

Time-dependent hepatic histopathological and biochemical changes following a single dose of methylprednisolone acetate in dogs

*Jevgenija Kondratjeva, Madara Nikolajenko, Aija Ilgaza

Latvia University of Life Sciences and Technologies, Lielā iela 2, Jelgava, Latvia

Abstract. Long-acting corticosteroids, such as methylprednisolone acetate, are commonly used in veterinary medicine due to their prolonged anti-inflammatory and immunosuppressive effects. Despite their extensive clinical use, evidence on the hepatic safety profile of long-acting corticosteroid formulations remains insufficient and requires further investigation. This study investigated the biochemical and histopathological changes in the liver following a single intramuscular injection of long-acting methylprednisolone acetate at 2 mg kg⁻¹ in seven healthy beagle dogs, monitored over a 56-day period. Serum enzyme activities – alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP) – were measured every seven days, while ultrasound-guided liver biopsies were obtained on days 0, 14, 28, and 56. Tissue samples were evaluated using haematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and immunohistochemical staining. Significant increases in GGT and ALP were observed, peaking at around day 14, followed by gradual normalisation by day 56. Histological evaluation revealed hepatocellular changes, including swelling, cytoplasmic vacuolation, and marginal nuclear displacement. Neutrophil counts and hepatic stellate cell numbers showed no significant differences across time points. This work extends current knowledge by documenting the time-dependent progression and partial recovery of hepatic alterations following a single dose of long-acting methylprednisolone acetate in dogs. The results highlight the importance of monitoring liver responses and provide new insight into the subclinical hepatic effects of depot corticosteroids, with potential implications for safer therapeutic decision-making in veterinary practice.

Key words: corticosteroid-induced hepatopathy, vacuolar hepatopathy, liver histopathology, hepatic enzymes.

Introduction

Corticosteroids are among the most frequently prescribed drugs in veterinary medicine because of their potent anti-inflammatory and immunosuppressive effects, as well as their ability to manage a wide range of immune-mediated and allergic conditions (Papich, 2016; Tilley *et al.*, 2021). Over recent decades, several formulations have been developed, allowing clinicians to tailor treatment protocols according to disease severity, patient compliance, and owner preferences. Among these, long-acting injectable preparations are widely used in small animal practice because they provide sustained effects and reduce the need for frequent dosing, which improves adherence to treatment regimens (Tilley *et al.*, 2021; Vargo & Banovic, 2021).

Methylprednisolone acetate, in particular, has become one of the most popular long-acting

corticosteroids due to its prolonged biological activity, which can extend over several weeks after intramuscular administration (Del Baldo *et al.*, 2024; Papich, 2016). However, despite its extensive use, data on the long-term systemic effects of depot formulations remain surprisingly limited compared to short-acting preparations (Del Baldo *et al.*, 2024; Elkholly *et al.*, 2020). Recent studies have highlighted that even a single intramuscular dose of methylprednisolone acetate can induce the marked suppression of the hypothalamic-pituitary-adrenal (HPA) axis, with recovery sometimes requiring up to seven weeks (Del Baldo *et al.*, 2024). These findings suggest that long-acting formulations may exert more profound and prolonged physiological effects than previously anticipated, warranting closer clinical monitoring, particularly in patients requiring repeated or high-dose corticosteroid therapy.

* Corresponding Author's email address:
 jevgenija.kondratjeva@lbtu.lv

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The liver plays a central role in the metabolism and elimination of corticosteroids, which makes it especially vulnerable to drug-induced histological and functional alterations (Tham *et al.*, 2024). Steroid-induced hepatopathy is a well-described phenomenon in dogs and is characterised by a combination of hepatocyte swelling, cytoplasmic vacuolation, nuclear marginalisation, and glycogen accumulation within the cytoplasm (Oo *et al.*, 2022; Sobczak-Filipiak *et al.*, 2014; Tinted *et al.*, 2023). In some cases, mild pericellular fibrosis and collagen deposition have also been reported, suggesting that even transient corticosteroid exposure can initiate structural remodelling within the hepatic parenchyma (Yehia *et al.*, 2013). Importantly, these changes appear to be largely species-specific and are typically reversible upon the discontinuation of corticosteroid treatment (Richter, 2003).

Nevertheless, the severity and persistence of hepatic alterations vary considerably and are influenced by multiple factors, including the choice of corticosteroid, formulation, dosage, treatment duration, and individual patient sensitivity. For instance, some dogs exhibit pronounced biochemical and histological changes even after a single administration, whereas others demonstrate minimal alterations despite prolonged treatment (Abraham *et al.*, 2006; Center *et al.*, 2005; Sobczak-Filipiak *et al.*, 2014). This inter-individual variability complicates clinical decision-making and highlights the need for evidence-based monitoring protocols during corticosteroid therapy (Abraham *et al.*, 2006; Budde & McCluskey, 2023; Del Baldo *et al.*, 2024; Sobczak-Filipiak *et al.*, 2014).

From a diagnostic perspective, serum biochemical markers are commonly used to detect liver injury in dogs receiving corticosteroid therapy. Increases in enzymes such as alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP) are frequently reported and may correlate with the extent of histological change (Fernandez & Kidney, 2007; Yehia *et al.*, 2013). However, enzyme activity alone is insufficient to fully characterise steroid-induced hepatopathy. Ultrasonography provides valuable, non-invasive information about parenchymal texture and structural changes, yet it often lacks sensitivity for detecting early or subtle lesions (Varshney, 2021). Consequently, liver biopsies remain the diagnostic gold standard, allowing the direct histopathological evaluation and characterisation of corticosteroid-associated changes (van den Ingh *et al.*, 2021). Routine haematoxylin and eosin (H&E) staining facilitates the assessment of hepatocellular architecture, while periodic acid-Schiff (PAS) staining is particularly useful for detecting

abnormal glycogen accumulation associated with steroid exposure (Young *et al.*, 2006). In addition to routine histological assessment, the evaluation of hepatic stellate cells can provide further insight into early hepatic responses to injury. Hepatic stellate cells are the principal mediators of hepatic fibrogenesis and play a central role in tissue remodelling. Upon activation, they transform into myofibroblast-like cells and begin producing extracellular matrix components, including collagen. Therefore, assessment of stellate cell activation, commonly performed using α -smooth muscle actin (α -SMA) immunohistochemistry, allows the detection of early fibrogenic activity that may not yet be evident on routine histological staining (Carpino *et al.*, 2005; Sobczak-Filipiak *et al.*, 2014).

Despite the widespread clinical use of methylprednisolone acetate, there is a lack of prospective studies documenting its time-dependent effects on hepatic histopathological changes and function. Most available data derive from investigations involving other corticosteroids or from cross-sectional studies with limited sampling intervals. To address this knowledge gap, the present study aimed to evaluate both biochemical and histopathological changes in the liver over a 56-day period following a single intramuscular administration of methylprednisolone acetate at a dose of 2 mg kg⁻¹ in healthy beagle dogs. Through combined biochemical and histopathological analyses, this study elucidates the progression and recovery of hepatic alterations following long-acting methylprednisolone acetate administration.

Materials and Methods

Seven clinically healthy female beagle dogs were used in this study (permission No. 70, Latvia Committee of Ethics). The dogs had free access to drinking water and were fed twice daily with a commercial lamb-and-rice diet (Josera, Germany). The daily food amount was calculated individually (13 g kg⁻¹ body weight), and consumption was recorded throughout the study. All animals were housed in individual indoor crates (150 × 100 × 280 cm). During daylight hours, the animals had access to an enriched and secure outdoor enclosure (500 × 500 cm). The study lasted 56 days. Daily clinical examinations were performed, including the assessment of behaviour, appetite, urination, defecation, and body weight. On day 0 (D0), all dogs received a single intramuscular injection into the biceps femoris muscle of methylprednisolone acetate at a dose of 2 mg kg⁻¹.

Blood samples (6 mL) were collected from the cephalic vein on D0, 7, 14, 21, 28, and 56. Serum was separated within 15 minutes of collection, and activities of alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase

(ALP) were measured using an automated biochemical analyser (Mindray BS-120, China).

Liver biopsies were obtained under ultrasound guidance on D0, 14, 28, and 56 using a disposable sterile 14G, 10 cm True-Cut biopsy needle (Baxter, Germany) mounted in a Vitesse biopsy gun (OptiMed, Germany). Sedation was induced with propofol (2 mg kg⁻¹ IV), and local anaesthesia was provided with 2% lidocaine at the biopsy site. Dogs were positioned in right lateral recumbency, and the needle was inserted through the caudal intercostal space. All biopsies were performed under sterile conditions: the hair was clipped, and the skin was washed with 4% chlorhexidine and disinfected with 70% alcohol. According to WSAVA histological sampling guidelines, liver tissue is considered painless to biopsy if major bile ducts are avoided (van den Ingh *et al.*, 2021). Samples were fixed in 10% neutral buffered formalin for 24 hours and processed using an automatic tissue processor (Shandon Citadel 69810040, USA). Sections were cut at 4 µm thickness (SLEE Mainz Cut, Germany) and stained with haematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). Immunohistochemistry for α -smooth muscle actin (α -SMA) was performed to identify hepatic stellate cells and assess their activation.

Histological evaluation included hepatocyte size, cytoplasmic reticulation, vacuolation, and nuclear position. All histological assessments were conducted by a single investigator blinded to treatment allocation. In cases of uncertainty, samples were re-evaluated to ensure the consistency of scoring.

Changes were graded semiquantitatively:

No change (0) – hepatocytes normal.

Mild (1) – up to one-third of the field affected; mild reticulation, few or no vacuoles, mostly central nuclei.

Moderate (2) – one-third to two-thirds of the

field affected; reticulation and vacuolation evident, marginal nuclei in about two-thirds of hepatocytes.

Severe (3) – more than two-thirds affected; diffuse reticulation, vacuoles of variable size, predominantly marginal nuclei.

The number of α -SMA-stained hepatic stellate cells was counted under a light microscope at 400x magnification per 100 hepatocytes: only the cells with visible nuclei on the section were considered (Carotti *et al.*, 2008; Carpino *et al.*, 2005). For each slide, ten microscopic fields were randomly chosen. Neutrophils were counted per 400x high-power field.

Data were analysed using SPSS Statistics, version 28.0 (IBM Corp., USA). Results are presented as mean $\pm$ standard deviation (SD). Differences in serum enzyme activities (ALT, ALP, and GGT) between time points and the baseline (D0) were analysed using paired Student's t-tests. Histopathological data, including hepatocyte change scores and PAS scores, were considered semiquantitative ordinal variables and were therefore analysed using the Wilcoxon signed-rank test. Neutrophil counts (per high-power field) and hepatic stellate cell counts (per 100 hepatocytes) were also compared between time points and the baseline using the Wilcoxon signed-rank test. A p-value < 0.05 was considered statistically significant.

Results

At the baseline (D0), blood samples were collected from all seven dogs to determine the initial serum values of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) before the administration of methylprednisolone acetate (2.0 mg kg⁻¹ IM). All measured values were within the generally accepted canine reference ranges (Willard & Tvedten, 2012).

Alanine aminotransferase (ALT)

Table 1

Changes in serum enzyme activities following a single intramuscular administration of methylprednisolone acetate

Parameter	Reference range, IU/L	D0 (IU/L)	D7 (IU/L)	D14 (IU/L)	D21 (IU/L)	D28 (IU/L)	D56 (IU/L)
ALT	10-94	45.83 $\pm$ 6.52	72.83 $\pm$ 7.99	75.54 $\pm$ 11.23	69.87 $\pm$ 10.18	51.10 $\pm$ 6.92	45.38 $\pm$ 6.61
ALP	0-102	89.33 $\pm$ 14.17	210.16 $\pm$ 30.74 ^a	213.47 $\pm$ 36.29 ^a	242.97 $\pm$ 54.54 ^a	189.74 $\pm$ 43.84 ^a	95.04 $\pm$ 23.00
GGT	0-6	5.20 $\pm$ 0.29	11.87 $\pm$ 0.86 ^a	14.36 $\pm$ 1.14 ^a	13.90 $\pm$ 1.61 ^a	11.37 $\pm$ 1.41 ^a	4.59 $\pm$ 0.55

^a $p < 0.05$

Data are presented as the mean $\pm$ SD.

Mean ALT activity remained stable throughout the study, showing no statistically significant differences compared with the baseline (D0) at any time point ($p > 0.05$; Table 1). A mild increase within physiological limits was observed from D7 to D21. From D28 onward, ALT levels normalised in all animals and remained within physiological limits.

Alkaline phosphatase (ALP)

Serum ALP activity demonstrated significant elevations from D7 through D28, with $p < 0.05$ at each time point, compared with D0 (Table 1). The highest mean value was recorded on D21 (242.97 ± 54.54 IU/L), approximately 2.7-fold above the baseline. By D56, ALP activity had declined substantially ($95.04 \pm$

23.00 IU/L) and was no longer statistically different from the baseline ($p > 0.05$).

Gamma-glutamyl transferase (GGT)

GGT activity showed a significant increase from D7 through D28, with $p < 0.05$ at each time point, compared with D0 (Table 1), peaking on D14 (14.36 ± 1.14 IU/L; Table 1). By D56, GGT levels returned close to the baseline (4.59 ± 0.55 IU/L), with no statistically significant difference compared with D0.

After the administration of methylprednisolone acetate, ALP and GGT values showed statistically significant elevations on multiple sampling days, whereas ALT activity showed no statistically

Table 2

Histopathological changes in the liver following a single intramuscular administration of methylprednisolone acetate

Parameter	D0	D14	D28	D56
Hepatocyte changes	0 ^b	2.57 ± 0.54^b	1.86 ± 0.69^b	2.00 ± 0.82^b
PAS reaction	0 ^b	2.15 ± 0.49^b	2.57 ± 0.54^b	1.57 ± 0.54^b
Neutrophilic leukocytes (per HPF)	2.87 ± 1.52	1.57 ± 0.79	2.71 ± 0.95	3.00 ± 1.63
Stellate cells (nuclei per 100 hepatocytes)	5.14 ± 2.19	4.43 ± 1.40	5.78 ± 1.51	5.00 ± 1.83

^b Scoring scale: 0 = none; 1 = mild; 2 = moderate; 3 = severe.

Data are presented as the mean $\pm$ SD.

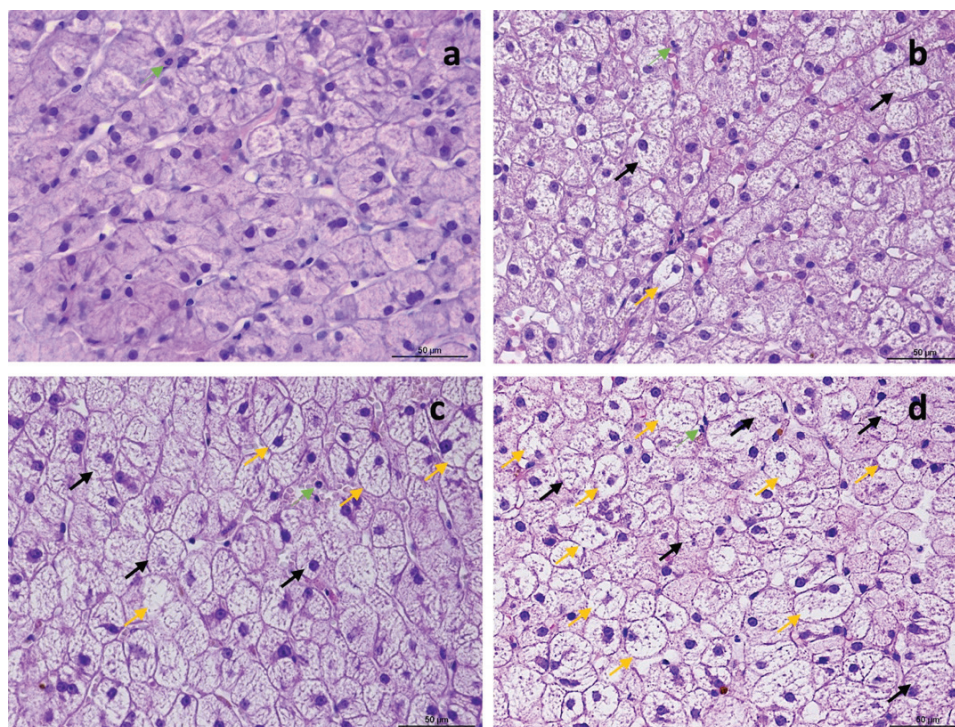


Figure 1. Representative images of (a) normal liver histology and steroid hepatopathy: (b) mild, (c) moderate, and (d) severe changes. A fine reticular cytoplasmic pattern (black arrows) with cytoplasmic vacuolation (yellow arrows) is present. Neutrophils are present within sinusoids (green arrow). H&E; 400x.

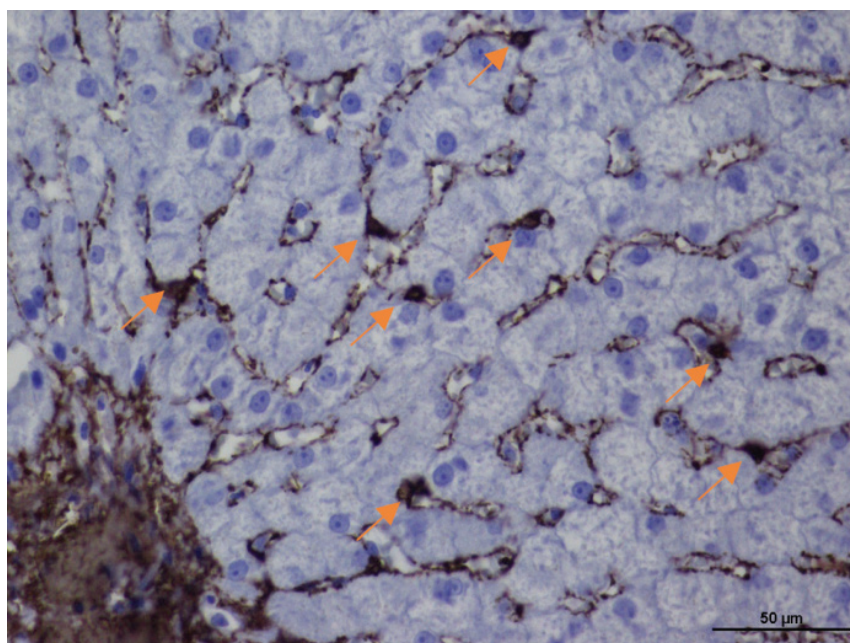


Figure 2. α -SMA-positive hepatic stellate cells with elongated cytoplasmic processes (orange arrows indicate nuclei, D28). Immunohistochemistry for α -SMA; 400x.

significant changes. By D56, all group mean enzyme values were comparable to the baseline levels.

Histopathological changes

Microscopic examination of liver tissue revealed time-dependent alterations following methylprednisolone acetate administration. The most pronounced changes were observed on Day 14, with a reduction in severity at later time points, although moderate alterations persisted until Day 56. No necrosis or fibrosis was observed at any time point. Due to the nature of needle biopsy samples, the zonal distribution of lesions could not be reliably assessed. A summary of histopathological findings is presented in Table 2, while representative microscopic images are shown in Figures 1 and 2.

Hepatocellular changes were characterised by cytoplasmic reticulation, vacuolation of varying sizes, and the marginal displacement of nuclei. These alterations affected a substantial proportion of hepatocytes and were reflected in increased hepatocyte change scores from 0 at the baseline to 2.57 ± 0.54 on Day 14 ($p < 0.05$), corresponding to severe changes according to the scoring scale, followed by values of 1.86 ± 0.69 on Day 28 ($p < 0.05$) and 2.00 ± 0.82 on Day 56 ($p < 0.05$), corresponding to moderate changes.

PAS staining demonstrated an increase in glycogen accumulation after treatment, with scores rising from 0 at the baseline to 2.15 ± 0.49 on Day 14 ($p < 0.05$), corresponding to moderate changes according to the scoring scale, and 2.57 ± 0.54 on Day 28 ($p < 0.05$), with some individuals reaching scores consistent with

severe changes. This was followed by a decrease to 1.57 ± 0.54 on Day 56 ($p < 0.05$), corresponding to mild to moderate changes.

Neutrophils were observed within hepatic sinusoids in low numbers across all time points and were not detected within the hepatic parenchyma. Neutrophil counts per high-power field showed no significant differences between sampling days ($p > 0.05$).

The number of hepatic stellate cells (expressed as nuclei per 100 hepatocytes) remained comparable across sampling points, with no significant differences detected ($p > 0.05$).

Discussion

Corticosteroids are indispensable in veterinary medicine due to their potent anti-inflammatory and immunosuppressive properties; however, their systemic effects, particularly on hepatic morphology and function, remain incompletely understood, especially when using long-acting depot formulations such as methylprednisolone acetate. In the present study, we investigated the biochemical and histopathological consequences of a single intramuscular administration of long-acting methylprednisolone acetate (2 mg kg^{-1}) in healthy dogs, focusing on temporal dynamics over a 56-day period.

The most prominent biochemical alterations involved significant elevations in serum alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) activities, while alanine aminotransferase

(ALT) remained largely stable and within the reference limits throughout the study. This pattern suggests that the hepatic response to methylprednisolone acetate is predominantly linked to enzyme induction and metabolic adaptation rather than hepatocellular necrosis. ALT, a cytosolic enzyme released during membrane disruption, showed only transient fluctuations, supporting the notion that a single exposure to this corticosteroid at the studied dosage does not produce acute hepatocyte injury. These findings are consistent with previous observations that corticosteroid-induced hepatic alterations are often functional rather than destructive in nature (Oikonomidis & Milne, 2023; Yehia *et al.*, 2013).

In contrast, the marked and sustained increases in ALP and GGT indicate enhanced enzymatic activity related to hepatocellular metabolic modulation. The rise in ALP is attributable to increased synthesis of corticosteroid-induced isoenzymes, associated with cytoplasmic vacuolation and glycogen deposition in hepatocytes, as documented in earlier studies (Fernandez & Kidney, 2007; Tinted *et al.*, 2023). Elevated GGT activity in dogs following corticosteroid administration is generally attributed to enzyme induction within hepatic microsomal pathways and, to a lesser extent, to the stimulation of bile duct epithelial cells (Oikonomidis & Milne, 2023).

In the present study, both ALP and GGT activities gradually declined towards the baseline by day 56. These findings suggest delayed hepatic adaptation after a single administration of long-acting methylprednisolone acetate rather than ongoing hepatocellular injury. Compared with previously published canine steroid hepatopathy models, the magnitude of enzyme elevation in the present study was relatively mild, not exceeding approximately 2-3-fold above the baseline, whereas markedly higher increases have been reported in experimental models using repeated or high-dose glucocorticoid administration (Badylak & Van Vleet, 1981). However, the temporal pattern was comparable, with enzyme elevations developing within the first week and peaking at around days 14 – 21. Notably, studies evaluating long-acting methylprednisolone acetate have demonstrated that serum ALP activity may remain elevated for more than four weeks following a single administration, indicating a more prolonged response compared with short-acting corticosteroids (Ginel, Lucena, & Fernández, 2002). This is consistent with the pharmacokinetic properties of methylprednisolone acetate, characterised by slow absorption and prolonged systemic exposure following intramuscular administration (Toutain *et al.*, 1986).

Histopathological evaluation provided important insight into the hepatic lesions underlying the observed

biochemical alterations. The most pronounced findings were detected on Day 14 and were characterised by cytoplasmic vacuolation, nuclear displacement, and reticulation, consistent with previously described corticosteroid-induced hepatocellular injury (Badylak & Van Vleet, 1981; Cullen & Stalker, 2016; van den Ingh *et al.*, 2021; Yehia *et al.*, 2013). Although partial improvement was observed at later time points, moderate changes persisted up to Day 56, indicating a relatively prolonged resolution phase following a single administration of long-acting methylprednisolone acetate.

PAS staining demonstrated increased glycogen accumulation after treatment; however, the extent was moderate and varied between individuals. This finding contrasts with reports describing more pronounced glycogenic hepatopathy following prolonged corticosteroid exposure (Badylak & Van Vleet, 1981; Sobczak-Filipiak *et al.*, 2014; Yehia *et al.*, 2013), suggesting that both the duration of exposure and formulation may influence the severity of histopathological changes.

Previous studies have reported a periportal predominance of hepatocellular alterations following corticosteroid exposure (Yehia *et al.*, 2013). However, in the present study, zonal distribution could not be reliably assessed due to the use of needle biopsy samples, and, therefore, no conclusions regarding lobular distribution can be drawn.

The absence of necrosis and fibrosis observed in this study is consistent with previous descriptions, indicating that corticosteroid-induced hepatopathy is primarily a reversible metabolic condition rather than a progressive fibrotic process (Cullen & Stalker, 2016; van den Ingh *et al.*, 2021).

Neutrophils were consistently observed within hepatic sinusoids in low numbers and showed no significant variation over time. Their localisation suggests that they represent circulating leukocytes rather than true inflammatory infiltration, in line with previous descriptions of minimal inflammatory involvement in steroid hepatopathy (Cullen & Stalker, 2016; Rothuizen *et al.*, 2006; van den Ingh *et al.*, 2021).

Hepatic stellate cells did not demonstrate significant changes in number or evidence of activation throughout the study period. Quantitative assessment using α -smooth muscle actin immunohistochemistry did not demonstrate detectable stellate cell activation under the conditions of this study. In contrast, previous studies have reported the activation and proliferation of hepatic stellate cells, accompanied by collagen accumulation, following corticosteroid administration (Sobczak-Filipiak *et al.*, 2014). This discrepancy may be explained by differences in treatment protocols, as

the present study evaluated a single administration of a long-acting corticosteroid, whereas previous studies used repeated administration or different corticosteroid regimens.

Conclusions

A single administration of long-acting methylprednisolone acetate at a therapeutic dose induces transient but measurable hepatic alterations in healthy dogs, characterised by reversible enzyme induction and moderate to severe vacuolar changes without evidence of persistent fibrosis or overt hepatocellular injury. While the biochemical parameters normalised by day 56, residual microscopic changes indicate that complete hepatic recovery may require a longer period. These findings emphasise the need for the careful monitoring of liver-related biomarkers during and after treatment with depot corticosteroids, especially in patients requiring repeated administration or with pre-existing hepatic compromise.

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