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# Efficacy of oral *Cynara scolymus* and *Silybum marianum* on toxicity of imidocarb dipropionate in horses

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## ABSTRACT

**Background** Despite hepatotoxic effects, imidocarb dipropionate is the drug of choice for treatment of equine piroplasmosis. It is important, therefore, to identify adjuvant therapies that may improve the safety of imidocarb dipropionate by reducing the risk of liver damage during its use. The aim of the present study was to evaluate the hepatoprotective and hepatoregulatory effects of treatment with *Cynara scolymus* and *Silybum marianum* during administration of imidocarb dipropionate.

**Methods** Ten healthy horses, seroconverted to *Theileria equi* by C-ELISA, were treated with 5 mg/kg/day of imidocarb dipropionate for three consecutive days. The study population was divided into two groups. The control group did not receive any complementary treatments. The treated group received a daily oral supplement containing *C scolymus* and *S marianum* for 30 days. Physical, haematological and histological examinations of hepatic fragments were performed.

**Results** All haematological values remained within normal range for the study population. Histological analysis revealed that treated group animals had 62 per cent less lobular inflammation, 55 per cent less pigment accumulation, 65 per cent less steatosis and 57 per cent less portal inflammation than control group animals, with an equivalent percentage of hydropic degeneration.

**Conclusion** *C scolymus* and *S marianum* supplements resulted in beneficial hepatoprotective effects in horses treated with imidocarb dipropionate.

## INTRODUCTION

Piroplasmosis, one of the main parasitic diseases that affects horses, can be found in South and Central America, the Caribbean, Africa, Southern and Eastern Europe, and the Middle East. Only the USA, Canada, Australia, Japan and Iceland are not considered endemic areas. It is a tick-borne disease caused by the haemoprotozoan parasites *Theileria equi* (formerly called *Babesia equi*) and *Babesia caballi*. More than 90 per cent of the world's total equine population is considered to live in regions infected with *T equi*,[1] underscoring the power of this disease agent's transmission system and the abundance of its vectors.[2]

Equine piroplasmosis, which is also known in the literature as equine babesiosis, theileriosis (concerning *T equi*) and biliary fever affects all equid species, including donkeys, mules and zebras.[3] Affected animals may have intermittent febrile spikes, anorexia, anaemia due to haemolysis, yellow mucous membranes with or without petechiae, limb oedema, abdominal discomfort, decreased athletic performance and even death. In addition to these serious health consequences, there are also important economic implications of the disease, including the high cost of treatment, high morbidity and restrictions on export of animals or participation in international equestrian events in disease-free countries.[2 4]

Of the drugs most commonly used for the treatment of equine piroplasmosis, imidocarb dipropionate is considered to be the safest and most effective. According to the US Department of Agriculture's Animal and Plant Health Inspection Service, treatment for piroplasmosis in horses must be performed with high doses of imidocarb dipropionate in order to ensure permanent elimination of the parasite from domestic animals. There is no doubt, however, that this drug has the potential for serious toxicity, especially in animals that have received multiple and consecutive treatments.[5] In general, the toxic effects related to imidocarb dipropionate are directly related to the wide and effective tissue distribution of the drug, the prolonged period required until its complete elimination, and the accumulation of the drug in vascular and extravascular compartments.[5]

Concurrently, the therapeutic use of medicinal plants has become widely accepted by the general public. The medical community has also begun supporting the uses of these agents, especially as their biological activities are being investigated more scientifically, proving both efficacy and safety.[6 7]

Considering the great diversity of constituents present in these medicinal plants, they carry great potential as a natural source of pharmacotherapies and prototypical molecules. It is important to note, however, that some medicinal plants used in herbal preparations require thorough quality control, as they may contain variable chemical compositions or even toxic substances.[8 9]

*Cynara scolymus* L, commonly known as artichoke, is a medicinal plant that has beneficial effects on diseases of the bile ducts and liver. Artichoke leaves contain up to 2 per cent of phenolic acids, including caffeic acid, chlorogenic acid and cinnarin; flavonoids (0.1 per cent–1 per cent); and volatile oils.[10] According to a meta-analysis by Salekzamani *et al*,[11] artichoke has health-promoting properties for a variety of diseases, with convincing evidence in animal models of its antioxidant ability to restore 'redox homeostasis'. Unfortunately, the authors of that meta-analysis were unable to suggest the best dosage or duration of treatment for artichoke due to the high heterogeneity between included studies and the equivalent antioxidant effects identified with lower (<1000 mg/kg) and higher dosages (≥1000 mg/kg).

In studies conducted in rats with hepatotoxicity induced by alpha-amanitin,[12] carbon tetrachloride[13–15] or paracetamol and, in mice with alcohol-induced acute liver damage,[16 17] it was observed that supplementation with artichoke leaf extract caused a significant decrease in the concentration of malondialdehyde (MDA) and a significant improvement in the activity of antioxidant enzymes, including superoxide dismutase, glutathione peroxidase and catalase. In addition, improvements were noted in the histopathological features of hepatocytes.[15] Using an artichoke leaf hydroalcoholic extract, El-Boshy *et al*[18] treated cadmium toxicity in rats and also observed a significant decrease in liver concentrations of MDA compared with a control group.

*Silybum marianum* L, also called milk thistle, is another medicinal plant that has been used for over 2000 years as a therapeutic herb to treat liver diseases. Silymarin is a standardised dry extract of milk thistle seeds containing mainly flavonolignans (about 70 per cent–80 per cent w/w), as well as polymeric and oxidised polyphenolic compounds, thus constituting a mixture of flavonoids.[19] The main flavonolignans of silymarin are silybin, silidianine and silichristine.[20 21]

Experimental studies in animals have shown protective effects of silymarin for hepatotoxicity induced by paracetamol, radiation, iron overload and carbon tetrachloride. These hepatoprotective effects may occur due to inhibition of lipid peroxide formation, elimination of free radicals and modification of the physical properties of cell membranes.[22]

In patients with liver diseases, several studies have demonstrated beneficial effects of treatment with silymarin, including its promotion of protein synthesis and its anti-inflammatory, immunomodulatory, antifibrotic and antioxidant activities.[19] Furthermore, silymarin has been shown to have regulatory actions on the permeability of the mitochondrial membrane and to increase cell membrane stability during xenobiotic injuries.[23] Silymarin also prevents the absorption of toxins by hepatocytes,[24] suggesting potential efficacy in the treatment of liver injuries induced by drugs or toxic substances. Schrieber *et al*[25] showed that the availability of silymarin and, consequently, its efficacy, varied with the type of liver disease. The systemic bioavailability of silymarin can be improved in a number of ways, including the addition of solubilising substances like vitamin E and phosphatidylcholine, micelle formation with bile salts, and, notably, self-emulsification, a drug-delivery system that uses a microemulsion to deliver hydrophobic drugs.[26 27]

The hepatotoxic effects of imidocarb dipropionate during treatment of horses with piroplasmosis are well established; therefore, it is important to identify adjuvant therapies that may reduce the rate of liver damage and increase the safety of this agent. Furthermore, the known hepatoprotective effects of *C scolymus* and *S marianum* suggest that these agents may be useful as an adjuvant treatment of liver diseases. The aim of the present study was to evaluate the hepatoprotective and hepatoregulatory effects associated with *C scolymus* (artichoke) and *S marianum* (milk thistle) in horses treated with imidocarb dipropionate.

## MATERIALS AND METHODS

### Animals

Ten healthy male and female Arabian cross horses with an average age of five years and weights ranging from 350 to 400 kg were used in this study. The horses were seroconverted to *T equi* using the C-ELISA technique. The horses were included in the study if they showed weight loss, or poor performance and condition or peripheral oedema. Horses presenting sudden onset of clinical signs which could lead to death, or signs as fever, inappetence, malaise and colic followed by diarrhoea were excluded.

The horses were treated at the Teaching and Research Support Centre/School of Veterinary Medicine and Animal Science - University of São Paulo (CAEP/FMVZ-USP). They were kept in a paddock without contact with ticks. Each horse was fed a total of 1 kg of a pelleted concentrate, twice a day, in addition to mineral salt. Tifton hay and water were offered ad libitum.

The horses were evaluated by a general physical examination, including assessments of heart rate, respiratory rate, rectal temperature, capillary perfusion time, skin turgor, auscultation of intestinal motility and mucosal staining; haematological tests, including blood count; biochemical assessments of the liver and muscles; and ultrasound assessments of the liver in order to exclude indicators of liver disease.

C-ELISA was used to measure antibody production resulting from an immune response to the parasite. The cut-off point for this response corresponded to a 40 per cent inhibition in colour formation, measured by reading the absorbance in a plate reader. Tested

sera that produced a 40 per cent inhibition percentage were considered positive and tested sera that produced a percentage of inhibition less than 40 per cent were considered negative.

### Application of imidocarb dipropionate

In order to eliminate the parasite from the horses and to maintain the characteristics of conservative protocols routinely used in equine medical clinics, a dosage of 5 mg/kg/day intramuscularly of imidocarb dipropionate was used for three consecutive days. This dosage was fractionated into two 2.5 mg/kg subdoses, with an hour-long interval between them.[28] The application was carried out on day 0 (D0), day 1 (D1) and day 2 (D2).

In animals that exhibited signs of parasympathetic hyperstimulation, including spasmodic colic or diarrhoea, scopolamine was administered intravenously at a dosage of 0.3 mg/kg.

### Supplementation group

The animals were randomly assigned to two groups with five animals each. To avoid order effects for each group (control or treated), 10 cards were kept in an envelope, and for each horse, the card was drawn at the time of the start of supplementation to address concealment. The control group did not receive any supplements after application of imidocarb dipropionate. The treated group received supplementation with a commercial product containing artichoke and thistle extracts (Hepvet, Vetnil, Louveira, SP, Brazil). The commercial product contained the following elements per kilogram: artichoke extract (11.53 g), milk thistle extract (96.15 g), nicotinic acid (5.385 mg), arginine (38.46 g), cynarin (61 mg), cysteine (1.154 mg), choline (7.692 mg), glycine (38.46 g), inositol (7.692 mg), glutamine (7.692 mg), calcium pantothenate (34.61 g), selenium (77 mg), taurine (7.692 mg), vitamin $B_1$ (38.46 g), vitamin $B_{12}$ (307.692 mcg), vitamin $B_2$ (5.385 mg), vitamin $B_6$ (15.38 g), vitamin C (7.692 mg), vtamin E (7.692 IU) and chelated zinc (1.154 mg).

The product was administered at a dosage of 13 g/day orally, which corresponds to 0.1503 g of artichoke extract, 0.8 mg of cynarin and 1.25 g of thistle extract. The treatment was given once a day in the early morning 1 hour before the first feeding for 30 days, starting on day D0.

Serological test samples for *T equi* were collected 30 days after the first dose of imidocarb dipropionate (D30).

### Haematological and biochemical evaluations

Venous blood samples (5 mL) from the animals were collected 3 days (D-3) and 1 day (D-1) prior to the first application of imidocarb dipropionate (D0), and 1 day (D1), 5 days (D5), 17 days (D17), 23 days (D23) and 30 days (D30) after the first application. The samples were stored in siliconised glass tubes containing 50 µl of EDTA K3 as an anticoagulant, as well as in a dry tube, and were placed on ice for further processing at the Multi-User Laboratory of Veterinary Clinical Analysis of the Faculty of Zootechnics and Food Engineer at USP.

Red blood cell and leucocyte counts, haemoglobin measurements, determinations of globular volume and haematimetric indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), as well as differential leucocyte counts, were carried out using an automatic haematology analyser (model BC 2800vet, Mindray, Shenzhen, Nanshan, China).

The following evaluations of liver and muscle functions were also performed: levels of total protein, albumin, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), direct and indirect bilirubin and creatine kinase (CK) using an automatic biochemical analyser (Randox's RX, Dublin, County Antrim, UK).

### Liver biopsies

Liver samples were collected using a percutaneous liver biopsy technique with a 14 G x 11.4 cm x 20 mm biopsy needle (Pro-Mag Ultra, Rio de Janeiro, Brazil) 3 days before imidocarb dipropionate (D-3) application, 10 days after the third application of imidocarb dipropionate (D12), and at the end of the experiment (D30).

The procedure was carried out with animals in season in a containment trunk after a 12 hours water and food fast. A 20 x 25 cm area was delineated over the right 12th and 14th intercostal spaces, at the intersection of a line established from the coxal tuberosity to the midpoint between the elbow and the tip of the scapula. This area was subjected to trichotomy and antisepsis with iodised alcohol. The animals were sedated using 0.02 mg/kg of 1 per cent detomidine, intravenously. Local infiltrative anaesthesia was then performed with 5 ml of 2 per cent lidocaine hydrochloride without vasoconstrictors. The skin incision was made using ultrasound assistance, and then the sharp biopsy needle was introduced by piercing the intercostal muscles and reaching the liver. The needle introduction was also guided by ultrasound, thus preventing the perforation of intestinal loops. The needle was directed towards the opposite elbow and, also with the aid of ultrasound, the depth necessary for the penetration of the needle into the liver parenchyma was determined (figure 1).[29 30]

### Histopathological evaluation

Liver biopsy samples were immediately fixed in a 10 per cent formaldehyde solution for 24 hours and then stored in a 70 per cent alcohol solution. Subsequently, the samples were sent to the Histopathology Laboratory of the Department of Pathology/FMVZ-USP for histological processing and manufacturing of paraffin blocks. Finally, 5 µm histological sections were stained using haematoxylin-eosin (HE) for histopathological evaluations of the liver.

Histopathological changes were graded according to their intensity, and classified as mild,[1] moderate,[2] or intense.[3] The following parameters were evaluated: presence of lobular inflammatory infiltrate, pigment

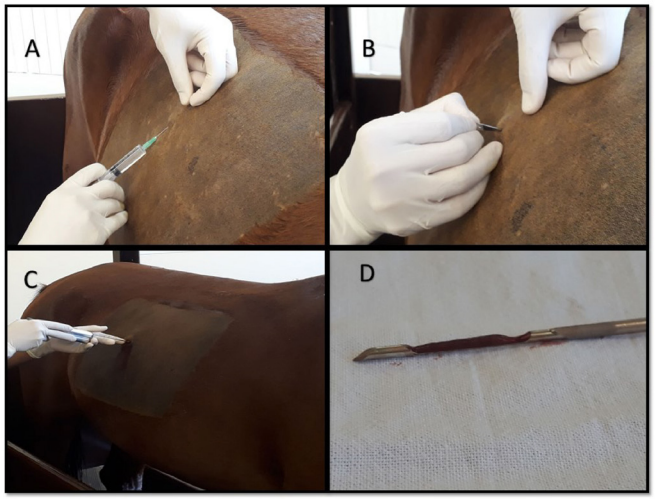


**Figure 1** (A) Local infiltrative anaesthesia. (B) Skin incision with number 15 scalpel blade. (C) TRU-CUT sharp needle piercing the intercostal muscles and reaching the liver. (D) liver biopsy.

accumulation, oedematous degeneration and portal inflammatory infiltrate.

### Statistical analysis

Statistical Package for Social Science V.19.0 was used for data analysis. For quantitative variables, normal distribution of the data was assessed using the Shapiro-Wilk test. Variables without a normal distribution were subjected to numerical transformation by the inverse, as described by Templeton.[31] Variables with normal distributions and those normalised by the inverse were submitted to the one-way test for analysis of variance over time, followed by the Bonferroni post hoc test for multiple comparisons between timepoints. The effect of time on variables with non-parametric distributions was assessed using the Friedman test, followed by the Wilcoxon post hoc test. For group comparisons within each timepoint, Student's t test for independent samples was used. For analysis of qualitative variables, the association of variables with the experimental groups was performed within each moment using the Fisher test and the $\chi^2$ test. Fisher's test was used when the variables were binary, whereas the $\chi^2$ test was used with variables that had more than two categories. For all tests, a P value of <0.05 was considered to be a significant result, with the trend >0.05 and<0.1.

## RESULTS

### C-ELISA

After study completion, another C-ELISA test was conducted on all animals to verify seronegativity for *T equi*. Despite the use of 5 mg/kg of imidocarb dipropionate, a relatively high dose, only four animals became seronegative (table 1).

### Physical examination

Four horses presented spasmodic colic and eight horses showed faeces softening on different days after 1 hour of the imidocarb diproprionate administration. All these horses were treated with scopolamine, and there was prompt remission of the clinical signs.

Heart rate, respiratory rate, rectal temperature, capillary perfusion time, skin turgor, auscultation of intestinal motility and mucosal colour did not change significantly in either group.

### Haematological evaluations

The number of red blood cells, the concentration of haemoglobin and the levels of haematocrit and platelets did not differ significantly between the groups or between different timepoints observed in the same group (P>0.05) (figure 2). MCV values increased in the treated group in relation to baseline values (D-3) on D23 and, in the control group, on D5 (P<0.05). MCH values increased in the treated group in relation to the baseline values (D-3) on D30 and, in the CG, from D17 to D30 (P<0.05). MCHC values increased in the treated group in relation to baseline values on D30 and, in the CG, on D0, D5, D17 and D30 (P<0.05) (figure 2). The values of

**Table 1** Seropositive and seronegative animals for *Theileria equi* at the beginning and after treatment with imidocarb dipropionate

| Animal | *T equi* Day 3 | % inhibition at antibody level | *T equi* Day 30 | % inhibition at antibody level |
|---|---|---|---|---|
| 1 | Positive | 85 | Positive | 82 |
| 2 | Positive | 84 | Positive | 81 |
| 3 | Positive | 87 | Positive | 81 |
| 4 | Positive | 81 | Positive | 73 |
| 5 | Positive | 53 | Negative | 4 |
| 6 | Positive | 52 | Negative | 6 |
| 7 | Positive | 72 | Positive | 62 |
| 8 | Positive | 85 | Positive | 82 |
| 9 | Positive | 55 | Negative | 10 |
| 10 | Positive | 54 | Negative | 6 |

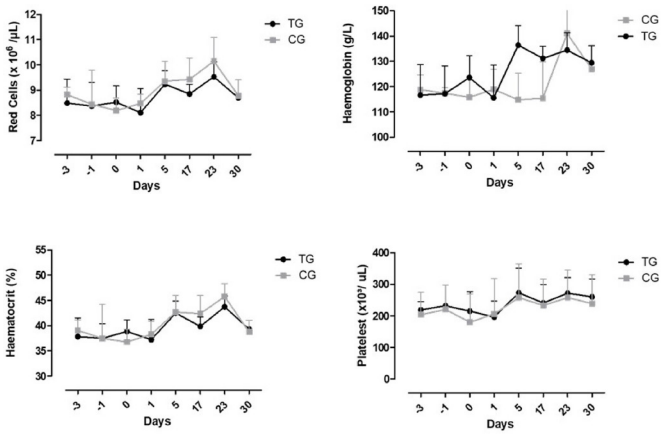


**Figure 2** Means and SD for the number of red blood cells ($10^6$/µl), haemoglobin concentration (g/dl), haematocrit values (%) and the number of platelets (x $10^3$/µl) of the animals in the TG and CG at different times of the experiment. CG, control group; TG, treated group.

MCV, MCH and MCHC did not show statistical difference between the groups.

The number of leucocytes decreased in the treated group compared with the CG on D17 and D23 (P<0.05) (figure 3). There were no significant differences in the number of eosinophils, monocytes or lymphocytes in relation to baseline values in both groups or between the groups (P>0.05).

## Biochemical evaluations

There were no significant differences in the total protein concentration in the two groups in relation to the baseline values (D-3). Albumin concentrations, however, decreased in the treated group in relation to baseline values from D0 to D30 and, in the GC, on D1, D5 and D30

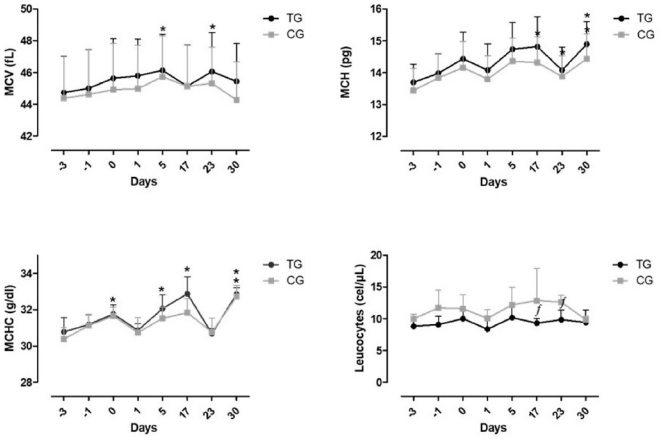


**Figure 3** Means and SDs of MCV (fl), MCH (pg), MCHC (g/dl) and leucocyte number (cell/µl) of animals in the TG and CG at different times of the experiment. * represents statistical difference in relation to the initial values; *ƒ* represents statistical difference between CG and TG (P<0.05). CG, control group; CHCM, mean corpuscular haemoglobin concentration; HCM, mean corpuscular haemoglobin; TG, treated group; VCM, mean corpuscular volume.

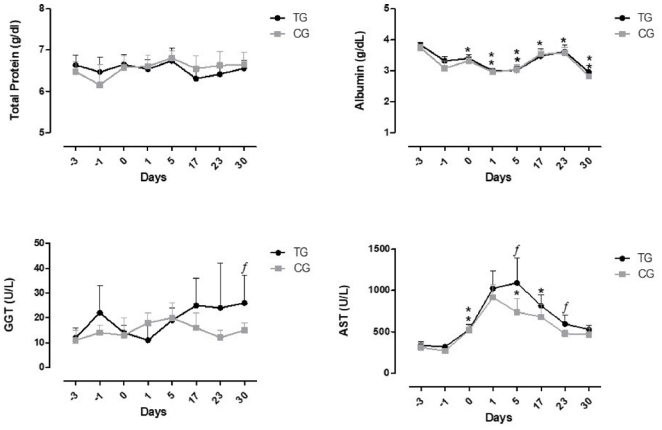


**Figure 4** Means and SDs of total protein concentration (g/dl), albumin concentration (g/dl), GGT concentration (U/l) and AST concentration (U/l) of animals in the TG and CG at different times of the experiment. * it means statistical difference in relation to the initial values; *ƒ* means statistical difference between CG and TG (P<0.05). AST, aspartate aminotransferase; CG, control group; GGT, gamma-glutamyl transferase; TG, treated group.

(P<0.05). There were no significant differences between the groups (P>0.05) (figure 4).

The concentration of GGT in both groups did not differ from baseline (P>0.05); however, an increase in the concentration of GGT was observed in the treated group in relation to the control group on D30 (P<0.05). The concentrations of AST increased in the treated group in relation to the baseline value on D0 and D17 (P<0.05) and, in the control group, on D0 and D5 (P<0.05). An increase in GGT in the treated group was observed in relation to the control group on D5 and D23 (P>0.05) (figure 4). The CK concentration increased in the treated group on D0 in relation to baseline values (P<0.05) and, in the control group, on D0 and D1 (P<0.05). The concentrations of total, direct and indirect bilirubin did not change significantly between the observed timepoints in each group or between the groups (figure 5).

## Histopathological evaluation

Liver fragments were evaluated for the presence of lobular inflammatory infiltrate, pigment accumulation, steatosis, hydropic degeneration and portal inflammatory infiltrate. These histopathological changes were graded according to intensity as mild,[1] moderate[2] or intense.[3] Histological analysis showed that animals in the treated group had 62 per cent less lobular inflammation, 55 per cent less pigment accumulation, 65 per cent less steatosis, 57 per cent less portal inflammation and an equal percentage of oedematous degeneration compared with the control group (figure 6).

## DISCUSSION

Imidocarb dipropionate is widely used in equine medicine as the drug of choice for control and prevention of infestation by the intraerythrocytic parasite, *T equi*. The

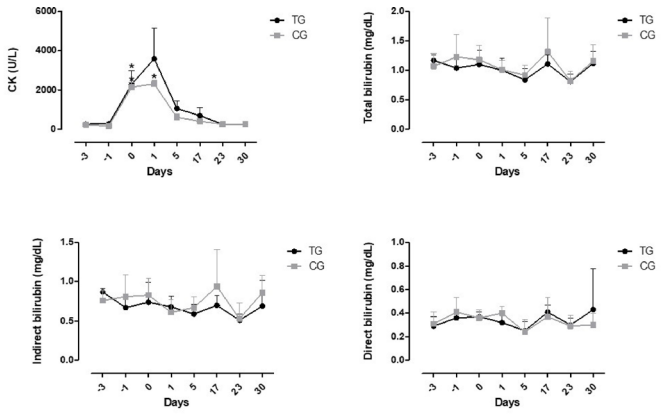


**Figure 5** Means and SDs of CK concentration (U/l), total bilirubin concentration (mg/dl), direct bilirubin concentration (mg/dl) and indirect bilirubin concentration (mg/dl) of animals in the TG and CG at different times of the experiment. * represents a statistical difference in relation to the initial values (P<0.05). CG, control group; CK, creatine kinase; TG, treated group.

toxic effects associated with imidocarb dipropionate are directly related to the wide and effective tissue distribution of the drug, the prolonged period required for its complete elimination, and the sequestration and accumulation of the drug in vascular and extravascular compartments.[5] Considering these drug properties, this study aimed to evaluate whether a supplement containing) *C scolymus* and *S marianum* could lead to a reduction in liver damage and an increase in safety during treatment of horses with imidocarb dipropionate. This was the first time that the use of these supplements has been studied in horses being treated with imidocarb dipropionate.

Of note, the animals did not exhibit any discomfort or changes in behaviour after using the formulation during the experimental period. The physiological constants also remained within normal limits throughout the treatment period.

Despite increased values for MCV, MCHC and MCH in both the treated group and control group in relation to baselines values, all values remained within the normal reference ranges for the equine species. Accordingly, the use of this formulation at the administered dosages did not affect the rheological function of the horses. Observed changes in these values may have been related to adequate control of the theileriosis, an effect that is expected after treatment with imidocarb dipropionate.

There was also an observed decrease in the number of leucocytes in the treated group at the end of the experiment compared with the control group; however, all values remained within the normal reference range for the species. The reduction of this parameter in the treated group may have been related to lower levels of liver inflammation, since it is known that silymarin has anti-inflammatory and immunomodulatory effects.[19]

GGT values remained stable in all biochemical analyses. Although most serum GGT activity occurs in the liver, this enzyme is also found in high concentrations in renal, intestinal, pancreatic and mammary gland diseases.[32 33] The GGT enzyme has a half-life of approximately 3 days in horses and is mainly associated with the membranes of the biliary epithelium. It is considered to be an excellent test to detect liver disease in horses, with greater sensitivity for chronic disease.[32 34 35] In the present study, the toxic effect of imidocarb dipropionate on the liver was considered to be acute, a feature that likely contributed to normal GGT concentrations.

Though AST has low specificity for liver disease, it is generally increased in most patients with liver disorders. In patients with chronic liver disease, AST levels are often found to be within normal range. The half-life of AST is long (7–10 days), and it may take more than 2 weeks for blood values to decrease after acute liver disease.[34] In the present study, the AST and CK enzymes increased in relation to baseline values in both groups, demonstrating that the obtained results were not exclusively indicative of liver damage. It can be assumed that the increase in these enzymes may have been due to the concomitant muscle and liver damage caused by the liver biopsy technique. It is also possible that imidocarb dipropionate, with its parenteral commercial formulation, may have caused muscle damage in the region where it is injected. This

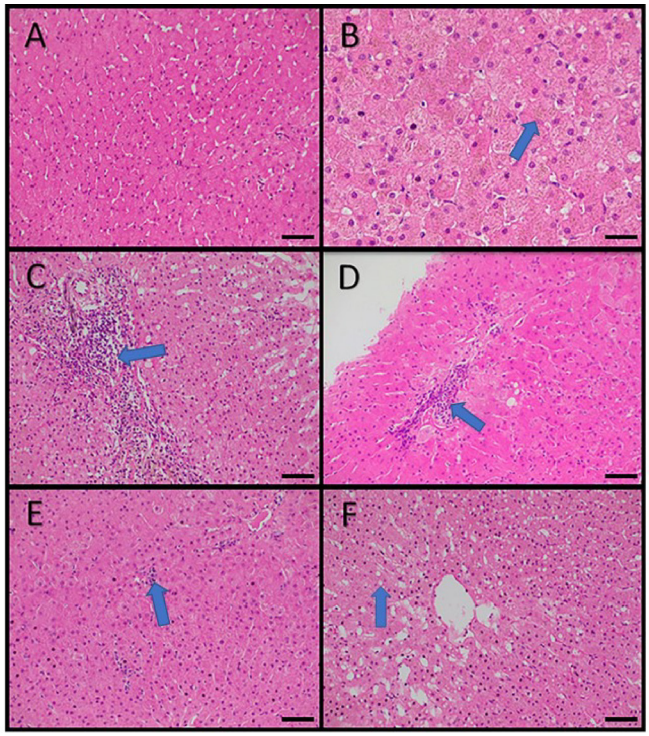


**Figure 6** (A) Liver parenchyma unchanged. Animal number 2, D3, TG. (B) Blue arrow points to the accumulation of pigment; animal number 7, D3, CG. (C) Blue arrow points to the inflammatory portal infiltrate; animal number 9, D12, CG. (D) Blue arrow points to the inflammatory portal infiltrate; animal number 8, D12, TG. (E) Blue arrow points to the lobular inflammatory infiltrate; animal number 3, D12, CG. (F) Blue arrow points to steatosis; animal number 4, D12, CG. Photos A, C, D, E and F (scale bar= 100 um) and photo B (scale bar= 50 µm). CG, control group; TG, treated group.

injury may also have been responsible for the increase in both enzymes observed in this study.[35]

The concentration of the GGT enzyme was higher in the treated group than in the control group on D5, and the concentration of AST was higher on D23 and D30 ($P<0.05$). When these two enzymes are concurrently increased, it strongly suggests liver damage. However, liver damage did not occur during this experiment, making it difficult to prove the origin of the increase in these enzymes. One possible alternative test would be the measurement of the enzyme sorbitol dehydrogenase (SDH), as it is specific for liver assessments and indicates leakage of the cytoplasmic contents of hepatocytes. In horses, SDH is present in high concentrations in the liver and in lower concentrations in other tissues, more specifically indicating hepatocellular damage than AST.[32] Unfortunately, the in vitro stability of this enzyme is much less than that of other liver enzymes, necessitating blood sample analysis within 5 hours after collection if stored at room temperature, or up to 48 hours if frozen.[32 33 36]

The assessment of serum bilirubin concentration is not a sensitive indicator of equine liver disease, as only one-fourth of horses with liver disease show an increase in the concentration of this substance.[34] In the present study, the concentrations of total, direct and indirect bilirubin remained within the normal range of values for the species.

Histopathological examinations of liver fragments obtained by ultrasound-guided or laparoscopic-guided percutaneous biopsies are the best diagnostic and prognostic means of evaluating liver disease, with more sensitivity and specificity than the biochemical tests previously listed.[37–39] In this study, histological assessments of liver samples showed an increase in portal inflammation in both groups in relation to the initial values, with lower levels of inflammation in the treated group compared with the control group on D12 (10 days after the third imidocarb dipropionate application). In the control group, lobular inflammation was also identified at different timepoints during the experiment; however, it was absent in the treated group after imidocarb dipropionate application (D12 and D30). It can be assumed that these results were mainly due to the inclusion of cinnarin (1,3-O-dicafeoylquinic acid) and the synergism of other products in the selected commercial product, with properties known to prevent aggravation of liver toxicity.

High-energy reactive metabolites derived from the toxic liver effect can form covalent bonds, or adducts, with other cellular constituents, including proteins and nucleic acids. In acute toxicity, adducts can lead to cell injury or death.[40]

It can be assumed that the animals in the treated group did not make these covalent bonds, decreasing the acute toxic effect. This supposition is supported by the known action of *C scolymus* (artichoke) in decreasing lipemic levels, including total cholesterol and low-density lipoprotein cholesterol, as proven in systematic reviews and randomised clinical trials.[40] Since the ideal concentration and dose-dependent effects of cinnarin are difficult and impractical to obtain by food,[41] artichoke extracts contained in supplements and medications may be a more practical source.

Steatosis outbreaks were identified in some animals in the treated group at the end of the experimental period (D30); however, all horses in the control group experienced steatosis throughout the duration of the experiment. Steatosis may be due to malfunctioning of the hepatocytes, which may lead to decreased energy for oxidation of fatty acids and the accumulation of triglycerides inside the cells.[40] The presence of steatosis in only some treated group animals on D30 suggests that the formulation used had antioxidant substances that helped to reduce this type of injury. It is known that both artichoke and thistle have these antioxidant activities.[11 19]

Pigment deposition was identified in the treated group animals before and at the end of the experiment (D-3 and D30) and, in the control group animals, at all times. Pigments are substances that give unusual colouring to the body or to specific tissues, and can be either exogenous or endogenous.[40] It is believed that the pigment accumulation observed in this study was mostly related to the injury caused at the time of the biopsy, leading to haematogenic pigment formation.

Hydropic degeneration cannot be identified without other concomitant histological lesions and is usually associated with inflammatory changes.[42] In this study, hydropic degeneration was found in animals in both the treated group and the control group at 10 days after the application of imidocarb dipropionate (D12). This finding may have been observed at this timepoint because imidocarb dipropionate had reached maximum blood concentration levels, and this drug is known to cause direct damage to the hepatocytes, which results in inflammation and, eventually, in hydropic degeneration.

According to Hackett *et al*,[42] portal inflammation and steatosis are the primary histopathological lesions of the liver identified in horses. Similar findings were observed in the present study. According to these authors, drugs with anti-inflammatory and antifibrotic properties would best facilitate hepatic metabolism and favour the regeneration of hepatocytes.

In the present study, it is inferred that the antioxidant and betaoxidation regulatory activities associated with *C scolymus* (artichoke) and *S marianum* (silymarin) resulted in effects that facilitated liver metabolism, contributing to the positive results observed in the treated group. Further studies using increasing dosages of these compounds are recommended for a more comprehensive evaluation.

## CONCLUSION

It is concluded that the use of a supplement formulation containing *C scolymus* and *S marianum* resulted in beneficial hepatoprotective effects in horses receiving imidocarb dipropionate.

**Contributors** The experiment was conceptualised by FMJ, RRC and RYAB; methodology: FMJ, FCP, BC and RYAB; validation of data was performed by FCP, RRC and BC; formal analysis was done by FMJ and RYAB; investigation was done by FMJ, DDVP, FCP and RRC; resources were looked up by RRC and RYAB; curation and preparation, visualisation of data and writing—original draft preparation were performed by FCJ and RYAB; supervision and writing of the review was done by all coauthors; editing was done by FCJ and RYAB; the project was administrated by FCJ and RYAB.

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