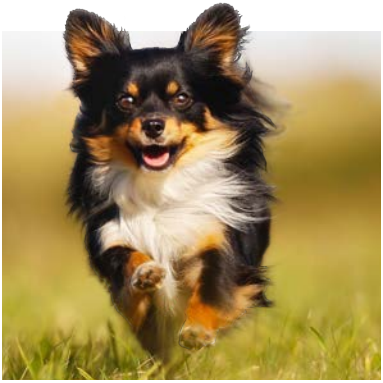
Antinol®

THE EXECUTIVE SUMMARY 2024

What is Antinol® (PCSO-524®)?

Antinol® is a natural anti-inflammatory supplement for the veterinary market containing a patented lipid extract isolated from the New Zealand green-lipped mussel, Perna canaliculus, called PCSO-524®.



Antinol® contains PCSO-524® in combination with olive oil, and a small amount of vitamin E added as an antioxidant.

The patented process involves extracting the oil from green lipped mussels which are first stabilised and freeze-dried.

Early in vitro and in vivo research indicated significant anti-inflammatory properties. However, this research also revealed inconsistencies in the results due to the poor stabilisation of the lipids. Subsequent research and further development culminated in the stabilised patented lipid extract PCSO-524® found in Antinol®.

PCSO-524® contains a unique combination of free fatty acids, sterol esters, polar lipids, and carotenoids, and has been shown to be a 5-lipoxygenase (LOX) [1] and cyclooxygenase 2 (COX-2) modulator [2] providing a potent anti-inflammatory effect. [3-6] The majority of the research using PCSO-524® has been in support of its anti-inflammatory activity, particularly in arthritis, where it has been compared to non-steroidal anti-inflammatory drugs (NSAIDs) in several animal clinical trials.

The manufacture and extraction process of PCSO-524® has been developed over many years and is covered by several international patents.

The extraction is undertaken using supercritical fluid extraction (SFE) technology which uses liquid carbon dioxide (CO_2) as a solvent. CO_2 is an ideal solvent as it becomes liquid under increased pressure and after extracting the oil the pressure is raised, and the CO_2 turns back into a gas leaving the extract intact.

PCSO-524® is produced in Nelson, New Zealand where the green-lipped mussels are farmed in pristine waters.

The stabilisation process and freeze drying of the mussels is conducted by Pharmalink International (PIL) shareholder and manufacturing partner MacLab and the extraction is completed in a state of the art facility owned by Pharmalink Extracts Limited. The patented PCSO-524® extract is sold in more than 40 countries around the world in soft-gel capsules, blister packed in aluminium foil, which are encapsulated and packaged by a number of GMP-approved facilities around the globe.

Unique features of

Antinol®

/ Point of difference

The unique lipid extract within Antinol® is different in structure compared with other marine oils (i.e. fish oils) in the chemical bonding of the omega fatty acids and incorporates a wide diversity of lipid classes.

91 individual fatty

There are over 91 individual fatty acids reported within the lipid extract with only 16 at concentrations greater than 1% of the total fatty acids [7]

Anti-inflammatory compound

Specific fractionation studies have shown a range of anti-inflammatory compounds found within the PCSO-524® matrix [8,9]

The Primary Component

The primary anti-inflammatory component of the lipid mix has not been identified but has been determined to be more complex than just the DHA / EPA component

Integrity

The anti-inflammatory action of the whole extract (PCSO-524®) is more effective than the sum of its parts

The importance of a stabilised extract

The patented extraction process has been refined over a number of years and produces an extract with consistent anti-inflammatory results due to the stable, consistent nature of the product. The early research informed us that a non-stabilised mussel powder could not consistently produce positive results, crude extracts were vulnerable to chemical change over time providing variable or unpredictable results.

- Most green-lipped mussel (GLM) extracts on the market are not stabilised extracts and therefore may be vulnerable to change over time. No other product has the stabilisation process used by PIL to manufacture Antinol®. Comparison of different mussel extracts for their anti-inflammatory activity reveal a wide variability in effectiveness, showing the stabilised lipid extract to be far superior [10]

- Comparison of similar clinical trials using a non-stabilised mussel extract [11] to Antinol® [12] for treatment of osteoarthritis in dogs have shown stronger outcomes for Antinol®

- Comparison of PCSO-524® to other available over the counter treatments for osteoarthritis in a rat trial, place it in the top two products of a group of 27, with the other top product being the stabilised GLM powder from which Antinol® is made [10]

- A clinical trial comparing Antinol® to fish oil for treatment of osteoarthritis in dogs has shown considerable clinical benefit for Antinol® over fish oil. In conjunction with this, the results showed a significantly decreased biomarker of cartilage breakdown in the Antinol® group, which was increased in the fish oil group [13]

- Clinical trials have also demonstrated the superiority of Antinol® to other nutraceuticals commonly used for arthritis or joint disease in dogs. These include glucosamine, chondroitin, and avocado soy-bean unsaponifiables [12, 14]

Analytical profile of Antinol®

- Analysis of the oil by thin layer chromatography shows several lipid classes including cholesterol esters, triglycerides, free fatty acids, diglycerides, cholesterol, phospholipids and monoglycerides [2,6] See Table 1

Table 1 Lipid class composition of Lyprinol [2]

Lyprinol lipid class	Solvent elution (% MTBE in hexane)	Total Lyprinol (%)
Sterol esters	1-5	5.8
Triglycerides	5-10	26.4
Free fatty acids	10-25	53.7
Sterols	25-20	8.0
Phospholipids (polar lipids)	50-100*	6.1

This includes the olive oil component of the capsules. MTBE = methyl-tert-butyl ether
*Includes 100% methanol fraction (to remove all remaining polar material from the column)

- The major fatty acids present in PCSO-524® are shown in Table 2.
- Of the 91 fatty acids reported, 16 were at concentrations greater than 1% of the total fatty acids, see Table 2.

In decreasing order of abundance, these were:
20:5n-3 (EPA),
C16:0 (Palmitic acid),
22:6n-3 (DHA),
C16:1n-7 (Palmitoleic acid),
C14:0 (Myristic acid),
C18:0 (Stearic acid),
18:1n-5 (Octadecenoic acid),
C18:4n-3 (Stearidonic acid),
C20:4n-6 (Arachidonic acid),
C18:2n-6 (Linoleic acid),
C18:3n- 3 (Alpha-linolenic acid),
and C20:1n-9 (Gondoic acid) [7].

	FA	Weight %
EPA	20:5n-3	21.2 ± 0.4
Palmitic	16:00	18.4 ± 0.2
DHA	22:6n-3	13.1 ± 0.1
Palmitoleic	16:1n-9,7,5	10.3 ± 0.2
Myristic	14:00	8.42 ± 0.14
Stearic	18:00	3.34 ± 0.05
Octadecenoic	18:1n-5	3.15 ± 0.03
Stearidonic	18:4n-3	2.70 ± 0.17
Arachidonic	20:4n-6	1.73 ± 0.09
Linoleic	18:2n-6/19:1	1.74 ± 0.03
Alpha-linolenic	18:3n-3	1.61 ± 0.04
Gondoic acid	20:1n-9	1.60 ± 0.09

Table 2
Composition of major fatty acids present in PCSO-524® [7]

Therapeutic uses

The primary recommendation for Antinol® use is for arthritis and other inflammatory conditions.

Anti-inflammatory Activity

There are two major inflammation pathways relevant to pain in osteoarthritis (OA). These are the cyclooxygenase pathways (COX-1 and COX-2) and the lipoxygenase pathway, which can be separated into 3-arms (15-LOX, 12-LOX and 5-LOX) of which the 5-LOX pathway is the best studied for OA.

COX and LOX pathways are inflammatory cascades that are initiated in ordinary cells from the lipid content that makes up the cellular membranes. Initiation and control of the inflammatory process are complex and governed by an array of biomolecular mechanisms. One important pro-inflammatory mechanism is associated closely with the cell-membrane-bound fatty acid arachidonic acid, which becomes converted into other compounds in the body that are potent pro-inflammatory substances. These pathways are shown in Figure 1.

The composition of the cellular fatty acids within cells is an area of particular interest when looking at the effects of dietary or supplemental fatty acids in the body (i.e. PCSO-524®). The composition can directly influence inflammatory processes in the body. In fact, research is now suggesting that fatty acids within the diet can alter OA risk and severity [15].

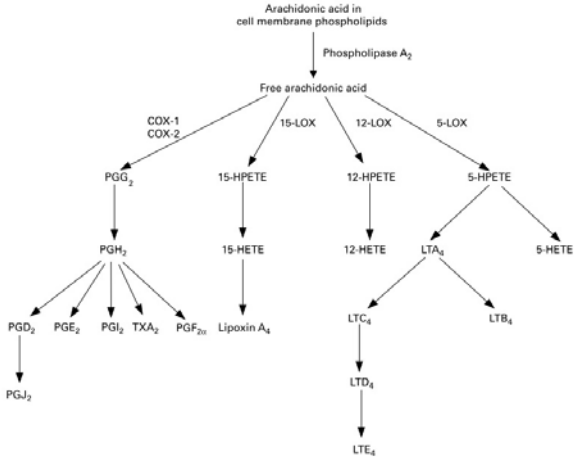


Figure 1 Pathways of eicosanoid synthesis from arachidonic acid. COX, cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroxyperoxyeicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane. [16]

Antinol®

working in conjunction with NSAIDs, or to replace NSAID use

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used drugs for arthritis conditions in humans and animals.

One of their main mechanisms of action is inhibition of the COX enzyme (shown with its link to the inflammatory mediators produced from arachidonic acid in Figure 1), which in turn, inhibits the production of prostaglandins, especially PGE2, one of the key inflammatory mediators known to cause inflammation and therefore pain, which is strongly implicated in OA [17].

NSAIDs are also well known for their potential gastrointestinal side effects, including the development of gastric ulceration. A review of the use of NSAIDs in dogs notes that monitoring them for gastric ulceration is difficult as there are no practical screening tests to detect early signs of gastric injury, with clinicians needing to be vigilant for signs of injury [18]. Ideally, treatment for OA should be as effective as NSAIDs without these debilitating side effects.

Supplementation with Antinol® has been shown in a canine study to be as effective as NSAIDs for improving the weight-bearing capacity of the affected limb, with superiority in clinical assessment seen with Antinol® only [12]. This study also saw a slightly enhanced effect when both NSAIDs and Antinol® were given together.

In one study examining the use of Antinol® post joint-surgery in canines, adverse events including vomiting and gastrointestinal irritation were seen in the NSAID group, but not the group that was prescribed Antinol® [14]. The same trial also saw a reduction in time to regain use of the affected limb with Antinol® in comparison to NSAID treatment.

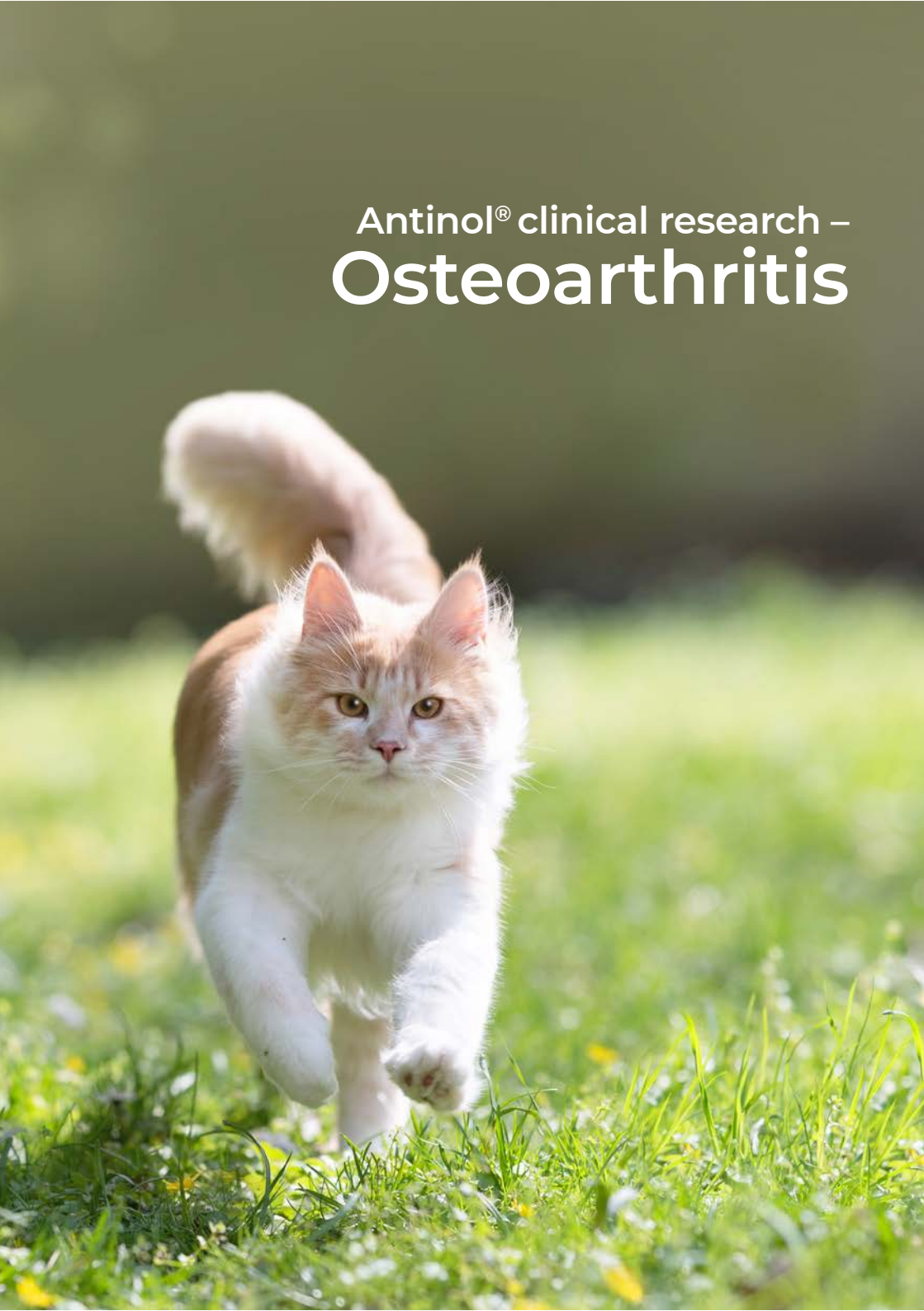
Studies in laboratory animals support these findings with PCSO-524® an effective treatment in rats for osteoarthritis [10]. The same researchers went on to demonstrate that unlike the NSAIDs tested (that were also effective), PCSO-524® was not gastro-toxic.

In 2003, it was shown that PCSO-524® could be used alongside either NSAIDs or low-dose prednisone for increased benefit[19].

The research demonstrated that the combined use of pharmaceuticals and PCSO-524® might be beneficial together to decrease the gastro-toxicity associated with the use of NSAIDs and to increase treatment effect.

Antinol® clinical research –

Osteoarthritis



Antinol® clinical research – Osteoarthritis

There have been several clinical trials looking at efficacy in **canine osteoarthritis of the hip, stifle, and shoulder joints**, as well as its effect in the **post-surgical treatment** of animals undergoing joint surgery. These trials are outlined in the following section.

1. The Preliminary study of the clinical outcome of using the PCSO-524 polyunsaturated fatty acid compound (Antinol®) in treatment of canine OA and degenerative spinal disease

Thai Journal of Veterinary Medicine. 2012. 42(3): 311-317 [19]

This trial included 84 mixed breed dogs with **OA of the hip, shoulder or stifle (n=64) or neurological signs of cauda equina syndrome** (n=20).

All dogs were given **50mg / 10kg per day of PCSO-524**, with primary outcome being improvement in clinical assessment after 4 weeks of treatment.

In both of the OA groups, between 88-90% of the dogs showed clinical improvements, see Table 3 , and in the cauda equina group, improvement of neurological signs was seen in 85% of the group.

Clinical outcomes (%(n))	Improve	Not improve	Progress (worse)
Clinical lameness	90.3% (28/31)	9.67% (3/31)	0% (0/31)
Owner preference	87.09% (27/31)	9.6% (3/31)	3.22% (1/31)
Radiographic findings	16.12% (5/31)	77.42% (24/31)	6.45% (2/31)

Table 3 Clinical outcomes of OA at hip and shoulder joints with PCSO-524 treatment after 4 weeks [19]

Specifically, on a 5-point lameness scale in dogs with hip and shoulder OA

35% improved by 1 point,
48% improved by 2 points, and
6% improved by 3 points,
whilst **none of the dogs scores decreased**, see Table 4.

Clinical outcomes (% (n))	Worse >1 score	No difference	Better 1 score	Better 2 score	Better 3 score
Lameness score	0% (0/31)	9.67% (3/31)	35.48% (11/31)	48.38% (15/31)	6.45% (2/31)
Radiographic score	6.45% (2/31)	77.42% (24/31)	9.6% (3/31)	6.45% (2/31)	0% (0/31)

Table 4 Lameness score of OA at hip and shoulder after four weeks of PCSO-524 [19]

Results from this study suggest improved clinical outcomes for the majority of dogs with PCSO-524 supplementation in OA, slightly less of an improvement was seen in the cauda equina group. Additionally, 85% of the owners stated that they were happy with the treatment.

2. PCSO-524 compared to NSAID for post-operative recovery in canines undergoing stifle surgery

Poster presentation: World Small Animal Veterinary Association 38th Annual Congress. 2013 [13]

This report includes 28 dogs given PCSO-524 (16 weeks) or NSAIDs (7 days) and glucosamine sulphate (16 weeks) post joint surgery.

Results suggest a large clinical improvement in lameness and a reduced amount of time to regain use of the affected limb in the PCSO-524 group in comparison to the NSAID/glucosamine group. There was also a considerable difference in the side-effects found between the two treatments, see Figure 2.

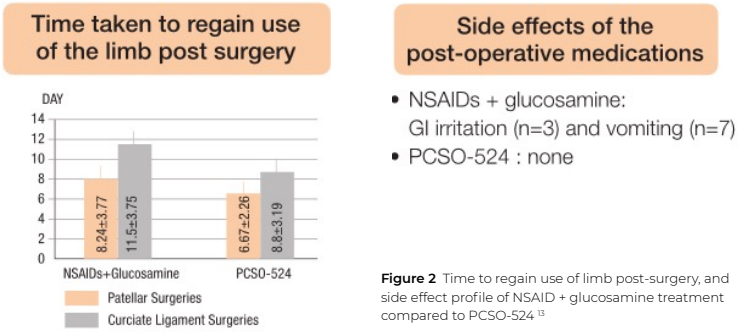


Figure 2 Time to regain use of limb post-surgery, and side effect profile of NSAID + glucosamine treatment compared to PCSO-524 [13]

This brief report suggests **clinical improvements using PCSO-524 post-surgery for 16 weeks**, superior to the use of NSAIDs for seven days and glucosamine sulphate for 16 weeks.

3. Effect of PCSO-524 on OA biomarkers and weight-bearing properties in canine osteoarthritis

Thai Journal of Veterinary Medicine 2015. 45(2): 157-165 [12]

This trial included 66 mixed breed dogs with x-ray confirmed OA split into two treatment groups for 24 weeks.

These were 1) PCSO-524 (5 mg/kg) and 2) fish oil (2,000 mg/d). The dogs were all placed on a standardised diet to minimise variability. The primary outcome was serum chondroitin sulphate epitope (WF6), which is an indicator of cartilage breakdown. Other measures were weight-bearing scores measured with peak vertical force gait analysis and range of motion (ROM).

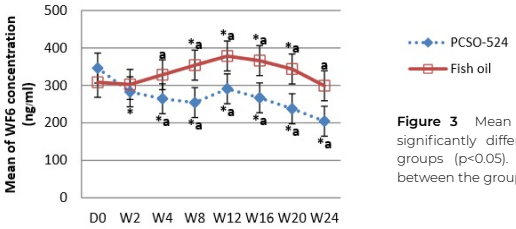


Figure 3 Mean of WF6 concentration *Values were significantly different compared with D0 within the groups (p<0.05). aValues were significantly different between the groups within the week (p<0.05) [12]

The results show an increase in WF6 in the fish oil group and a decrease in the PCSO-524 group. The mean of serum WF6 of the PCSO-524 group (262.46±118.06 ng/ml) was significantly (p<0.05) less than that of the fish oil group (324.76±133.65 ng/ml) after 24 weeks of administration, see Figure 3.

Clinical outcomes also improved significantly for the PCSO-524 group, with weight-bearing improving above 25% and up to 88% of dogs improving their lameness scores, as opposed to 32% of dogs in the fish oil group.

See Table 5 for changes in weight-bearing properties.

Joint	Patients with OA in hip joints (percentage of mean [SD])					Patients with OA in shoulder joints (percentage of mean [SD])				
Time	D0	W2	W8	W16	W24	D0	W2	W8	W16	W24
PCSO-524	64.5 (6.43)	79.3 (4.32)	82.1 (8.42)	88.7 (5.11)	89.2 (5.81)	68.1 (4.18)	81.4 (7.12)	86.7 (3.84)	89.3 (6.18)	91.2 (2.12)
Fish oil	66.8 (8.41)	68.4 (5.31)	70.1 (9.21)	71.5 (8.76)	72.3 (3.14)	67.8 (9.42)	68.9 (7.63)	70.4 (8.43)	71.2 (9.44)	71.6 (4.56)

Table 5 Weight bearing properties measured by peak vertical force gait analysis. Data were analysed as percentage of expected weight bearing in each leg: 60:40, forelimbs: hind limbs [12]

This study suggests very positive clinical effect and a significant reduction in a measure of cartilage breakdown for dogs with OA treated with PCSO-524. The treatment was far superior to treatment with a standard dose of fish oil. The results suggest that PCSO-524 is protective against cartilage breakdown in dogs with osteoarthritis.

4. PCSO-524 compared to the NSAID firocoxib for recovery post-surgery in canines undergoing medial patellar luxation repair

Thai Journal of Veterinary Medicine 2015. 639-643[20]

This trial included 30 Pomeranian dogs undergoing surgery for medial-patellar luxation repair. The dogs were split into 3 groups post-surgery,

1) firocoxib (5 mg/kg/d); 2) PCSO-524 (10 mg/kg, twice daily),
or 3) a combination of both firocoxib and PCSO-524.

All treatments were given for 14 days from the day of surgery. The primary outcome measure was **interleukin 1-beta (IL-1β)** chosen for its role as a biomarker for joint inflammation and bone loss especially in rheumatoid arthritis [22].

The results show a very similar pattern of decline for IL-1β levels in the firocoxib and the combined firocoxib/PCSO-524 group. The levels of PCSO-524 alone fell more slowly than the other groups over the 14 days recorded.

The role of IL-1β in OA remains unclear[22] and this study suggests that **effect of PCSO-524 is not mediated via this cytokine pathway.**

5. The effectiveness of marine based fatty acid compound and NSAIDS for treatment of canine osteoarthritis: A preliminary study:

WSAVA Congress 2015

A prospective block-randomized single-blinded study. **Thirty client-owned dogs** that had clinical and radiographic evidence of hip or stifle osteoarthritis. Dogs were assigned randomly into 3 treatment groups of 10 dogs each:
1) PCSO-524, 2) NSAIDs and 3) The combination of NSAIDs and PCSO-524 (NSAIDs-PCSO). Each group received the therapeutic agents orally for 4 weeks.

Peak vertical force (PVF) was measured by **force plate gait analysis**. Hematology and blood chemistry values were evaluated prior to treatment and at the 2nd and 4th week post treatment. Comparisons between groups by repeated measurement demonstrated indifferences of PVF, hematology and blood chemistry values.

Analyses within each group determined that the PVF prior to treatment and the second week of study and the end of study increased significantly in all groups ($p < 0.05$).

The mean changes of PVF at the fourth week of treatment were;
3.88 ±1.35, 4.23 ± 0.74, 5.36 ± 0.94 % BW, in PCSO-524, NSAIDs and NSAIDs-PCSO groups respectively. The BUN value in NSAIDs group tended to increase when compared to baseline values ($p<0.05$), however all BUN values were within the normal range.

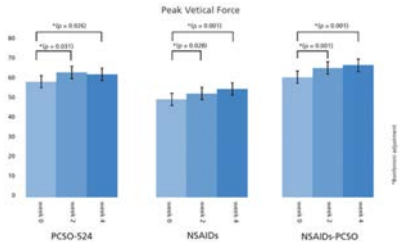


Figure 4 Multiple comparison of PVF in each group between prior to treatment, the 2ndand the 4th week post treatment at p-value <0.05

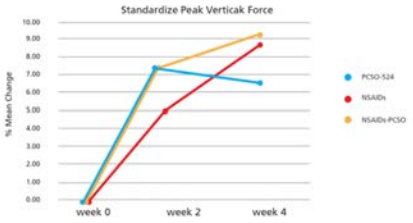


Figure 5 Percent mean change of PVF in each group between to prior treatment, the 2nd and the 4th week post treatment

6. The effectiveness of disease modifying osteoarthritis agents and carprofen for treatment of canine osteoarthritis:

Thai Journal of Veterinary Medicine. 2016. 46(3): 363-371 [11]

This trial included 40 mixed breed dogs with x-ray confirmed OA split into four different treatment groups for four weeks.

1) PCSO-524 (200 mg/d); 2) carprofen (2.2 mg/kg b.d.); 3) combination of PCSO-524 and carprofen; and 4) a nutraceutical supplement containing a combination of glucosamine HCL, chondroitin sulphate and avocado-soybean unsaponifiables (GC-ASU).
Outcome measures were **changes in kinetic force plate gait analysis - peak vertical force (PVF); gait observation and complete orthopaedic examination (OAS)**.

Results show overall that **the combination of PCSO-524 / carprofen was superior to all of the other treatments.** The GC-ASU performed the worst of all the treatments.

The PVF within-group analysis showed a significant increase (improvement) for three of the four groups, with no significant improvement seen in the CG-ASU group. After four weeks, the adjusted PVF was increased in the PCSO-524/carprofen group up to above 9% mean change. The results were slightly lower for carprofen only, and around 6.5% change for the PCSO-524 only group, see Figure 6.

The combined measures of the OAS showed a significant decrease in symptom score across these same groups with a clinical improvement equivalent to between 7.8-9.4%.

PCSO-524 performed better in this assessment than carprofen alone, and the combination of the two was superior to either alone.

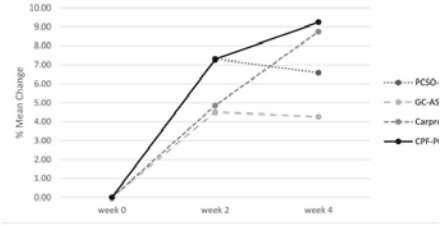


Figure 6 Adjusted PVF values across treatment groups [11]

This study suggests that the combination of PCSO-524 and carprofen together was superior to other treatments for management of canine OA.
Results were seen in both objective and clinical assessment.

7. PCSO-524 report clinical results of nineteen dogs with tracheal collapse that received PCSO-524,A lipid extract of New Zealand green lipped mussels (Perna canaliculus)

Journal of Modern Veterinary Medicine (MVM) 2016[43] 25: 79-82

Nineteen dogs presented with cough and diagnosed with **tracheal collapse** based on inspiration / expiration chest radiography between December 2013 and April 2015; The recruitment was only small dogs ;7 Chihuahuas, 4 Yorkshire terriers, 4 Pomeranians, 2 Papillons, 1 Pug, and 1 Maltese respectively. The age range of these 19 dogs were from 63 months (5 years and 3 months) to 199 months (16 years and 7 months) in which considered as the senior life stage.

At baseline the body condition score of 12 dogs were scored as obese, 5 dogs were lean, and 2 dogs were at normal weight. **Weight control protocol had been instructed to each Pet Owner to follow throughout 12 months of study period.**

Antinol were applied as the conjunctive supplementation to the supportive treatment of each dog. Antinol dosage was 2 caps per day as the loading dose in first 14 days and on maintenance dose 1 cap per day starting from Day 15 onwards. The evaluation of coughing were done at the home environment which dog were most comfort and least stress.

According to the study had been found the change of incidence rate of Cough from D0 to D360 were **improved in 16 dogs that survived for 12 months** the results as shown in Table 6.

Three dogs were excluded from the study: 1 died of pulmonary edema, 1 died of advanced valvular heart disease, and 1 discontinued PCSO-524® due to dosing difficulty by the owner.

Table 6 The results of Coughing score of each case by the time of post treatment with Antinol

	Dogs	baseline*	0.5 month	1 month	2 months	3 months	6 months	12 months
1	Chihuahua A	100	90	70	50	40	50	5
2	B	100	80	80	40	0	50	20
3	C	100	100	100	100	100	50	0
4	D	100	90	50	-	-	-	-
5	E	100	0	0	0	0	0	0
6	F	100	0	10	25	25	10	0
7	G	100	100	50	50	50	50	-
8	Yorkshire terrier A	100	0	0	0	10	0	0
9	B	100	0	10	0	10	10	0
10	C	100	95	10	10	10	10	-
11	D	100	50	60	60	100	0	50
12	Pomeranian A	100	40	80	80	80	30	50
13	B	100	15	40	30	40	40	20
14	C	100	50	10	10	20	10	0
15	D	100	0	0	0	0	0	0
16	Papillon A	100	0	0	0	0	0	0
17	B	100	50	50	40	20	10	10
18	Pug	100	70	50	40	40	50	50
19	Maltese	100	100	120	90	60	20	60

*Cough at baseline is assumed to be 100

8. The Effectiveness of marine based fatty acid compound (PCSO-524) and firocoxib in the treatment of canine osteoarthritis:

Poster Presentation: Veterinary Orthopaedic Society Conference, March 2018

This randomized study involved **31 mixed breed dogs with x ray confirmed OA of stifle joint.** They were split into 2 groups for four weeks of treatment;

1) PCSO-524 200 mg (4 caps) q24hr PO and
2) Firocoxib (FCX) 5 mg/kg q24hr PO & PCSO-524 200 mg (4 caps) q24hr PO.

Outcome measures were **Computer-assisted Force Plate gait analysis** (OR6-7,AMTI, Watertown MA) and **Owner assessment Canine brief pain inventory score** (CBPI).

The results showed **a non-significant effect of the treatment on the adjusted Peak Vertical Force (PVF) value (p=0.447) among the 2 treatment groups.** The comparison within group revealed significant increases in the PVF value at week 2 and week 4 in both treatment groups compared to their pretreatment values ($p<0.05$). Change in mean PVF of 7.81(±1.27) and 6.19(±1.8%BW) [mean(±SE)] were detected in PCSO-524 and FCX+PCSO-524 groups respectively.

CBPI values compared between the two groups was non-significant (p=0.4359). CBPI comparison within both groups showed significant decreases at 2 and 4 weeks compared with their pretreatment value ($p<0.05$).

9. The clinical study of PCSO-524 (Antinol®) as nutraceutical in canine allergic skin disease:

Oral Presentation: The World Veterinary Congress 2018

This study included **31 allergic dogs with chronic pruritis with other skin diseases excluded.** Dogs were split into 3 treatment groups based on degree of pruritis, distribution, extension of the lesions and skin and coat condition; Group 1: mild, Group 2: moderate, and Group 3: severe.

All dogs received **PCSO-524 loading dose 10 mg/kg q12hr PO for 2 weeks** followed by **a maintenance dose of 10 mg/kg q24hr PO.**

All dogs were evaluated at 2, 4, 8, 12 and 16 weeks for clinical response.

The assessment made by the owners (PVAS Score) and Vets (CADESI-03 score).

The results demonstrated that **PCSO-524 had beneficial effects on improvement in skin and coat condition and a reduction in PVAS score in Group 1.** Similarly, vet evaluation revealed greater reduction in pruritic score in Group 1 (50%) with less effects in Group 2 (44.44%) and Group 3 (40%) as in Table 7 below.

The results of this study suggested **PCSO-524 had beneficial effect in atopic skin allergy with a greater effect in mild cases.** The effect was less in more severely affected atopic dogs, probably due to the severity degree of inflammation they experienced. PCSO-524 may be used as treatment of canine atopic dermatitis.

	Number of dogs with improvement (%)			Time at improvement seen (weeks)		
	Group 1 PCSO-524 (n=12)	Group 2 PCSO-524 +AH (n=9)	Group 3 PCSO-542 +Pred (n=10)	Group 1 PCSO-524 (n=12)	Group 2 PCSO-524 +AH (n=9)	Group 3 PCSO-542 +Pred (n=10)
Skin & coat condition	9/12 (75%)	3/9 (33.33%)	5/10 [b,c] (50%)	5.33±1.56 [c]	7.33±2.70	12±2.09 [a]
Degree of pruritus (PVAS)	6/12 (50%)	4/9 (44.44%)	4/10 (40%)	4±0.95	4.67±1.40	5±1.17
Lesion score (CADESI-03)	11/12 [b,c] (91.67%)	3/9[a] (33.33%)	4/10[a] (40%)	4.55±1.28[c]	4.67±2.45	10.50±2.12[a]

Table 7 The Skin&coat,PVAS and CADESI score after treatment and the time point of improvement seen by group in weekly basis.

10. The effectiveness of marine based fatty acid compound (pcso-524) and firocoxib in the treatment of canine osteoarthritis:

Journal of BMC Veterinary Research 2019 15:349

This randomized double blinded study included **79 mixed breed dogs that had hip and/or stifle OA** with X-ray confirmation. The dogs were randomly split into 3 different treatment groups;

1) Firocoxib 5mg/kg q24hr PO & PCSO-524 placebo (4 caps a day q24hr PO);
2) PCSO-524 4 caps a day, q24hr PO & Firocoxib Placebo; and
3) combination of Firocoxib and PCSO-524 for 28 days of treatment.

Outcome measures were changes in Kinetic force plate gait analysis-Peak Vertical Force (PVF), the Orthopedic Assessment Score (OAS), Canine Brief Pain Inventory score (CBPI), and serum prostaglandin E2 concentration (PGE2).

Results showed overall that within-group comparisons saw a significant increase in PVF over the 4-week treatment period for all three treatments (Firocoxib, PCSO-524 and the combination group) ($p<0.05$).

The mean increase in PVF were 3.25 (±4.13), 2.01 (±3.86), and 4.11 (±4.69)%BW [mean (±SD)] respectively, see Table 8.

Table 8 PVF values at pre treatment (day0), day 14, day 28 for each group and the change from baseline

	Time	Firocoxib	PCSO-524	Combination	*P*-value*
PVF (%BW)	Day 0 (PVF)	64.79 ± 5.98 [a]	62.01 ± 6.85 [a]	59.97 ± 9.38 [a]	0.069
	Day 14 (PVF)	67.82 ± 6.70 [b]	63.82 ± 6.15 [b]	62.71 ± 9.15 [b]	
	Mean change±SD	3.03 ± 4.67	1.82 ± 3.22	2.74 ± 4.41	
	Day 28 (PVF)	68.05 ± 6.29 [b]	64.01 ± 6.52 [b]	64.08 ± 9.99 [b]	
	Mean change±SD	3.25 ± 4.13	2.01 ± 3.86	4.11 ± 4.69	

SD standard deviation
[a, b] *p*-value < 0.05 from Tukey's Studentized Range within each treatment
* *p*-value from repeated measurement analysis for 3 treatments

The OAS showed non-significant changes in all treatment groups.

There were no significant decreases in CBPI Pain severity score (PSS) and CBPI interference scores (PIS) between groups. The concentration of serum PGE2 significantly decreased ($p<0.05$) in the combination group only, see Table 9.

The study also found **significant increases in BUN and creatinine ($p<0.05$) compared to pre-treatment in the Firocoxib and combination groups, but** not in the PCSO-524 group at day 28, but all other values in all dogs remained within the normal range.

The results of the study suggest that within each group of a combination (PCSO-524 and Firocoxib), PCSO-524, Firocoxib showed the significant improvement of weight bearing ability but not in the comparison between.

Table 9 PGE2 level adjusted baseline (MEAN+/- SE at pre treatment(day 0), day 14 and day 28

PGE2 level after adjusted baseline (Mean + SE) at pre-treatment (day0), day14 and day28					
Time		Firocoxib	PCSO-524	Combination	P-value**
Adjusted PEGE2a (pg/mL)	Day 0	1815.795±0.00	1815.795±0.00	1815.795±0.00[a]	0.639
	Day 14	1721.642±88.889	1780.773± 85.645	1791.691±82.430[a]	
	Day 28	1717.807±94.695	1805.974±91.239	1589.641±87.814[b]	

** p-value from repeated measurement ANCOVA for 3 treatments
[a, b] p-value <0.05 from multiple comparison within each treatment using Bonferroni test
[a]Covariates appearing in the model are evaluated at PEG2 of every group at week 0= 1815.795 pg/mL

11. Use of PCSO-524 and cyclosporin for treatment of immune-mediated polyarthritis in dogs:

The Journal of Thai Veterinary Practitioners 2020 32:66-77

An 8 years old Chihuahua dog was admitted to the Small Animal Hospital at Chulalongkorn University due to signs of depression, lethargy, lameness and joint effusion. Hematological examination and test of synovial fluid indicated **Immune-Mediated Polyarthritis (IMPA);** see Figure 7 for x-rays. Prednisolone, Gabapentin and Amoxillin-clavulanic acid were prescribed for the treatment. **Abnormally increase of ALT, ALK and AST enzyme was observed 1 week later,** therefore, the treatment was changed to Cyclosporin, Gabapetin, and PCSO-524®.

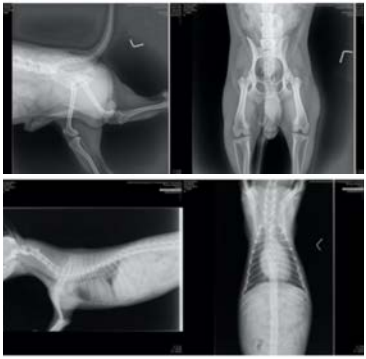


Figure 7 The radiographic images of immune mediated Polyarthritis in this case

The clinical signs continued to improve after the adjustment of prescription and **Cyclosporin and Gabapetin were terminated after 3 and 4 months, respectively.** A follow-up examination at 4 months later showed no signs of lameness and joint effusion and no adverse effects caused by the long-term use of PCSO-524®.

The success of IMPA treatment is a result of rapid diagnosis and appropriate treatment protocol. Follow-up for treatment evaluation is necessary during the treatment course in order to reduce the impact on health and minimize the medication dosage without risk of causing adverse effects or recurrence of the disease.

12. PCSO-524 (Antinol®) Use in Fighting Cocks for Treatment of Chronic Pododermatitis

The Journal of Thai Veterinary Practitioners 2020 32:83-93

2-year old fighting cock had been showing sign of swollen left plantar and left tarsometatarsal joint and lameness of the left leg for 2 months; see Figure 8. Radiographic examination found lysis of the left tarsometatarsal bone. Significant amount of red and white blood cells was found at the lesion and bacterial culture showed negative result.

The treatment consisted of NSAID; 0.5 mg/kg meloxicam sid for 2 weeks, antibiotic; 125 mg/kg Amoxicillin-clavulanic acid bid for 4 weeks, and 1 capsule of PCSO-524 (Antinol®) sid for 5 weeks. The kinetic gait analysis was performed weekly during the follow-up to evaluate the improvement and pressure on the foot pads was measured by determining **Peak vertical force (PVF) of each leg**.

Figure 8 The image of fighting cock with swollen left plantar and left tarsometatarsal

After 2 weeks, the animal started to show improvement of clinical signs as partial weight bearing was restored and increased by approximately 30%, but the pad swelling was still present. Increased weight bearing of the left leg was observed after 3 weeks and the animal was able to run and chase female chickens. **After 5 weeks,** walking mobility and weight bearing was increased from 30% to 45% and the swelling of the left foot was reduced.

This clinical study demonstrated efficacy of PCSO-524 (Antinol®) in treatment of foot-pad inflammation in chickens. The antibiotic was for infection control and the NSAIDs and PCSO-524 (Antinol®) were used to reduce pain and inflammation. **Long-term use of NSAIDs was limited due to adverse effects on kidney and gastrointestinal tract, therefore it was discontinued after 2 weeks of the treatment.** Only nutraceutical supplementation, PCSO-524 (Antinol®), was prescribed for 5 weeks. Omega-3, one of the major components of PCSO-524 (Antinol®), is widely accepted for its anti-inflammatory effect

Safety in canines:

Thai Journal of Veterinary Medicine 2014 44(4):505-511

A clinical trial specifically examining the safety of Antinol use in canines was conducted **using a range of doses over a period of 8 weeks** in a group of healthy Beagle dogs [23]. The experiment used laboratory conditions and standardised all aspects of feeding, activity etc. of the dogs. Antinol was given in four even groups amongst 40 dogs in doses of either 0, 100, 300, or 1000 mg/day. The highest dose is **5X the normal recommended dose** for Antinol.

The study measured a range of biochemical tests including liver and kidney function tests, glucose, cholesterol levels, and tests of prolonged bleeding time. The dogs were also closely observed for clinical changes such as weight, feed intake, faecal excretion, physical examination and behavioural change.

Over the 8 week period there was no significant difference in any of the parameters tested in any of the groups of dogs and the authors concluded that **Antinol did not have a harmful effect.**

Safety in Felines:

Poster Presentation the 10th VPAT REGIONAL VETERINARY CONGRESS 2017

A clinical trial specifically examining the safety of Antinol use in canines was conducted **using a range of doses over a period of 28 days in 3 group of healthy Mixed breeds cats (n=7)**, aged 1 to 5 years, body weights 3 to 5 kgs.
The Antinol dosage given are as following: 1x the recommended maximum dose (2 caps daily), 2x the recommended maximum dose (4 caps daily) and 3x the recommended maximum dose (6 caps daily), with daily food for **28 days.**

Individual feline health and physical examinations, including vital signs, ocular, nervous, musculoskeletal, and integumentary systems, were conducted. Signs of illness and behavioural changes were recorded, as were changes in hematology and blood chemistry values. Food and water consumption, and body weight were measured every day.

The results of this study demonstrated that CBC and blood chemistry levels were within the normal reference ranges among clinically healthy normal cats administered two to four capsules of PCSO-524 per day for 28 days.

At a dose of six capsules per day, feline blood samples revealed lipemia, which may have been due to the fact of PCSO-524 comprises triglycerides (TG, 10-25%), free fatty acids (FFA, 7-12%) and sterols (ST, 12-18%) (1). **PCSO-524 supplementation at two to four capsules per day for 28 days in clinically healthy normal cats has no adverse effects.** Further study is needed to investigate the role of PCSO-524 in geriatric cats with DJD and other degenerative diseases in the future.

Keep happiness in motion

Research in laboratory animals

Testing in laboratory animals (predominantly rats) has also demonstrated either **inhibition of arthritic development** (prophylaxis) or **improvement in induced arthritic symptoms** (treatment) with PCSO-524®.

The classic test for osteoarthritis uses an arthritic adjuvant injected into the animal to produce arthritic changes in the joints. A series of Chinese studies utilising this model has shown excellent results both in the arthritic signs as well as the cytokines and inflammatory protein expression in rats [3, 33, 34]. These results have been supported by an Australian study showing treatment results with up to 52% greater effect than controls and an equivalence to pharmaceutical treatment [4].

In laboratory studies, PCSO-524® had a substantial effect on the production of cytokines known to be associated with inflammation (IL-6, IL-1α TNF-α, IFN-α).

Feeding with PCSO-524® was associated with significantly decreased expression levels of TNF-α and IFN-α when compared to the NSAID naproxen (positive control) and, even more, when compared with extra-virgin olive oil (negative control), see Figure 11 [3].

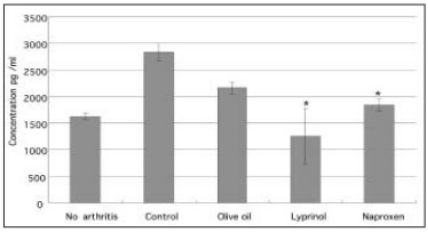


Figure 12 Amount of pro-inflammatory cytokine TNF-α – 14 days after arthritis induction in rats [3]

PCSO-524® was found to be effective in both prophylactic or therapeutic treatment of induced arthritis in rats but showed **little or no activity in acute irritation assays** (carrageenan, kaolin, histamine) indicating it is not mimicking rapid-acting NSAIDs [5]. Sub fractions of the oil also inhibited leukotriene-B4 and prostaglandin E2 (PGE2) biosynthesis in vitro. **The other significant finding across these studies was that PCSO-524® was not gastro-toxic like the drugs of the NSAID series [5].**

In vitro research

Anti-inflammatory effect

A significant study showing PCSO-524® to be an effective COX inhibitor was conducted in 2007 [2]. This study showed that PCSO-524® moderately inhibited ovine COX-1 and COX-2 pure enzymes in vitro. The PCSO-524® was fractionated to find the most active fraction of the oil (which was determined to be the free fatty acids) and compared with fish oil for inhibition at 1µg/ml, see Figure 13.

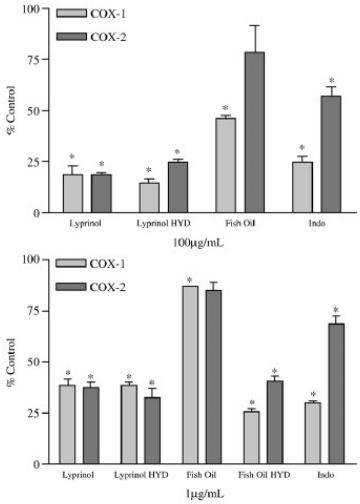


Figure 13 Inhibition of COX-1 and COX-2 by Lyprinol, fish oil and indomethacin at 100 and 1 ug/mL. Lyprinol HYD = hydrolysed Lyprinol complex and Fish oil HYD = hydrolysed fish oil complex [2]

The study suggests that PCSO-524® can be used as an arachidonic acid (AA) substrate substitute in the production of prostaglandins and that therefore it can be used as an alternative for conventional NSAIDs and fish oil treatment for the relief of the symptoms of arthritis.
Additionally, the study showed that PCSO-524® was able to selectively inhibit the different forms of COX, which highlighted the effect for longer term pain management and set it apart from the NSAIDs.

Earlier, the inhibition of the 5-LOX pathway had been demonstrated by showing the decreased activation of this pathway in monocytes incubated with PCSO-524[1]. There have been several more recent studies looking into the anti-inflammatory properties and mechanisms of action.

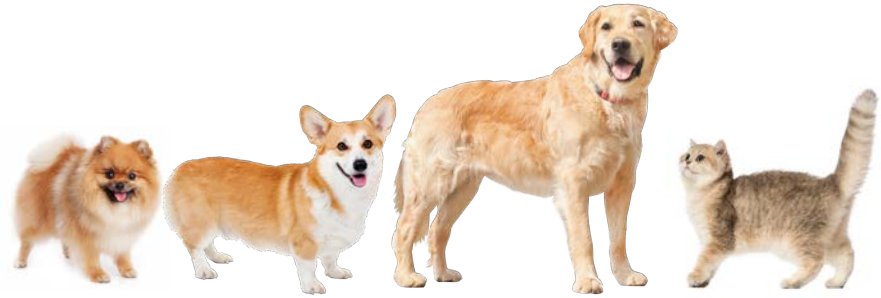
Important new research in 2007 showed that the isolated fatty acids of P. canaliculus have significant anti-inflammatory activity, being effective in the LOX pathway [6]. When these free fatty acids from PCSO-524 were fractionated, a novel class of fatty acids was found called furan fatty acids [36]. This research group then demonstrated the anti-inflammatory activity of these isolated constituents in a rat model of arthritis.

Chondroprotective effect

There is more than just an anti-inflammatory effect that has been attributed to this unique lipid extract. In vitro simulated digestion of green-lipped mussel has demonstrated not only **effective inhibition of inflammatory PGE2 production**, but also an increased level of glycosaminoglycan (GAG) release [37]. These GAGs are important for the maintenance and repair of cartilage, and may be linked to the reduction in the marker of cartilage destruction - WF6, seen in animal OA research [20]. A separate study has shown that the anti-inflammatory action of this extract in chondrocytes was similar to the pharmaceutical deracoxib when studied in vitro using canine chondrocytes [38].

Dosage

The recommended dosage for oral administration is based on bodyweight.
Recommended dosage for dogs & cats: 1 Antinol capsule per 20 kg. body weight
A loading dose of twice the usual dosage is recommended for **the first 14 days of treatment.**



Required caps	Dog < 10 kgs	Dog < 20 kgs	Dog > 20 kgs	Cat
First 2 weeks	2 caps daily	2 caps daily	3-4 caps daily	2 caps daily
After 2 weeks	1 cap daily	1 cap daily	2 caps daily	1 cap daily

Maintenance dose: applied after loading dose period under Vet supervision.

APPLICATION – Antinol may be fed by hand or placed on food during or before feeding. Care should be taken to ensure that the dog consumes the complete dose to maximise benefits of efficacy.

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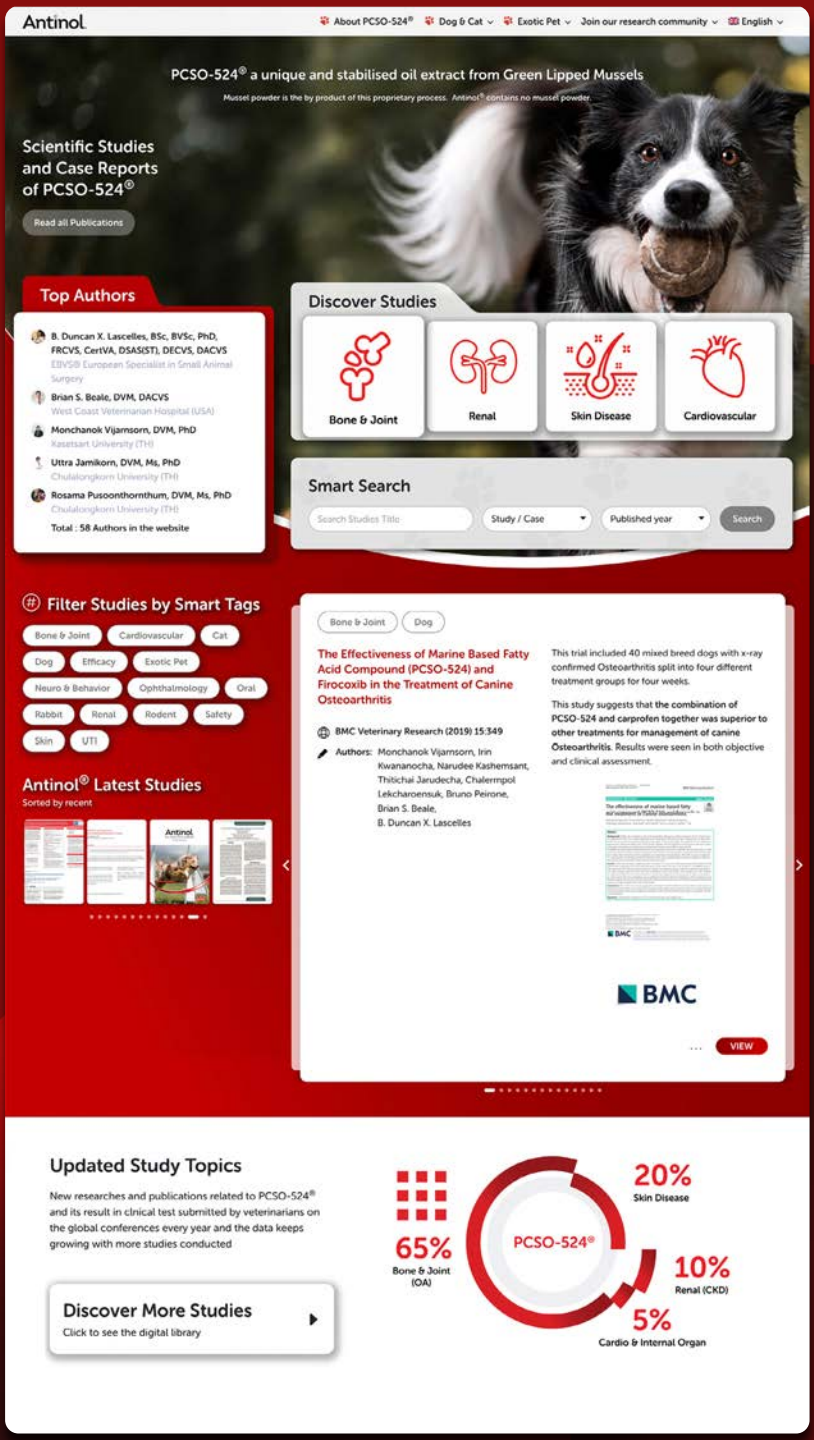

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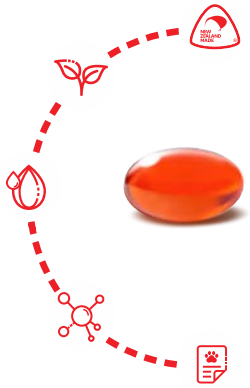

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by Scientific Researches both invitro and invivo

The two marine lipids used in Antinol® Rapid are proprietary and exclusively produced. The exact combination of 30mg lipid fractions from **Perna canaliculus (New Zealand green lipped mussel)** and 20mg **high phospholipid krill oil** is the result of years of research combining and isolating lipid groups and essential fatty acids to find the optimal nutrient synergy.
Our proprietary high phospholipid krill oil is high in polar lipid enrichment which enhances bioactivity "Potency" of this marine oil blend formula as a result of proven efficacy.
The Antinol® Rapid EAB-277® blend has been proven via laboratory tests to be **more effective than either of the individual lipids alone** in inhibiting inflammation markers such as nitric oxide, TNFα, and IL-6.

+ Perna canaliculus
(New Zealand)

+ High Phospholipid Krill Oil
(Antarctic)

Backed by science and extensive research

REVIEWED INTERNATIONALLY BY VETERINARIANS

RECOMMENDED BY VETERINARIANS GLOBALLY

SAFE TO USE + DOES NOT INTERACT WITH OTHER MEDICATIONS

TESTED FOR STABILITY + PURITY

THE LEADING JOINT AND MOBILITY SUPPORT PRODUCT IN JAPAN

- Suitable for long-term use for disorders or as a preventative + wellness supplement
- Patented and stabilized oil extracts
- 100% natural, free from Heavy metal such as Mercury, Cadmium etc. Clear from toxins
- No synthetic preservatives or fillers
- Fully tracable and sustainable
- No report of contraindications*
- According to safety study no known adverse effects have been reported.

*The safety of this product has not been tested in pregnant or lactating animals.
May contain a minute amount of tropomyosin protein

Antinol® Rapid Animal Clinical Studies:

1.

Study of the effectiveness of glucosamine and chondroitin sulfate, marine based fatty acid compounds (PCSO-524® and EAB-277®), and carprofen for the treatment of dogs with hip osteoarthritis:

A prospective, block-randomized, double-blinded, placebo-controlled clinical trial

Study Design:

There were 15 dogs per group under treatment over 6 weeks.

1. Dasuquin
2. Carprofen
3. PCSO-524®
4. EAB-277®
5. Placebo

This 75 dogs' study at Khon Kaen University, Thailand was investigating the effectiveness of these compounds for the treatment of dogs with degenerative osteoarthritis and also to demonstrate the significantly superior therapeutic effect of PCSO-524® and EAB-277® compared with a placebo.

The study used **objective force plate gait analysis, subjective orthopedic assessment scores, hematology and blood chemistry profile analysis and subjective owner assessment scores**.

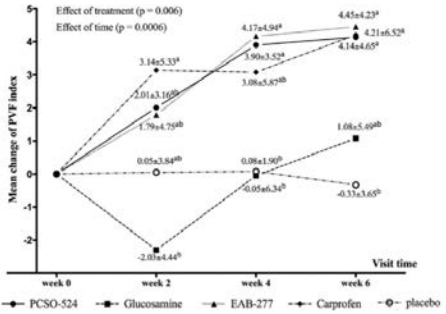


Figure 1

Graphic representation of the mean (± standard deviation) change from baseline in PVF for each group during the study period. Different superscripts (a, b) indicate significant differences between groups for change in PVF.

Results;

Overall, the results of this study indicate that **there appear to be benefits of carprofen, PCSO-524®, and EAB-277® for the treatment of OA-pain in dogs based on the measurement of PVF.** Glucosamine/chondroitin and placebo (sunflower oil) did not appear to be associated with positive treatment effects based on the measurement of PVF.

At 4 and 6 weeks after treatment, **the change in PVF of both PCSO-524® and EAB-277® were similar to that of the carprofen group.** The PVF of placebo group remained unchanged (−0.33 ± 3.65) as expected after study completed (6 weeks).

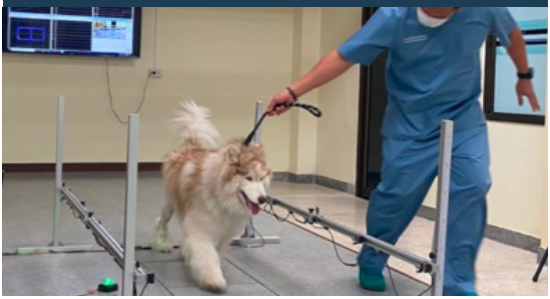
The changes in PVF (significant when compared to baseline) in this study with carprofen after 2, 4, and 6 weeks were 3.14 ±5.33, 3.08 ±5.87, and 4.21 ±6.52, respectively.

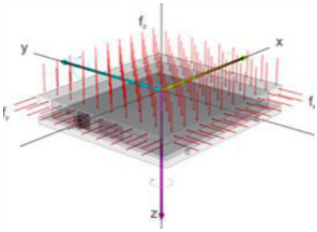
These values are similar to those of a recent study [28] which found an increase in PVF of 3.2 ±0.8 after 2 weeks treatment of carprofen. In that study, most enrolled dogs had hip OA, and the baseline PVF for index limb was similar in both studies (62.1 ±13.5 and 60.7 ±13.5).

Our result is also similar to another OA study [22] using the NSAID treatment firocoxib where the change in PVF of the index limb was reported to be 3.03 ±4.67 and 3.25 ±4.13 at 2 and 4 weeks. treatment, respectively.

Other Diagnostic Tools:

1) Gait analysis: Objective measurement
2) Provide quantitative weight bearing force
- Force plates
- Pressure sensitive mats





2.

Randomized placebo-controlled trial to evaluate the efficacy of Oil extract of the seed of Biota Orientalis (4CYTE™ Epiitalis® Forte) compared with: (i) placebo, (ii) NSAID: Meloxicam (Boehringer Ingelheim) and (iii) the marine based fatty acid compound, EAB-277® ("Antinol® Rapid") for the treatment of osteoarthritis in dogs.

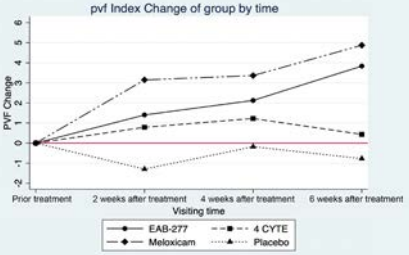
This 100 dogs' study at Khon Kaen University, Thailand is investigating **the effectiveness of these compounds for the treatment of dogs with degenerative osteoarthritis** and also to demonstrate the significantly superior therapeutic effect of EAB-277® compared with a placebo.

The study is using objective force plate gait analysis, subjective orthopedic assessment scores, hematology and blood chemistry profile analysis and subjective owner assessment scores.

Study Design:

There were 25 dogs per group were under treatment over 6 weeks.

1) Meloxicam
2) EAB-277®
3) 4 CYTE
4) Placebo



Results;

The peak vertical force (PVF); gait analysis between group over 6 weeks after treatment indicate that **the Carprofen and EAB-277 group showed scientifically significant increased (improvement)** compared between group with 4 Cyte and Placebo groups. In addition, the performances of 4 Cyte and Placebo groups showed no significant difference between group and also within group based on PVF mean change of each group. This could imply that the 4 CYTE performance is equal to Placebo.

Remarks: This short summary is aimed to brief on 4 Cyte study results. Limited data provided as it is unpublished data and the outcomes of this study is under the manuscript preparation process.

3.

Randomized placebo-controlled trial to evaluate the effects of PCSO-524® (Antinol®) and EAB-277® (Antinol® Rapid) on weight bearing in the treatment of canine osteoarthritis by comparison with the administration of the compounds:
(i) NSAID: Meloxicam (Metacam, Boehringer Ingelheim Animal Health), (ii) PCSO-524®, (iii) EAB-277®, (iv) a combination of PCSO-524® with Meloxicam and (v) placebo.

This clinical trial was conducted by Kasetsart University, Thailand, and supervised and co-authored by **Dr. Duncan X. Lascelles** in the USA. The study investigated the hypothesis that **the combination of PCSO-524® and Meloxicam would result in a superior therapy compared with the use of Meloxicam or PCSO-524® or EAB-277® alone.**

Study Design:

There were a total of 194 dogs enrolled on this study over 4 week period by splited into 5 treatment groups as follows;

1) Combined treatment: Meloxicam+PCSO-524®
2) Meloxicam
3) PCSO-524®
4) EAB-277®
5) Placebo

The study also investigated whether the administration of PCSO-524® and EAB-277® to dogs with osteoarthritis demonstrated a significantly superior therapeutic effect compared with a placebo and whether the combined administration of PCSO-524® with the NSAID, Meloxicam, **resulted in a significantly superior therapeutic effect than with Meloxicam alone.**

- The study used objective force plate gait analysis
- Hematology and blood chemistry profile analysis
- Subjective orthopedic assessment scores
- Owner assessment scores.

Results show overall that **the combination of PCSO-524® and Meloxicam was superior to all the other treatments.** The PVF within-group analysis showed a significant increase (improvement) in over 4 weeks, seen in the Combined treatment, PCSO-524® and EAB-277® group but in The Placebo group worst in performances with no significant improvement. As the outcomes of this study is under manuscript preparation process to submit peer review journal.

Remarks; Unpublished data

4.

A Randomized Placebo Controlled Trial - Preliminary Study of the effects of PCSO-524® and EAB-277® on Renal Protective Function in the case of long term NSAID use for osteoarthritis in dogs.

This study at Kasetsart University, Thailand is designed to **determine the potential efficacy of PCSO-524® on renal (kidney) protection in the long term (4 week) NSAID treatment of dogs.**
This clinical trial is being conducted on 100 owned client dogs divided into five groups: (i) NSAID: Meloxicam (Metacam, Boehringer Ingelheim Animal Health), (ii) PCSO-524®, (iii) EAB-277®, (iv) a combination of PCSO-524® with Meloxicam and (v) Placebo.
The full parameters of renal function evaluation will be conducted to monitor the renal function of the dogs in each group including the newly innovative biomarkers (SDMA test, Idexx) and the CRP inflammatory cytokine test. The study also will show the effects of EAB-277® on renal function.

Results;

The results showed that the combination of PCSO-524® with the various NSAID formulas **provided no negative effect to the dogs' kidneys** during their treatment with NSAIDs.
The results of this Renal study will include in the Meloxicam study manuscript which is under preparation process to submit Peer Review Journal.

Remarks; Unpublished data

5.

The Effect of EAB-277® on Heart Rate Variability (HRV) in Dogs with Tracheal Collapse.

This preliminary project funds veterinary students at Chiang Mai University, Thailand.
The hypothesis is **to study the effects of EAB-277® to see if it will help decrease HRV impairment due to systemic inflammatory processes** which could induce the dysfunction of the sympathetic nervous system as well as the parasympathetic nervous system.

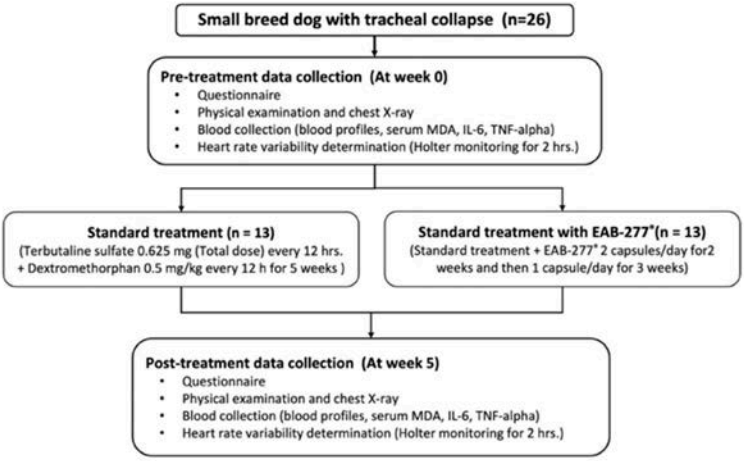


FIGURE 1 | Schematic representation of the study protocol. At pre-treatment, all dogs underwent chest X-ray, blood collection, Holter recording for 2-h. Then, dogs were divided into two groups to receive the standard treatment or standard treatment combined with polyunsaturated fatty acid EAB-277® from Green-Lipped Mussel blend for the 5 weeks. At post-treatment, all dogs underwent the re-assessment of chest X-ray, blood collection, Holter recording for 2-h to investigate cardiac sympathovagal balance, plasma oxidative stress, and inflammatory marker measurement. MDA, Malondialdehyde.

Ten small breed dogs will be used in this study and will be divided into two groups.

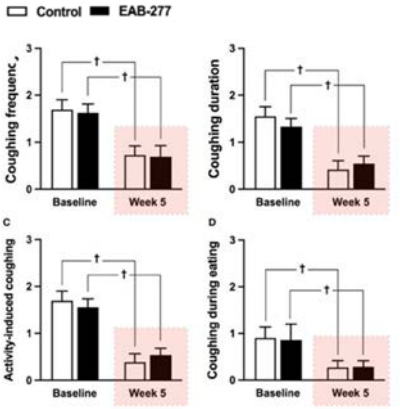
- The first group of five dogs comprises canine tracheal collapse patients given **standard treatment** for one month.
- The second group of five dogs comprises canine tracheal collapse patients given the **standard treatment plus EAB-277®** for one month.

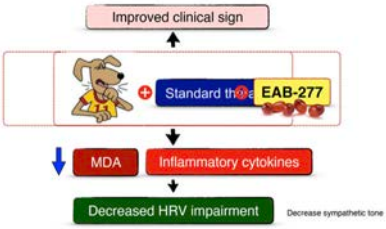
Blood from all dogs will be collected for testing of morphology, blood chemistry and measurement of heart rate variability.

The project has been completed. However, since the results showed improvement in the EAB-277® group based on owner assessments, the study has been expanded to add objective inflammatory biomarker data which is expected to show the relation between clinical improvement and the reduction in the level of inflammation in the body based on scientific evidence developed in the study.
This expanded study was completed at the end of the third quarter of 2021 and has been published in Frontiers in Veterinary Science, a peer reviewed journal, in 2022.

This study demonstrated the beneficial effects of **EAB-277® supplementation combined with standard therapy on heart rate variability** in dogs with tracheal collapse.

Both standard treatment alone and standard treatment combined with EAB-277® **improved all clinical signs** as evaluated by the questionaire.





The major findings of this study are as follows.

- First, EAB-277® supplementation for 5 weeks **did not affect the physical examination results, radiographic findings, or blood profile parameters** in the tracheal collapse dogs.
- Second, EAB-277® supplementation for 5 weeks **decreased oxidative stress and inflammatory marker compared to standard therapy** as indicated by serum MDA, canine serum IL-6 and canine TNF-α in tracheal collapse dogs.
- Third, EAB-277® supplementation for 5 weeks attenuated sympathovagal imbalance by **increasing parasympathetic activity** in tracheal collapse dogs.
- Fourth, using a questionnaire or evaluating TD/TI by chest X-ray was not accurate in evaluating the improvement of tracheal collapse in dogs.

Regarding clinical signs evaluated by questionnaire, **this study found improvement in clinical signs in tracheal collapse dogs after treatment compared to pre-treatment in both groups**, including a decrease in the frequency and duration of coughing, coughing induced by activities such as exercise, and eating and drinking.

A limitation of the questionnaire used in this study was the wide range of response scores and had much personal information, which could affect the reliability of the results.

This suggests that a questionnaire might not be appropriate as a prognostic tool for routine follow-up and routine examination in tracheal collapse dogs.

Frontiers in Veterinary Science | www.frontiersin.org 8 July 2022 |

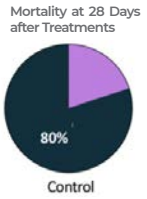
6.
Supplementary effect of EAB-277® in Combination with Prednisolone against Immune-Mediated Hemolytic Anemia in Dogs.

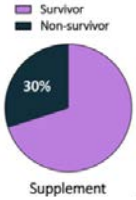
This preliminary project funds veterinary students at Chiang Mai University, Thailand to determine the effects of EAB-277® in combination with Prednisolone against immune-mediated hemolytic anemia in dogs. Ten dogs will be separated into two groups (five dogs in each group).

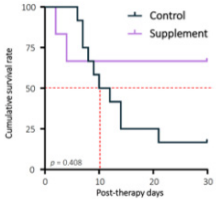
- *The first group of dogs* with immune-mediated hemolytic anemia will be treated with **Prednisolone.**
- *The second group of dogs* with immune-mediated hemolytic anemia will be treated with **EAB-277® as the adjunctive treatment combined with the standard therapy.**

The results are expected to result in the reduction of the Prednisolone dosage in immune-mediated hemolytic anemia cases.

The results demonstrate that The mortality rate of dogs in the control group (80%) was higher than the supplement group (30%) (Figure 1a,b).







Immune-mediated hemolytic anemia (IMHA) is a common autoimmune disorder in dogs with a high fatality rate and it remains a therapeutic challenge.
The marine lipid extract, EAB-277®, is a natural anti-inflammatory nutraceutical product.
However, the effects of EAB-277® in IMHA dogs has rarely been investigated.

The objective of this study is to assess the clinical effects of EAB-277® and prednisolone dose-tapering for supplemental therapy in IMHA dogs.

Prednisolone was given to 18 anemic IMHA dogs according to a standard regimen.
Six dogs were supplementally treated with EAB-277® for 28 days and the remaining twelve dogs were a control group of untreated supplementations.

The Kaplan–Meier curve was used to demonstrate the survival time from a certain date to the time of IMHA dog death (Figure 1c).
The results show that the killing ability of IMHA was time-dependent.
In the control group, the survival time was 11 post-therapeutic days and the survival rate markedly declined to 16.7 ± 10.7% at 28-day post-therapeutic observation, whereas the survival rate of dogs in the supplement group was 66.7 ± 19.2% throughout the study period (log-rank test, p = 0.408).

When compared to pre-therapy,
the supplement group's blood profiles improved (p < 0.05). The EAB-277® treated group showed a moderate decrease in the incidence rate (4.20 times) of prednisolone tapering compared to the control group.
The dosage reduction of prednisolone in supplement group was more than that in the control group (p < 0.0001).
Our results suggest that
EAB-277® supplementation may enhance clinical outcomes and lessen prednisolone dose-tapering in canine IMHA therapy.

7.
Tracheal Collapse Study in Small Dogs.

These 40 small dog studies at the Mahidol Research Centre of the Mahidol Veterinary School, Thailand is designed to evaluate the efficacy of EAB-277® (Antinol® Rapid) in reducing inflammation in tracheal collapse cases which is very common in small dog breeds causing the dog to have difficulty in breathing.

At present the common treatments prescribed for canine tracheal collapse are cough depressants, bronchodilators and steroids to control inflammation.

The objective of this study is to prove the anti-inflammatory efficacy of Antinol® Rapid as an adjunctive supplement to the standard treatments in tracheal collapse cases to demonstrate Antinol's superior therapeutic effects, including increased exercise tolerance and less coughing.

Study Design:

There were 40 client owned dogs enrolled over 8 weeks in this study by splited into 2 treatment groups as followed

- Control Group:
Standard treatment + Placebo

- Treatment group:
Standard treatment + EAB-277® supplementing

Results;

The treatment group showed improvement in respiratory effort and appetite compared to the placebo group after received the supplement on day 28 compared to day 14 (P=0.017 and 0.021). Fluoroscopically, comparisons of diameter changing of tracheal height within groups showed a significant improvement in the treatment group at thoracic inlet region (P =0.047) when compared the changing diameter on day 14 to day 0.

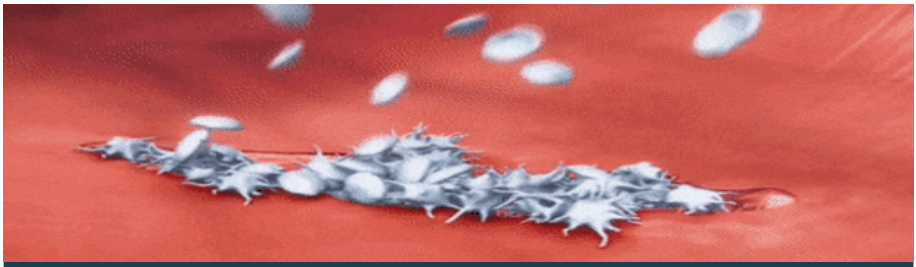
Conclusion and clinical importance;

EAB-277®, the additional supplement for manage tracheal collapse in dogs can improve the quality of life and decrease percent change obtained from fluoroscopy.

Remarks; Unpublished data

8.
Effects of marine based fatty acid compound EAB-277® on coagulopathy in dogs.

This preliminary project funds veterinary students at Kasetsart University, Thailand to investigate **whether long term supplementation of the marine based fatty acid compound EAB-277® affects blood coagulation.** If there is an effect, the study seeks to learn how long the canine patient needs to be withdrawn from the supplementation of EAB-277® prior to a surgical operation to reduce the risk of bleeding during the operation.



This preliminary study is being conducted by veterinary students on **nine dogs with a recommended dose** based on lean body weight and will **continue for three months.**

Blood samples will be collected to record

- The base line of hematology coagulation parameters
- Activated prothrombin time (apTT)
- Prothrombin time (PT)
- Thrombin time (TT)
- Collagen-epinephrine closure time (CEPI-CT)
- Collagen-ADP closure time (CADP-CT)

before prescribing EAB-277® at the outset and one, two and three months after treatment.

If there is no coagulopathy effect shown, all dogs will continue with EAB-277® and will be monitored for the above parameters on a weekly basis.
If coagulopathy is shown, then from that point EAB-277® will be withdrawn and the dogs will be followed on a weekly basis until their coagulation parameters have returned to normal.

Results;

The study demonstrated that **consumption of EAB-277® at 20 mg per kg (4 times of loading dose) continuously for 3 months did not prolong coagulation.** Although some dogs showed the higher collagen-epinephrine closuretime (CEPI-CT) than Human reference range, it can not conclude that EAB-277® prolong coagulation in dogs as there is no report of Canine CEPI-CT morover the delayed coagulation was interpreted based on > 300 sec. However the normal reference range for dogs and cats should be defined by studing in larger samples sizes.

Remarks; Unpublished data

9.
Safety Study of EAB-277® (Antinol® Rapid) in Dogs.

This study at Chulalongkorn University, Thailand was proposed to evaluate the safety of EAB-277® in dogs which is required for veterinary product registration purposes. Initially a total of 40 mature beagles of 8 months in age or older were divided equally into four groups:
(i) placebo, (ii) recommended loading dose of two capsules per day, (iii) administration of six capsules once per day and (iv) administration of twenty capsules once per day

10.
Safety Study of EAB-277® (Antinol® Rapid) in Cats.

This study at Chulalongkorn University, Thailand has been proposed to evaluate the safety of EAB-277® in cats which is required for veterinary product registration purposes. A total of 36 cats of 8 months in age or older were divided equally into four groups: (i) placebo, (ii) recommended dose of 1 capsule per day, (iii) administration of 3 capsules once per day and (iv) administration of 10 capsules once per day.

Results;

Animals used in the current study composed of **twenty healthy mature beagle dogs** (12 female and 8 male), body weight 9.50+0.75 kg and twelve healthy mature domestic cats (8 female and 4 male), body weight 3.25+0.50 kg. All animals had the body condition scores of 2 to 3 (on a 5-point scale) at the beginning of study.
Physical examination of all studied population composing of body temperature (38.4-38.,8oC), capillary refill time (CRT, 1 or 2 second) and fecal score (2.5 to 3.5) (on a 5-point scale) were all normal. No animals had neither constipation nor diarrhea.
Studied dogs and cats maintained their initial body condition without significant weight loss or gain. No abnormalities and illness sign of any system (ocular, nervous, musculoskeletal and integumentary systems) were observed. Blood parasite was not found for both dogs and cats.
Both dogs and cats of all four treatment groups preferred consumption of the PUFAs supplement capsules even the empty ones. No association was detected between the dose treatments and the dependent variables including blood hematology, blood chemistry, blood clotting factors, and PLI values.

Remarks; Unpublished data

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