drugs, with the help of their hepatoprotective antioxidant and anti-inflammatory properties, enhance recovery in dogs with hepatic dysfunction. The findings of the present study can contribute to the selection of good therapeutic medicines that can be used in combination with urosdeoxycholic acid in dogs with hepatic dysfunction.

4. References

1. **Cooper J.** Symposium on liver function, *Veterinary Medicine*. 2006; 277-310.
2. **Lathamani VS, Nalinikumari K.** Studies on therapeutic efficacy of Liv-52 in dogs suffering from hepatic disorders. *International Journal of Innovative Research in Science in Engineering and Technology*. 2015; 4(8): 7361-7365. DOI:10.15680/IJIRSET.2015.0408124
3. **Johnson V, Gaynor A, Chan, DL.** Multiple organ dysfunction syndrome in humans and dogs. *Journal of Veterinary Emergency Medicine and Critical Care*. 2004; 14(3):158–66.
4. **Craig DG, Lee A, Hayes PC.** The current management of acute liver failure. *Alimentary Pharmacology and Therapeutics*. 2010; 31(3):345–358p
5. **Melissa S, Yonaire C.** Hepatic encephalopathy: Diagnosis and treatment liver inflammation damage and repair. *Journal of Physiology and Pharmacology*. 2013; 59(1): 107-117.
6. **Ming H, Li S, Tan HY, Wang N, Tsao SW, Feng Y.** Current status of herbal medicines in chronic liver disease therapy: The biological effects, molecular targets and future prospects. *International Journal of Molecular Science*. 2015; 29(35): 28705-45. doi: 10.3390/ijms161226126
7. **McCord KW, Webb CB.** Hepatic dysfunction. *Veterinary Clinics of North America Small Animal Practice*. 2011 41(4): 745-75 <https://doi.org/10.1016/j.cvsm.2011.04.002>
8. **Sameeksha C, Bhojne G R, Dhoot V M, Upadhye SV, Panchbhai CK, Somkuwar AP, Khan WA, Kriti D.** Age, Breed and Gender-Wise Prevalence of Hepatic Disorders in Dogs of Nagpur City. *International Journal of Current Microbiology and Applied Sciences*. 2021; 10(02): 1685-1689. <https://doi.org/10.20546/ijcmas.2021.1002.199>

9. **Schlesinger D P, Rubin SI.** Serum bile acids and the assessment of hepatic function in dogs and cats. *Canadian Veterinary Journal.* 1993; 34(4), 215–220. PMID: [17424198](#)
10. **Alvarez L, Whittemore J.** Liver enzyme elevations in dogs: physiology and pathophysiology. *Compendium (Yardley, PA).* 2009; 31(9): 408-10. PMID: 20180207
11. **Hauptman K, Tichý F, Knotek Z.** Clinical diagnostics of hepatopathies in small mammals: evaluation of importance of individual methods. *Acta Veterinaria Brno.* 2001; 70(3): 297-311. <https://doi.org/10.2754/avb200170030297>
12. **Schotanus BA, Penning LC, Spee B.** Potential of regenerative medicine techniques in canine hepatology. *Veterinary Quarterly.* 2013; 33(4): 207-216. DOI: [10.1080/01652176.2013.875240](#)
13. **Saravanan M, Mondal DB, Sarma K, Mahendran K, Vijayakumar H, Sasikala V.** Comprehensive study of haemato-biochemical, ascitic fluid analysis and ultrasonography in the diagnosis of ascites due to hepatobiliary disorders in dog. *Indian Journal of Animal Science.* 2014; 84(5): 503-506.
14. **Pyleris E, Giannikopoulos G, Dabos K.** Pathophysiology and management of acute liver failure. *Annals of Gastroenterology.* 2010; 23(4): 257-265.
15. **Honeckman A.** Current concepts in the treatment of canine chronic hepatitis. *Clinical Techniques in Small Animal Practice.* 2003; 18(4): 239-244. DOI: [10.1016/S1096-2867\(03\)00050-1](#)
16. **Meyer DJ, Thompson MB, Senior DF.** Use of ursodeoxycholic acids in a dog with chronic hepatitis: effects on serum hepatic tests and endogenous bile acid composition. *Journal of Veterinary Internal Medicine.* 1997; 11(3), 195-197. DOI: [10.1111/j.1939-1676.1997.tb00090.x](#)
17. **McGrotty YL, Ramsey IK, Knottenbelt CM.** Diagnosis and management of hepatic copper accumulation in a Skye terrier. *Journal of Small Animal Practice.* 2003; 44(2): 85-89. DOI: [10.1111/j.1748-5827.2003.tb00126.x](#)
18. **Jain NC.** Schalm's Veterinary Haematology, Lea and Febiger, Philadelphia. 1986; 6: 31-65.
19. **Kozat S, Sepehrizadeh E.** Methods of diagnosing in liver diseases for dog and cats. *Turk Bilimsel Derlemeler Dergisi.* 2017; 10(2): 36-46.
20. **Patel MD, Parikh PV, Barot HM.** Clinical, Ultrasonographic and Haemato-Biochemical Assessment of Liver and Gall Bladder Affections in Dogs. *Indian Journal of Veterinary Science and Biotechnology.* 2022; 18(1): 66.

21. **Balagoni H, Devani K, Phemister J, Young MF.** Ursodiol-associated paroxysmal atrial fibrillation. *American Journal of Therapeutics*.2017; 24(5): 612.
DOI: [10.1097/MJT.0000000000000570](https://doi.org/10.1097/MJT.0000000000000570)
22. **Singh SK, Shukla SK, Bhatt P, and Singh AK.** Clinico therapeutic studies on ascites in canines. *International Journal of Current Microbiology and Applied Sciences*. 2019; 8(9): 1120-1137.
23. **Kircheis G, Stefan L.** Pharmacokinetic and Pharmacodynamic Properties of L-Ornithine L-Aspartate (LOLA) in Hepatic Encephalopathy. *Drugs*. 2019; 79 (1): 23-29.doi: [10.1007/s40265-018-1023-2](https://doi.org/10.1007/s40265-018-1023-2)
24. **Hudyma TM,SlivinskaLG.** Therapeutic efficacy of Hepatiale® Forte in treatment of the dogs with hepatodystrophy. *Science and Education a New Dimension. Natural and Technical Science*. 2015; 5(41): 51-53
25. **Abenavoli L, Aviello G, Capasso R, Milic N,Capasso F.** Milk thistle for treatment of non-alcoholic fatty liver disease. *Hepatitis monthly*. 2011; 3 (32), 173 - 177.
26. **Kumar M, Mondal DB, Saravanan M, SharmaK.** Therapeutic management of hepatobiliary dysfunction in canines. *IntasPolivet*. 2013; 14(1), 117–120.
27. **Fieten H, Dirksen K, van den Ingh, TS, Winter EA, Watson AL, LeegwaterPA, Rothuizen J.** D-penicillamine treatment of copper-associated hepatitis in Labrador retrievers. *VeterinaryJournal*. 2013; 196(3): 522-527.DOI: [10.1016/j.tvjl.2012.12.013](https://doi.org/10.1016/j.tvjl.2012.12.013)
28. **Norton Rebecca D, Lenox Catherine E, Manino P, Vulgamott, James C.** Nutritional Considerations for Dogs and Cats with Liver Disease. *Journal of American Animal HospitalAssociation*.2016; 52(1): 1–7. DOI: [10.5326/JAAHA-MS-6292R2](https://doi.org/10.5326/JAAHA-MS-6292R2)

Table1: Effect of different therapeutic protocol on hematological profile of dogs with hepatic dysfunction

	GROUP A	Group B		Group C		GROUP D	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Hemoglobin	12.72±0.30	8.92±0.94 ^a	11.13±0.55 ^a	9.03±0.72 ^a	11.57±0.35 ^b	9.15±0.87 ^a	12.08±0.43 ^b
PCV	47.50±1.87	26.50±4.62 ^a	39.17±3.93 ^a	27.83±3.50 ^a	43.00±2.25 ^b	25.83±4.87 ^a	43.67±3.41 ^b
TEC	6.40±0.15	4.21±0.50 ^a	5.44±0.39 ^a	3.96±0.3 ^a	5.59±0.21 ^b	4.08±0.49 ^a	5.81±0.25 ^b
TLC(10 ³ /μl)	10.48±1.23	19.85±3.06 ^a	13.15±1.15 ^a	19.30±2.86 ^a	12.32±0.65 ^a	20.75±3.22 ^a	12.40±0.72 ^a
Neutrophil(%)	63.50±1.95	81.67±4.72 ^a	68.17±2.69 ^b	82.50±5.04 ^a	65.50±2.26 ^b	83.33±6.08 ^a	64.67±1.86 ^b
Lymphocyte(%)	29.67±0.33	13.67±3.99 ^a	24.50±2.67 ^b	13.00±3.78 ^a	27.50±2.09 ^b	12.33±4.85 ^a	29.00±1.69 ^b
Monocyte(%)	3.50±0.89	1.83±0.31 ^a	3.00±0.31 ^a	1.67±0.33 ^a	2.83±0.31 ^a	1.66±0.21 ^a	3.00±0.37 ^a
Platelets(10 ⁶ /μl)	3.32±0.20	1.63±1.02 ^a	2.52±0.98 ^a	1.78±1.10 ^a	2.83±1.09 ^a	1.68±1.16 ^a	3.03±1.17 ^a

Mean ±S.E. with different alphabet in superscript(a,b,c) differ significantly($p \leq 0.05$).

Table 2: Effect of different therapeutic protocol on biochemical profile of dogs with hepatic dysfunction

	GROUP A	Group B		Group C		GROUP D	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
ALT (IU/L)	67.33±2.99	157.17±20.50 ^a	92.17±7.73 ^b	160.67±20.60 ^a	87.17±7.25 ^b	168.50±21.75 ^a	84.67±6.78 ^b
AST(IU/L)	43.83±3.06	109.83±17.18 ^a	57.33±6.15 ^b	111.50±17.54 ^a	51.67±4.39 ^b	117.00±19.71 ^a	50.67±3.99 ^b
ALP(IU/L)	62.67±3.25	212.67±34.36 ^a	99.33±13.71 ^b	217.83±35.07 ^a	86.33±12.12 ^b	220.83±35.51 ^a	79.50±10.20 ^b
GGT(IU/L)	4.52±0.29	17.87±4.29 ^a	7.62±0.88 ^b	19.32±4.02 ^a	7.17±0.91 ^b	22.02±4.31 ^a	6.17±0.63 ^b
Total bilirubin(mg/dl)	0.25±0.02	1.86±0.37 ^a	0.52±0.13 ^b	1.74±0.41 ^a	0.37±0.12 ^b	1.89±0.44 ^a	0.36±0.09 ^b
Total protein(g/dl)	6.68±0.08	5.33±0.37 ^a	6.12±0.23 ^b	5.38±0.34 ^a	6.27±0.23 ^b	5.43±0.42 ^a	6.37±0.22 ^b
Albumin(g/dl)	3.60±0.07	2.12±0.26 ^a	3.05±0.17 ^b	3.22±0.19 ^a	2.10±0.21 ^b	2.25±0.32 ^a	3.38±0.19 ^b
Globulin(g/dl)	3.08±0.07	3.22±0.36 ^a	3.07±0.19 ^a	3.28±0.29 ^a	3.05±0.16 ^a	3.18±0.27 ^a	2.98±0.11 ^a
A: G(g/dl)	1.17±0.04	0.72±0.13 ^a	1.02±0.08 ^a	0.67±0.08 ^a	1.07±0.09 ^a	0.70±0.11 ^a	1.13±0.05 ^a
BUN(mg/dl)	18.00±0.73	16.83±4.59 ^a	18.67±1.31 ^a	16.00±4.37 ^a	19.17±1.30 ^a	15.17±4.25 ^a	19.67±1.15 ^a
Creatinine(mg/dl)	1.20±0.12	1.10±0.28 ^a	1.20±0.08 ^a	1.15±0.37 ^a	1.22±0.11 ^a	1.13±0.34 ^a	1.25±0.06 ^a
Glucose (g/dl)	93.33±1.5	66.33±7.06 ^a	84.00±2.83 ^b	62.67±6.24 ^a	86.17±2.50 ^b	60.00±7.80 ^a	88.17±2.57 ^b
Cholesterol(mg/dl)	159.33±2.44	227.83±25.27	205.33±23.24	231.50±22.62	202.67±20.45	236.33±26.54	198.83±18.65

Mean \pm S.E. with different alphabet in superscript(a,b,c) differ significantly($p \leq 0.05$).

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