Preliminary results on the efficacy of a dietary supplement combined with physiotherapy in dogs with osteoarthritis on biomarkers of oxidative stress and inflammation

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**ABSTRACT**

The purpose of this study was to evaluate the effects of a supplement containing Cannabis sativa oil and other plant derivatives (Boswellia serrata Roxb. and Zingiber officinalis phytosomized extracts) combined with physiotherapy treatments in dogs with osteoarthritis. We evaluated the response of serum inflammatory and oxidative stress biomarkers to the treatment. We found a significant reduction in the levels of serum markers reflecting the strong anti-inflammatory and antioxidant properties of the supplement under study.

**HIGHLIGHTS**

- Diet supplements are a valid alternative therapy in dogs with osteoarthritis.
- Evidence of anti-inflammatory and antioxidant properties of a supplement containing Cannabis sativa oil and other plant derivatives (Boswellia serrata Roxb. and Zingiber officinalis).

**Introduction**

Osteoarthritis (OA) is a chronic, degenerative and inflammatory disease that affects the synovial joints and leads to loss of mobility (Zhang 2018).

There are several markers of oxidative stress and the level of antioxidant enzymes can change in the blood of dogs affected by OA. Glutathione (GSH) is an elective serum marker (Goranov 2007) together with the serum C-reactive protein (CRP). The concentration of these markers was found elevated in patients with OA (Zhang 2018). The use of natural supplements to help pets overcome several clinical conditions including OA is becoming very appealing to dog owners.

The purpose of this randomised placebo-controlled, double-blind clinical trial was to evaluate the effects of a novel nutritional supplement (Evexia Plus, Candioli s.r.l., Italy) containing Cannabis sativa oil and other plant derivatives, Boswellia serrata Roxb. and Zingiber officinalis phytosomized extracts combined with physiotherapy treatments in dogs with OA and to generate preliminary data for future studies.

**Materials and methods**

Dogs included in the study had the following characteristics: age >6 months, weight 10–60 kg, body condition score 2–4 (on a scale of 1–5), and muscle condition score (WSAVA Global Nutrition Committee) between ‘normal’ and ‘moderate loss’, radiographic signs of OA in their elbow, knee, or hip at the first visit. Animals with acute pain, signs of recent trauma or surgery, neurological conditions, treated with corticosteroids or NSAIDs or other supplements 14 days before the beginning of the study, allergy to any of the ingredients included in the tested supplement were excluded. The dogs’ owners were informed and signed informed consent. The study was performed in compliance with the guidelines of the Italian Minister of Health for the care and use of animals (D.L. n 26 2014) and the use of supplements was regulated by the Regulation (EC) No 767/2009.

This study was designed as a randomised placebo-controlled, double-blind clinical trial. The duration of the entire trial was 80 days.
A total of 27 dogs were randomly assigned to two groups: Control (CTR, \( n = 13 \)) and Treated (TRT, \( n = 14 \)). The CTR group was given a placebo while the treated group was tested with the supplement. A dose of one tablet (2 g) every 10 kg of body weight was orally administered once daily for a total of six weeks (Supplementary Appendix 1). The animals were under physiotherapy treatment once a week.

Dogs were evaluated at the baseline (T0), then after 20 (T1), 40 (T2), 60 (T3), and 80 (T4) days. The veterinarian recorded at T0 data on sex, age, breed, body weight, and radiological assessment. Body condition score was calculated four times (T0–T3). Blood analysis (complete blood count and serum biochemical analysis) was performed at T0 and T4. In particular, the response of serum inflammatory and oxidative stress biomarkers was evaluated using two blood parameters: C-reactive protein (CRP) and Glutathione (GSH). The owner evaluated at each time point, the chronic pain of its animal, using a validated Helsinki Chronic Pain Index (HCPI) questionnaire translated in Italian (Martello et al. 2018). The statistical analysis was performed using ANOVA 2-way (treatment; time) with repeated measures on the factor ‘time’, followed by the Sidak’s test for post-hoc comparisons (when appropriate) (GraphPad Prism, Version 6.05). The \( p \)-value was set at <.05.

**Results and discussion**

The ages of dogs ranged from 8 to 15 years (TRT: mean 10 ± 2.4 SD; CTR mean 9.5 ± 1.7 SD), males were 13 (eight TRT and five CTR group) and female 14 (six TRT and eight CTR groups). Several dog breeds were included, with their weight ranging from 5 to 54 kg (TRT: mean 33.9 kg ± 16.3 SD; CTR 29.5 kg ± 14.5 SD). No dog was withdrawn during the study and no adverse clinical effects as vomiting or diarrhoea were reported by owners. Regarding the BCS and values of all blood parameters, no statistically significant changes were reported. The marker for oxidative stress levels (GSH) was found significantly lower in the TRT group at T0 but significantly higher at T4. On the other hand, serum CRP concentrations were similar at T0 in both groups but significantly lower in the TRT group at T4. Biomarkers for oxidative stress and inflammation show statistical differences when comparing the two groups. These results support our hypothesis being the supplement positively effective on metabolic parameters. Changes in the values of CRP can be linked to the *Boswellia serrata* activity. In fact, recent studies on animals and humans showed its potential in the treatment of a variety of inflammatory disorders like OA (Umar et al. 2014).

The increase in the GSH levels may be attributed primarily to *Cannabis sativa* oil. In literature, the antioxidant activity of *Cannabis sativa* is linked to the presence of (E)-caryophyllene and caryophyllene oxide in high concentrations (Nafis et al. 2019). In recent years, the Food and Drug Administration has required companies to rely on owner assessments as a primary variable tested in studies evaluating drugs for OA in dogs. Using data on HCPI scores, the ANOVA test indicated a highly significant effect of time \([F_{(3,75)} = 77.90, p < .0001]\), no effect of treatment \([F_{(1,25)} = 1.23, p > .05]\), and a significant interaction \([F_{(3,75)} = 3.33, p < .05]\) on HCPI scores. However, no difference was observed at post-hoc analysis. This result shows that providing the supplement in combination with physiotherapy progressively reduces the pain in dogs with OA. No significant difference in HCPI scores was found between TRT and CTR groups, and this could be due to the fact that clinical signs and behaviour associated with orthopaedic pain in dogs can be undetected or misinterpreted by the owners who completed the questionnaire. In general, owners show difficulty in identifying the initial condition and progression of chronic pain associated with OA, with a delay in providing treatment to the animal (Gilbert et al. 2019). On the other hand, a significant reduction in the levels of both serum markers (CRP and GSH) reflects the strong anti-inflammatory and antioxidant proprieties of the supplement under study. A significant progressive reduction of dog pain was also observed even if no significant difference in the HCPI scores was highlighted. This is probably the consequence of a faster effect of physiotherapy on patients never treated before. As reported in the literature, multiple approaches are used for the management of OA in dogs. Studies showed positive results on the progression of OA in dogs using classical drugs (i.e. Meloxicam*, Carprofen*) but also nutraceutical (i.e. Dinamic*, Confis Ultra*) (Aragon et al. 2007; Comblain et al. 2016; Martello et al. 2018; Musco et al. 2019).

**Conclusion**

This preliminary data helps to better plan future trials where a longer administration of the tested supplement in association with physiotherapy could also reveal a stronger effect on chronic pain together with
the confirmation of the effect on objective blood parameters like CRP and GSH. The effect of animal breed and size could be also evaluated.

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Disclosure statement

One of the authors is an employee of the Candioli Pharma S.r.l. Two of the authors are scientific consultants for the Candioli Pharma S.r.l. Candioli Pharma S.r.l is a company that may be affected by the research reported.

Ethical approval

The dogs’ owners were informed and signed informed consent. The study was performed in compliance with the guidelines of the Italian Minister of Health for the care and use of animals (D.L. n 26 2014) and the use of supplements was regulated by the Regulation (EC) No 767/2009.

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author (EM).

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