Research Articles

Effects of a Standardized Extract of *Centella asiatica* ECa 233 on Clinical Parameters and Radiography Using Computed Tomography in Dogs with Osteoarthritis

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Received: 23 May 2023; Revised: 16 July 2023; Accepted: 17 July 2023

Abstract

Osteoarthritis in dogs is a chronic disease that causes pain from inflammation of the joints. For treatment, medication is only used for the relief of the pain and inflammation of the joints. Side effects of long-term treatment affect multiple systems. This study aimed to evaluate the effects of a standardized extract of *Centella asiatica* (ECa 233) in dogs with osteoarthritis by examining the clinical score, C-reactive protein (CRP), radiographs, and computed tomography (CT) imaging. Eight dogs were diagnosed with osteoarthritis. The Eca 233 was given to the dogs orally at a dose of 10 mg/kg once a day for 28 days. Results showed that the clinical evaluation found that most parameters showed improvement compared to pretreatment. The mean clinical lameness score after treatment was found to have decreased significantly from 2.13±1.36 to 0.5±0.93 (p=0.01). Both the mean CRP values and radiographic score had decreased insignificantly. The average CT Hounsfield unit (HU) value was also decreased significantly, which indicated that ECa233 improved the soft tissue around the joint and the function of the osteoarthritis joint. These results showed that ECa233 reduced the pain and inflammatory reaction resulting in better walking ability and life quality in dogs with osteoarthritis.

Keywords: Computed tomography, dogs, ECa233, Osteoarthritis, Radiography

ผลของสารสกัดมาตรฐานบัวบกอีซีเอ 233 ต่อการประเมินทางคลินิกและภาพทางรังสีโดยใช้เครื่องเอกซเรย์คอมพิวเตอร์ในสุนัขที่มีภาวะข้อเสื่อม

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Received: 23 May 2023; Revised: 16 July 2023; Accepted: 17 July 2023

บทคัดย่อ

โรคข้อเสื่อมในสุนัขเป็นโรคเรื้อรังที่ทำให้เกิดความเจ็บปวดจากการอักเสบของข้อ การรักษาทำได้โดยการใช้ยาเพื่อลดอาการเจ็บปวดและการอักเสบของข้อ แต่เมื่อใช้ยาไปนานๆอาจเกิดผลข้างเคียงต่อระบบต่างๆตามมาได้ การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของสารสกัดมาตรฐานบัวบกอีซีเอ 233 ต่อโรคข้อเสื่อมในสุนัขโดยประเมินค่าทางคลินิก ค่าโปรตีนซี-รีแอกทีฟ ภาพทางรังสีโดยใช้เครื่องเอกซเรย์คอมพิวเตอร์ สุนัข 8 ตัวที่มีภาวะข้อเสื่อมได้รับสารมาตรฐานอีซีเอ 233 ขนาด10 มิลลิกรัมต่อกิโลกรัมน้ำหนักตัวสุนัข โดยการกินวันละ 1 ครั้ง ติดต่อกันเป็นเวลา 28 วัน ผลการวิจัยพบว่าการประเมินทางคลินิกส่วนมากพบว่ามีอาการดีขึ้นหลังได้รับสารสกัดมาตรฐานบัวบกอีซีเอ 233 ค่าเฉลี่ยคะแนนความเจ็บปวดขณะเดินลดลงอย่างมีนัยสำคัญทางสถิติจาก 2.13±1.36 เหลือ 0.5±0.93 (p=0.01) ค่าโปรตีนซี-รีแอกทีฟและค่าเฉลี่ยคะแนนภาพรังสีลดลงแต่ไม่มีนัยสำคัญทางสถิติ ค่าเฉลี่ย HU ของเนื้อเยื่อรอบๆข้อสะโพกจากภาพเอกซเรย์คอมพิวเตอร์ลดลง อย่างมีนัยสำคัญทางสถิติ ซึ่งชี้แสดงให้เห็นว่าสารสกัดมาตรฐานบัวบกอีซีเอ 233 ทำให้เนื้อเยื่อรอบๆข้อมีการอักเสบลดลงช่วยให้ข้อทำงานดีขึ้น ผลการทดลองแสดงให้เห็นว่าสารสกัดมาตรฐานบัวบกอีซีเอ 233 ช่วยลดความเจ็บปวดและการอักเสบ ส่วนหนึ่งดูได้จากการเดินและคุณภาพชีวิตที่ดีขึ้นในสุนัขที่มีภาวะข้อเสื่อม

คำสำคัญ : เอกซเรย์คอมพิวเตอร์ สุนัข สารสกัดมาตรฐานบัวบกอีซีเอ 233 โรคข้อเสื่อม ภาพทางรังสี

Introduction

Osteoarthritis (OA) in dogs is a chronic disease that causes pain from inflammation of the joints resulting in poor life quality. The most common breeds are Labrador Retrievers and German Shepherds. In particular, German Shepherds that are overweight have a higher incidence (Anderson et al., 2018). No proper treatment for OA leads to long-term abnormality of the structure and function of the joints. Supportive treatment also only uses medication for the relief of the pain and inflammation of the joints. Furthermore, long-term use of anti-inflammatory drugs may cause the malfunctions of the liver and kidneys (Alhassani et al., 2021).

Diagnosis of OA by history taking, physical examination, radiography (X-rays), and computed tomography (CT-scan) determined the joint structure, bone formation, and subchondral bone sclerosis. Previous studies reported the same sensitivity and specificity for damage to an intervertebral disc diagnosis (Marino and Loughin 2010). However, it had been reported that a CT-scan could enable quantitative assessment of the soft tissues and materials using the Hounsfield unit (HU) (Barber and Dockery 2011). The HU is also referred to as CT numbers, which correspond to the average amount of radiation absorbed by the tissue (Ai et al., 2018). The absorption is measured for any given slice relative to water, which is one unit. In comparison, the bone is 700-3,000 units and air 0.001 units (Ai et al., 2018). Therefore, the HU value could be used to analyze the quality of the tissues around the joint. Serum markers, such as C-reactive protein (CRP) would indicate the severity, prognosis, and follow-up of treatment in dogs (de Bakker et al., 2017).

The goals of the OA treatment consist of improvement or preservation of the function of the joints, progressive prevention of the disease, pain control, and maintenance of the body weight and health conditions. There is differential management with individualized medication that includes a combination of non-medication (Ashford and Williard 2014). The recommended drugs used for treatment in OA are Non-Steroidal Anti-Inflammatory drugs (NSAIDs) such as Carprofen, Meroxicam, Deracoxib, Ketoprofen, etc. In patients with resistance to NSAIDs, adjunctive pain medication is used to control pain, such as Amantadine, Gabapentin, Tramadol, Codeine, Corticosteroid, etc. However, there is unclear information on their effectiveness in pain control in OA.

The herb *Centella asiatica* has anti-inflammatory effects and other properties (Somchit et al., 2004). The anti-inflammatory effect is the property of Eca233 extract, contributes to the wound healing process (Wannarat 2009; Anukunwithaya et al., 2017). Moreover, it enhances the healing effect on second-degree burns with the shrinkage of the wound, increasing of the blood flow to the skin, and increasing of the hair follicle, thus making the wound become dry and smooth, as well as reducing vascular inflammation and inflammatory cell migration. In addition, research has confirmed that ECa233 contributes to the healing of oral ulcers in humans by reducing inflammation, pain level and wound size (Ruengprasertkit et al., 2010). The anti-inflammatory effect of ECa233 is a good candidate for the prevention and treatment of inflammatory-related diseases (Sukketsiri et al., 2019). In rat and mouse experiments, it was found that up to 10 g/kg of ECa233 extract could be administered without acute toxicity (Chivapat et al., 2011).

However, no study has evaluated the ECa 233 for anti-inflammation purposes in OA dogs using the clinical score, CRP, radiography, and CT-scan imaging. The

purpose of this study was to investigate the effects of ECa233 on anti-inflammation by evaluating the clinical score, CRP, radiography, and CT-scan imaging in osteoarthritis dogs.

Materials and Methods

Animals

The experimental protocol was approved by the Faculty of Veterinary Science and the Ethics committee, Mahidol University, Thailand (MUVS-2021-05-20). Eight dogs were diagnosed with osteoarthritis, recruited from the Prasuarthorn Animal Hospital. All dogs fulfilled the inclusion criteria and were enrolled. All dogs did not receive any medication. All were prescribed with ECa233 for 28 days.

Inclusion/exclusion criteria

Dogs with clinical signs of chronic lameness (more than 1 month), 3-5 stiffness and joint pain, and radiological evidence of OA of the hip were eligible. Dogs were examined by veterinarian to confirm OA, previously entrance to this study. All osteoarthritis dogs were categorized with 1-4 lameness grades according to Table 1 (Nganvongpanit et al., 2014). Dog with the conditions of pregnancy or hepatic, cardiovascular, gastrointestinal, neurological disease were excluded. Dog with lameness due to lumbosacral instability, infection, immune disease, or fractures, including dogs which had previously received drug or dietary supplements for osteoarthritis treatment were also excluded.

The standardized extract ECa233

The standardized extract ECa233 containing madecassoside (51% w/w) and asiaticoside (38% w/w) was obtained from Siam Herbal Innovation Co. Ltd, Thailand). It is given to dogs orally at a dose of 10 mg/kg once a day after meal for 28 days. ECa233 10 mg/kg by conversion dose from the resulting reduce inflammation in mice (Nair and Jacob 2016).

Methods for assessing osteoarthritis

Clinical score

Efficacy of treatment for relieving inflammation in dogs with osteoarthritis can also be assessed using a clinical lameness scoring system which was examined by walking and trotted 12 meters (6 meters for evaluation) on a flat floor in the Prasuarthorn Animal Hospital. The test was conducted 3 times and evaluated by the veterinarian based on the criteria were modified from (Nganvongpanit et al., 2014) in (Table 1).

Table 1. Lameness scores for assessing dogs with osteoarthritis.

Grade	Clinical evaluation
1	Walks normally
2	Slightly lame when walking
3	Moderately lame when walking
4	Severely lame when walking
5	Reluctant to rise and will not walk more than five paces

Blood collection

Blood samples were collected from the vein of dogs (cephalic, saphenous or jugular vein) for 3 ml from each dog on before and after the treatment. Blood chemistry and serum CRP were performed at the laboratory of Prasuarthorn Animal Hospital, Faculty of Veterinary Science, Mahidol University.

Radiography

Radiographs of the participating dogs were taken before and after 28 days of ECa233 administration by the same radiologist technician and standard computed radiography machine. Radiographs of dogs were performed in lateral and extended-ventrodorsal view of hip and stifle. Repositioning of the dog for subsequent radiographs was guided by the original film, and the same radiographic settings were used. Therefore, hip and stifle of x-rays were taken and evaluated by a veterinarian based on the criteria were modified from (Nganvongpanit et al., 2014) in Table 2

Table 2. Radiographic scoring system for assessing dogs with osteoarthritis.

Grade		Radiographic evaluation
0	Normal	Not affected
1	Mild	Doubtful narrowing of joint space and possible osteophytic lipping
2	Moderate	Definite osteophytes and possible narrowing of joint space
3	Severe	Moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour
4	Very severe	Large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour

Computed tomography (CT) scan

General anesthesia

Feed and water are withheld for 12 and 6 hours respectively prior to being anesthetized. Moreover, a thorough physical examination, which includes body temperature, heart rate, respiratory rate, dehydration status, mucous membrane color, capillary refilling time, neuromuscular, integumentary and cardio-respiratory system, is performed by a veterinarian in order to evaluate risk of anesthetic complications.

After the patient is clinically evaluated that the anesthesia could be performed, the premedication and sedative agents are administered by a veterinarian. The satisfactory depth of anesthesia should be stage 3 planes 2 or 'surgical' anesthesia in order to perform a computed tomography. During anesthesia, the intravenous fluid is administered according to individual physical status. The veterinarian monitors animal's health status during the anesthesia which takes approximately 10-15 mins or throughout the CT-scan process. A veterinarian closely

monitors the patient until all the vital signs become stable within normal range and it is certain that the dog has no side effects of general anesthesia.

Computed tomography examination

CT-scan is to be performed on the area of hip joint with OA condition using Optimums 64 slice by positioning the joint with the caudal aspect of the limb on the scanning plane. Extend both hind limbs while the dog is in the ventral recumbency position. Then, place tibial plateau parallel to the scanning plane using scout view in order to take overlapping transverse pre-arthrography CT images of 1.25 mm from the distal third of the femur to the proximal third of the tibia/fibula, using a bone algorithm, 100 kVp, 140 mA, a field of view of 96 mm and a pitch of 0.75. A second scan was performed from the proximal aspect of the patella to the tibial plateau, with a slice thickness of 0.625 mm thickness. These images were acquired in a bone algorithm, 100 kVp, 180 mA, and a field of view of 96 mm and a pitch of 1. The contrast Iohexol (Omnipaque 350) was injected using a volume of 2 to 2.4 mL (median 2.2 mL) at a concentration 120 mg I/mL. The CT protocol was repeated for all stifles after intra-articular positive contrast injection (Van der Vekens et al., 2019). Every CT image is analyzed by veterinary diagnostic imaging specialist.

Statistical analysis

Clinical score, radiographs, CT-scan, CRP were calculated in the formulation of mean ± SD. The difference between day 0 and day 29 was compared using Non-parametric, Kruskal-Wallis Test. The data was analyzed with SPSS program version 21.0 and 95% level of confidence or < 0.05 statistical significance.

Results

Animals

Eight dogs were diagnosed with osteoarthritis, fulfilled the inclusion criteria and were enrolled. All dogs were 6-15 years old. The 8 dogs of experimental group consist of 5 males and 3 females. There were 2 Pomeranians, 2 mixed breeds, 2 Chihuahuas, 1 Beagle and 1 Poodle. Demographic data of OA dogs were summarized in Table 3. All dogs showed normal complete blood count and no blood parasites.

Table 3. Demographic data of osteoarthritis (OA) dogs.

Signalment	OA group (n=8)	*p*-value
Age (years old)	9.6 (6-15)	NA
Body weight (kg)	19.50 (8.20-38.56)	0.64
Sex	Male 5, Female 3	NA
Breed	Pomeranian (n=2), Mixed breed (2), Chihuahua (2), Beagle (1), Poodle (1)	NA

NA, Not applicable

CRP level

The experimental results showed decreasing of CRP value in 4 out of 8 dogs after receiving of ECa 233. The mean CRP before and after the administration of ECa 233 was 10.38±2.01 mg/L (range 9.00-14.63, n=8) and 9 mg/L (all dogs CRP value was 9 mg/L, n=8) respectively. The mean serum CRP was as shown in Table 4.

Clinical lameness score

Clinical evaluation in lameness showed significant improvement in the before the administration of ECa 233 compared with after the administration of ECa 233 (2.13±1.36, 0.5±0.93) respectively (p=0.01). The mean clinical lameness score was showed in Table 4.

Table 4. The C-reactive protein (CRP) value and clinical lameness score of osteoarthritis (OA) dogs before and after ECa233 administration.

Parameters	OA group (mean±SD)		*p*-value
	Before	After	
CRP value	10.38±2.01	9.00±0.00	0.06
Lameness score	2.13±1.36	0.5±0.93*	0.01*

Radiographic evaluation

Eight dogs from the OA group were diagnosed with OA of the hip and stifle joint and were classified as grades 1-3 according to the radiographic scoring system. The mean stifle and hip radiographic score as shown in Table 5. However, the results showed that there were no statistically significant differences (p>0.05) of the radiographic score between before and after the administration of ECa 233 in the stifle and hip joints. The radiographic score was 1, 2, and 3 of OA in the stifle joints and hip joints (Figure 1, 2), respectively.

Table 5. The radiographic score between before and after the administration of ECa233 in the stifle and hip joints of osteoarthritis (OA) dogs.

Radiographic score	OA group (mean±SD)		*p*-value
	Before	After	
Stifle joint	1.5±0.84	1.33±0.82	0.19
Hip joint	2.5±0.55	2.5±0.55	1.00

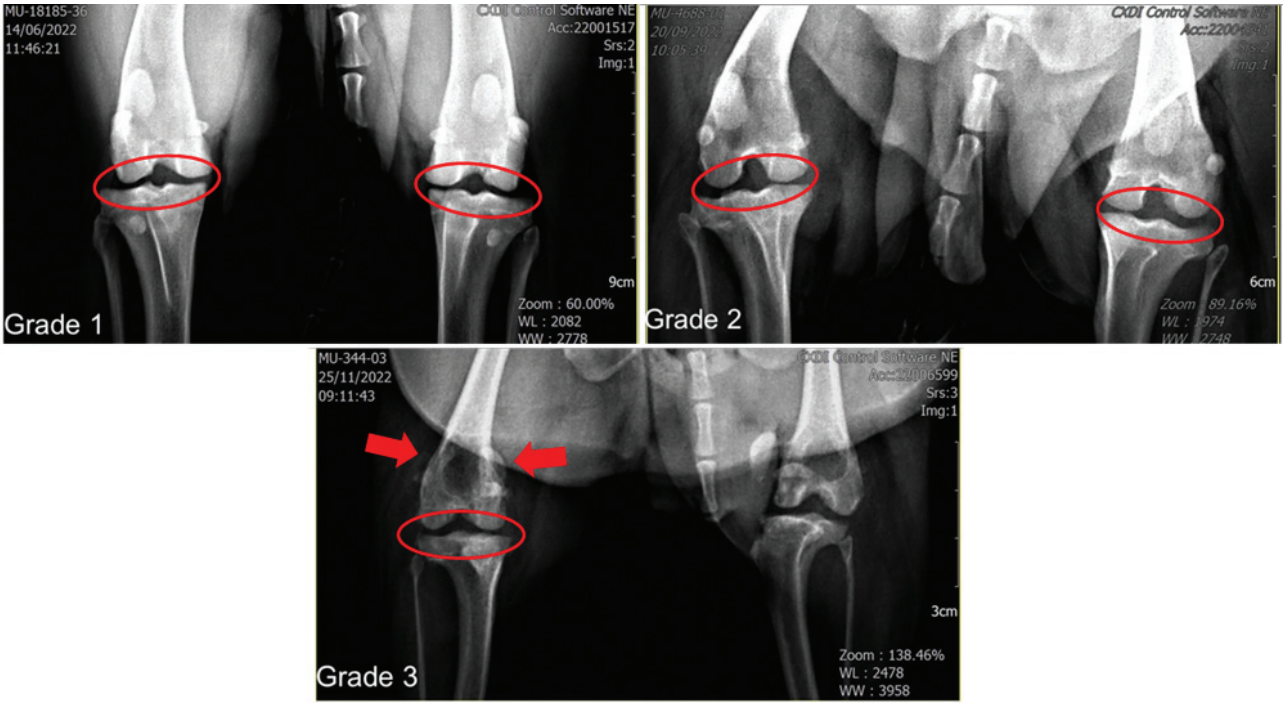

Figure 1. The radiographic score was 1, 2, and 3 of osteoarthritis in the stifle joints.

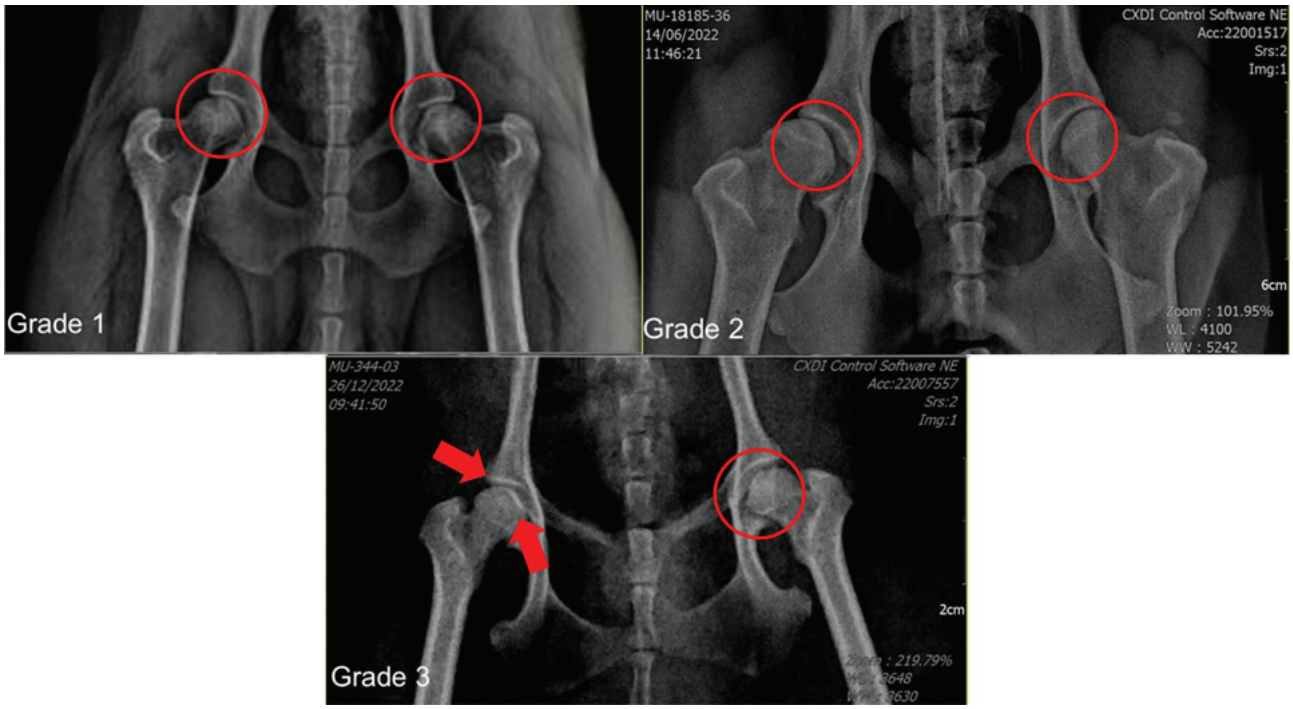

Figure 2. The radiographic score was 1, 2, and 3 of osteoarthritis in the hip joints.

Computed tomography evaluation

In this research study, a CT-scan was performed on six dogs before and after the administration of ECa 233 in order to evaluate the HU which would determine the radiographic density of the tissue of hip joint. The HU was measured from three random areas within three planes (sagittal, dorsal, and transverse plane). Two phases of the CT-scan were evaluated: pre-contrast and post-contrast phase.

From the two phases of the CT-scan result after the administration of ECa 233, the mean HU of the hip joint decreased from before ECa 233 administration and significantly different in post-contrast phase (p=0.037) as shown in Table 6. It means that ECa 233 improved tissue quality around the hip joint. CT images of hip joint of dog as shown in Figure 3.

Table 6. Hounsfield unit of pre-contrast and post-contrast of computed tomography imaging before and after the administration of ECa233.

Hip	Pre-contrast phase		Post-contrast phase	
	Before	After	Before	After
1	57.44	36.27	86.02	71.72
2	106.97	74.94	114.78	59.71
3	64.84	63.88	68.58	83.14
4	70.38	59.43	78.34	62.54
5	107.81	75.46	120.09	69.17
6	50.18	37.42	77.52	56.47
Mean±SD	76.27±25.05	57.9±17.46	90.89±21.36	67.12±9.71*
p-value	0.24		0.037*	

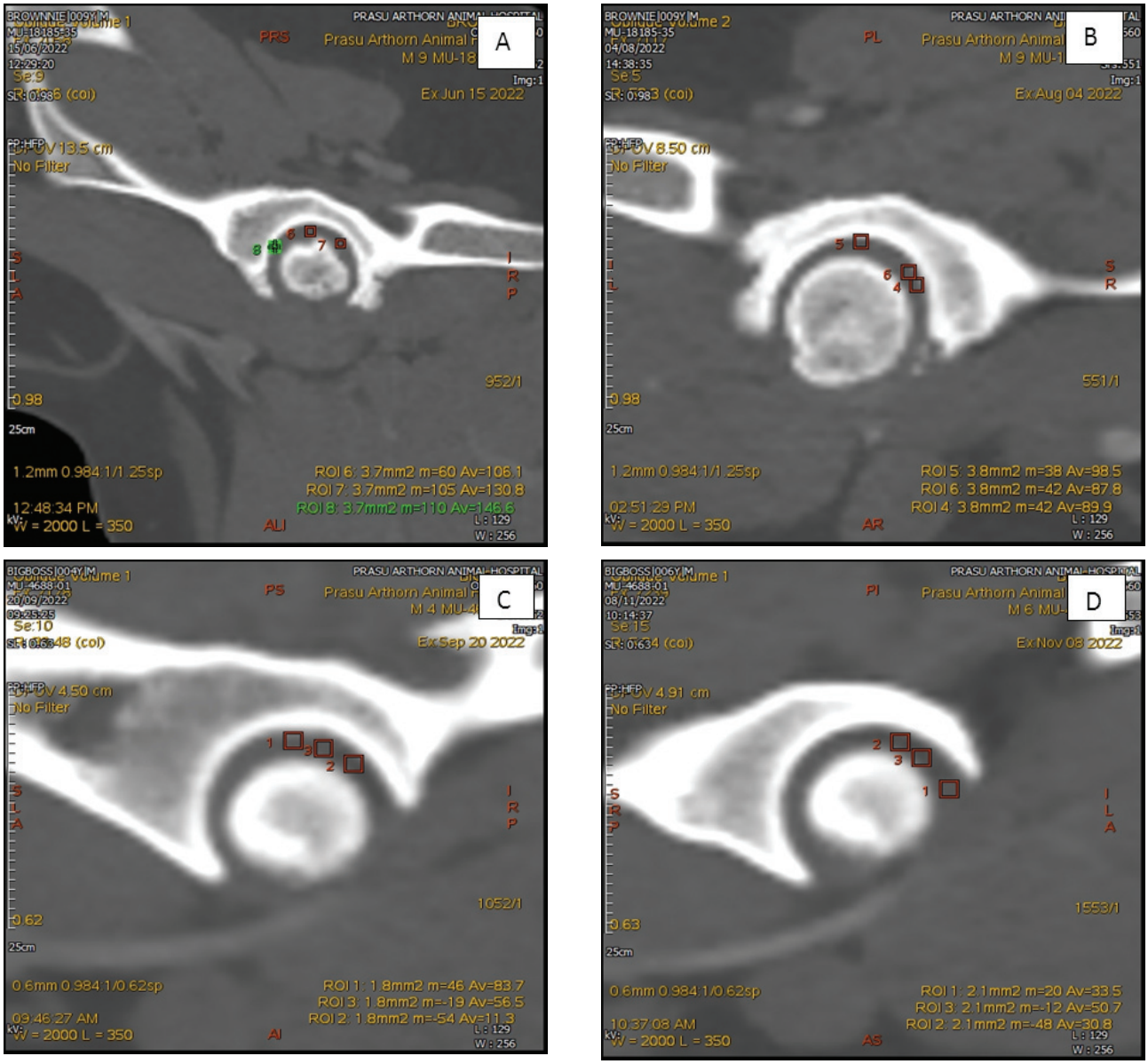

Figure 3. Computed tomography images of the hip joint of dog. Hounsfield unit was measured from 3 random areas of interest within the tissue around the right hip joint of dog (A, C) before and (B, D) after the administration of ECa 233.

Discussion

The research effects of ECa 233 in OA dogs were conducted by using the clinical lameness score, radiography, CT-scan, and CRP. The result of the clinical lameness score found that all dogs showed improvement in this parameter. Nevertheless, the radiographic score, CRP values, and CT-scan evaluation in some dogs did not improve.

Serum markers such as CRP indicate severity, prognosis, and follow-up plan of treatment in dogs (de Bakker et al., 2017). CRP values of the dogs with OA in this study were not high. No significant differences were found in the before and after treatment groups. The previous research has found that the CRP and IL-6 values are similarly correlated and used to differentiate between dogs with OA and suppurative arthritis. However, CRP values were not different in OA and normal joint dogs (Hillström et al., 2016). The increase in CRP values could not identify the inflammatory site (Christensen et al., 2014). In addition, synovial CRP was not correlated with serum CRP (Bennett et al., 2013).

An X-ray had the limited condition in the sense of the difficulty in holding the dog in the desired position. Additionally, the CT-scan required the animals to be anesthetized; consequently, the number of dogs participating in the project that went through the CT-scan was low. The radiographic findings between day 0 and 29 in the treatment group showed no significant change. Nevertheless, the radiographic images could not provide as much information about the tissues around the joint as MRI.

The diagnosis of OA by history taking, physical examination, X-rays, and CT-scan determined the joint structure, bone formation, and subchondral bone sclerosis. Previous studies reported the same sensitivity and specificity for damage to the intervertebral disc (Marino and Loughin 2010). However, it was reported that a CT-scan enabled quantitative assessment of the soft tissues and materials using the HU (Barber and Dockery 2011). The HU is also referred to as CT numbers, which correspond to the average amount of radiation absorbed by the tissue (Ai et al., 2018). Therefore, the HU value could be used to analyze the quality of tissues around joint.

Apart from CRP, there are other methods of indicating arthritis in order to follow-up the dog's condition. For example, other inflammatory markers such as IL, and TNF alpha, have been reported to be noninvasive markers of joint inflammation in dogs with idiopathic immune mediated polyarthropathy (Foster et al., 2014). Furthermore, the synovial fluid evaluation of the pH and glucose values was found to be significantly higher in degenerative joint disease dogs than in normal dogs (de Bakker et al., 2021). Therefore, further studies would be needed to determine the effect of ECa233 on specific inflammatory biomarkers like serum chondroitin sulfate and hyaluronan: biomarkers for osteoarthritis in canine hip dysplasia and other cytokines (Nganvongpanit et al., 2008). If possible, longer treatment of ECa 233 should be undertaken for three or six months to determine the bone structure and side effects.

The study design had limitation. Because this was a clinical study, the animals could not be controlled by using the same breed, sex, and/or age. Moreover, not all dogs in the study had the same OA grade. The total number of animals in this study was not large. Only 6/8 dogs were performed CT scan. Two dogs did not CT scan; one dog had an accident running into a door, causing a strained neck that required him to stay in the hospital for 2 weeks. In another dog, the owner had work commitments that made it inconvenient to appointment.

In conclusion, the result of the clinical lameness score found that all dogs showed improvement in this parameter. This could be a result that possibly the bone structure had not changed within 28 days. However, a s ignificant decrease in the lameness score of all dogs indicated that ECa 233 reduced the pain and inflammatory reaction, thus resulting in better walking ability and life quality. In addition, the decreased HU value from the CT imaging after treatment indicated that ECa 233 decreased the damage of tissues around the joint and the function of the osteoarthritis joint in dogs.

Acknowledgements

This work was supported by Faculty of Veterinary Science, Mahidol University. The authors would like to express their sincere gratitude to all the staffs of CT unit at Prasuarthorn Animal Hospital as well as the owners' permission and patients whose support and collaboration.

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