



Comparison of Ellagic Acid and NSAID Agents in the Treatment of Achilles Tendon Lacerations: An Experimental Study in Rabbits

Ertuğrul Allahverdi¹, Tülay Diken Allahverdi², Sevil Vural³

¹*Department of Orthopedics and Traumatology, Kafkas University, Kars, Turkey*

²*Department of General Surgery, Kafkas University, Kars, Turkey*

³*Department of Pathology, Ankara University, Kars, Turkey*

Objective: Although Achilles tendon ruptures can have many causes, they are known to develop most commonly with trauma. Nonsteroidal anti-inflammatory drugs (NSAID) and low doses of corticosteroids are used in the medical treatment of tendon ruptures. Ellagic acid (EA), which also has an anti-inflammatory effect, has been reported to show its effect via cyclooxygenase 2 (COX2) inhibition as well. The effects of EA and diclofenac sodium on tendon healing were compared in this study.

Methods: We used a total of 18 male New Zealand rabbits in 3 groups with 6 in each. The study was performed under general anesthesia with a xylazine-ketamine combination. After a defect was created in the right Achilles tendon of all the rabbits, group I was administered diclofenac sodium and group II was administered EA for 1 week, whereas the control group (group III) was not administered anything. Postoperative follow-up was provided for all groups.

Results: Euthanasia was performed in all subjects at the end of the eighth week, and the tendons were compared in terms of macroscopic and histopathologic features and tensile resistance. Although there was no statistically significant difference in the tensile resistance Newton values of group I and group II, these values were higher than in the control group, and the NSAID group values were statistically significantly higher than in the control group.

Conclusions: We concluded that EA and NSAIDs could be effective in the recovery of tendon integrity and tensile strength and increasing the movement capacity in pathology caused by tendon damage because of their anti-inflammatory features.

Key words: Rabbit – Achilles tendon – Ellagic acid – NSAID

Although Achilles tendon ruptures can have many causes, they are known to develop most commonly with trauma.¹⁻³ Partial or total tears can develop during trauma with the extension of the Achilles tendon. Treatment for this condition is planned according to whether the tear is partial or total. Conservative or surgical treatment has been recommended for partial tears in the literature. A duration of 6 to 8 weeks can be sufficient for conservative treatment.¹⁻⁶ Nonsteroidal anti-inflammatory drugs (NSAIDs) and low doses of corticosteroids are used in the medical treatment of tendon ruptures.⁶ NSAIDs are preferred for preventing adhesions and providing postoperative analgesia, eliminating edema, shortening the healing process, and preventing periarticular ossification.⁷⁻⁹ Diclofenac sodium, an NSAID, has an important place among the commonly used clinical agents, and its anti-inflammatory mechanism of action is cyclooxygenase (COX2) inhibition. Ellagic acid (EA), which also has an anti-inflammatory effect, has been reported to show its effect via COX2 inhibition as well. EA is a natural antioxidant that is found in significant quantities in walnuts, carrots, tomatoes, pomegranates, grape juice, grape wine, blueberries, blackberries, and strawberries.¹⁰⁻¹³ It is a phenolic acid derivative that inhibits lipid peroxidation besides its anti-inflammatory, antiproliferative, anti-angiogenesis, and anticarcinogenic effects.^{14,15}

We aimed to compare the healing effects of EA, with its anti-inflammatory and antiadhesive effects, and diclofenac sodium, which is widely used for tendon repair, in the postinjury period in an experimental animal model in this study.

Materials and Methods

This study was conducted after obtaining permission from the Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK-2012-54).

The animal material in the study consisted of 30 3- to 3.5-kg male New Zealand rabbits. The 30 rabbits were divided into 3 different groups of 10 each. However, 4 rabbits from each group died after the experiment started because of infection (pneumonia) or altered feeding habits because of torticollis. We therefore continued the experiment with a total of 18 rabbits consisting of 6 rabbits in each group. Group I was the diclofenac sodium group, group II was the EA group, and group III was the control group. All the rabbits in all study groups were kept in the same laboratory environment for 1

week before the experiment and fed standard rabbit food and water ad libitum. The operations were performed on the right Achilles tendon under general anesthesia provided by xylazine 10 mg/kg, intramuscular (IM; Rompun 2%, Bayer, Istanbul, Turkey) and ketamine HCl 80 mg/kg IM (Ketasol, Interhas, Turkey). Once routine preparations were completed and the operation region covered with sterile drapes, the Achilles tendon of the rabbits in all groups was accessed by passing through the skin, subcutaneous ligaments, and tendon sheath using a 5-cm vertical incision 3 cm proximal to the tarsal joint. A partial rupture was created in the Achilles tendon midline by creating a Z incision with a number 15 scalpel. We made sure the scalpel exited the other side when the incision was being made. The area was then closed routinely without sutures, and no external support was applied to the relevant extremity.

A first-generation cephalosporin (Sefazol 250 mg, Mustafa Nevzat, Istanbul, Turkey) was administered IM to the animals in all groups at a dose of 25 mg/kg for 3 days for prophylaxis against infection. Group I was administered diclofenac sodium 5 mg/kg (Dikloron 50-mg tablet, DEVA, Tekirdağ, Turkey) by orogastric gavage. EA 85 mg/kg was mixed with 10 mL drinking water, and group II was administered this mixture in the same way for 7 days. No drug administration except prophylaxis was performed in group III. All rabbits were observed for 8 weeks and whether they used the relevant extremities or not was noted. The rabbits were euthanized with a high dose of sodium pentobarbital (nembutal sodium 100 mg/kg, 50 mL, intravenously, Ovation Pharmaceuticals, Inc, Deerfield, Illinois) at the end of the eighth week.

The right leg Achilles tendon was excised to include the region of the gastrocnemius muscle near the tendon origin proximally and the calcaneus distally following death. The tissue samples were kept at -20°C for 2 weeks. Biomechanical tests followed by histopathologic evaluation were conducted at the end of this duration.

Biomechanical tests were performed using an axial tensile force and proximal and distal connections for each operated and control tendon. The AG IS-50 kN (Shimatzu, Kyoto, Japan) tensile test device was used at the Istanbul University Mechanical Engineering Laboratory for the biomechanical tests (Figs. 1-4). A computer connected to the device was used to record the biomechanical force measurements and endurance power scale graphs (Fig. 5).

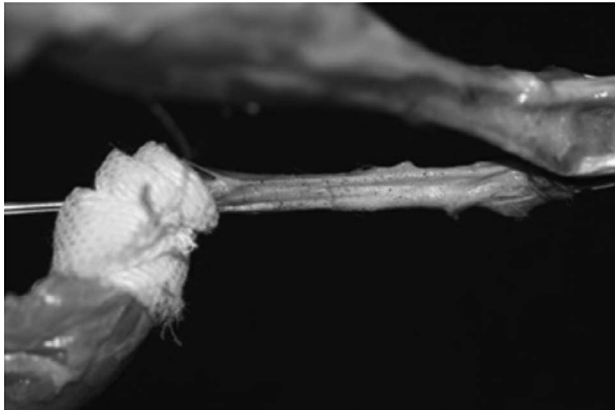


Fig. 1 Initiation of tensile force application to the Achilles tendon in the mechanism.

Following the biomechanical tests, samples were taken and transferred for histopathologic examination performed with the hematoxylin-eosin stain (H&E) and 100× magnification. The cellular morphology, degenerative changes in the muscle, nerve and collagen structures, and the vascularization features were evaluated during this analysis (Figs. 6–9).

SPSS version 18 software was used for the statistical analyses of all data obtained from the study. The mean and standard deviations, which are the central distribution measures, were calculated, and the intragroup differences of the nominal values were studied using Fisher’s exact χ^2 test for the analysis of nonparametric data. $P < 0.05$ was accepted as significant. We used the Kruskal-Wallis test for the analysis of the measurement values, whereas statistical significance between 2 groups was evaluated with the Mann-Whitney U test. $P <$



Fig. 2 Tendon stress caused by tensile force application in the mechanism.



Fig. 3 Rupture of tendon fibers with increased force on Achilles tendon in the mechanism.

0.015 was accepted as significant with Bonferroni correction.

Results

The rabbits were followed up for 8 weeks regarding their use of the relevant extremity as the clinical finding. None of the rabbits used the relevant extremity in the first 3 days. We observed that 4 of the rabbits in group II started to use their operated legs on the third day, and all group II rabbits on the fourth day. Group I and III rabbits were seen to start using their extremities after the seventh day. We used the visual analog scale (VAS) and pain simple descriptive scale (SDS) for the follow-ups during the study.^{16–20}

The mean tensile force was 169.93 ± 50.4 N (minimum, 112 N; maximum, 253 N) in group I, 131.5 ± 49.2 N (minimum, 75 N; maximum, 223 N)



Fig. 4 Total rupture of the Achilles tendon in the mechanism.

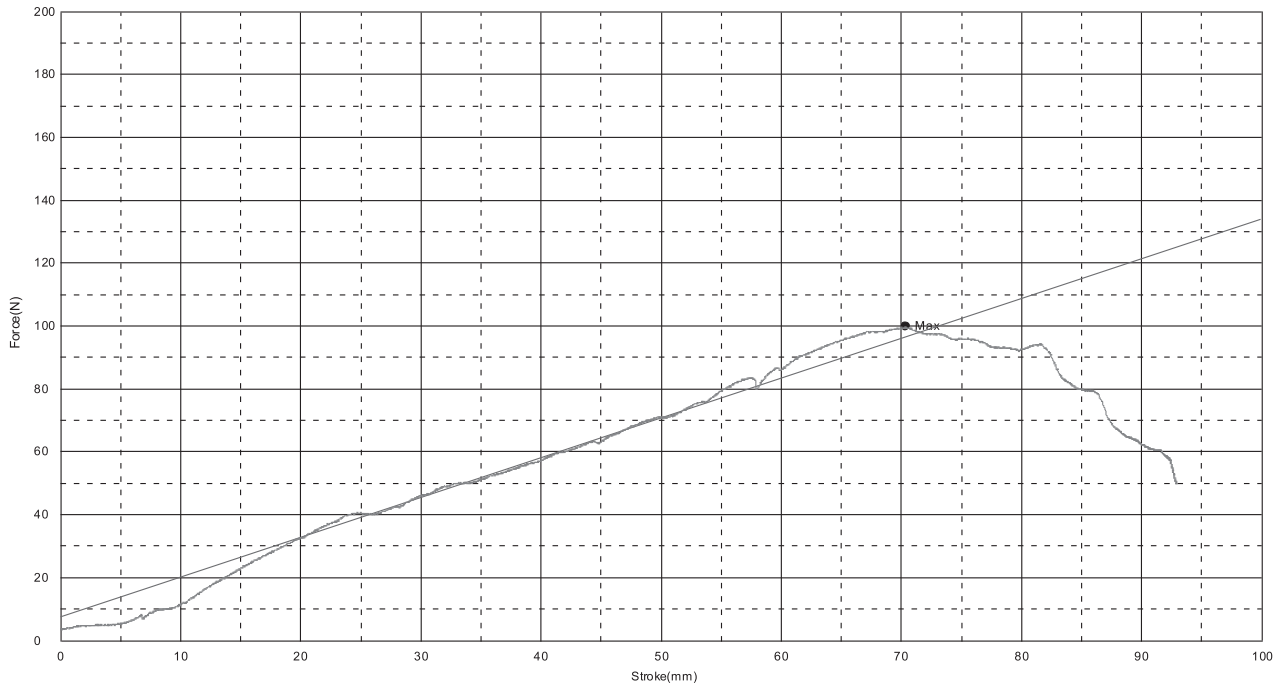


Fig. 5 Graphical representation of tensile force application in the mechanism.

in group II, and 89.80 ± 10.37 (minimum, 80 N; maximum, 101 N) in group III in Newton’s units on biomechanical evaluation. A statistically significant difference was not found between group I and group II ($P > 0.05$) but was present between group I and group III ($P < 0.05$; Tables 1 and 2).

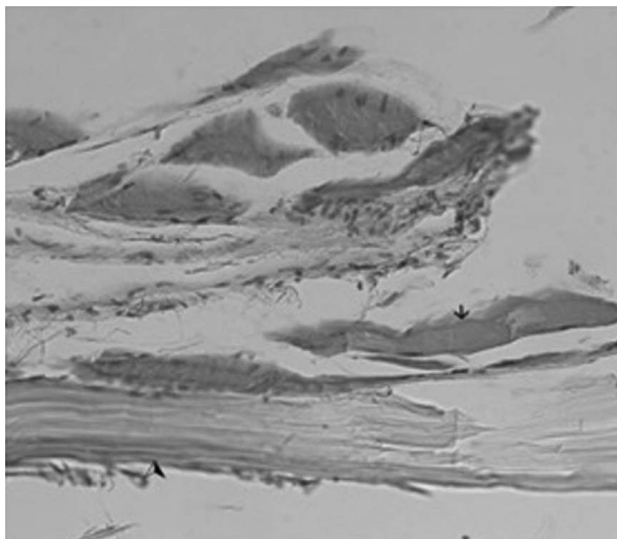


Fig. 6 Collagen (arrowhead) and muscle (arrow) bundle hyalinization, H&E, 200X. Control group.

Cellular growth, vascularization, and degenerative changes in the muscle and collagen were investigated in each section during the histopathologic evaluation. The vessels had a normal appearance in all groups. Generally mild hyalinization was usually noticed in collagen bundles and some muscle bundles in all cases (Fig. 6). Inflammatory cell infiltration was observed in a single case in all groups (Fig. 7). Although this cell infiltration was composed of mononuclear cells and predominantly of lymphocytes, neutrophil leukocytes were also noted. The axons in sections passing through nerve bundles were found to be swollen and the myelin granular, whereas the Schwann cells were swollen and hyperchromatic in appearance, especially in the control and NSAID groups (Figs. 7 and 8). Tenocytes were swollen in all 3 groups (Figs. 7–9).

Discussion and Conclusion

We aimed to investigate the effects of EA, which has many effects, but especially is antioxidant and anti-inflammatory, on tendon laceration and peritendon adhesion development and compared them with diclofenac, which is commonly used for similar purposes.

Diclofenac has a nonsteroidal anti-inflammatory effect, and various studies using various methods on

Table 1 Mann-Whitney U test results for the experimental and control groups

Group	n	Mean		χ^2	P	Significant difference
		rank	SD			
Operated NSAID (I)	6	14.0	2	9.977	<0.007	1-3
Operated Antioxidant (II)	6	10.17				
Control (III)	6	4.33				

experimental animals and humans have shown it to be an inhibitor of selective COX2 activity and to have various effects on the tendon structure as with other selective COX2 inhibitors.¹⁵⁻¹⁷ Although the antioxidant, anti-inflammatory, and antiangiogenesis effects of EA that were the subject of our comparisons are already known, its antiadhesive or other positive or negative effects on tendon healing are not fully recognized. The anti-inflammatory effect of EA by inhibiting COX2 is also known.²⁴ NSAIDs are recommended for the treatment of the acute pain of tendinopathy but do not provide effective treatment for the tendon tissue with long-term use.^{3,21-23} Early period exercise that does not place stress on the ankle joint in addition to mobilization through small movements has been found to increase Achilles tendon vascularization, decrease muscle atrophy, and support tendon healing.^{3,5} The lack of adhesion and scar formation in the Achilles tendon and the presence of peritenon vascularization enable early repair and functional improvement in the early period in Achilles tendon injuries.

The oral forms of both substances were administered postoperatively, and the comparative effects were evaluated in this study.

Experimental animal tests have shown that NSAIDs usually have an antiadhesive effect by decreasing leucocyte migration, edema, granulation tissue, collagen deposits, and fibrosis and have a positive effect in ensuring tendon healing and functional stability with COX1 and COX2 inhibition at specified doses and use durations with their anti-inflammatory activity.^{8,9,21-25} An experimental study on induced Crohn's disease has found that EA prevented hyperemia, edema, and necrosis in

Table 2 Mean tensile strength by group

	Group I (n = 6)	Group II (n = 6)	Group III (n = 6)
Tensile strength (N)	169.93 ± 50.4 ^a	131.5 ± 49.2 ^{ab}	89.80 ± 10.37 ^b

Different superscript letters indicate a statistically significant difference at $P < 0.05$.

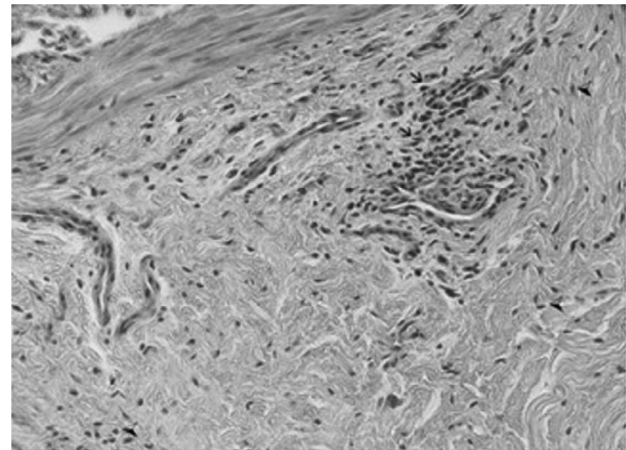


Fig. 7 Inflammatory cell infiltration (arrows) and swollen tenocytes (arrowheads), H&E, 200×. Control group.

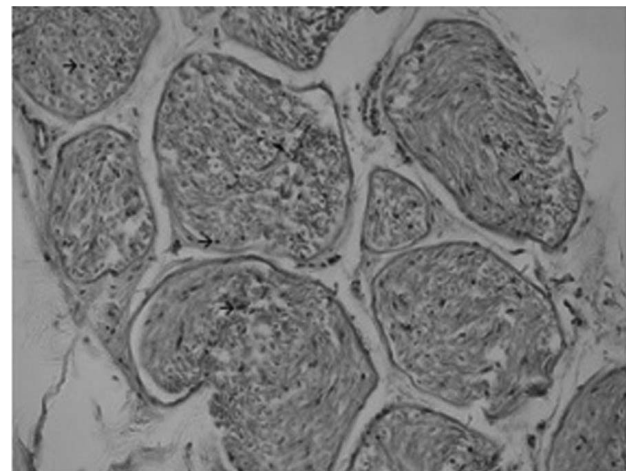


Fig. 8 Swollen Schwann cells (arrows) and axons (arrowheads) in nerve bundles, H&E, 200×. Operated NSAID group.

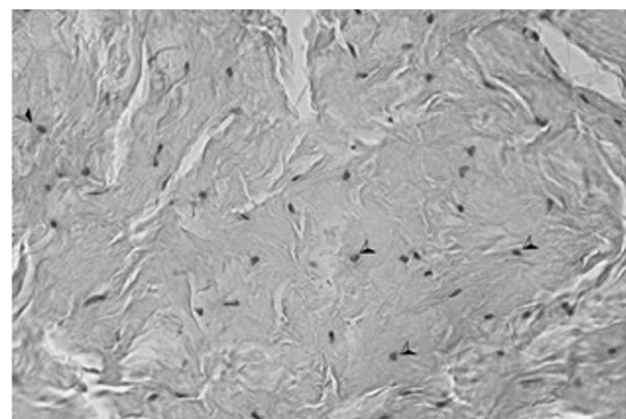


Fig. 9 Swollen tenocytes (arrowheads), H&E, 200×. Operated EA group.

intestinal tissue and started re-epithelialization in the healing process by inhibiting COX2 and nitric oxide synthase (NOS) activity.²⁶ Other experimental models on liver toxicity have found decreased liver necrosis, fattening, and inflammatory reactions.^{27,28} We observed the effects of NSAI and EA in groups of rabbits receiving these substances and determined their effect on pain, movement capacity, and feeding using the VAS and SDS scales throughout the study period. We believe that our clinical observation of the early period increases in movement capacity in the EA groups subjects compared with group I and group III is because of the decrease in both pain and inflammation.

We also observed mild necrotic changes in the collagen and muscle bundles in the tendons on histopathologic evaluation of all 3 groups in this study. Despite the low levels of edema in the tenocytes in all groups, edema was less in the EA group than the NSAI group. Inflammatory cell infiltration was similar in the EA and NSAI groups. This finding was also similar in the control group. Although edema and inflammation were histologically observed in the tendons in all 3 groups, there was no edema or granular appearance of myelin in the EA group in contrast to the marked edema and granular appearance of myelin in the NSAI and control groups (Figs. 7 and 8). These findings indicate that the EA group experienced better recovery of the tendons than the other two groups.

We also did not find a significant difference between the biomechanical stress test results of the operated NSAI and EA groups. We can therefore state according to our study results that they have beneficial effects on the tendon just like their effects on the gastrointestinal system as reported previously thanks to the inhibition of COX2 and NOS activity.^{14,26} The fact that these effects were similar to those from the NSAI group also indicates that EA has an anti-inflammatory effect similar to that of NSAIs, again according to our study results.

Tendon mechanical behavior evaluation and biomechanical analysis enable us to understand the mechanism of the damage created and also give us important data about the tendon. Tendons continue working under normal and overload conditions until specific stress forces develop. When the load damages the tendon, the degree of damage is related to the load amount and application frequency. Various studies have demonstrated that appropriate mechanical loading contributes to the building processes of the tendon such as matrix

protein and collagen synthesis. It has also been reported that overloading can damage the tendon by supporting destructive processes such as matrix structure degradation.^{15,29-32} Tendon immobilization or disuse is known to have a destructive effect. Mechanical loading at appropriate intervals for hemostasis to develop and adhesion to decrease is required for tendon damage to recover.^{31,32}

We did not use any bandage or limit the motility of the animals in any way in this study. We found that besides the anti-inflammatory effect, early mobilization or force application resulted in early recovery of tendon functions and tendon healing. Biomechanical tests in group I (NSAI) and group II (EA) showed favorable results in the comparison of our anti-inflammatory applications. The biomechanical tensile resistance results showed that damaged tendons were weaker than normal tendons with the resistance to applied force significantly decreased and the tendon integrity suffering mechanical disruption, according to the maximum deformation amount, the hardness and decreased stored energy that indicate the structural features of the tissue and the maximum rupture force that best indicates the structural integrity. Our tensile resistance results in group I and group II were higher than in the control group. It was observed that the structural integrity of the tendons in group I and group II were better protected and more resistant to mechanical tensile forces.

In conclusion, we found that the use of EA and NSAIs was effective in the recovery of tendon integrity and tensile strength and increases in motion capacity in tendon damage-related pathology because of their anti-inflammatory features, in addition to the positive effect of early mobilization on tendon healing. However, more advanced immunohistologic and bimolecular investigations and follow-up are needed to support the effect of EA on tendon recovery. The required dose and use duration also need to be evaluated with various methods using in vivo models.

References

1. Price AE, Evanski PM, Waugh TR. Bilateral simultaneous Achilles tendon ruptures. *Clin Orthop Relat Res* 1986;**213**:249-250
2. Arner O, Lindholm A. Subcutaneous rupture of the Achilles tendon. A study of 92 cases. *Acta Chir Scand Suppl* 1959;239
3. Jarvinen TAH, Kannus P, Paavola M, Jarvinen TLN, Jozsa L, Jarvinen M. Achilles tendon injuries. *Curr Opin Rheumatol* 2001;**13**:150-155

4. Anagnostopoulou S, Mavridis I. Achilles death: anatomical considerations regarding the most famous trauma of the Trojan War. *J Trauma Acute Care Surg* 2013;**74**:946Y947
5. Longo UG, Ronga M, Maffulli N. Acute ruptures of the Achilles tendon. *Sports Med Arthrosc Rev* 2009;**17**:127–138
6. Chung-Kuo H, Fu CC, Wai KC. Advances in treatment of Achilles tendon injury. *J Reparative Reconstruct Surg* 2033;**17**(4):343.5
7. Taylor TJ, Crowell D, Assor RD. The effect of indomethacin on Achilles tendon healing in rabbits. *Clin Orthop* 1991;**272**:308–311
8. Forslund C, Bylander B, Aspenberg P. Indomethacin and celecoxib improve tendon healing in rats. *Acta Orthop Scand* 2003;**74**(4):465–469
9. Tsai WC, Hsu CC, Chang HN, Lin YC, Lin MS, Pang JH. Ibuprofen upregulates expressions of matrix metalloproteinase-1,-8,-9, and-13 without affecting expressions of types I and III collagen in tendon cells. *J Orthopaed Res* 2010;**28**(4):487–491
10. Zhang L, Tran N, Chen HQ, Khan CJ, Marchal S, Groubatch F *et al.* Time-related changes in expression of collagen types I and III and of tenascin-C in rat bone mesenchymal stem cells under co-culture with ligament fibroblasts or uniaxial stretching. *Cell Tissue Res*. 2008;**332**(1):101–109
11. Ireland D, Harral R, Curry V, Holloway G, Hackney R, Harleman B *et al.* Multiple changes in gene expression in chronic human Achilles tendinopathy. *Matrix Biol* 2001;**20**(3):159–169
12. Alfredson H, Lorentzon M, Backman S, Backman A, Lerner LH. cDNA-arrays and real-time quantitative PCR techniques in the investigation of chronic Achilles tendinosis. *J Orthop Res* 2003;**21**(6):970–975
13. Zhang G, Young BB, Ezura Y, Favata M, Soslowsky LJ, Chakravarti S *et al.* Development of tendon structure and function: regulation of collagen fibrillogenesis. *J Musculoskeletal Neuronal Interact* 2005;**5**(1):5–21
14. Rogerio AP, Fontanari C, Borducchi E, Keller AC, Russo M, Soares EG *et al.* Anti-inflammatory effects of *Lafoensia pacari* and ellagic acid in a murine model of asthma. *Eur J Pharmacol* 2008;**580**:262–270
15. Lee W-J, Ou H-C, Hsu W-C, Chou M-M, Tseng J-J. Ellagic acid inhibits oxidized LDL mediated LOX-1 expression, ROS generation and inflammation in human endothelial cells. *J Vasc Surg* 2010;**52**(5):1290–1300
16. Mich PM, Hellyer PW. Objective, categoric methods for assessing pain and analgesia. In: Gaynor JS, Muir WW III, eds. *Handbook of Veterinary Pain Management*. 2nd ed. St Louis, MO: Mosby; 2009:78–109
17. Kuzucu O. *Comparison of Postoperative Analgesic Effects of Flunixin and Carprofen in the Dog* [PhD thesis]. Ankara, Turkey: Ankara University, Graduate School of Health Sciences, 2005
18. Gültekin Ç. *Comparison of the Analgesic Effects of Morphine and Tramadol in Canine Oncosurgery* [PhD thesis]. Ankara, Turkey: Ankara University, Graduate School of Health Sciences, 2012
19. Holton L, Reid J, Scott EM, Pawson P, Nolan A. Development of a behaviour-based scale to measure acute pain in dogs. *Vet Rec* 2001;**148**:525–531
20. Nolan A, Reid J. Comparison of the postoperative analgesic and sedative effects of carprofen and papaveretum in the dog. *Vet Rec* 1993;**133**:240–242
21. Schwarting T, Pretzsch S, Debus F, Ruchholtz S, Lechler P. The effect of cyclooxygenase inhibition on tendon-bone healing in an in vitro coculture model. 2015;**2015**:926369
22. Dimmen S, Nordsletten L, Engebretsen L, Steen H, Madsen JE. The effect of parecoxib and indometacin on tendon-to-bone healing in a bone tunnel: an experimental study in rats. *J Bone Joint Surg Br* 2009;**91**(2):259–263
23. Cabuk H, Avci A, Durmaz H, Cabuk FK, Ertem F, Muhittin Şener I. The effect of diclofenac on matrix metalloproteinase levels in the rotator cuff. *Arch Orthop Trauma Surg* 2014;**134**(12):1739–1744
24. Lisete R, Teixeira LR, Vargas FS, Acencio M, Antonangeio L, Vaz MAC *et al.* Influence of antiinflammatory drugs (methylprednisolone and diclofenac sodium) on experimental pleurodesis induced by silver nitrate or talc. *Chest* 2005;**128**(6):4041–4045
25. Lardinois D, Vogt P, Yang L, Hegyi I, Baslam M, Weder W. Non-steroidal anti-inflammatory drugs decrease the quality of pleurodesis after mechanical pleural abrasion. *Eur J Cardiothorac Surg* 2004;**25**(5):865–871
26. Rosillo MA, Sanchez-Hidalgo M, Cárdeno A, de la Lastra CA. Protective effect of ellagic acid, a natural polyphenolic compound, in a murine model of Crohn's disease. *Biochem Pharmacol* 2011;**82**(7):737–745
27. Özkaya A, Çelik S, Yüce A, Şahin Z, Yılmaz Ö. The effects of ellagic acid on some biochemical parameters in the liver of rats against oxidative stress induced by aluminum. *Kafkas Univ Vet Fak Derg* 2010;**16**(2):263–268
28. Jaeschke H. Molecular mechanisms of hepatic ischemia reperfusion injury and preconditioning. *Am J Physiol Gastrointest Liver Physiol* 2003;**284**(1):G15–G26
29. Maganaris CN, Narici MV, Almekinders LC, Maffulli N. Biomechanics and pathophysiology of overuse tendon injuries: ideas on insertional tendinopathy. *Sports Med* 2004;**34**(14):1005–1017
30. Wang JH, Iosifidis ML, Fu FH. Biomechanical basis for tendinopathy. *Clin Orthop Relat Res* 2006;**443**:320–332
31. Wang JH, Guo O, Li B. Tendon biomechanics and mechanobiology: a minireview of basic concepts and recent advancements. *J Hand Ther* 2012;**25**(2):133–140
32. Nordin M, Frankel VH. *Basic Biomechanics of the Musculoskeletal System: Biomechanics of Tendons and Ligaments*. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:102–125