SUMMA

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Treatment with Complementary Hepatoprotector Feed

Experience in 14 dogs with phenobarbital hepatopathy

Tiziana Cocca, Freelance Veterinarian, Naples

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Treatment with Complementary Hepatoprotector Feed

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Experience in 14 dogs with phenobarbital hepatopathy

Acknowledgements
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Abstract
Phenobarbital is a first choice drug in controlling idiopathic epilepsy in dogs. It can cause secondary hepatopathy (reversible), which can develop into primitive hepatopathy (irreversible) in ca 14% of cases. This clinical trial studies an early approach to the evaluation of complementary hepatoprotector feed during long-term administration of phenobarbital through one year testing of the levels of enzymic hepatopathy markers ALT, AST, PA and γGT in 14 adult dogs treated with phenobarbital and concurrently administered complementary hepatoprotector feed.

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It is common knowledge that phenobarbital is the choice treatment in controlling idiopathic epilepsy in dogs. It is generally administered for life to achieve control of seizures. The drug's side-effects count its well known and documented effect on the liver (Jaggy, 2003; Ettinger & Feldman, 2008).
A 2-12-fold increase caused by microsomial induction in some enzymic hepatopathy markers (PA - ALT - AST - γGT - figures 1, 2, 3, 4, 5, 6, 7, 8)

(Meyer & Harvey, 1998), compared to the normal range, is noticed soon after treatment commences (2 – 3 weeks). This ongoing induction leads to hepatic cellular hyperplasia, which, in turn, causes metabolic hyperactivity in the liver and activates transcription of genes that encode various degrading enzymes (box 1). Following this hyperactivity, the liver degrades the various substances at a faster pace, especially corticosteroids, metronidazole, β-blockers and phenobarbital (Kakizaki S. et al., 2003). This biochemical mechanism is called "tolerance" and its practical outcome is the need to increase some of these drugs' doses to maintain effective doses. Some months after the treatment's commencement there can be signs of toxicity with hepatocellular damage, periportal inflammatory response and, in time, fibrosis and cirrhosis (box 2). It has been extensively proved that this occurs both in human medicine and in at least 14% of dogs under treatment for minimum 6 months (Levine, 1990; Kinze et al, 1980). Hence, hepatopathies resulting from the ongoing use of phenobarbital can be distinguished in two phases:

Phase 1 – REVERSIBLE

Phase 2 – IRREVERSIBLE.

The clinician must be able to distinguish the two phases of phenobarbital hepatopathy. Many protocols have been proposed to detect the hepatopathy phase on the basis of liver function tests, especially bile acid tests (Jaggy, 2003; Ettinger & Feldman, 2008).

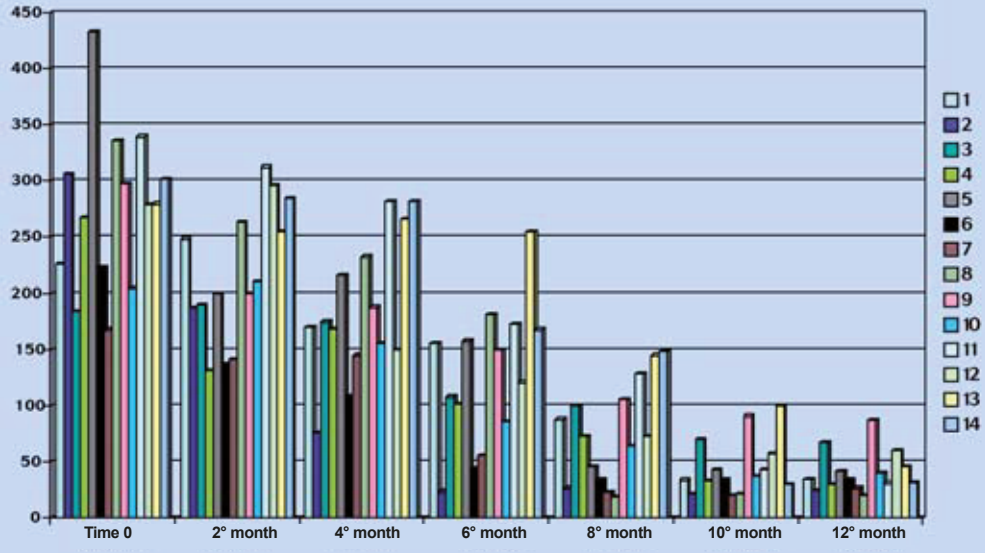
It is also essential to understand whether there are tools to prevent the transition of hepatopathy from the reversible phase (secondary) to the irreversible one (primitive).

This study focuses on describing the changes – in a 12-month period – in the levels of enzymic hepatopathy markers (PA – ALT – AST – γGT) in 14 dogs with idiopathic epilepsy and secondary hepatopathy that can be associated with ongoing phenobarbital treatment. The dogs were concurrently administered complementary hepatoprotector feed.

Material and methods

The study selected 14 dogs with hepatopathy associated with antiepileptic drug treatment for idiopathic epilepsy.

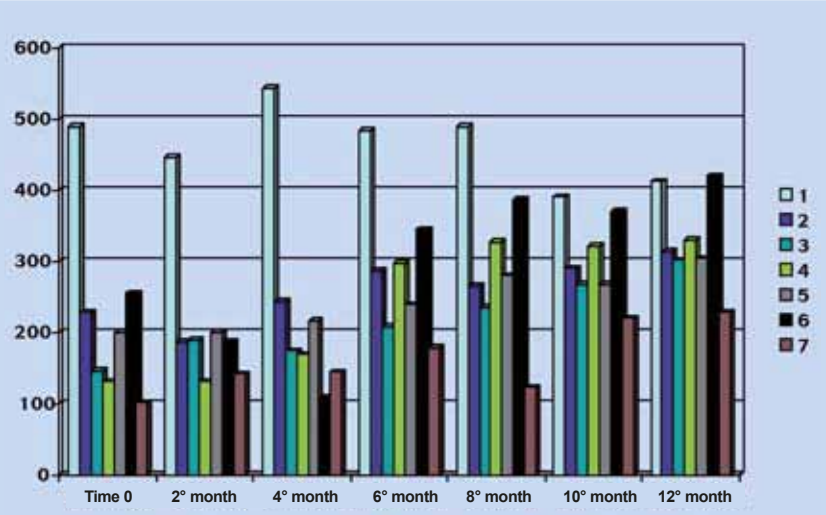
Inclusion criteria in the trial:
1. adult (min. 3 years);
2. diagnosis of idiopathic epilepsy;
3. under phenobarbital treatment for min. 6 months;



Progress in time of test results (ALT) obtained in all subjects studied during treatment with complementary hepatoprotector feed.

Figure 1

4. normal hepatopathy marker levels (alkaline phosphatase – ALT – AST – γ GT) before the treatment's commencement;
5. alteration of at least 3 on 4 hepatopathy markers (which at least double) for min. 3 months;
6. normal bile acids (before meals <25 m.mol/l; after meals <50 m.mol/l).
Subjects presenting altered liver function (bile acid levels before and/or after meals > 25% of our clinical laboratory's reference range) or other concurrent diseases that increase enzymic hepatopathy markers during the study were removed from the same. All cases were administered complementary hepatoprotector feed (at recommended doses, concurrently with phenobarbital treatment and never discontinued).
Samples were taken to test the total blood count, urinalysis and biochemical profile every 30th day following administration of complementary hepatoprotector feed for 12 months.

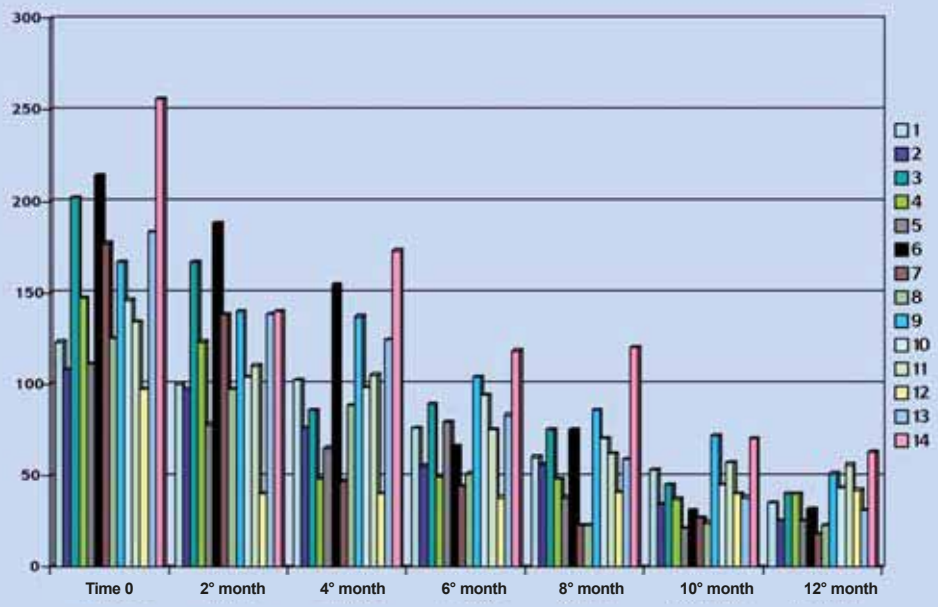


Progress in time (ALT) in subjects not treated with complementary hepatoprotector feed (controls).

Figure 2

CONTROL GROUP – 7 dogs chosen at random from the clinic's patients that met the same criteria as those included in the trial were not treated with complementary hepatoprotector feed.
All subjects included in the study attended our clinic for an examination over a five-year period (2001–2006).

Phenobarbital hepatopathy monitoring protocol (Jaggy, 2003):
- test blood levels of phenobarbital (every 3 months – 2 hours after the drug's administration – starting 2 –3 weeks after the treatment's commencement);
- test bile acids every 6 months.



Progress in time of test results (AST) obtained in all subjects examined during treatment with complementary hepatoprotector feed.

Figure 3

The protocol adopted to diagnose idiopathic epilepsy in subjects classified as “epileptics” following the clinical examination and neurological test was the following (Bernardini, 2004 – Ettinger & Feldmann, 2008):

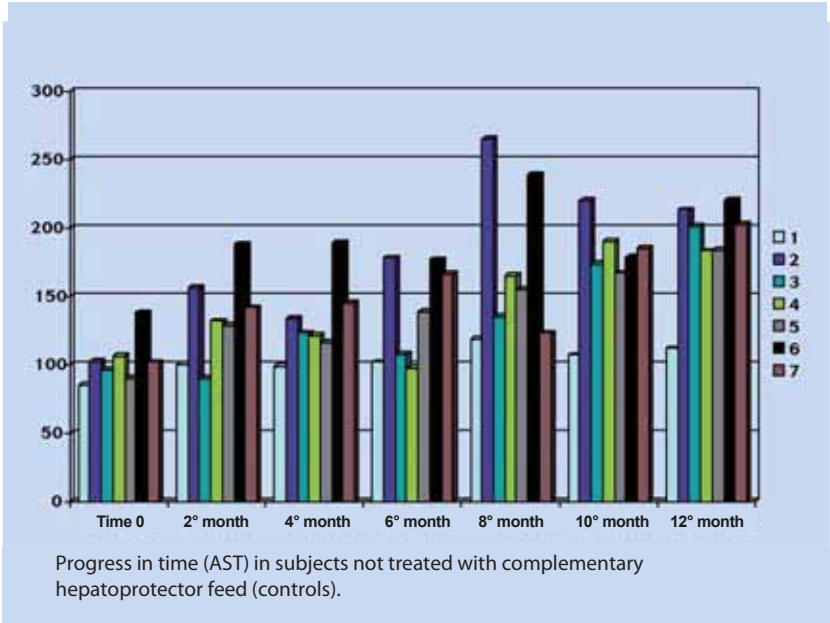
1. report of onset at a young age (from 1 to 4– 6 years);
2. no infectious disease that could be diagnosed with common tests (i.e. serum tests for: distemper,
neosporosis, toxoplasmosis, ehrlichiosis, leishmaniosis);
3. exclusion of severe metabolic diseases or disorders that could cause seizures (hypercorticism, hypothyroidism, diabetes mellitus, hyperinsulinaemia, renal failure, hypocalcaemia);
4. abdominal ultrasound scan within normal limits;
5. chest X–ray within normal limits;

CT scan of the CNS within normal limits.

Results

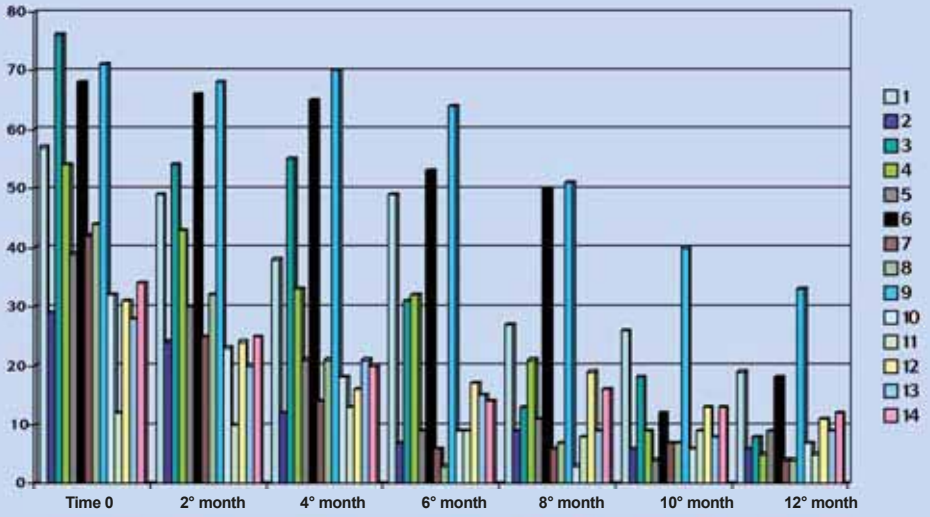
Table 1 summarises the reference range for laboratory dogs that underwent the said biochemistry tests.

Tables 2, 3, 4 and 5 summarise the biochemical profile of treated subjects before commencing treatment with complementary hepatoprotector feed, and on the 4th, 8th and 12th month.



Progress in time (AST) in subjects not treated with complementary hepatoprotector feed (controls).

Figure 4



Progress in time of test results (γGT) obtained in all subjects examined during treatment with complementary hepatoprotector feed

Figure 5

Discussion and conclusions

The complementary hepatoprotector feed administered contains titred sylmarin and mannan oligosaccharides (MOS).

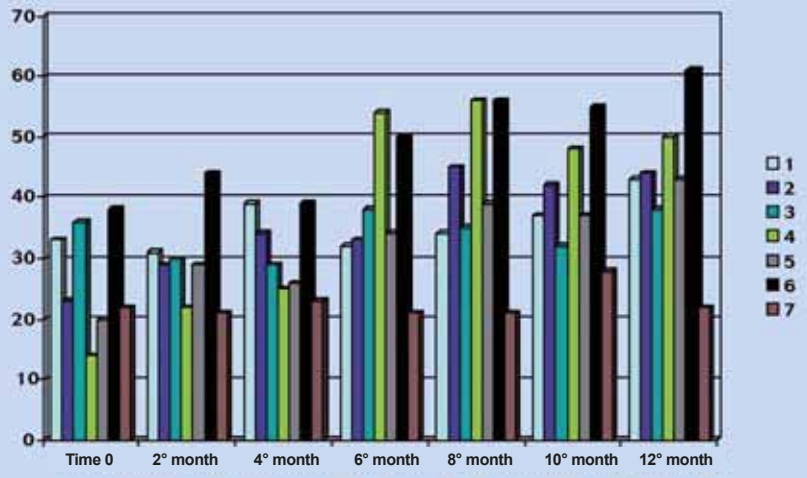
The effects of sylimarin – standard mixture of flavonolignans extracted from cardo mariano (Silybum marianum) seeds and of one of its structural components, sylibin (pure, chemically defined substance) (Laeckeman et al) – are well known in the treatment of various types of liver diseases in humans; the substance has been long adopted by human medicine (Flora et al., 1998; Wellington et al., 2001; Lirussi & Okolicsanyi, 1992). Its medical importance clearly results from the exponential rise in the number of papers published (i.e. over 800) on the topic in the past 5 years (Saller et al., 2007). Various trials on humans have proved that administering sylmarin to patients with various types of liver diseases considerably reduces serum levels of hepatopathy markers AST, ALT and γGT. (Wellington & Jarvis, 2001) The mechanism behind these substances' activity has not been entirely explained from a biochemical perspective. (Valenzuela et al., 1994).

However, three essential action mechanisms have been defined and studied, especially in laboratory animals (Saller et al, 2007; Zuber et al, 2002; Medina & Moreno-Otero, 2005; Fiebrich & Koch, 1979; Fiebrich & Koch, 1979; Basaga et al, 1997):

1) antioxidant activity performed by increasing intracellular glutathione concentration and eliminating preoxidant free radicals; the latter depends on the activation of the enzyme lipoxygenase;

2) membrane permeability regulation and stability enhancement (i.e. protection from foreign agents);

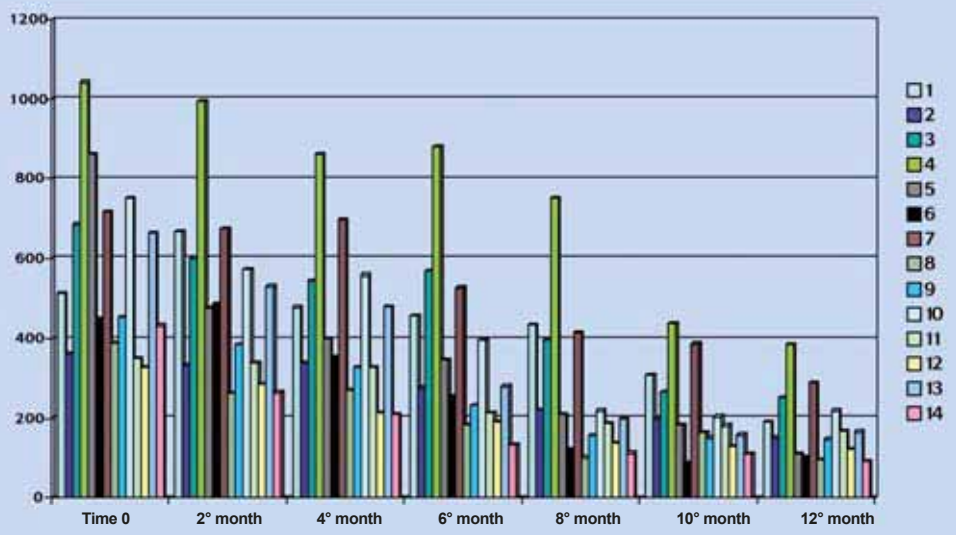
3) increased synthesis of ribosomial RNA by stimulating DNA polymerase and exerting a regulatory action even on DNA transcription.



Progress in time (γGT) in subjects not treated with complementary hepatoprotector feed.

Figure 6

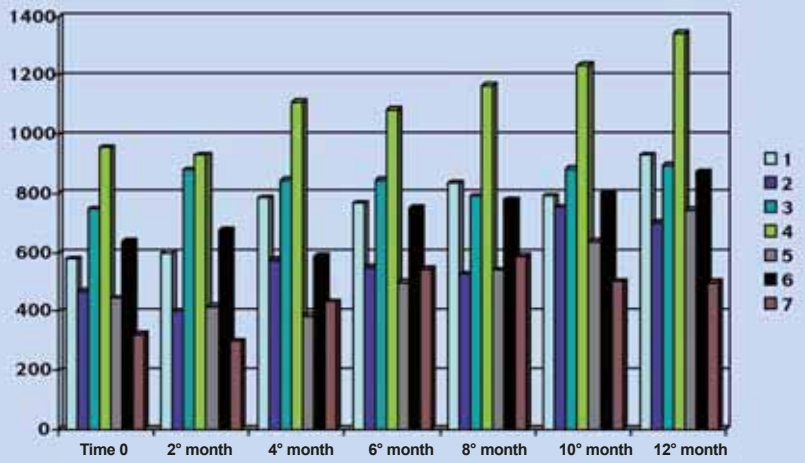
The liver protective action depends both on the aforementioned biochemical processes and on some specific actions, especially ethanol toxicity (Par, 2000; Lieber, 2005), acetaminophene, galactosamine (Meyer-Burg, 1972) and the Amanita phalloides toxin (Vogel & Temme, 1969). In the latter case the action consists in competing for receptor sites on hepatocytes – this has been clearly proved even by veterinary medicine (Floersheim et al., 1978; Vogel et al., 1984). Ethanol's toxic effect on the liver – a key issue in human medicine – mainly depends on the activation of the enzyme alcohol-dehydrogenase.
This enzyme converts nicotinamide adenine dinucleotide (NAD) into reduced NAD, which causes hyperuricaemia, hypoglycaemia and hepatic steatosis by inhibiting lipid oxidation and activating lipogenesis (Platt & Schnorr, 1971). Moreover, ethanol is metabolised in the liver through the MEOS route (Microsomal Ethanol Oxidizing System), whose main enzyme is cytochrome P450. Both this cytochrome and its encoding gene's activity is enhanced by chronic intake of alcohol and leads to metabolic tolerance to ethanol (Zuber et al., 2002). Cytochrome P450 detoxicates many other substances, and its activity is often associated with the production of an excess of free radicals, which leads to membrane lipid peroxidaton with subsequent cell damage and secondary inflammatory reaction (Bosisio et al., 1992; Vitaglione et al., 2004). Oxidative stress is a common pathogenic process both in the initial and progressive phase of many liver diseases that either increase free radicals or reduce antioxidant substances (Feher et al., 1998).



Progress in time of test results (PA) obtained in all subjects examined.

Figure 7

It is clear that, on the basis of such evidence, antioxidants are a logical therapeutic strategy in the treatment of many liver diseases. (Medina & Moreno-Otero, 2005). With its antioxidant activity, sylmarine merges well with the treatment of chronic alcohol-related hepatitis and the many pathological conditions related with cytochrome p450 hyperactivation



Progress in time in subjects not treated with complementary hepatoprotector feed (controls).

Figure 8

(Zhang et al., 2005). It has also been proved that Sylmarine can inhibit collagen synthesis by means of DNA/RNA mediated effects and this indicates its use as an antifibrotic agent. (Saller et al, 2007).

Phenobarbital hepatopathy

PHASE 1 (reversible):
enzymic microsomial induction >ALP
ALT > AST (2 -12 F)

↓

hepatocellular hyperplasia

↓

metabolic hyperactivity

↓

faster elimination of some drugs and/or toxic substances (corticosteroids, metronidazole, β-blockers...).

Figure 1

Phenobarbital hepatopathy

PHASE 2 (irreversible):
It occurs in ca 14% of dogs under treatment for min. 6 months.

↓

Hepatocellular damage

↓

Inflammation (periportal)

↓

Fibrosis

Figure 2

It also possesses an evident hypocholesterolemising activity most likely by inhibiting the synthesis (Gazak et al. 2007; Skottovà & Krecman, 1998) that indirectly but essentially enhances sylmarine's sustaining activity in the liver metabolism. In fact, considering the liver's essential role in plasma lipoprotein metabolism regulation, the various types of liver damage often cause secondary dyslipoproteinaemia associated with hypercholesterolaemia and even severe consequences (atherosclerosis). Sylmarine promotes and improves the liver's regenerative capacity by stimulating protein synthesis in the liver. (Magliulo, 1994; Wellington & Jarvis, 2001)

Many in vitro and in vivo studies on cancer models suggest this substance's potential use both in controlling the side-effects of some chemotherapeutic agents (Bokemeyer et al., 1996) and in treating (Aggarwal & Shishodia, 2006; Mc Kllop & Schrum, 2005) and preventing them (Wright et al., 2006; Tyagi et al., 2002).

Reference values applied to tested laboratory dogs		
BUN	mg/dl	15-45
Creatinine	mg/dl	0.5-1.8
Glucose	mg/dl	50-100
Total proteins	g/dl	6-7.5
Albumin	mg/dl	2.5-4.2
Globulins	mg/dl	2.2-4.5
A/G ratio		0.5-1.3
Bilirubin	mg/dl	0.0-0.7
AST	UI/lt a 25 °C	7-40
ALT	UI/lt a 25 °C	7-40
Gamma GT	UI/lt a 25 °C	1-10
PA	UI/lt a 25 °C	5-110
Total cholesterol	mg/dl	140-240
Triglycerides	mg/dl	50-200

Table 1

Especially sylmarin's topical and/or systemic use reveals protective properties against UV-induced skin damage and up regulation of tumour-suppressor genes p-53 and p21cp1. (Singh & Agarwal, 2005)
Studies on the use of sylmarine in veterinary medicine, especially in dogs, are doubtless fewer and more recent (Fillburn et al., 2007). Anyhow, even in this species the hepatoprotective activity performed seems to be confirmed. (Paulova et al., 1990)
The synergic effects shown by sylmarine towards certain drugs are of considerable interest, since it improves, for instance, the efficacy of phenbendazole in giardiasis in dogs (Chon & Kim, 2005) or that of amiodarone in preventing atrial flutter (Konia et al., 1993; Vereckei et al., 2003). This data suggests possible uses in veterinary medicine that are not envisaged by human medicine.
MOS originate from yeast (Saccaromices cerevisiae) wall lysis and produce well known effects in veterinary medicine. They can influence ileal and faecal bacterial composition by increasing bifidobacteria and lactobacilli content to encourage balanced intestinal ecology (Weese et al., 2004). They can also modulate certain immune functions by influencing immunocompetent cell balance in circulation (Grieshop et al., 2004).

References are available on request.

No studies have evaluated the MOS–sylmarine combination's efficacy in controlling liver diseases. In this regard, the consideration that substances may cooperate metabolically is of considerable interest, since maintaining the intestinal ecosystem's homeostasis prevents proliferation of pathogenic flora that is potentially harmful for the liver both as such and due to any toxins produced. Furthermore, MOS' capacity to compete for intestinal receptor sites that allow absorption of certain food toxins (i.e. aphlatoxins, micotoxins), which are a potential cause of both acute and chronic severe liver damage (Swanson et al., 2002), form an interesting synergic action with sylmarine in preventing liver damage associated with chronic toxicity.

Biochemical profile of subjects treated before starting treatment with complementary hepatoprotector feed

case n°	BUN	Crea	Glu	P. T.	Alb	Glo	A/G	Bil	Ast	Alt		γGt	PA	Col	Tri
1 -	21	0,8	108	6,7	3,7	3,0	1,2	0,14	123	226	57	514	185	89	
2 -	34	1,2	98	6,9	4,0	3,9	1	0,20	108	306	29	361	170	103	
3 -	27	1	89	7,4	3,2	4,2	0,8	0,12	202	184	76	684	132	78	
4 -	31	0,6	97	7,2	3,6	3,6	1	0,10	147	267	54	1042	145	88	
5 -	24	0,8	100	6,8	3,8	3	1,2	0,14	111	432	39	861	165	76	
6 -	25	1,1	97	7,1	3,4	3,7	0,9	0,11	214	223	68	449	134	108	
7 -	30	0,7	99	7,5	3,4	4,1	0,80	0,13	177	168	42	717	136	90	
8 -	22	1	107	7,1	3,8	3,3	1	0,14	125	335	44	388	150	75	
9 -	17	0,9	100	7,7	3,8	3,9	1	0,18	167	297	71	453	128	86	
10 -	26	0,9	96	7,4	3,9	3,5	1,1	0,10	146	204	32	751	176	75	
11 -	18	1	99	7,7	3,4	4,3	0,80	0,11	134	339	12	350	124	77	
12 -	29	0,8	102	7,2	3,1	4,1	0,7	0,20	97	278	31	328	130	81	
13 -	18	0,7	106	7,5	3,5	4	0,9	0,13	189	275	28	664	144	65	
14 -	22	1	87	7,8	4,0	3,8	1	0,15	256	301	34	432	153	100	

Table 2

Biochemical profile of subjects treated 4 months after starting treatment with complementary hepatoprotector feed

case n°	BUN	Crea	Glu	P. T.	Alb	Glo	A/G	Bil	Ast	Alt		γGt	PA	Col	Tri
1 -	21	0,8	84	6,8	3,4	3,4	1	0,15	102	170	38	478	162	90	
2 -	37	1,1	101	7,0	3,9	3,1	1,1	0,21	76	76	12	340	174	121	
3 -	21	0,8	100	7,3	3,1	4,2	0,8	0,12	86	175	55	546	148	65	
4 -	28	0,9	88	7,6	3,6	4,0	0,9	0,10	48	169	33	860	145	88	
5 -	24	0,8	100	6,8	3,8	3	1,2	0,14	65	216	21	400	165	76	
6 -	25	1,1	97	7,1	3,4	3,7	0,9	0,16	154	109	65	355	134	98	
7 -	32	0,8	98	7,6	3,3	4,3	0,8	0,18	47	145	14	698	109	91	
8 -	28	1	100	7,3	3,7	3,6	1	0,14	88	232	21	271	133	79	
9 -	23	0,7	109	7,6	3,8	3,8	1	0,16	137	188	70	328	108	81	
10 -	23	0,8	102	7,4	3,8	3,6	1	0,16	98	156	18	558	155	90	
11 -	27	1,2	82	7,5	3,2	4,3	0,80	0,10	105	281	13	327	104	73	
12 -	33	1,1	107	7,4	3,5	3,9	0,9	0,19	40	150	16	213	139	66	
13 -	22	0,7	97	7,6	3,9	3,7	1	0,10	124	266	21	479	121	86	
14 -	28	1,1	103	7,6	3,8	3,8	1	0,18	173	281	20	210	144	73	

Table 3

Sylmarine's capacity to effectively control the increase in enzymic hepatopathy markers during phenobarbital treatment has long been proved in human medicine (Carrescia et al., 1980). Results obtained by this trial suggest that the addition of complementary hepatoprotector feed to the therapeutic protocol of subjects under phenobarbital treatment could reduce enzymic hepatopathy marker (PA, ALT, AST, γGT) levels evaluated in this trial even in dogs. Hence, the prospect of reducing the percentage of cases in which reversible liver damage develops into irreversible primitive hepatopathy and its consequences seems interesting.

Biochemical profile of subjects treated 8 months after starting treatment with complementary hepatoprotector feed

case n°	BUN	Crea	Glu	P. T.	Alb	Glo	A/G	Bil	Ast	Alt		γGt	PA	Col	Tri
1 -	27	0,7	88	7,1	3,6	3,5	1	0,14	60	88	27	433	132	96	
2 -	35	1,1	97	7,2	3,9	3,3	1,1	0,20	56	27	9	220	123	80	
3 -	22	0,6	106	7,5	3,3	4,2	0,8	0,17	75	100	13	397	112	77	
4 -	24	0,7	80	7,8	3,8	4,0	1	0,14	48	73	21	751	99	85	
5 -	29	1,1	103	6,9	3,8	3,1	1,2	0,11	38	46	11	210	144	70	
6 -	26	1,1	97	7,1	3,4	3,7	0,9	0,16	75	34	50	120	134	98	
7 -	26	0,9	101	7,0	3,1	3,9	0,8	0,2	23	23	6	415	133	87	
8 -	28	1	100	7,3	3,7	3,6	1	0,14	23	19	7	100	133	79	
9 -	32	1,3	98	7,4	3,6	3,8	1	0,12	86	106	51	231	141	74	
10 -	21	0,8	91	7,4	3,6	3,8	1	0,15	70	65	3	397	125	92	
11 -	30	1	101	7,8	3,5	4,3	0,8	0,15	62	129	8	212	139	86	
12 -	36	1	104	7,5	3,5	4,0	0,9	0,14	41	73	19	190	121	94	
13 -	25	0,9	110	7,4	3,9	3,5	1	0,17	59	145	9	280	148	59	
14 -	23	1	95	7,9	3,8	4,1	1	0,2	120	149	16	132	103	88	

Table 4

Biochemical profile of subjects treated 12 months after starting treatment with complementary hepatoprotector feed

case n°	BUN	Crea	Glu	P. T.	Alb	Glo	A/G	Bil	Ast	Alt		γGt	PA	Col	Tri
1 -	27	0,7	88	7,1	3,6	3,5	1	0,14	35	35	19	190	132	96	
2 -	35	1,1	97	7,2	3,9	3,3	1,1	0,20	25	25	6	150	123	80	
3 -	22	0,6	106	7,5	3,3	4,2	0,8	0,17	40	67	8	250	112	77	
4 -	24	0,7	80	7,8	3,8	4,0	1	0,14	40	30	5	384	99	85	
5 -	29	1,1	103	6,9	3,8	3,1	1,2	0,11	25	42	9	110	144	70	
6 -	26	1,1	97	7,1	3,4	3,7	0,9	0,16	32	34	18	102	134	98	
7 -	26	0,9	101	7,0	3,1	3,9	0,8	0,2	18	27	4	290	133	87	
8 -	28	1	100	7,3	3,7	3,6	1	0,14	23	10	4	96	133	79	
9 -	32	1,3	98	7,4	3,6	3,8	1	0,12	51	87	33	148	141	74	
10 -	21	0,8	91	7,4	3,6	3,8	1	0,15	43	40	7	219	125	92	
11 -	30	1	101	7,8	3,5	4,3	0,8	0,15	56	31	5	167	139	86	
12 -	36	1	104	7,5	3,5	4,0	0,9	0,14	42	60	11	121	121	94	
13 -	25	0,9	110	7,4	3,9	3,5	1	0,17	31	46	9	165	148	59	
14 -	23	1	95	7,9	3,8	4,1	1	0,2	63	32	12	90	103	88	

Table 5

REFERENCES

References
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