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Mefepronic acid is associated with a decrease in serum liver enzyme activities in dogs with suspected hepatopathy

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Abstract

Although suspected hepatopathy in dogs can be assessed by the blood levels of both liver enzyme activities and functional liver parameters, very often the precise diagnosis of primary or secondary hepatobiliary diseases can remain uncertain. Therefore, in a number of patients, the therapeutic intervention has the purpose of slowing the progression of fibrosis and provide for optimal hepatic support. Recently the PPARs (peroxisome proliferator-activated receptors) have been identified as a family of hepatic nuclear hormonal receptors, involved in the regulation of lipid and glucose metabolism. The aim of this work is to assess the effect of mefepronic acid (PMPA), a PPAR agonist, on liver enzyme markers in blood samples of dogs with suspected hepatopathies. Twenty dogs, with suspected hepatopathies, were divided into two groups: ten of them received subcutaneously daily 10 mg/kg of PMPA for 7 days (treated, group T), while the remaining dogs were treated with a conventional supportive treatment for hepatopathies consisting of ursodeoxycholic acid (UDCA) 10 mg/kg PO SID for 45 days (control, group C). PMPA yielded a faster decrease in liver enzyme activities compared to UDCA, that in most cases was maintained after the suspension of the treatment. These data suggest that PMPA might be considered as supportive treatment for dogs with suspected hepatopathy.

KEYWORDS

dog, liver disorders, liver enzymes, mefepronic acid, PMPA, ursodeoxycholic acid

1 | INTRODUCTION

In veterinary practice, an important diagnostic role for the assessment of hepatic function is the evaluation of several blood markers. In the dog, the most relevant are those that evaluate hepatocellular damage (alanine aminotransferase-ALT and aspartate aminotransferase-AST), in combination with cholestasis markers (alkaline phosphatase-ALP and gamma-glutamyltransferase-GGT) and other metabolites characterizing both the hepatic metabolism (total serum bilirubin, ammonia, and bile acids) and hepatic synthetic function (lipids, albumin, urea, and coagulation inhibitors). Recently, in the dog, Raghu et al., (2018) proposed the evaluation of the 'AST/ALT ratio' as a diagnostic tool to distinguish moderate from severe hepatic fibrosis. In addition, these markers can show variations also in the absence of primary liver disease, as for example with pharmacologic induction, endocrinopathies, hypoxia/hypotension, muscle injury, neoplasia, bone disorders (Comazzi et al., 2004). The best way to arrest, or resolve, the progression of hepatic fibrosis

would comprise the identification and the removal of the underlying cause (Eulenberg & Lidbury, 2018). However, because often the exact aetiology cannot be determined with certainty, the therapeutic approach remains to be supportive care. In the dog, supportive treatment in potentially reversible liver disorders, involves the use of molecules able to counter inflammation, to reduce the progression of fibrosis and to protect against the damage induced by bile acids and oxidative damage (Honeckman, 2003). Some active substances, also known as the term hepatoprotectant drugs, are often recommended in canine liver disorders. These include: S-adenosyl-L-methionine (SAMe), silymarin (*Silybum marianum* or milk thistle), ursodeoxycholic acid, *N*-acetylcysteine, vitamin E (α-tocopherol), vitamin C (Lawrence & Steiner, 2015; Sartor & Trepanier, 2003; Vanderweerd et al., 2013) and probiotics (Lucena et al., 2019). Recently the PPARs (peroxisome proliferator-activated receptors) have been identified as a family of hepatic nuclear hormonal receptors which act as transcription factors involved in the regulation of lipid and glucose metabolism. Moreover, they can modulate certain inflammatory responses which accompany the development of atherosclerosis (Norata et al., 2003 and Grygiel-Gòrniak, 2014). The name attributed to these receptors derives from initial observations on drugs, such as the fibrates that, in rodents, led to the proliferation of peroxisomes, which are intracellular corpuscles responsible for the oxidation of various xenobiotics and fatty acids (Norata et al., 2003). The PPARs family includes three isoforms: PPARα, PPARβ/δ and PPARγ. PPARα exerts most of its effects in the liver, by activating the transcription of genes linked with the mechanisms of lipid peroxidation; while in this same tissue the activation of PPARγ indirectly modulates carbohydrate metabolism (Grygiel-Gòrniak, 2014; Norata et al., 2003). Various natural and synthetic PPAR agonists are used in human patients for the treatment of glucose and lipid disorders (Grygiel-Gòrniak, 2014; Ibabe et al., 2005). Recently, similar drugs have also been proposed in veterinary medicine for the activation of the PPARs in several pathologies, including some hepatopathies; these include clofibrate, bezafibrate, fenofibrate and mefepronic acid (2-methyl-2-phenoxy propionic acid or PMPA) (Aparicio-Cecilio et al., 2012; De Marco et al., 2017; Litherland et al., 2010; Rizzo et al., 2014; Yang et al., 2013; Yuksek et al., 2013). Starting from the observation that fibrates have an anti-hyperlipidemic effect, we hypothesized that their use could improve hepatopathies, where the lipid metabolism is probably altered. On this basis, the objective of the present work is to assess the possible beneficial effect of mefepronic acid on the levels of serum enzyme activities whose abnormal variations are currently detected in clinical cases of dogs presenting either primary or secondary hepatopathies.

2 | MATERIALS AND METHODS

This study was reviewed and approved by the Committee for Animal Ethics of the University of Parma (approval number PROT. 02/CE/2018 del 07/12/2018), and the experiments were conducted in accordance with the approved guidelines, including the written informed consent by the owners.

2.1 | Study design and animals

Twenty dogs of different ages, breed, and sex were included in the study (Table 1). Although the owners had not referred any clinical symptom, all the animals had shown, for over 4 months, both the levels of several liver leakage markers (transaminases, alkaline phosphatase, total bilirubin, gamma-glutamyltransferase, etc.) in the blood above the reference intervals, and changes to the hepatic parenchyma during the abdominal ultrasound scan. When the dogs were referred to us (T0), after a fasting period of 12 hr, they were subjected to a careful clinical examination that included blood sampling from the cephalic vein and a complete abdominal ultrasonography. If the clinical condition of the patient required it, and with agreement from the owner, a pericutaneous ultrasound-guided needle biopsy (BD Spinal Needle 22G x 3.00" 0.7 x 75 mm, Becton Dickinson S.A., Madrid-Spain) of the liver and a cytological analysis were performed. The study did not consider euthanasia of the enrolled dogs at the end of the investigation. Reasons for exclusion from enrolment were the recent administration in the previous fifteen days of drugs with hepatotropic action (e.g., silymarin, B group vitamins, vitamin E, ursodeoxycholic acid, reduced glutathione), as well as glucocorticoids or NSAIDs, immunomodulatory drugs (e.g., cyclosporine) and/or antibiotics.

Once ascertained the fulfilment of the inclusion criteria of the study, the patients of group T were treated with 10 mg/kg of mefepronic acid (Hepagen© 100 mg/ml injectable solution for dogs- A.T.I. s.r.l., Ozzano Emilia-BO Italy) once a day, subcutaneously, for 7 consecutive days, while those of group C (controls) were treated SID for 45 days orally with ursodeoxycholic acid (Deursil© - Sanofi-Aventis S.p.A., Milano Italy) as compounded powder prepared by a pharmacist from the capsules, at a dose of 10 mg/kg. The treatment was administered by the owner at home, and the compliance was verified by a phone call every two days. During the course of the study (from T0 to T3), no other treatment than PMPA or UDCA was administered to the patients enrolled. A comprehensive clinical evaluation with blood sampling, together with ultrasound examination of the abdominal cavity, was performed in the patients of both groups twice 7 days apart (T1 and T2) from T0; a further control was finally (T3) 30 days after T2. Since a liver diet was not prescribed, the dogs maintained their usual feeding regimen for the entire duration of the trial (from T0 to T3).

2.2 | Blood collection for haematology and serum biochemistry

Blood samples were collected both in plastic test-tubes for the preparation of the serum and in test-tubes containing K_3EDTA and

TABLE 1 Animal enrolled and treated with mefepronic acid (treated group, T) and ursodeoxycholic acid (control group, C)

Group	N° dog	Breed	Age	Sex	Body weight (kg)
T	1	Mongrel	10y	M	9.5
	2	Shih-tzu	11y	F	7.8
	3	Dachshund kurzhaar	16y, 6m	M	14
	4	Mongrel	11y	NM	13.2
	5	Mongrel	13y, 6m	SF	6.8
	6	Jack Russell terrier	14y, 10m	SF	5.4
	7	Italian hound	11y, 3m	F	26.4
	8	Yorkshire terrier	15y	M	5
	9	Labrador retriever	2y	M	35
	10	Mongrel	12y, 9m	M	7.5
C	1	Mongrel	12y	M	9.8
	2	Labrador retriever	9y, 6m	F	36.8
	3	Jack Russell terrier	2y	M	7.6
	4	Cavalier King Charles spaniel	12y	M	8
	5	Mongrel	10y, 4m	F	10.9
	6	Basenji	13y, 6m	M	9.4
	7	Mongrel	10y, 5m	F	11.3
	8	Mongrel	13y, 1m	F	14
	9	Pug	10y	M	10.4
	10	Maltese	8y	M	6.2

Abbreviation: y = year, m = months, *M* = male, NM = neuterd male, *F* = female, SF = sterilized female

sodium citrate, then they were immediately subjected to the following tests:

- complete haemogram test for the evaluation of the following parameters: red blood cells (RBC, number/μl), haematocrit (HCT, %), haemoglobin (Hb, g/dl), white blood cells (WBC, number/μl) and differential blood count (neutrophils, lymphocytes, monocytes, eosinophils, basophils, numbers/μl) and platelets (PLT, number/μl);
- plasmatic coagulation times: prothrombin time (PT, sec.), activated partial thromboplastin time (aPTT, sec.);
- serum biochemical parameters: total bilirubin (mg/dl), alanine aminotransferase (ALT,U/L), alkaline phosphatase (ALP, U/L), gamma-glutamyltransferase (GGT, U/L), aspartate aminotransferase (AST, U/L), total proteins (g/dl), albumin (g/dl), globulins (g/dl), creatinine (mg/dl), BUN (mg/dl), glucose (mg/dl), sodium (Na, mEq/L), chloride (Cl, mEq/L), potassium (K, mEq/L), phosphorus (P, mg/dl), calcium (Ca, mg/dl), cholesterol (mg/dl), triglycerides (mg/dl), α-amylase (U/L), lipase (U/L).

All the analyses were performed at the laboratory of the 'Ospedale degli Animali S.R.L.', Ferrara (Italy), that is certified as UNI EN ISO 9001–2015.

2.3 | Statistical analysis

In view of performing a reliable *t*-test for the assessment of significant differences between the treatments with PMPA and UDCA, the 'within-group' variations of the biochemical indicators at the different sampling times were first evaluated by ANOVA test. The statistical evaluation was performed using SPSS version 25 (IBM Statistics).

3 | RESULTS

The administration of both mefepronic and UDCA were well tolerated in all animals.

With regard to ultrasonography, the patients of both groups at T0 showed mostly hypo-to isoechoic areas, heterogeneous liver parenchyma and slight/mild hepatomegaly. In some of them, the liver appeared hyperechoic and the gallbladder was distended, with hyperechoic walls, and without any stones or sediment (Table 2). The ultrasound scans, repeated at the different blood sampling times, showed a gradual improvement of the hepatic sonographic findings for both C and T groups, even if the complete *restitutio ad integrim* could not be observed for all the patients.

TABLE 2 Hepatic echographic findings of the dogs belonging to group T (treated) and C (control) at T0

Dog number	Group T	Group C
1	Diffuse hyperechoic liver parenchyma with a slight hepatomegaly. Normal vascular pattern. Gallbladder with normal wall and content.	Severe hepatomegaly with large amount of biliary sludge.
2	Inhomogeneous parenchyma with hypoechoic areas with different size (max. 1.79 cm diameter), mild hepatomegaly with regular borders. With contrast enhanced ultrasound (CEUS), hypoechoic areas showed slight hyperenhancement in the arterial phase, isoenhancement during the portal phase, hypoenhancement during the late phase.	Moderate hepatomegaly and moderate homogeneous hyperechoic liver parenchyma.
3	Hepatomegaly, homogeneous hyperechoic liver parenchyma, multifocal iso/hypoechoic nodular lesions (4 mm in diameter). Mild thickening of intrahepatic biliary wall. Gallbladder and common bile duct dilation.	Normal liver size and echogenicity, normal porto-biliary pattern. Gallbladder normally distended with little amount of biliary sludge.
4	Moderate hepatomegaly, inhomogeneous parenchyma with multifocal ill-defined nodules (8 mm in diameter). Porto-biliary pattern within normal limits. Normal gallbladder wall and content	Moderately distended gallbladder with hyperechoic wall, biliary sludge.
5	Normal shape and size, coarse liver parenchyma, hyperechoic thickened intrahepatic biliary duct wall. Gallbladder with irregular and thickened wall (2 mm).	Mild hepatomegaly, normal liver parenchyma. Porto-biliary pattern within normal limits. Normal gallbladder wall and content.
6	Normal shape and size, two nodular ill-defined hypoechoic lesions. Porto-biliary pattern within normal limits.	Moderate hepatomegaly, inhomogeneous texture, multiple nodules from a few mm up to 2 cm in diameter, with different echogenicity. Gallbladder normally distended with thickened wall small polypoid formations.
7	Moderate hepatomegaly, homogeneous hyperechoic parenchyma. Porto-biliary pattern within normal limits. Normally distended gallbladder with normal content.	Moderate/severe hepatomegaly, coarse hyperechoic parenchyma. Normal vascularity. Intrahepatic biliary ducts within normal limits. Over distended gallbladder with large amount of biliary sludge and thickened wall. No extrahepatic biliary dilation. A vascularized, ill-defined nodular lesion in the left caudal lobe, and 2.74 x 2.45 cm in size.
8	Inhomogeneous liver parenchyma, kiwi like pattern of the gallbladder indicating gallbladder mucocele.	Shape and volume within normal limits. Slightly hyperechoic homogeneous parenchyma. Normal porto-biliary and venous vascular pattern. Normally distended gallbladder with normal wall and content.
9	Severe hepatomegaly with dilatation of the hepatic veins. Free fluid in the peritoneal cavity indicating ascites.	Shape, size, echogenicity of the liver parenchyma within normal limits. Normal gallbladder.
10	Hepatomegaly, multifocal nodules, hyperechoic biliary wall, indicating cholangitis/cholecystitis, biliary sludge.	Moderately hepatomegaly, hyperechoic coarse liver parenchyma with multifocal nodules. Gallbladder within normal limits. Normal intrahepatic portal veins.

Only two dogs in group T (dogs 2 and 8) were subjected to liver biopsy at T0, but these same patients did not undergo the same exam at T3. In one patient (dog 2), the presence of numerous hepatocyte aggregates differentiated with binucleated cells drove the histopathological diagnosis towards a hyperplastic/adenomatous process, while for the other dog (n°8), the cytological examination showed that it was affected by a hepatic carcinoma. Moreover, in this same dog, and in two others of group T (n°1 and 9), the hepatopathy was associated with hyperadrenocorticism, an immune-mediated haemolytic anaemia, and cor triatriatum dexter with concomitant tricuspid dysplasia. For the remaining dogs, on the basis of both clinical biochemistry and echography, it was only possible to arrive at a presumptive diagnosis of hepatopathy.

The values of at least one of the biochemical parameters (i.e ALT, ALP, GGT, AST, total protein, albumin, cholesterol and triglycerides) were outside of the normal ranges in all the patients enrolled, while complete hemogram (except for dog n° 1 of group T, that showed moderate monocitosis), plasmatic coagulation times and the other biochemical parameters considered, were not altered in any of the dogs for the duration of the study. Nevertheless, it must be underlined that the high variability of the values of the altered biochemical indicators 'within-groups', as determined by ANOVA test (not

shown), did not allow to perform a statistical evaluation of the data by *t*-test 'between groups' (not shown); therefore, their levels were drawn in graphs as box plots, reporting median and interquartile range for each group at the different sampling times (Figure 1).

Regarding the ALT values, their median in both groups at T0 was above the highest reference limit, with an extremely higher variability for the dogs treated with mefepronic acid. For these patients, at the end of the therapy (T1) a reduction of the median of the values was seen, that was accompanied by a decrease of their variability. The decrease of ALT over time was assessed for the entire duration of the study, and at T3 its median value was inside the reference interval in group T. The profile of the ALT values of the dogs belonging to group C was similar to that of group T, although the median remained slightly above the upper reference limit and a less marked reduction was seen.

The variation of the AST time profile for both groups was similar to that of ALT, with the difference that a highest variability at T0 was measured, in this case, for the controls. As shown for ALT, at the end of the treatment with mefepronic acid, the AST in most of the subjects was within the reference limits, which persisted until the end of the investigation. Also, the treatment with UDCA showed a similar result, with a reduction at T1 that was maintained until the end of the study.

As shown in Figure 1, the median of the 'AST/ALT ratio', except for the dogs of group C at T1 and T2, was between 0 and 1. Although single AST and ALT values changed in both groups, their ratio was unchanged for the entire duration of the study, a trend that was clearer for the dogs treated with mefepronic acid (red boxes).

ALP values in both groups showed a similar time profile, but different from ALT and AST, a decrease of the parameter over time could be observed only at T3. Although the median of the parameter started to decrease from T1, for both C and T patients it remained slightly above the upper reference limit.

The initial values of GGT were markedly different for the dogs of the two groups. The median GGT of group T at T0 was inside the

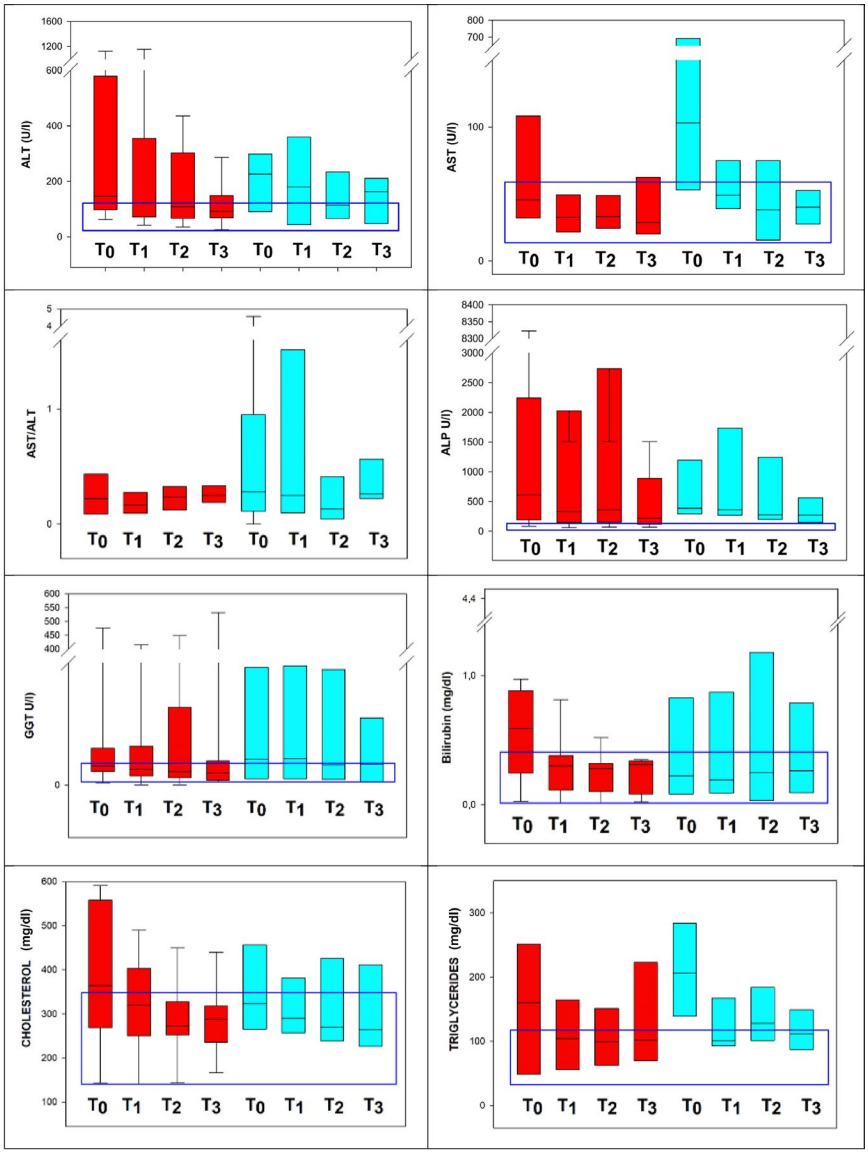

FIGURE 1 Changes over time of the serum enzyme activities and functional liver parameters of the dogs treated with mefepronic acid (red boxes) and with ursodeoxycholic acid (cyan boxes). The data are reported as medians and interquartile ranges of the values of the parameters of all dogs of groups T and C at each blood sampling time. T1, T2 and T3 correspond to 7, 14 and 45 days after T0. The treatment with mefepronic acid was discontinued at T1, while the control group was treated until T3. The blue frames represent the reference values of each parameter. Enzymatic activity and metabolite levels are reported as U/L and mg/dl, respectively

reference interval and, with the exception of one subject far above the upper limit that did not decrease during the study, the values in the remaining patients in this group were much lower than those of group C. Although less marked than for ALT and AST, a decrease of the values could be seen at T3 for both groups.

For bilirubin, the decrease over time was more evident in the dogs of group T compared to the controls. For the subjects treated with mefepronic acid, this could be already observed at T1, continued after the suspension of treatment, and at T3 most dogs were normobilirubinemic, with a limited variability in serum bilirubin concentrations.

Figure 1 shows also the time profile of hematic cholesterol. Cholesterol concentrations were variable in the dogs of group T and in most cases were above the upper reference limit of the parameter, showed a decrease at the end of the treatment (T1) that continued for the remaining time of the study. At T3 most of the dogs had normocholesterolemia with a low variability in individual cholesterol concentrations. The dogs of group C at T0 showed a median value inside the reference limit of the parameter, with less ariability in cholesterol concentrations compared to those of group T; at T3 the variability was maintained, but the median of the parameter had decreased.

Triglyceride concentrations in dogs treated with mefepronic acid decreased from T0 to T1, but these levels were not maintained after the suspension of the therapy. The dogs of group C showed an initial decrease at T1 that was maintained for the entire duration of UDCA treatment (T3).

4 | DISCUSSION

PPARα is a receptor expressed at high concentrations within hepatocytes, where it plays a crucial role in fatty-acid metabolism by increasing the rate of β-oxidation. The fibrates, that are synthetic ligands of the PPARα, have been administered experimentally in obese dogs (Serisier et al., 2006), in Beagle dogs fed on a diet rich in fats (Tsunoda et al., 2008) for the treatment of metabolic dysfunction in type 2 diabetes (Kasai et al., 2008) as well as for the treatment of lipid metabolism disorders (Sato et al., 2018). 2-methyl-2-phenoxy propionic acid (PMPA), a substance belonging to the fibrate family, is an activator of energy metabolism that shows a marked therapeutic effect in rats with hepatic steatosis (Gumus et al., 2013). On the basis of this experimental evidence, since no clinical studies with mefepronic acid have been conducted in the canine species, we considered this substance for the treatment of 10 dogs with suspected hepatopathies. Their biochemical outcomes were then compared with those of a control group that were treated with a conventional hepatoprotective therapeutic protocol based on ursodeoxycholic acid (3α, 7β-dihydroxy-5β-cholanic acid or UDCA). This compound has a combination of cytoprotective and anti-apoptotic, membrane stabilising, antioxidant and immunomodulatory effects (Kumar & Tandon, 2001). In dogs, UDCA diffusing into the bile from hepatic cells, has a choleretic effect due to a bile acid-dependent mechanism (Yanaura & Ishikawa, 1978). Moreover, by interfering with ileal motility it could play a role in bile acid diarrhoea (Kruis et al., 1986). The evaluation of the blood levels of several enzyme activities may suggest hepatic leakage (Dirksen et al., 2017; Fuentealba et al., 1997), therefore in the present study their alterations in serum, in combination with ultrasonography, led us to presume the presence of hepatopathies in the dogs enrolled. These subjects, in agreement with observations by other authors (Eulenberg et al., 2018; Kortum et al., 2018; Raghu et al., 2018), often did not show specific clinical manifestations and signs of chronic progressive hepatopathy, except for abnormal values at T0 in the blood of some enzyme activities related either to liver cytolysis (ALT and AST) or cholestasis (ALP, GGT) (Fuentealba et al., 1997; Rothuizen et al., 2006). Moreover, other blood parameters of hepatic synthetic function, including albumin, bilirubin, cholesterol and triglycerides were considered. Although the "gold standard" for the diagnosis and for the evaluation of the response to treatment of liver disorders is the a histopathological examination of the hepatic tissue biopsies ultrasound-guided fine-needle aspirate (FNA) have limitations, due mainly to the risk of haemorrhage, the small size of the sample obtained, and the increased cost (Lidbury & Suchodolski, 2016), this exam could be realized only for two of the clinical cases enrolled in group T, and only at T0, since dog owners did not give the permission for a second liver biopsy. Therefore, the lack of cytological examination represents a limitation to this study because, on one hand it was not possible to diagnose the various pathologies affecting the patients, and on the other to monitor the therapeutic response. With regard to hepatic blood parameters, a general trend for values within the reference intervals was observed for both groups already starting from T1. Nevertheless, it must be emphasized that T1 corresponds to the suspension of the treatment with mefepronic acid, while UDCA was administered to the patients of group C until the end of the study. The decrease of blood parameters over time seen in the dogs of group C is in contrast with previous investigations in healthy dogs, where UDCA, administered at a slightly higher dose than in the present study, but for a shorter time (15 mg/kg PO once daily for 15 days), caused an increase of serum ALT, AST and GGT activities after 7 days of treatment (Lucena et al., 2013). Serum ALT is the most specific enzyme marker of hepatic tissue damage; being largely present in the cytosol of hepatic cells, with cellular damage it passes rapidly in large amounts into the blood stream. In fact, in dogs, ALT is the gold-standard marker of hepatocellular lesions (Oosthuyzen et al., 2018). Therefore, the reduction in serum activity of ALT suggests a better integrity of the hepatocellular membrane (Meyer et al., 1997); moreover, it has been reported that decreases in serum ALT activity of 50% every 2 to 3 days suggest that hepatocyte damage is resolving (Center, 2007). In our study, ALT showed a marked decrease, as also observed with bezafibrate by De Marco and colleagues in dogs presenting hyperlipidemia (De Marco et al., 2017). In this study, it was hypothesized that the improvement in ALT is likely

related to a decrease in circulating triglycerides, which we also observed, and amelioration of hepatic lipidosis. UDCA yielded similar result but for mefepronic acid a more rapid decrease of these parameters was observed, which continued also after the suspension of the treatment for another 37 days, until T3. AST is found in both cytosol and mitochondria of the hepatocytes and may indicate more severe hepatocellular necrosis. In agreement with a previous investigation (Weingarten & Sande, 2015), in this study, the changes in AST activity were parallel to those of ALT. The 'AST/ALT' ratio has been considered to distinguish between mild and severe fibrosis (cirrhosis) in human patients, but this index does not differentiate between hepatic fibrosis or necroinflammatory scores in dogs (Raghu et al., 2018). The dogs we enrolled, with few exceptions among the patients of group C at T1 and T2, did not exceed an 'AST/ALT' ratio of 1. This might suggest that the dogs did not have hepatic fibrosis, but this presumption can only be confirmed by liver biopsies that, due to the low severity of clinical signs in most of these dogs, could only be realized for the two most clinically affected cases. The increased activity of ALP, a metalloprotein enzyme, parallels with that of ALT in most chronic liver diseases, reflecting on one end the inability of inflamed hepatic cells to recycle the bile acids and on the other side cholestasis deriving from the morphological changes in the biliary system (Konstantinidis et al., 2015). Unfortunately, the lack of the determinations of both bile acids and ammonia levels represents a limitation of this study since these markers would have been helpful to further evaluate liver function. In liver tissue, ALP and GGT are anchored to the hepatocyte membrane by glucosyl phosphatidylinositol linkages. Fibrates were first noticed to reduce hepatic ALP isoenzyme levels in humans, when developed as a cholesterol-lowering agent (Zumoff, 1977). Similar results were obtained in the present study, where following an up and down profile, the ALP activities reached their minimum at T3, a time that corresponds to 37 days after the suspension of treatment with PMPA. Comparable results were obtained with UDCA administered for the duration of the study, which confirms that both treatments lead to a similar response, although acting on different metabolic pathways. The trends in total bilirubin concentrations allows to suspect a cholagogue and choleretic effect of mefepronic acid which in our study was not seen with UDCA. Moreover, for this parameter the improvement obtained with mefepronic acid was maintained after the suspension of the treatment for the following 37 days, till the end of the investigation. Interestingly a similar effect was not observed in group C, thus letting us hypothesize an additional beneficial effect of PMPA. An increase in serum total cholesterol and/or triglyceride concentrations are indicative of diseases associated with endocrine disorders (hypothyroidism, hyperadrenocorticism, diabetes mellitus), obesity, protein-losing nephropathy, pancreatitis, and liver disease (De Marco et al., 2017; Li et al., 2014; Sato et al., 2018). Hyperlipidemia in dogs has been associated with several clinical complications such as insulin resistance, increased liver enzyme activity, gallbladder mucocele, behavioural changes, peripheral neuropathies, and seizures (De Marco et al., 2017). The fibrates are currently considered the most effective antihyperlipidemic drugs available, whereas the statins are a class of LDL-cholesterol lowering drugs (Li et al., 2014). Mefepronic acid in the present investigation produced a significant decrease in cholesterol concentrations over time, while triglycerides, after a first reduction from T0 to T2, showed a tendency to increase again at T3. This partly differs from what has been observed in other animal species. In fact, although the activation of PPARα in the liver of rodents and in primary hepatocyte cultures promotes hepatocyte proliferation and regeneration (Bayly et al., 1994; Hasmall et al., 2000), the concentrations of triglycerides, cholesterol, albumin, total proteins, AST and creatine kinase after administration of PMPA did not significantly change in cows treated with PMPA (Aparicio-Cecilio et al., 2012). Due to this discrepancy with other animal species, the results obtained in the dogs in this study warrant confirmation by further investigations. Interestingly, although acting through different mechanisms, a comparable decrease of the considered parameters over time was obtained also with UDCA, but it must be underlined that PMPA was administered only for the first seven days following T0. Finally, Cheung and coworkers reported that the combined administration of UDCA and fenofibrate in primary biliary cholangitis in human patients with incomplete UDCA responses may improve the outcome (Cheung et al., 2016). A similar therapeutic protocol could be proposed for further investigations on the same or similar pathology in dogs.

5 | CONCLUSIONS

In liver disorders, supportive treatment with hepatoprotective agents and antioxidants is an important part of the therapeutic plan in veterinary patients. Although the limited number and heterogeneity of the clinical cases have not allowed a statistical treatment of the data, and moreover the lack of biopsies has prevented to establish a definitive diagnosis in most of the patients, this study shows that the use of fibrates, and in particular mefepronic acid is safe and can be used in dogs with suspected hepatopathies.

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CONFLICT OF INTERESTS

The authors declare that they have no competing interest.

AUTHOR CONTRIBUTION

Fausto Quintavalla: Conceptualization; Investigation; Methodology; Project administration; Supervision; Writing-original draft; Writing-review & editing. Elisa Gelsi: Conceptualization; Investigation; Methodology; Project administration; Writing-original draft. Luca Battaglia: Investigation. Raffaella Aldigeri: Data curation; Methodology. Roberto Ramoni: Conceptualization; Writing-original draft; Writing-review & editing.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Informed consent about the nature of diagnostic and experimental procedures to be performed was obtained from each dog owner. The trial was conducted in compliance with institutional guidelines for research on animals, and was approved by the Ethics Committee of the University of Parma (Prot. N. 02/CE/2018).

CONSENT FOR PUBLICATION

Not applicable.

DATA AVAILABILITY STATEMENT

The datasets supporting the conclusions of this article are included within the article.

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