Antinol®

THE EXECUTIVE SUMMARY

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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.

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

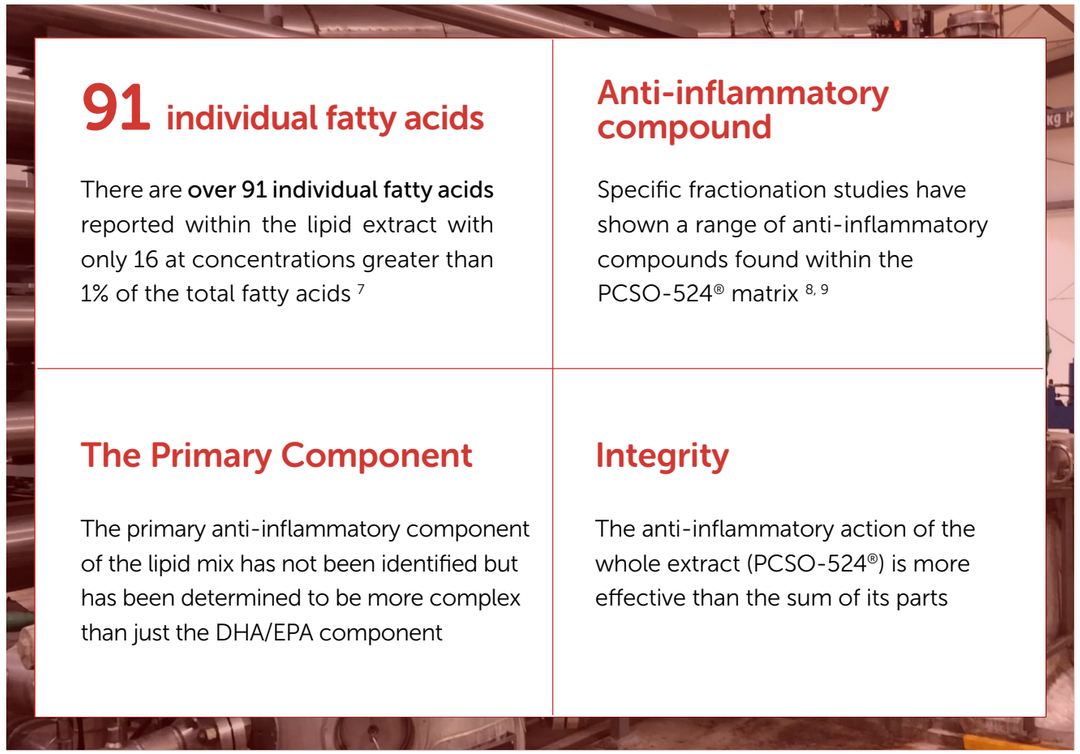
The extraction is undertaken using supercritical fluid extraction (SFE) technology which uses liquid carbon dioxide (CO_2) as a solvent. CO_2 is an ideal solvent as it becomes liquid under increased pressure and after extracting the oil the pressure is raised, and the CO_2 turns back into a gas leaving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

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

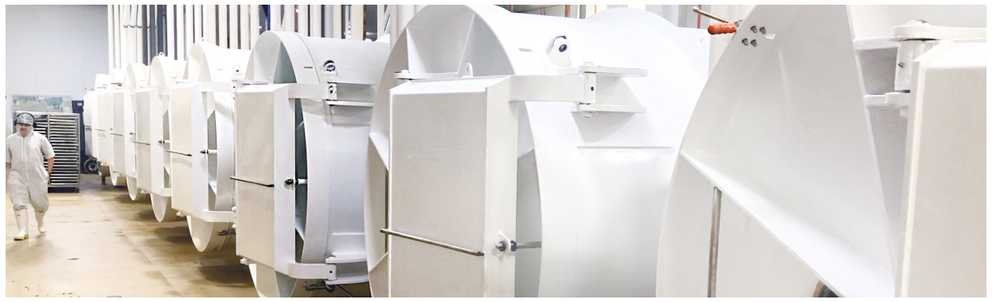
The primary anti-inflammatory component of the lipid mix has not been identified but has been determined to be more complex than just the DHA/EPA component

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].

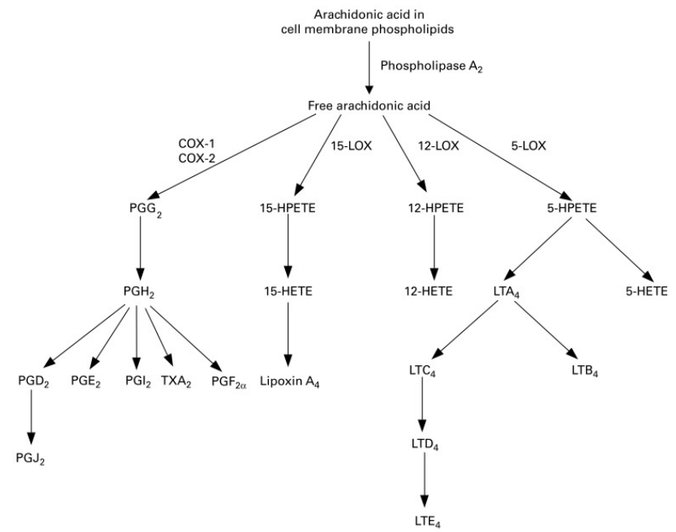


Figure 1 Pathways of eicosanoid synthesis from arachidonic acid. COX, cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroxyperoxyeicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane. [16]

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.**

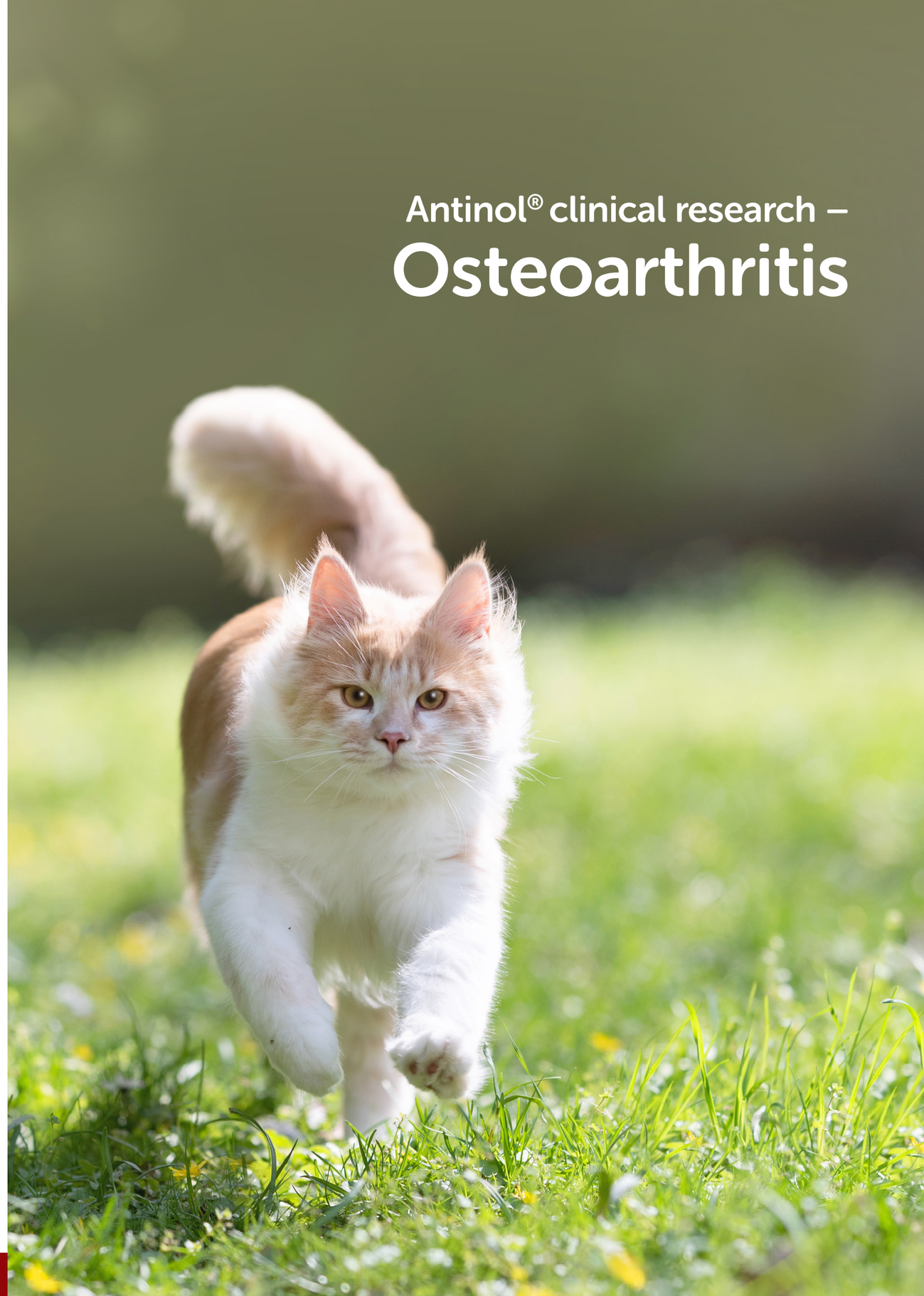
Supplementation with Antinol® has been shown in a canine study to be as effective as NSAIDs for improving the weight-bearing capacity of the affected limb, with superiority in clinical assessment seen with Antinol® only [12]. This study also saw a slightly enhanced effect when both NSAIDs and Antinol®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

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) PCSO-524 in Comparison the NSAID Carprofen for Treatment of Canine Osteoarthritis Thai Journal of Veterinary Medicine. 2016. 46(3): 363-371[12]

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 2.

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.

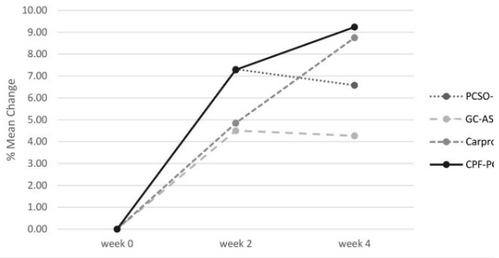


Figure 2 Adjusted PVF values across treatment groups [12]

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.

2) Effect of PCSO-524 on OA Biomarkers and Weight-bearing Properties in Canine Osteoarthritis Thai Journal of Veterinary Medicine 2015. 45(2): 157-165[13]

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).

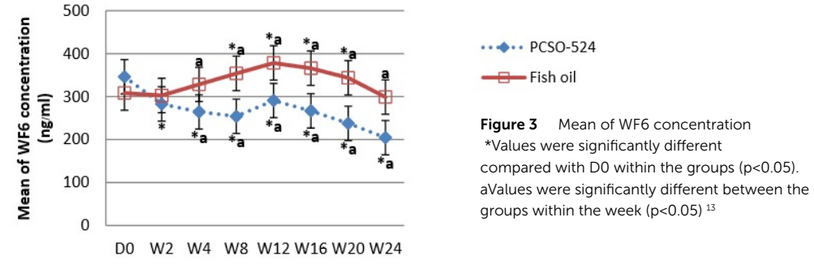


Figure 3 Mean of WF6 concentration
*Values were significantly different compared with D0 within the groups ($p<0.05$).
aValues were significantly different between the groups within the week ($p<0.05$) [13]

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 Figure 4 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)

Figure 4 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 [13]

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.

3) PCSO-524 for Treatment of Canine OA and Degenerative Spinal Disease Thai Journal of Veterinary Medicine. 2012. 42(3): 311-317 [20]

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 the primary outcome being an improvement in clinical assessment after four weeks of treatment. In both of the OA groups, between 88-90% of the dogs showed clinical improvements, see Figure 5, 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)

Figure 5 Clinical outcomes of OA at hip and shoulder joints with PCSO-524 treatment after 4 weeks[20]

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

35% improved by 1 point,
48% improved by 2 points,
6% improved by 3 points,
while **none of the dog's scores decreased**, see Figure 6.

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)

Figure 6 Lameness score of OA at hip and shoulder after four weeks of PCSO-524 [20]

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.

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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. 45(4): 639-643 [21]

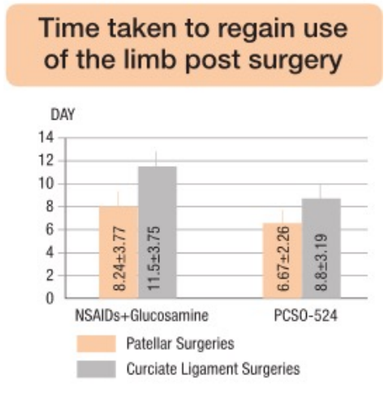
This trial included 30 Pomeranian dogs undergoing **surgery for medial-patellar luxation repair**. The dogs were split into 3 groups post-surgery, 1) firocoxib (5 mg/kg/d); 2) PCSO-524 (10 mg/kg, twice daily), or 3) a combination of both firocoxib and PCSO-524.

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. improvement of neurological signs was seen in 85% of the group.

5) 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 [14]

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 7.



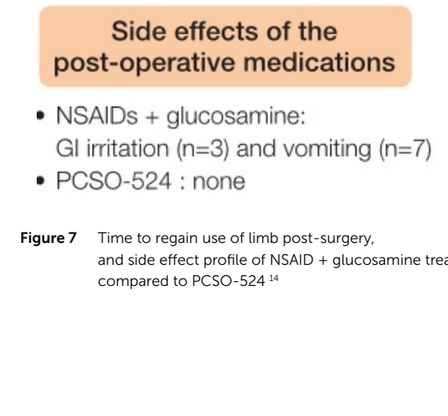


Figure 7 Time to regain use of limb post-surgery, and side effect profile of NSAID + glucosamine treatment compared to PCSO-524 [14]

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.

6) The Effectiveness of Marine Based Fatty Acid Compound (PCSO-524) and Firocoxib in the Treatment of Canine Osteoarthritis. Beale et al. Poster Presentation Veterinary Orthopedic 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

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 were slightly lower for carprofen only, and around 6.5% change for the PCSO-524 only group, see Figure 2.

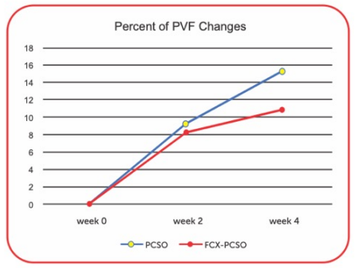


Figure 1 Changes in Peak Vertical Force values over time expressed as a percentage.

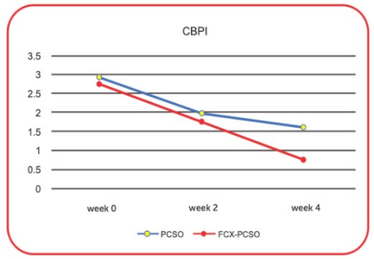


Figure 2 Overall mean CBPI scores (combined pain and pain interference sections) over time.

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($\pm$1.27) and 6.19($\pm$1.8%BW) [mean($\pm$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 the both groups showed significant decreases at 2 and 4 weeks compared with their pretreatment value ($p<0.05$).

7) The Effectiveness of Marine Based Fatty Acid Compound (PCSO-524) and Firocoxib in the Treatment of Canine Osteoarthritis. Vijarnsorn et al. Veterinary Research: 2019. 15:349 http://doi.org/10.1186/s12917-019-2110-7

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;

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).

Table 2 PVF values at pre-treatment (day0), day14 and day28 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

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 ($\pm$4.13), 2.01 ($\pm$3.86), and 4.11 ($\pm$4.69) % BW [mean ($\pm$SD)] respectively.

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. 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.

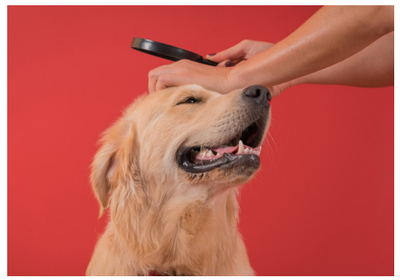
The results of the study suggest that within each group of a combination (PCSO-524 and Firocoxib), PCSO-524, Firocoxib showed the significant improvement of weight bearing ability but not in the comparison between.

8) The Clinical Study of PCSO-524 (Antinol®) as Neutraceutical in Canine Allergic Skin Disease. Chaiyot et al. Oral Presentation at 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 Group1. 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%).

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 severe degree of inflammation they experienced. PCSO-524 may be used as treatment of canine atopic dermatitis.

Safety in canines

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].

Human clinical research – Osteoarthritis

There are seven human clinical trials that have been conducted in osteoarthritis using PCSO-524® or a pre-cursor Green-Lipped Mussel extract (GLMe), with a combined total of 368 OA patients [25-31]. The trials are all slightly different in design and cover mild to severe OA. However, the key areas of investigation across the studies support a reduction of pain and an increase in function with PCSO-524® supplementation.

(1) Pain relief for osteoarthritis of the knee and/or hip from PCSO-524 in comparison to fish oil. Rheumatologia. 2011. 49(4): 244-252 [25]

This clinical trial compared the effects of PCSO-524® (400 mg/day) to a standardised fish oil (1200 mg/day) in 50 older patients with **OA of the knee and/or hip**. The authors evaluated the difference in the level of pain and quality of life, as well as the patient's perception of their clinical response [25].

What they found was that those treated with fish oil showed significantly less improvement in all areas and had a greater level of physical discomfort during the study.

Those taking the PCSO-524® treatment found a substantial reduction in their pain over the twelve week treatment period (from 66 to 6.9 on a 100 point scale), while the pain levels in the fish oil group decreased from 64 to 60 on the same scale. This equates to an almost ten-fold improvement in the level of pain that was experienced in the PCSO-524® group. Additionally, 91% of people in the PCSO-524® group reported an improvement in their quality of life.

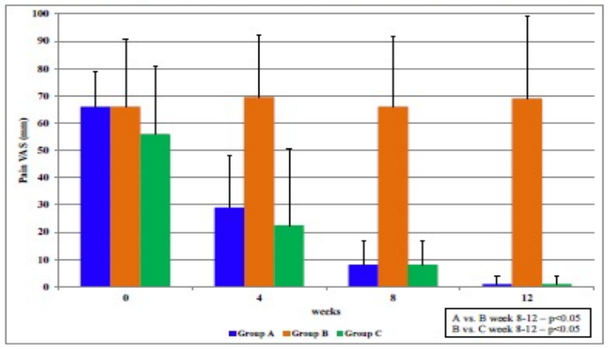


Figure 8
Changes in visual analogue scale (VAS) pain measurement in osteoarthritis.

Group A = PCSO-524® (400mg) stage 1;

Group B = fish oil (1200mg);

Group C + PCSO-524® (400mg) stage 2 (n=50) [26]

Due to the positive results seen in the cohort taking PCSO-524®, a second phase of the trial was undertaken in those that had been randomised to the fish oil group. The fish oil group was switched to PCSO-524® for a further 12 weeks and saw similar levels of pain reduction as the first group [26].

Figure 8 shows the changes in pain scores for the initial PCSO-524® group as Group A; fish oil group as Group B; and the second PCSO-524® cohort as Group C. **The results very strongly support PCSO-524® effect in reducing pain in OA of the knee and/or hip.**

(2) Pain relief for osteoarthritis of the knee and/or hip from PCSO-524 in comparison to fish oil. International Journal of Pharmaceutical Sciences Review and Research. 2014. 27(1): 209-216 [29]

A randomised controlled trial in 45 people evaluated the effect of 2 capsules per day of PCSO-524 (100 mg PCSO-524/day) for six weeks in a group of older adults with **OA in various joints**. All of the patients in this trial were given PCSO-524, with half of the group given an additional supplement (D-002-a standardised beeswax alcohol) and the other half was given placebo as an adjunct.

A standard measure of OA severity known as WOMAC 32 was used to test the treatments across three subscales of pain, stiffness and physical function [29]. Using this scale, they found a total reduction from 29.6 to 6.2 over a six week treatment period in people supplemented with PCSO-524 and placebo. The individual subscales showed improvement in total score, pain, stiffness, and physical scores of WOMAC of 79.1%, 73.6%, 84%, and 82% respectively. These results are shown in Figure 9. The pain was also assessed separately and decreased in this group from 62 to 11 on a 100 point scale.

This study used a dose of only 100 mg/day of PCSO-524, which is much less than that used in the previously described research.

A)

WEEK	GME + Placebo
0 (baseline)	29.6 ± 0.9
1	18.8 ± 1.0*
2	13.5 ± 1.1*
3	9.2 ± 1.3*
4	7.9 ± 1.2*
5	7.0 ± 1.1*
6	6.2 ± 1.0*

B)

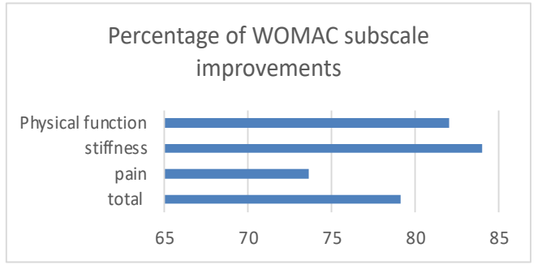


Figure 9 a) WOMAC scores from baseline to 6 weeks in Lyprinol plus placebo group (GME + Placebo).
b) Percentage change in WOMAC scores over 6 weeks with 100 mg/day Lyprinol) in people with osteoarthritis (n=25) [29]

Other human OA studies

Similarly, a Korean study in 60 OA patients with **hip and knee OA** found reductions in a joint function index from 13.7 to 8.6 after eight weeks of treatment with PCSO-524® (200 mg/day), and reductions in pain from 64 to 39 on a 100 point scale [27]. Positive results were also seen in a Chinese study assessing the use of PCSO-524® in pain and functional scales for OA, with significant differences between PCSO-524® and placebo at several time points throughout a six month treatment period [33]. Results from this study show a steady decline in pain associated with OA through to week 12, see Figure 10.

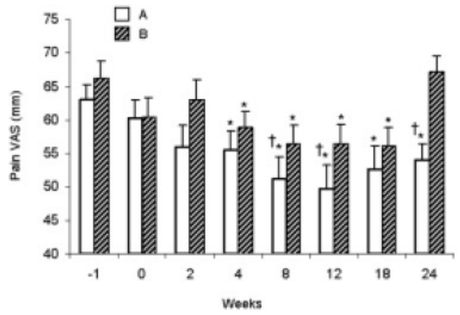
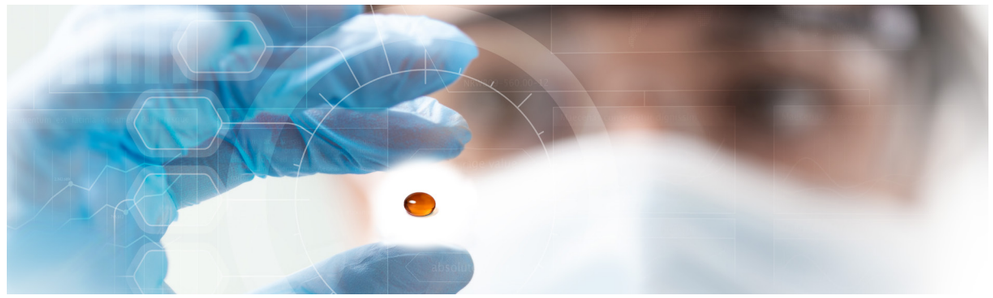


Figure 10 Changes in pain over 6 months with 200 mg/day Lyprinol in people with osteoarthritis31 A = Lyprinol group; B = placebo group



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-γ).

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

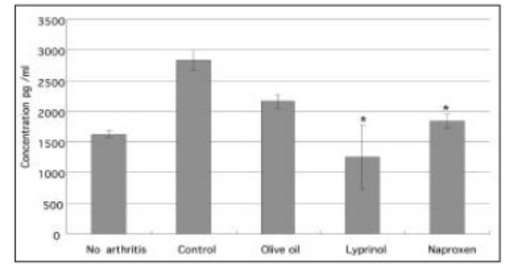


Figure 11 Amount of pro-inflammatory cytokine TNF-α – 14 days after arthritis induction in rats [3]

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 12.

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.

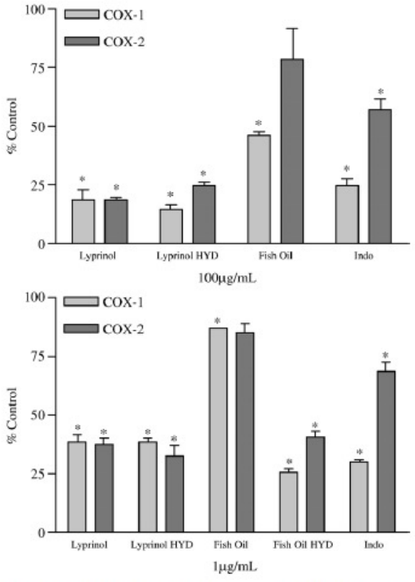


Figure 12 Inhibition of COX-1 and COX-2 by Lyprinol, fish oil and indomethacin at 100 and 1 ug/mL.
Lyprinol HYD = hydrolysed Lyprinol complex and Fish oil HYD = hydrolysed fish oil complex [2]

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 [35].

This research group then demonstrated the anti-inflammatory activity of these isolated constituents in a rat model of arthritis.

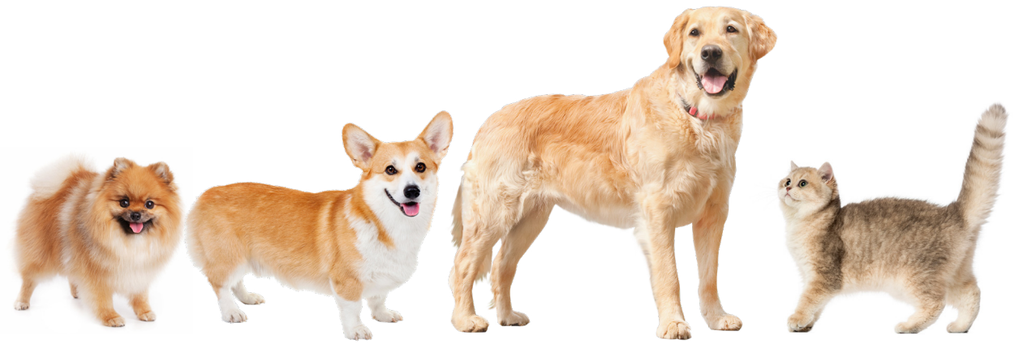
Dosage

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

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

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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