Kafkas Univ Vet Fak Derg
20 (3): 383-392, 2014
DOI: 10.9775/kvfd.2013.10239

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Clinical Study on the Effects of Diacerein and Diacerein Combined with Chondroitin Sulfate on Canine Hip Osteoarthritis [1]

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[1] *This research was financially supported by the National Research University Project under Thailand's Office of the Higher Education Commission*

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Makale Kodu (Article Code): KVFD-2013-10239

Summary

This study aimed to evaluate the effects of diacerein (DAR) and DAR combined with chondroitin sulfate (CS) for treatment of canine hip osteoarthritis (OA) in a 6-month clinical trial. Client-owned dogs included in the study consisted of 27 males and 25 females, aged 59.43±17.05 months old and weighing 17.63±5.19 kg. The dogs were randomly divided into five groups: DAR50 (administration of DAR 50 mg daily); DAR100 (DAR 100 mg daily); DAR50/CS (DAR 50 mg + CS 525 mg daily); DAR100/CS (DAR 100 mg + CS 525 mg daily); and CS (CS 525 mg daily). Dogs were re-examined monthly for 6 months after initiation of treatment. The assessment protocol included clinical scores and radiographic findings. Blood samples were collected three times (pre-treatment, and after 3 and 6 months) for evaluation of the serum biomarker, CS-WF6. Dogs treated with DAR showed statistically significant improvements ($P<0.05$) in lameness, joint mobility, pain on palpation, weight-bearing, and overall clinical score at 3, 6, 5, 4, and 4 months, respectively, after the start of treatment. Side effects, including diarrhea and dark-colored urine, were found in all groups receiving DAR. After the 3rd month, the level of serum CS-WF6 in the CS group was significantly elevated ($P<0.05$), while the other four groups showed a significant decrease ($P<0.05$). The results showed that DAR 50 or 100 mg had a similarly positive therapeutic effect on dogs with osteoarthritis. The use of DAR alone or in combination with CS resulted in decreased degradation of OA cartilage.

Keywords: *Condroitin sulfate, Chondroprotective drug, Diacerein, Dog, Osteoarthritis*

Diacerein ve Kondroitin Sülfat Eklenmiş Diacerein'in Köpek Kalça Osteoartritisindeki Etkileri Üzerine Klinik Bir Çalışma

Özet

Bu çalışma Diacerein (DAR) ve Kondroitin Sülfat (CS) Eklenmiş Diacerein 'in tedavi amaçlı olarak Canin Kalça Osteoartritisinde (OA) 6 aylık klinik denemedeki etkisini değerlendirmeyi amaçlamaktadır. Çalışmada yaşları 59.43±17.05 ay ve kiloları 17.63±5.19 kg arasında değişen 27 erkek ve 25 dişi sahipli kopek kullanıldı. Köpekler rastgele olarak 5 gruba ayrıldı; DAR50 (günlük 50 mg DAR uygulanan); DAR100 (günlük 100 mg DAR); DAR50/CS (günlük 50 mg DAR + 525 mg CS); DAR100/CS (günlük 100 mg DAR + 525 mg CS) ve CS (günlük 525 mg CS). Köpekler tedavinin başlangıcından itibaren aylık olarak 6 ay boyunca yeniden muayene edildi. Protokol değerlendirmesi klinik skoru ve radyografik bulguları içerdi. Kan örnekleri üç defa (tedavi öncesi, tedavinin 3. ve 6. aylarında) serum biyomarkırı CS-WF6'nın değerlendirilmesi amacıyla toplandı. DAR ile tedavi edilen köpeklerde topallık, eklem hareketliliği, dokunmaya karşı acı, ağırlık taşıma ve genel olarak sırasıyla 3, 6, 5, 4 ve 4 aylarda klinik skorda tedavinin başlangıcından sonra istatistiksel olarak önemli derecede iyileşmeler gösterdi. İshal ve koyu renkli idrar yan etki olarak DAR alan tüm gruplarda gözlendi. Üçüncü aydan sonra CS grubunda serum CS-WF6 düzeyi önemli ölçüde yükselirken ($P<0.05$) diğer dört grupta önemli oranda düşme gösterdi ($P<0.05$). Elde edilen sonuçlar osteoartritisde 50 veya 100 mg DAR uygulamasının benzer olarak pozitif tedavi edici etkiye sahip olduğunu göstermektedir. DAR'ın tek başına veya CS ile birlikte kullanılması OA kartilajın degredasyonunda azalma ile sonuçlanmıştır.

Anahtar sözcükler: *Kondroitin sülfat, Kondrokoruyucu ilaç, Diacerein, Köpek, Osteoartritis*

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INTRODUCTION

In recent years, a variety of pharmacological agents have been introduced into veterinary medicine and human medicine as well for treatment of one of the classical musculoskeletal diseases, osteoarthritis (OA). However, no one agent has demonstrated a superior therapeutic effect over the others, based on several published meta-analysis studies [1-3]. To increase the efficiency of chondroprotective agents, using them in combination is one choice: for example, glucosamine combined with chondroitin sulfate or with other effective agents [4,5].

It is well documented that the catabolic effect of interleukin-1β (IL-1β) plays a major role in the pathophysiology of OA. This cytokine is stimulated to increase the synthesis of catabolic factors, and also decreases cartilage proteoglycan synthesis [6-8]. Diacerein (9,10-dihydro-4,5-bis(acetyloxy)-9,10-dihydro-9,10-dioxo-2-anthracene carboxylic acid) acts as an IL-1β blocker [9], and was introduced for use as a chondroprotective drug in OA patients. After entering the body, diacerein is converted entirely into rhein before reaching the systemic circulation. About 20% of rhein is eliminated from the body directly by renal function. The other 80% is conjugated in the liver into rhein glucuronide (60%) and rhein sulfate (20%); these metabolites are then eliminated via the renal system [9]. The potential OA-modifying properties of diacerein have been demonstrated both *in vitro* and *in vivo*. A recent *in vitro* study showed that diacerein inhibited IL-1β secretion [10]. Diacerein can reduce the production of matrix metalloproteinase 13 (MMP-13) in subchondral bone via inhibition of extracellular signal-regulated kinase 1/2 (ERK1/2) and p38. Moreover, it can inhibit cathepsin K as well [11]. In a cartilage explant model, it was found that diacerein and rhein could reduce the levels of caspase-3 and iNOS [12]. Previous studies have reported that diacerein is safer than non-steroidal anti-inflammatory drugs [1,13,14]. Moreover, it can even be used in cases of renal and liver impairment [14]. No significant difference has been found in the pharmacokinetics of rhein between patients with liver and kidney impairment and healthy control subjects [15,16].

Diacerein has been widely investigated in human *in vivo* studies [1,3,10,13,17]; however, research has been limited in dogs [18]. In a human study that graded the efficacy of chondroprotective drugs, diacerein was classified as 'platinum', which indicated that there was good evidence for its effectiveness in the treatment of OA [19]. A report in 1999 used a canine cruciate-deficiency model of OA [20]. A daily diacerein dose of 40 mg/kg was administered for 32 weeks after surgery. Diacerein treatment significantly reduced the severity of morphological changes of OA, compared with a placebo. Based on a search of the PubMed database, no studies have been conducted on the clinical use of diacerein for OA in dogs since this report by Smith and colleagues in 1999 [20]. Therefore, it is still unclear whether orally administered diacerein improves the symptoms of OA. To clarify this issue, we designed a 6-month clinical study to compare the effects of diacerein, chondroitin sulfate, and a combination of diacerein plus chondroitin sulfate.

MATERIAL and METHODS

Animals

Of the 60 client-owned dogs originally included in the study, 52 completed the 6-month trial: 27 males and 25 females with an average age of 59.43±17.05 months and average weight of 17.63±5.19 kg. Informed owner consent was obtained, and the trial protocol was approved by the Faculty of Veterinary Medicine and the Ethics Committee of Chiang Mai University, Chiang Mai, Thailand, in 2011.

Inclusion/Exclusion Criteria

Dogs weighing less than 25 kg and showing clinical signs of chronic lameness, stiffness and joint pain, and radiological evidence of OA of the hip (grades 1–3) were considered eligible for the study. Animals were excluded if they were classified as OA grade 4, weighed more than 25 kg, were pregnant, receiving medication, or had hepatic, cardiovascular, gastrointestinal or neurological disease. Dogs with lameness due to lumbosacral instability, infection, immune disease or fractures, and dogs which had previously received drugs or dietary supplements for OA treatment were also excluded.

Treatment Protocol

Dogs were randomly assigned to five treatment groups. The first group (DAR50) received diacerein (Artrodar®; TRB Chemedica, Vouvry, Switzerland) 50 mg daily; the second group (DAR100) received diacerein 100 mg daily; the third group (DAR50/CS) received diacerein 50 mg plus chondroitin sulfate (Fortiflex®; Virbac, Carros, France) 525 mg daily; the fourth group (DAR100/CS) received diacerein 100 mg plus chondroitin sulfate 525 mg daily; and the fifth group (CS) served as a control group, and received chondroitin sulfate 525 mg daily. Animals were re-assessed monthly for clinical evaluation and blood collection, while radiographs were taken every 2 months. Treatment was stopped at the end of the 6th month.

Assessment Protocol

Three veterinarians (blinded to group classification) recorded the severity of clinical signs at each monthly visit using an ordinal scoring system *(Table 1)*. Radiographs of hip joints were taken every 2 months (three times per animal) and were interpreted by two veterinarians using a radiographic scoring system *(Table 2)*. Side effects of the medicine - diacerein alone, or diacerein plus chondroitin sulfate - included diarrhea, discoloration of urine, and

Table 1. *Clinical scoring system for assessing dogs with osteoarthritis*
Tablo 1. *Osteoartritisli köpeklerin değerlendirilmesi için klinik skorlama sistemi*

Criterion	Grade	Clinical Evaluation
Lameness	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
Joint mobility	1	Full range of motion
	2	Mild limitation (10-20%) in range of motion; no crepitus
	3	Mild limitation (10-20%) in range of motion; crepitus
	4	Moderate limitation (20-50%) in range of motion; ± crepitus
	5	Severe limitation (>50%) in range of motion; ± crepitus
Pain on palpation	1	None
	2	Mild signs; dog turns head in recognition
	3	Moderate signs; dog pulls limb away
	4	Severe signs; dog vocalizes or becomes aggressive
	5	Dog will not allow palpation
Weight-bearing	1	Equal on all limbs standing and walking
	2	Normal standing; favors affected limb when walking
	3	Partial weight-bearing standing and walking
	4	Partial weight-bearing standing; non-weight-bearing walking
	5	Non-weight-bearing standing and walking
Overall score of clinical condition	1	Not affected
	2	Mildly affected
	3	Moderately affected
	4	Severely affected
	5	Very severely affected

Table 2. *Radiographic scoring system for assessing dogs with osteoarthritis*
Tablo 2. *Osteoartritisli köpeklerin değerlendirilmesi için radyografik skorlama sistemi*

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 joint 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

vomiting. Two ml of blood was collected from the cephalic vein monthly for determination of the level of the biomarker for OA, chondroitin sulfate epitope WF6.

Clinical Score

Efficacy of the treatment was determined by means of a clinical scoring system [21,22], which assessed the animal's lameness, joint mobility, pain on palpation, weight-bearing, and overall score. The dogs had to walk and trot 6 m three times for evaluation of lameness by three veterinarians; this was followed by palpation of the hip joint for joint mobility and pain evaluation. Palpation was performed by three veterinarians, 30 min apart.

Radiographs

Structural joint changes were assessed from serial radiographs performed according to standardized technique [21,23]. Radiographs were taken for each animal at enrollment and after 3 and 6 months of treatment by the same technician using a standard X-ray machine (KELEX; Kongsak X-Ray Medical Industry Co., Ltd., Bangkok, Thailand). Ventrodorsal radiographs were obtained of the dog's hip and the leg in full extension position. Repositioning of the dog for subsequent radiographs was guided by the original film, and the same radiographic settings (i.e. kV, mA and ms) were used. All radiographs in a set (three films) were evaluated by two veterinarians using the criteria in *Table 2*.

Competitive Immunoassay Using Monoclonal Antibody WF6

A mouse monoclonal antibody WF6 was raised against a shark cartilage aggrecan preparation. A quantitative ELISA method for recognition of the WF6 epitope by the monoclonal antibody was modified from previous studies [21,24,25]. The antibody was specific for intact chondroitin sulfate chains, and showed no interaction with other sulfated glycosaminoglycans, hyaluronan, or other polyanions such as DNA, RNA or dextran sulfate. The standard used in the assay was shark cartilage aggrecan (A1 fraction) at concentrations of 19-10.000 ng/ml in 6% bovine serum albumin (BSA) with TE buffer (0.1 M Tris HCl, pH 7.4, containing 0.15 M sodium chloride, 0.1% Tween 20 and 0.1% BSA). Diluted canine serum samples (1:5 in 6% BSA-TE) were added to 1.5 ml plastic tubes containing an equal volume of WF6 (cell culture supernatant, 1:200 dilution in TE buffer). They were incubated at 37°C for 1 h, and then added to the microtiter plate, which was pre-coated with shark aggrecan (A1 fraction). Non-specific protein binding was blocked with BSA. The plates were incubated at 37°C for 1 h; the wells were then washed, and peroxidase-conjugated anti-mouse IgM antibody (1:2.000) was added (100 µl/well in TE buffer). The bound conjugate was detected by adding ortho-phenylenediamine (o-PD) substrate (100 µl/well in 0.05 M citrate buffer, pH 5.0). The reaction was stopped after 10 min with 50 µl/well of 4 M sulfuric acid. Absorbance was determined using a microplate reader

at 492/690 nm. The concentration of the WF6 epitope in supernatant samples was calculated by reference to a standard curve.

Data Collection and Statistical Analysis

The results of CS-WF6 serum level were calculated based on relative change compared to pre-treatment (month 0). Clinical sign scores, radiographic scores, and relative change of the serum biomarker were presented as mean ± SD. A non-parametric two-sample Mann-Whitney procedure was used to test for differences before and after treatment. The side effects were report as percentages. Data analysis was performed using the SAS version 8.0 software package (SAS Institute, USA). A *P*-value of ≤0.05 was considered to be statistically significant.

RESULTS

Table 3 shows a summary of age, sex and body weight data of the 60 dogs that entered the trial. All dogs enrolled in the trial had hemogram and biochemical profile results within the reference range (data not shown). Comparison of pre-treatment disease scores found no significant difference (*P*>0.05) between the DAR and CS groups *(Table 4)*.

However, during the study 8 dogs were withdrawn (2 dogs in the DAR50 group, 1 dog in DAR100, 2 dogs in DAR50/CS and 3 dogs in DAR100/CS) due to various adverse responses, including failure to attend an assessment appointment, illness, and death from a car accident. Clinical evaluations and radiographic scores of those animals were excluded from the data analysis.

Lameness scores *(Table 5)* in the DAR50 and DAR100 groups were significantly improved (*P*<0.05) after 3 months of receiving the medicine; however, the scores of the DAR50/CS, DAR100/CS and CS groups showed earlier significant improvement (*P*<0.05), after only 2 months of treatment. When groups were compared for the same month, it was found that the lameness scores of the DAR50/CS, DAR100/CS and CS groups were significantly better (*P*<0.05) than those of the DAR50 and DAR100 groups from the 2nd through the 6th month of the study.

Joint mobility scores *(Table 6)* in the DAR50 and DAR100 groups were significantly improved (*P*<0.05) by the final month of the study; but the DAR50/CS, DAR100/CS and CS groups showed significant improvement (*P*<0.05) earlier, after 4 months. When groups were compared for the same month, it was found that after 5 months the joint mobility scores of the DAR50 and DAR100 groups were significantly worse (*P*<0.05) than those of the DAR50/CS, DAR100/CS and CS groups.

Pain on palpation scores *(Table 7)* in the DAR50/CS, DAR100/CS and CS groups showed the fastest significant improvement (*P*<0.05), after 2 months of receiving medicine. This was followed by the DAR100 group, which

Table 3. *Sex, age and body weight distribution for all 60 dogs*
Tablo 3. *Altmış köpeğin cinsiyet, yaş ve vücut ağırlığı dağılımı*

Group	Total	Gender		Age (months)	Weight (kg)
		Male	Female		
DAR50	12	5	7	57.33±18.62	18.58±5.66
DAR100	12	6	6	60.17±21.27	16.75±5.33
DAR50/CS	12	6	6	55.25±15.36	16.92±5.21
DAR100/CS	12	5	7	64.42±15.10	17.00±4.86
CS	12	5	7	60.00±5.60	18.92±6.26

Age and weight data are expressed as mean ± SD; neither were significantly different among the five groups (P>0.05)

Table 4. *Comparison of pre-treatment clinical and radiographic scores*
Tablo 4. *Tedavi öncesi klinik ve radyografik skorların karşılaştırılması*

Parameters	Groups				
	DAR50	DAR100	DAR50/CS	DAR100/CS	CS
Lameness	3.7±0.5	3.7±0.5	3.6±0.7	3.7±0.7	3.7±0.9
Joint mobility	3.1±0.6	3.0±0.6	3.1±0.6	3.1±0.6	3.0±0.6
Pain on palpation	2.3±0.5	2.3±0.5	2.5±0.5	2.6±0.5	2.3±0.5
Weight-bearing	3.6±0.5	3.5±0.5	3.4±0.7	3.2±0.7	3.5±0.5
Overall score	3.3±0.5	3.1±0.5	3.1±0.6	3.3±0.5	3.3±0.7
Radiography score	2.5±0.5	2.5±0.5	2.5±0.5	2.6±0.5	2.4±0.5

Data are expressed as mean ± SD. There were no significant differences among the 5 groups

showed significant improvement ($P<0.05$) after 4 months; while the DAR50 group was slowest to improve, beginning after the 5th month of the study. When group scores were compared for the same month, a significant difference ($P<0.05$) was found in months 2 and 3. The DAR50/CS, DAR100/CS and CS groups showed a significant ($P<0.05$) improvement in pain on palpation compared with the DAR50 and DAR100 groups.

Weight-bearing scores *(Table 8)* in the DAR100/CS and CS groups showed the fastest significant improvement ($P<0.05$), after 2 months of treatment. This was followed by the DAR100 and DAR50/CS groups, whose scores significantly improved ($P<0.05$) after the 3rd month; while the DAR50 group was slowest to improve, beginning after the 4th month of the study. When groups were compared for the same month, a significant difference in weight-

Table 5. *Comparison of lameness scores*
Tablo 5. *Topallık skorlarının karşılaştırılması*

Months	Groups				
	DAR50	DAR100	DAR50/CS	DAR100/CS	CS
Pre-treatment	3.7±0.5	3.7±0.5	3.6±0.7	3.7±0.7	3.7±0.9
1st month	3.4±0.5	3.5±0.5	3.1±0.7	2.9±0.9	2.7±0.7
2nd month	3.4±0.5[a]	3.4±0.5[a]	2.5±0.8*,[b]	2.3±1.0*,[b]	2.3±0.9*,[b]
3rd month	3.2±0.6*,[a]	2.6±0.5*,[a]	2.0±0.7*,[b]	1.7±0.7*,[b]	1.7±0.7*,[b]
4th month	2.8±0.8*,[a]	2.5±0.5*,[a]	1.5±0.5*,[b]	1.3±0.5*,[b]	1.3±0.5*,[b]
5th month	2.5±0.7*,[a]	2.4±0.5*,[a]	1.2±0.4*,[b]	1.1±0.3*,[b]	1.2±0.4*,[b]
6th month	2.2±0.6*,[a]	2.2±0.8*,[a]	1.2±0.4*,[b]	1.1±0.3*,[b]	1.2±0.4*,[b]

*Data are expressed as mean ± SD. *A significant difference (P<0.05) compared with pre-treatment in the same group. Superscripts [a,b] indicate a significant difference (P<0.05) between groups within the same time period*

Table 6. *Comparison of joint mobility scores*
Tablo 6. *Eklem hareketliliği skorlarının karşılaştırılması*

Months	Groups				
	DAR50	DAR100	DAR50/CS	DAR100/CS	CS
Pre-treatment	3.1±0.6	3.0±0.6	3.1±0.6	3.1±0.6	3.0±0.6
1st month	3.1±0.6	3.0±0.6	3.1±0.6	3.1±0.6	3.0±0.6
2nd month	3.1±0.6	3.0±0.6	3.0±0.7	2.9±0.6	2.8±0.6
3rd month	2.9±0.6	2.9±0.7	2.6±0.7	2.4±0.5	2.5±0.7
4th month	2.7±0.5	2.6±0.5	2.3±0.5*	2.3±0.5*	2.3±0.5*
5th month	2.7±0.5[a]	2.5±0.5[a,b]	2.1±0.6*,[b]	2.2±0.4*,[b]	2.1±0.5*,[b]
6th month	2.2±0.6*	2.1±0.5*	2.0±0.5*	2.2±0.4*	2.0±0.4*

*Data are expressed as mean ± SD. *A significant difference (P<0.05) compared with pre-treatment in the same group. Superscripts [a,b] indicate a significant difference (P<0.05) between groups within the same time period*

Table 7. *Comparison of pain on palpation scores*
Tablo 7. *Dokunmaya karşı acı skorlarının karşılaştırılması*

Months	Groups				
	DAR50	DAR100	DAR50/CS	DAR100/CS	CS
Pre-treatment	2.3±0.5	2.3±0.5	2.5±0.5	2.6±0.5	2.3±0.5
1st month	2.3±0.5	2.3±0.5	2.2±0.4	2.0±0.7	1.9±0.7
2nd month	2.2±0.6[a]	2.2±0.6[a]	1.6±0.5*,[b]	1.6±0.5*,[b]	1.3±0.5*,[b]
3rd month	2.2±0.4[a]	2.0±0.4[a]	1.5±0.5*,[b]	1.2±0.4*,[b]	1.2±0.4*,[b]
4th month	1.9±0.6	1.7±0.5*	1.3±0.5*	1.2±0.4*	1.2±0.4*
5th month	1.6±0.5*	1.5±0.5*	1.1±0.3*	1.1±0.3*	1.1±0.3*
6th month	1.4±0.5*	1.4±0.5*	1.1±0.3*	1.1±0.3*	1.1±0.3*

*Data are expressed as mean ± SD. *A significant difference (P<0.05) compared with pre-treatment in the same group. Superscripts [a,b] indicate a significant difference (P<0.05) between groups within the same time period*

bearing scores ($P<0.05$) was found in months 2-6. The DAR50/CS, DAR100/CS and CS groups showed significantly ($P<0.05$) improved weight-bearing scores compared with the DAR50 and DAR100 groups.

Overall scores *(Table 9)* were significantly improved ($P<0.05$) fastest in the DAR50/CS, DAR100/CS and CS groups, after 2 months of receiving medicine. The DAR100 group showed significant improvement ($P<0.05$) after 3 months, while the DAR50 group only showed significant improvement after 4 months. When comparing scores of groups within the same month, significant differences ($P<0.05$) were found in months 2-5. The DAR50/CS, DAR100/CS and CS groups had significantly ($P<0.05$) improved scores compared with the DAR50 and DAR100 groups after 2 and 3 months. But after the 4th and 5th months, the score in the DAR50 group was significantly higher ($P<0.05$) than those of the other groups.

Radiographic scores *(Table 10)* were found to significantly increase ($P<0.05$) in the DAR50 and DAR100 groups, while the other three groups showed no significant change. Most of the clinical scores improved after dogs had received diacerein for 5-6 months; there was no difference between 50 mg or 100 mg per day.

Table 8. *Comparison of weight-bearing scores*
Tablo 8. *Ağırlık taşıma skorlarının karşılaştırılması*

Months	Groups				
	DAR50	DAR100	DAR50/CS	DAR100/CS	CS
Pre-treatment	3.6±0.5	3.5±0.5	3.4±0.7	3.2±0.7	3.5±0.5
1st month	3.6±0.5	3.5±0.5	3.3±0.8	2.9±0.9	2.9±1.0
2nd month	3.5±0.5[a]	3.5±0.5[a]	2.7±0.5[b]	2.0±0.9*,[b]	2.3±0.9*,[b]
3rd month	3.1±0.6[a]	3.0±0.6*,[a]	2.2±0.8*,[b]	1.9±0.8*,[b]	2.0±0.9*,[b]
4th month	2.9±0.6*,[a]	2.8±0.6*,[a]	1.9±0.7*,[b]	1.6±0.7*,[b]	1.7±0.8*,[b]
5th month	2.8±0.6*,[a]	2.7±0.6*,[a]	1.5±0.5*,[b]	1.3±0.5*,[b]	1.3±0.5*,[b]
6th month	1.9±0.6*,[a]	1.8±0.6*,[a]	1.3±0.3*,[b]	1.1±0.3*,[b]	1.3±0.5*,[b]

*Data are expressed as mean ± SD. *A significant difference ($P<0.05$) compared with pre-treatment in the same group. Superscripts [a,b] indicate a significant difference ($P<0.05$) between groups within the same time period*

Table 9. *Comparison of overall scores*
Tablo 9. *Tüm skorların karşılaştırılması*

Months	Groups				
	DAR50	DAR100	DAR50/CS	DAR100/CS	CS
Pre-treatment	3.3±0.5	3.1±0.5	3.1±0.6	3.3±0.5	3.3±0.7
1st month	3.3±0.5	3.1±0.5	3.0±0.7	3.1±0.6	2.9±0.5
2nd month	3.3±0.5[a]	2.9±0.5[a]	2.6±0.5*,[b]	2.4±0.5*,[b]	2.6±0.5*,[b]
3rd month	2.9±0.3[a]	2.4±0.5*,[a]	2.2±0.4*,[b]	2.2±0.4*,[b]	2.4±0.5*,[b]
4th month	2.7±0.5*,[a]	2.3±0.5*,[b]	1.8±0.4*,[b]	1.8±0.4*,[b]	2.0±0.6*,[b]
5th month	2.4±0.5*,[a]	2.0±0.9*,[a,b]	1.5±0.5*,[b]	1.6±0.5*,[b]	1.7±0.5*,[b]
6th month	1.9±0.6*	1.6±0.7*	1.5±0.5*	1.6±0.5*	1.6±0.5*

*Data are expressed as mean ± SD. *A significant difference ($P<0.05$) compared with pre-treatment in the same group. Superscripts [a,b] indicate a significant difference ($P<0.05$) between groups within the same time period*

Table 10. *Comparison of radiographic scores*
Tablo 10. *Radyografik skorların karşılaştırılması*

Months	Groups				
	DAR50	DAR100	DAR50/CS	DAR100/CS	CS
Pre-treatment	2.5±0.5	2.5±0.5	2.5±0.5	2.6±0.5	2.4±0.5
3rd month	2.5±0.5	2.6±0.5	2.6±0.5	2.7±0.5	2.7±0.5
6th month	2.7±0.5*	3.0±0.4*	2.8±0.6	2.7±0.5	2.8±0.4

*Data are expressed as mean ± SD. *A significant difference ($P<0.05$) compared with pre-treatment in the same group*

Table 11. *Three side effects observed in all 5 groups (data expressed as mean ± SD)*
Tablo 11. *5 grupta gözlenen üç yan etki (veri, ortalama± standart sapma olarak verilmiştir)*

Weeks	DAR50	DAR100	DAR50/CS	DAR100/CS	CS
Diarrhea					
1	5/12 (41.67%)	7/12 (58.33%)	4/12 (33.33%)	6/12 (50.00%)	0/12 (0.00%)
2	1/12 (8.33%)	2/12 (16.67%)	1/12 (8.33%)	3/12 (25.00%)	0/12 (0.00%)
3	1/12 (8.33%)	2/12 (16.67%)	1/12 (8.33%)	2/12 (16.67%)	0/12 (0.00%)
4	1/11 (9.09%)	1/12 (8.33%)	1/12 (8.33%)	1/10 (10.00%)	0/12 (0.00%)
5	0/11 (0.00%)	1/12 (8.33%)	0/12 (0.00%)	0/10 (0.00%)	0/12 (0.00%)
6	0/11 (0.00%)	1/12 (8.33%)	0/12 (0.00%)	0/10 (0.00%)	0/12 (0.00%)
Discoloration of Urine					
1	10/12 (83.33%)	11/12 (91.67%)	10/12 (83.33%)	11/12 (91.67%)	0/12 (0.00%)
2	9/12 (75.00%)	11/12 (91.67%)	10/12 (83.33%)	10/12 (83.33%)	0/12 (0.00%)
3	9/12 (75.00%)	11/12 (91.67%)	10/12 (83.33%)	10/12 (83.33%)	0/12 (0.00%)
4	8/11 (72.73%)	11/12 (91.67%)	9/12 (75.00%)	9/10 (90.00%)	0/12 (0.00%)
5	8/11 (72.73%)	11/12 (91.67%)	9/10 (90.00%)	8/10 (80.00%)	0/12 (0.00%)
6	9/11 (81.82%)	10/12 (83.33%)	8/10 (80.00%)	8/10 (80.00%)	0/12 (0.00%)
Vomiting					
1	0/12 (0.00%)	1/12 (8.33%)	0/12 (0.00%)	2/12 (16.67%)	0/12 (0.00%)

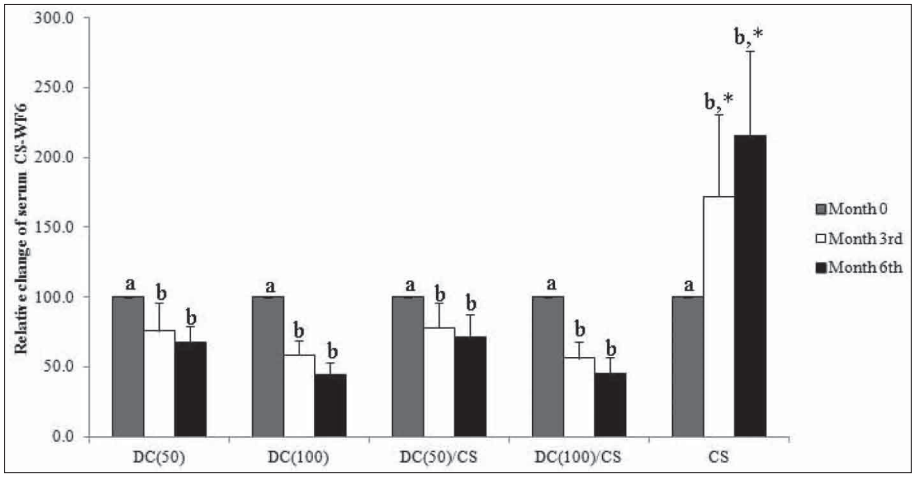

Fig 1. Mean (± SD) of relative change (%) of serum chondroitin sulfate (CS) epitope WF6. Gray bar = pre-treatment; white bar = 3 months after treatment; and black bar = 6 months after treatment. [a,b] Values were significantly different compared to month 0 within groups ($P<0.05$) **Values were significantly different between groups within a particular month ($P<0.05$)*

Şekil 1. Serum kondroitin sülfat (CS) epitop WF6'nın orantısal değişiminin (%) ortalaması (± standart sapma). Gri bar = tedavi öncesi; beyaz bar = tedaviden sonra 3 ay; ve siyah bar = tedaviden sonra 6 ay. [a,b] Değerler gruplar arasında 0. aya kıyasla önemli oranda farklılık göstermektedir ($P<0.05$)
** Değerler belli aylar arasında önemli oranda farklılık göstermektedir ($P<0.05$)*

The major side effects found in the study included soft stools, mild diarrhea, dark-colored urine, and vomiting *(Table 11)*. Dark urine was the major side effect after the 1st month of the study; the highest number was found in the DAR100 and DAR100/CS groups (91.67%), followed by the DAR50 and DAR50/CS groups (83.33%); however, no evidence of discoloration of urine was found in the CS group. Diarrhea or soft stools were also found in all groups that received diacerein. After the first month of the study, this side effect was found to be highest (58.33%) in the DAR100 group, followed by the DAR100/CS group (50.00%); the other two groups (DAR50 and DAR50/CS) were found to have 41.67 and 33.33%, respectively. Diarrhea slightly decreased after receiving medicine for a few months. Vomiting was found only in the 1st month of the study, in the DAR100/CS group (16.67%) and DAR100 group (8.33%).

The results of serum CS-WF6 are shown in *Fig. 1*. The level of CS-WF6 in the CS group was significantly higher ($P<0.05$), while the levels of this biomarker in the other four groups were significantly decreased ($P<0.05$) compared with pre-treatment. After 3 and 6 months of treatment, serum CS-WF6 in the CS group was significantly higher ($P<0.05$) than in the other four groups.

DISCUSSION

The results of the study showed that dogs with OA had significant ($P<0.05$) improvements in clinical evaluation scores after treatment with diacerein, chondroitin sulfate, and diacerein plus chondroitin sulfate. However, all of the effects appeared more slowly in the diacerein groups compared with groups treated

with chondroitin sulfate and diacerein plus chondroitin sulfate.

Previous *in vitro* studies have reported that diacerein inhibits the secretion of IL-1β in a dose-dependent manner [9,19]. But in clinical usage, there was no significant difference between 50 and 150 mg per day for pain score and clinical signs of OA [19]. The present study used 50 mg and 100 mg in the experimental design because previous human studies reported that the effective dose of diacerein was between 50–150 mg per day [13,19]. However, because the weights of all animals in this study were not more than 25 kg, we limited the dosage to 50 and 100 mg per day, while in human studies the highest dose was 150 mg for an average body weight of 60 kg. The results from this study showed that there was no significant difference in clinical sign scores for all five categories, regardless of whether 50 or 100 mg of diacerein was administered per day. But this study found that dogs receiving 100 mg per day had a higher percentage of side effects: 25% and 58% of dogs receiving diacerein 100 mg per day had diarrhea and discoloration of urine, respectively, while the corresponding percentages for dogs receiving diacerein 50 mg per day were 17% and 33%.

Human studies have found diacerein to be effective for treating OA [13,17,26]. In 2007, Louthrenoo and colleagues [26] reported on the efficacy, safety and carry-over effect of diacerein, in comparison to piroxicam, in the treatment of Thai patients with symptomatic knee osteoarthritis. Ninety percent of the patients treated with diacerein showed significantly reduced pain compared with 70% baseline. Diacerein was as effective as piroxicam in reducing pain and improving function, with longer carry-over effect and a better safety profile. Although the present study did not compare the effects of diacerein with NSAIDs, the effects were compared with another chondroprotective drug, chondroitin sulfate, as in a previous study [21]. Moreover, we studied the efficiency of diacerein when combined with chondroitin sulfate. Diacerein combined with chondroitin sulfate had a greater effect than diacerein alone from the 2nd through the 5th month of the experiment, while after 6 months there was no significant difference. Moreover, no significant difference was found when comparing the effects of 3 groups; diacerein 50 mg plus chondroitin sulfate, diacerein 100 mg plus chondroitin sulfate, and chondroitin sulfate alone. However, after 6 months of the experiment, no significant difference was found between all five groups. This study found that using chondroitin sulfate alone or in combination with diacerein could improve clinical signs faster than using diacerein alone.

The limitations of this clinical study included a lack of instruments to assess joint mobility (e.g. arthroscopy) and instruments to evaluate motion (e.g. faceplate analysis or motion analysis). However, to increase confidence in the subjective assessment, clinical scores and radiographic scores were evaluated by three and two blinded veterinarians, respectively. Another limit of the study was the lack of a negative control group receiving a placebo. The university's ethics committee did not permit the use of a negative control group, because all dogs enrolled in this study were pets of the owners.

This study found dogs that received only diacerein (both DAR50 and DAR100 groups) showed steady improvement of clinical signs compared to dogs that received chondroitin sulfate (CS group) or diacerein plus chondroitin sulfate (DAR50/CS and DAR100/CS groups). Administration of 100 mg per day was slightly more effective than 50 mg per day for pain on palpation, weight-bearing and overall score; there was no difference in the effect on lameness and joint mobility. Diacerein combined with chondroitin sulfate showed similar effects to the administration of chondroitin sulfate alone. However, when comparing the radiographic scores between pre-treatment, 3 and 6 months of treatment, dogs that received only diacerein (50 and 100 mg) showed significantly increased radiographic scores after 6 months of treatment. No significant increase in radiographic scores was found in the other three groups. The study results may indicate that diacerein alone (either 50 or 100 mg) does not prevent pathophysiological changes of OA in dogs weighing less than 25 kg. On the other hand, chondroitin sulfate and diacerein plus chondroitin sulfate both showed preventive effects against pathological change. To conclude that, the other study has to done in particular using biomarker for osteoarthritis to evaluate the micromolecular or biochemical changes while receiving these medicines [21,24,25].

Two previous studies [18,20] from the same research group reported conflicting results from using diacerein. In 1997 [18], Brandt and colleagues induced OA in 14 adult mongrel dogs; 7 dogs received diacerein (15-20 mg/kg) daily, while the other 7 dogs served as OA controls. At the end of the study (8 weeks), no statistical significance was found between the two groups. In a 1999 study [20], Smith and colleagues induced OA in 20 adult mongrel dogs by transection of the anterior cruciate ligament, and then provided a total daily dose of 40 mg/kg for 32 weeks. At the end of the study, a significant reduction in the severity of morphological changes of OA (under arthroscopic evaluation) was found for diacerein compared with the placebo group. This is accordance with the results of the present study, which found that there was a positive effect from administration of diacerein for at least 4 to 5 months, and that there was no significant difference between 50 and 100 mg per day. This study showed that the use of 50 mg diacerein had similar effects to 100 mg diacerein in dogs weighing not over 25 kg; this result was comparable to a previous study using doxycycline in canine hip OA [21], where the efficiency was similar but with a slower affect compared with chondroitin sulfate.

Because of the increased use of diacerein in small animal clinics, the side effects of this medicine must be

considered. In humans, the main side effects that have been reported are diarrhea/soft stools and dark urine [19]. Our study is the first to report on the side effects of diacerein in dogs. The main side effect was found to be dark urine (89-90%) which highest number during study. The second most common side effect was diarrhea, which was also found in higher numbers (30-50%) during the first few months, and then decreased. Vomiting was found in a few dogs receiving 100 mg diacerein, but this occurred only during the first month of the study. Although the pharmacokinetics of diacerein in dogs has not been established, the results of human studies may explain the causes of diarrhea and dark urine after receiving diacerein. The cause of diarrhea after receiving diacerein is not well understood; however it is believed that this may be due to the chemical structure of diacerein and rhein, which are anthraquinone derivatives [14]. Anthraquinone is a laxative agent, and for this reason diacerein has a laxative effect as well. Darkening of urine is caused by chemical reactions occurring in an acidic medium, and is directly linked to the antraquinonic structure of the molecule [14].

Previous studies have reported that in chronic OA, the level of CS-WF6 is higher than normal because the native CS chain in cartilage is degraded and released into the blood system [24,27]. The finding of changed levels of serum CS-WF6 after treatment reflects the alteration of cartilage metabolism. This study found that serum CS-WF6 was elevated after dogs received chondroitin sulfate alone; however, the level of this biomarker decreased after dogs received diacerein or diacerein plus chondroitin sulfate. It possible that diacerein blocked the action of IL-1β, causing downregulation of other degradation enzymes such as matrix metalloproteinases and the ADAMTS family [9]. Moreover, the suppression function of IL-1β resulted in nitric oxide (NO) synthesis [8]. For this reason, the levels of serum CS-WF6 in dogs receiving diacerein were decreased, with a significant decrease (25%) after 3 months of receiving medication. This effect also occurred in the groups receiving a combination of diacerein and chondroitin sulfate. However, when comparing administration of 50 and 100 mg per day of diacerein, no significant differences in serum CS-WF6 levels were observed.

In conclusion, this study showed similar effects for diacerein doses of 50 and 100 mg; dogs showed improvement in metabolism after 3 months and in clinical signs 4-5 months after the start of treatment. The clinical signs improved faster after receiving diacerein, when combined with chondroitin sulfate. Moreover, using chondroitin sulfate alone gave efficacy similar to that of diacerein plus chondroitin sulfate. However, 100 mg doses increased the side effects, including diarrhea, dark-colored urine, and vomiting. Pet owners should be informed in advance about the two main side effects, diarrhea and dark urine, to prevent any unnecessary feelings of fear or anxiety.

List of Abbreviations

BSA: bovine serum albumin
CS: chondroitin sulfate
DAR: diacerein
ELISA: enzyme-linked immunosorbent assay
ERK1/2: extracellular signal-regulated kinase 1/2
IL-1β: interleukin-1β
MMP: matrix metalloproteinase
NOS: nitric oxide syntheses
NSAIDs: non-steroidal anti-inflammation drugs
OA: osteoarthritis
o-PD: ortho-phenylenediamine
SD: standard derivation

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