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ORIGINAL ARTICLE

The effect of caffeic acid on tendon healing in rats with an Achilles tendon injury model

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ABSTRACT

Objectives: This study aims to examine the effect of caffeic acid on tendon healing histopathologically and biomechanically in rats with an Achilles tendon injury model.

Materials and methods: Twenty male Wistar-albino rats were used in this study. The rats were divided into two groups as the experimental group and control group. All rats underwent a bilateral achillotomy injury model and then surgical repair. Postoperatively, for four weeks, the experimental group was given intraperitoneal caffeic acid (100 mg/kg/day suspended in saline), while the control group was given only intraperitoneal saline. At the end of four weeks, after sacrificing each rat, right Achilles tendons were subjected to biomechanical analysis and the Achilles tendons were subjected to histopathological analysis. Bonar and Movin scores were used for histopathological analysis. In biomechanical analysis, tensile test was applied to Achilles tendons until rupture. For each tendon, failure load, displacement, cross-sectional area, maximum energy, total energy, length, stiffness, ultimate stress and strain parameters were recorded.

Results: According to Bonar and Movin scoring, the experimental group had lower scoring values than the control group (p=0.002 and p=0.002, respectively). Bonar scoring parameters were analyzed separately. Vascularity, collagen, and ground substance scores were lower in the experimental group compared to the control group (p=0.001, p=0.003, and p=0.047, respectively). No significant difference was found for tenocyte (p=0.064). In biomechanical analysis, failure load, displacement, ultimate stress, strain, and stiffness values were found to be higher in the experimental group compared to the control group (p=0.049, p=0.005, p=0.028, p=0.021, and p=0.049, respectively).

Conclusion: The caffeic acid contributed positively to tendon healing histopathologically and biomechanically in rats with an Achilles tendon injury model.

Keywords: Achilles tendon, antioxidant, caffeic acid, phenolic, tendon injury model, tendon healing.

With the increasing interest in sports and the average life expectancy, tendon injuries particularly Achilles tendon injuries, appear more frequently in developing countries.[1-3] Many reasons such as slow regeneration rate, low blood perfusion, and inability to use the implants used in the fixation of bone fractures for these tissues cause the healing process of injured tendons to prolong with respect to other tissues.[4] Studies are continuing on the effects of various antioxidant or anti-inflammatory substances in this difficult and long healing process of tendons.[5,6]

Caffeic acid is one of the phenolic compounds synthesized by plants and commonly found in nature and in the human diet.[7] Recent studies have shown that caffeic acid has multiple biological activities such as antioxidant, anti-inflammatory, antibacterial and anticancer.[8-11] Although there are studies on the scavenging activities of free radicals and antioxidant

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effects of caffeic acid on the musculoskeletal system, there is no study on the tendon healing effect of caffeic acid in the literature. In the present study, we hypothesized that caffeic acid could accelerate and strengthen tendon healing with its antioxidative activity in rats with Achilles tendon injury. We, therefore, aimed to examine the effect of caffeic acid on tendon healing histopathologically and biomechanically in rats with an Achilles tendon injury model.

MATERIALS AND METHODS

In this study, 20 male Wistar-Albino rats with an average weight of 486 g (range, 457 to 543 g) and age of 12 weeks were used. To perform histopathological and biomechanical analyses of tendon healing in rats, the rats were divided into two equal groups as the experimental group (n=10) and the control group (n=10).

Surgical technique

All surgical procedures applied to the rats were performed after controlling the pedal reflex under general anesthesia (intraperitoneal injection of 10 mg/kg xylazine) (XylazinBio® %2, Bioveta PLC, Ivanovice na Hane, Czech Republic) and 80 mg/kg ketamine hydrochloride (Narkamon 50 mg/mL, Bioveta PLC, Ivanovice na Hane, Czech Republic). The rats were prevented from feeling pain by controlling their reflexes at intervals throughout the procedure and by administering an additional dose of anesthesia when necessary. After shaving, the incision areas were washed with povidone-iodine (Batix®, Denizpharma, Istanbul, Türkiye) and all surgical procedures were performed under sterile conditions.

A well-known Achilles tendon injury model, which has been used in many studies in the literature,

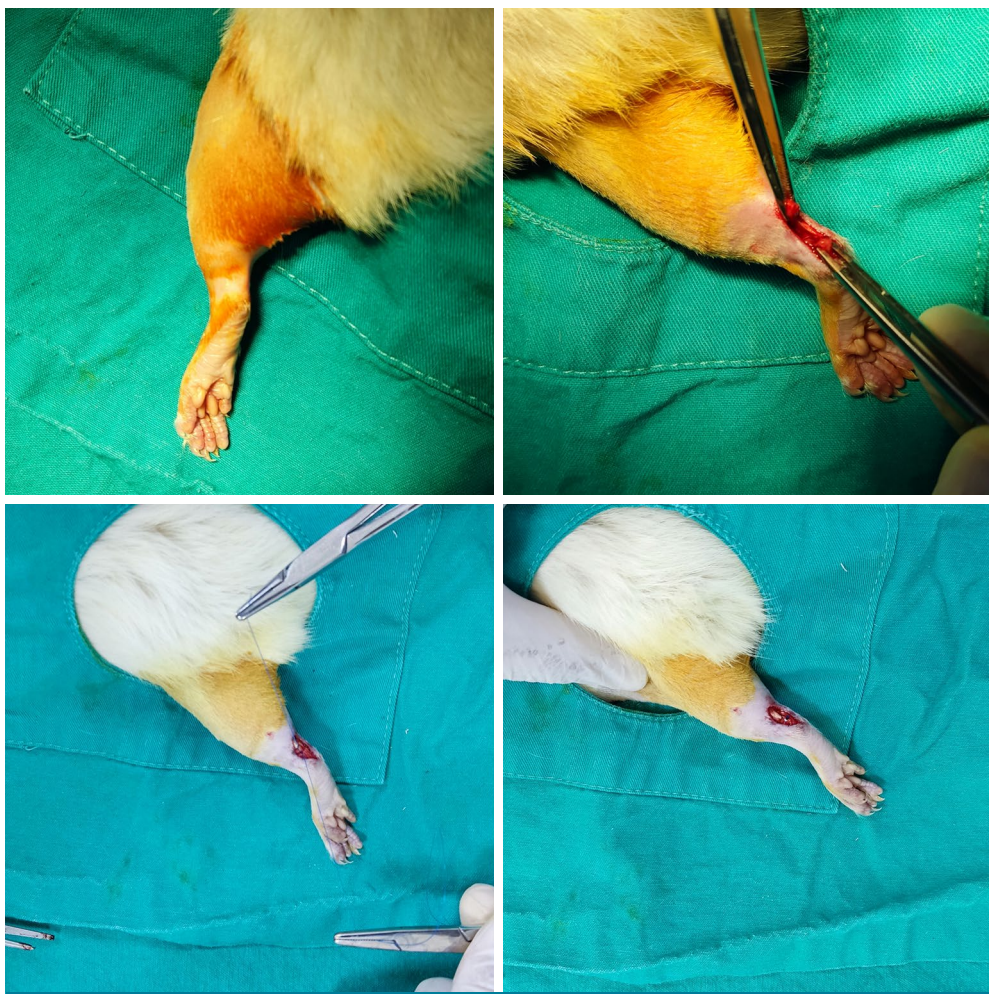

FIGURE 1. Surgical procedure for Achilles injury tendon model.

was applied.[2,3] After the surgical preparation of the lower extremities, ankle posterior line incisions were made and the Achilles tendons and plantaris tendons were exposed. Tenotomies were performed 0.5 cm proximal to the calcaneus insertion of the Achilles tendons, by using a No. 15 scalpel. Tendons were repaired end-to-end by using the modified Kessler method with 4/0 round polypropylene monofilament sutures (TıpKimSan Limited Company, Istanbul, Türkiye). After washing the wounded areas with saline, the incisions were closed by using 3/0 polypropylene monofilament sutures (TıpKimSan Limited Company, Istanbul, Türkiye) and integrity of skins were achieved (Figure 1).

No dressings, bandages, or casts were applied to the rats postoperatively. Starting from the early period, all rats were allowed to make free joint movements and to load their lower extremities. The rats in the experimental group were given 100 mg/kg/day caffeic acid (Sigma-Aldrich Inc., St. Louis, MO, USA) suspended in saline intraperitoneally at the same times during the day for four weeks. In the control group, only saline was given intraperitoneally. The dose of caffeic acid was determined according to previous studies.[12-14] The rats were euthanized by cervical dislocation after high-dose anesthesia. Achilles tendons of the sacrificed rats were removed distally from the bone-tendon junction in the calcaneus and proximally from the bone-tendon junctions in the femur and tibia. Histopathological analysis was performed on one Achilles tendon of each rat and biomechanical analysis was performed on the other.

Histopathological evaluation

After removing the Achilles tendons of the rats, they were kept in 10% formalin fixative solution for three days and, then, left under running water

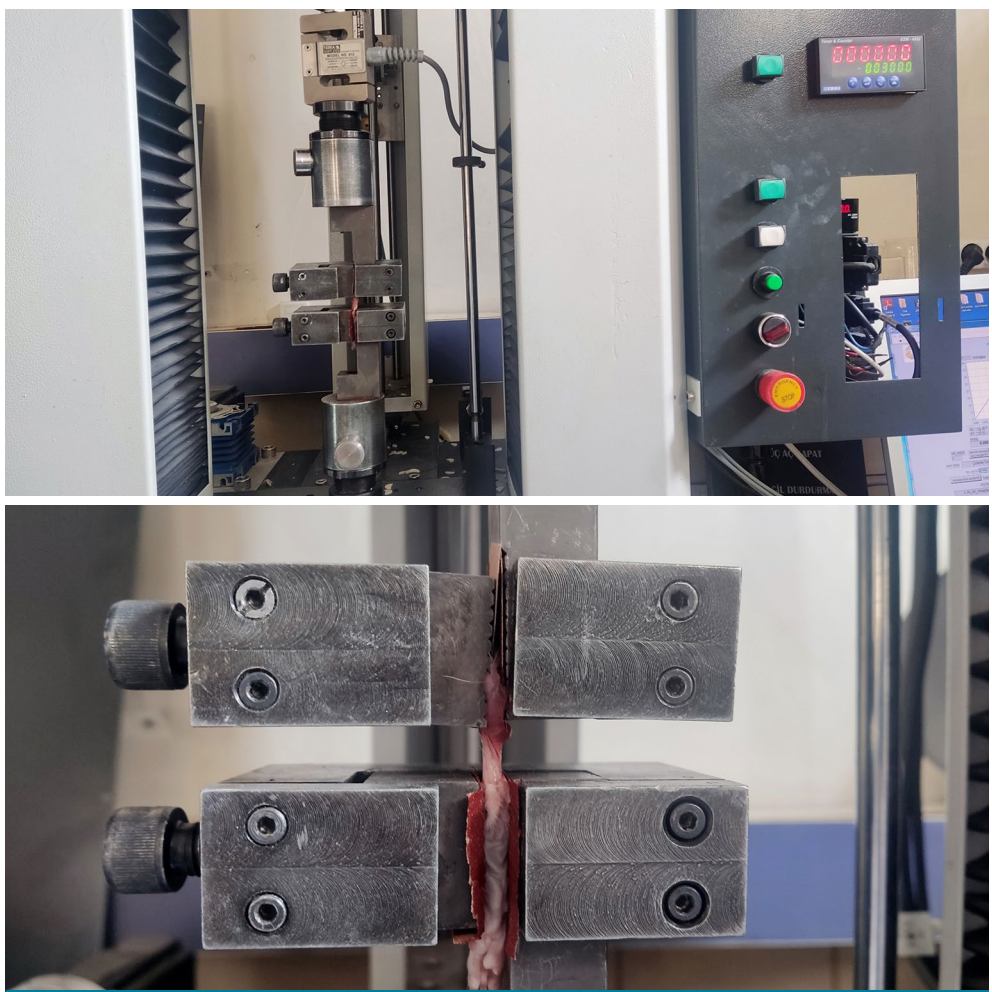

FIGURE 2. Tensile test for biomechanical analysis.

overnight. The next day, they were passed through a series of 60%, 70%, 80% and 100% alcohol, respectively and taken into xylene. Tissues were embedded in paraffin and 3-μm sections were taken with a microtome. The sections were kept in an oven at 60°C overnight and deparaffinized. Then, routine hematoxylin and eosin (H&E) and Masson trichrome (MT) staining was performed as the first step of the morphological evaluation. The completed preparations were examined under the microscope and captured.

Tenocytes, collagen, ground substance, and vascularization were assessed by using the Bonar scoring system, each out of four points (0, 1, 2 and 3). Zero (0) points were used for normal tissue structure and 3 points for abnormal appearance. Total points were scored from 0 (normal tendon appearance, strong healing) to 12 (most severe detectable pathology, very poor healing).[15] Fiber structure, fiber arrangement, rounding of the nuclei, regional variations in cellularity, increased vascularity, decreased collagen stainability, hyalinization, and glycosaminoglycan (GAG) content were assessed by using the Movin scoring system, each out of four points (0, 1, 2, and 3).[15] In both scoring systems, a lower value indicates better recovery, while a higher value indicates poor recovery. Zero (0) points

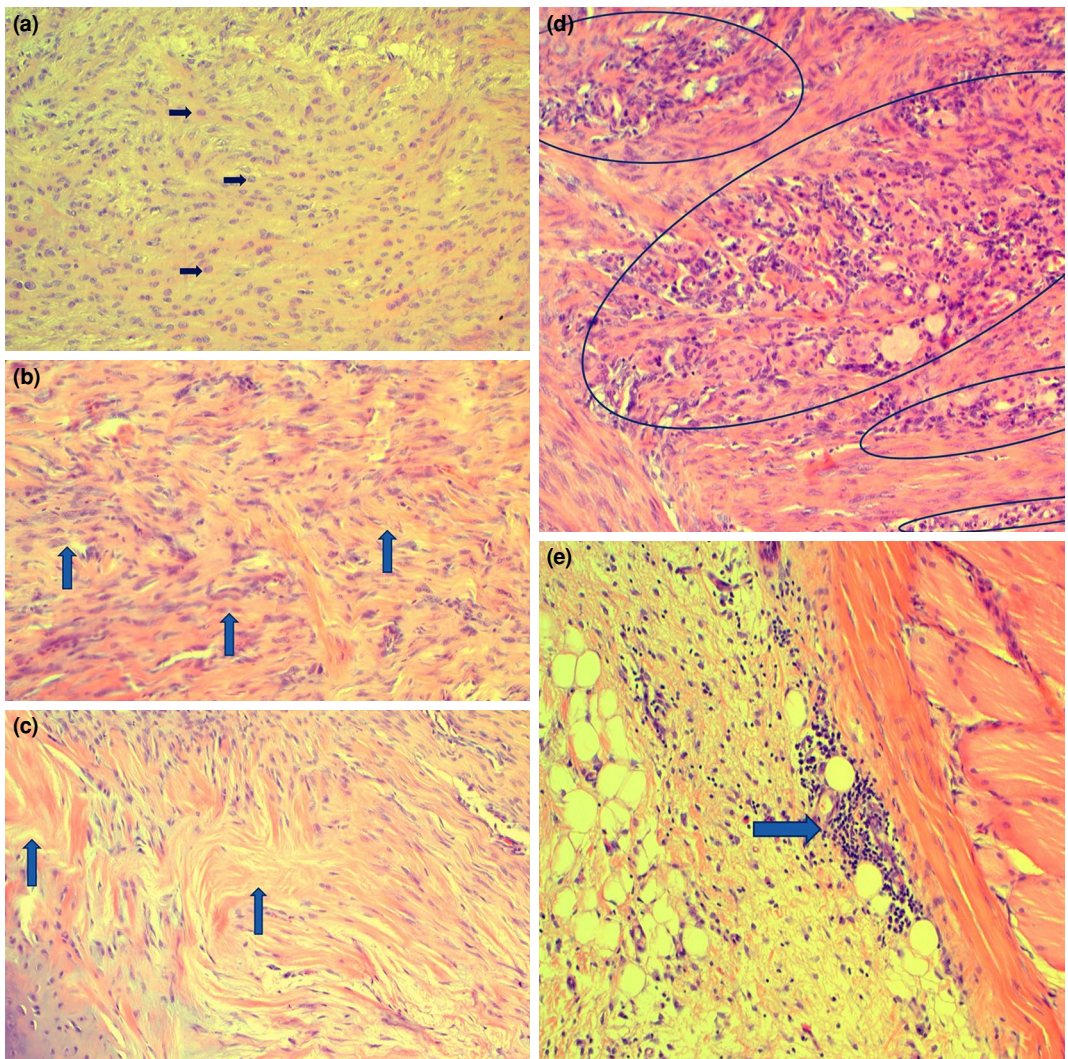

FIGURE 3. (a) Tenocyte group with prominent rounding of nuclei in the control group (indicated by arrow), **(b)** Collagen structures showing polarization and misalignment in the experimental group (indicated by arrow), **(c)** Clusters of capillaries formed in more than two groups (indicated by the round), **(d)** Hyalinization focus observed in the control group (indicated by arrow), **(e)** Grouped lymphoplasmacytic inflammatory cell infiltration in focal areas (indicated by arrow) (H&E, ×200).

were used for normal tissue structure and 3 points for abnormal appearance. Total points were scored from 0 (normal tendon appearance, strong healing) to 24 (most severe detectable pathology, very poor healing).

Biomechanical evaluation

After sacrification, distraction force was applied to the right Achilles tendon of each rat with a material testing machine (ELISTA, TST 2500 mxe). One end of the Achilles tendon was fixed from the calcaneus and the other end from the musculotendinous junction with the help of apparatus and the test was performed at a speed of 1 mm/min (Figure 2). Each Achilles tendon was tested, until it was ruptured from the tenotomy site. For each tendon, failure load (N), displacement (mm), cross-sectional area (mm^2), maximum energy (J), total energy (J), length (mm), stiffness (N/mm), ultimate stress (MPa), and strain (%) during the tendon rupture parameters were recorded.

Statistical analysis

Statistical analysis was performed using the SPSS version 23.0 software (IBM Corp., Armonk, NY, USA). Data were expressed in mean ± standard deviation (SD), median (min-max) or number and frequency, where applicable. The goodness-of-fit of the data to normal distribution was evaluated with the Shapiro-Wilk test (n<50). The Mann-Whitney U test was used because the data did not show normal distribution. Correlation analysis was performed using the Mann-Whitney U test and Spearman. A p value of <0.05 was considered statistically significant.

RESULTS

No death was observed in any of the rats during the experiment. No operative infection, re-rupture, or any other complication occurred in the Achilles tendons.

Histopathological findings

While tenocyte groups and hyalinization foci with prominent rounding were observed in the nuclei in the control group, collagen structures with misalignment and hyalinization foci that formed more than two groups were observed in the experimental acid group (Figure 3). Collagen bundles were observed with the MT staining as in Figure 4, and mucinous staining with histochemical Alcain Blue staining as in Figure 5. In both Bonar scoring and Movin scoring, the control group had a significantly higher score compared to the experimental group (p=0.002, p=0.002, respectively). In addition, the scores of the four parameters (i.e., tenocytes, vascularity, collagen,

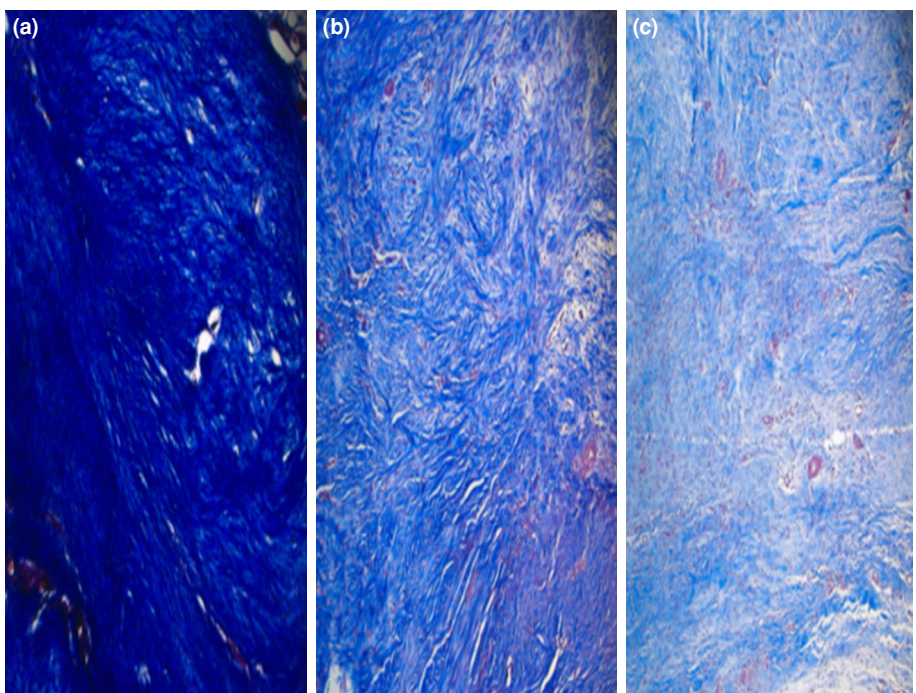

FIGURE 4. **(a)** Staining of 3+ collagen with MT, **(b)** Staining of 2+ collagen with MT, **(c)** Staining of 1+ collagen with MT (×100) (MT: Masson-Trichrome, decreased collagen stainability).

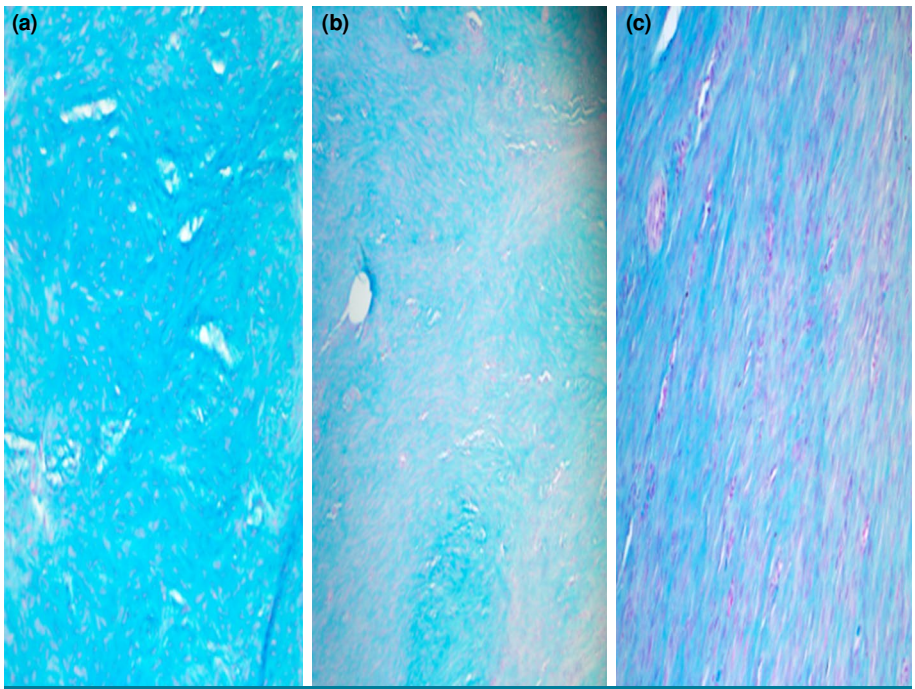

FIGURE 5. Mucinous staining with histochemical Alcein Blue Ph 2.5: **(a)** 3+ staining, **(b)** 2+ staining, **(c)** 1+ staining (×100) (MT: Masson-Trichrome) (Movin, GAG content; Bonar, Ground substance).

basic substance) in the Bonar scoring system were compared between the two groups, and there was a significant difference in the other three parameters, except for the tenocyte number (p=0.064, p=0.001, p=0.03, and p=0.047, respectively). Histology, which looked similar with morphometric scoring, also showed significant differences in terms of these parameters (Table I and Figure 6).

Biomechanical findings

In five of the nine biomechanical parameters (i.e., failure load, displacement, ultimate stress, strain and stiffness), significantly better results were obtained in the experimental group compared to the control group. There was no significant difference between the two groups in terms of other four parameters (i.e., cross-sectional area, maximum energy, total energy, length) (p>0.05) (Table II and Figure 7).

DISCUSSION

Our study suggested that caffeic acid, which is a powerful antioxidant, might positively affects the Achilles tendon healing after tendon repair

TABLE I
Tenocyte morphology and proliferation, presence or absence of ground substance. collagen bundle properties and vascularity were evaluated using Bonar and Movin scoring systems in H&E and MT staining

	Experimental group (n=10)			Control group (n=10)			
	Mean±SD	Median	Min-Max	Mean±SD	Median	Min-Max	*p*
Tenocytes	0.3±0.67	0	0-2	0.7±0.48	1	0-1	0.064
Ground substance	1.2±0.42	1	1-2	1.9±0.87	2	1-3	0.047
Collagen	0.2±0.63	0	0-2	1±0.47	1	0-2	0.003
Vascularization	0.2±0.42	0	0-1	2.4±0.69	2.5	1-3	0.001
Bonar score	2.5±1.84	2	1-7	5.3±0.67	5	4-6	0.002
Movin score	5.5±2.5	5	2-11	9.8±1.39	10	8-12	0.002

SD: Standard deviation; Scoring was made between 0 (best) and 3 (worst).

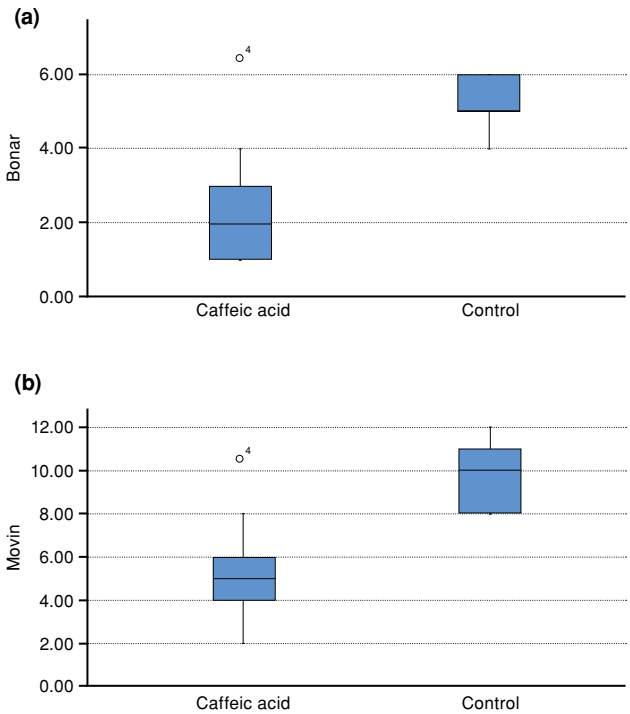

FIGURE 6. The comparison of Bonar and Movin scores in both groups **(a)** Histological Bonar score **(b)** Histological Movin score.

surgery in the rats with a ruptured model of the Achilles tendon. We speculate that caffeic acid, an antioxidant agent, increased tendon healing by reducing oxidative stress. In our literature review, there is no study on the effect of caffeic acid on tendon healing.

In the histological examinations of the rats with the Achilles tendon injury given intraperitoneal caffeic acid, tendon healing was better than the control group according to Bonar and Movin scoring. In addition, we subjected the repaired Achilles tendons to the biomechanical tensile test performed until rupture. In which nine different parameters were examined, we found that the caffeic acid group was more resistant to rupture than the control group in terms of five parameters (i.e., failure load, displacement, ultimate stress, strain, and stiffness). There was no significant difference between the two groups in terms of other four parameters (i.e., cross-sectional area, maximum energy, total energy, length). The fact that failure load, displacement and ultimate stress parameters, which are more important among these nine parameters, were statistically higher in the experimental group, provides us with valuable clinical data.[6] We believe that the histopathological and biomechanical tendon healing being significantly higher in the caffeic acid group compared to the control group would reduce the complication of tendon re-rupture and allow early movement. We consider that better range of motion can be created with early mobilization with the use of the caffeic acid and it can be an effective agent in preventing adhesion.

Reactive oxygen derivatives produced in the body can damage cell structures and cause no or less healing in tissues with low blood supply, such as tendons. Phenolic compounds such as caffeic acid can be used to prevent or treat this pathophysiological condition. Onat et al.,[16] in their study on the caffeic acid and some other phenolic compounds, showed primary antioxidant activity by inhibiting the chain reactions and by making complexes with heavy metals, it inhibited the formation of free radicals by inhibiting peroxide decomposition and

TABLE II
Comparison of biomechanical parameters between the experimental and control groups

	Experimental group (n=10)			Control group (n=10)			
	Mean±SD	Median	Min-Max	Mean±SD	Median	Min-Max	*p*
Failure load (N)	37.2±5.76	38.5	26-43	31.8±5.90	31.5	24-41	0.049
Displacement (mm)	10.4±2.06	10	8-14	7.1±2.18	6.5	4-11	0.005
Maximum energy (J)	104.6±35.88	111.5	54-142	78.7±22.78	76	41-121	0.121
Total energy (J)	203.7±54.87	189.5	123-294	157.2±44.2	167	96-211	0.225
Cross section area (mm^2)	6.32±1.28	1-6	4.8-8	5.63±1.49	5.45	3.8-8.1	0.141
Length (mm)	10.39±2.13	10.30	7.5-13.1	9.62±1.93	10.55	7.1-11.9	0.404
Stiffness (N/mm)	4.08±1.04	3.85	2.5-5.5	3.29±0.72	3.25	2.4-4.9	0.049
Ultimate stress (MPa)	7.31±0.63	7.5	6.1-8.1	6.59±0.64	6.3	5.9-7.8	0.028
Strain (%)	69.1±6.43	67	59-78	61.8±5.76	62.5	51-71	0.021

SD: Standard deviation.

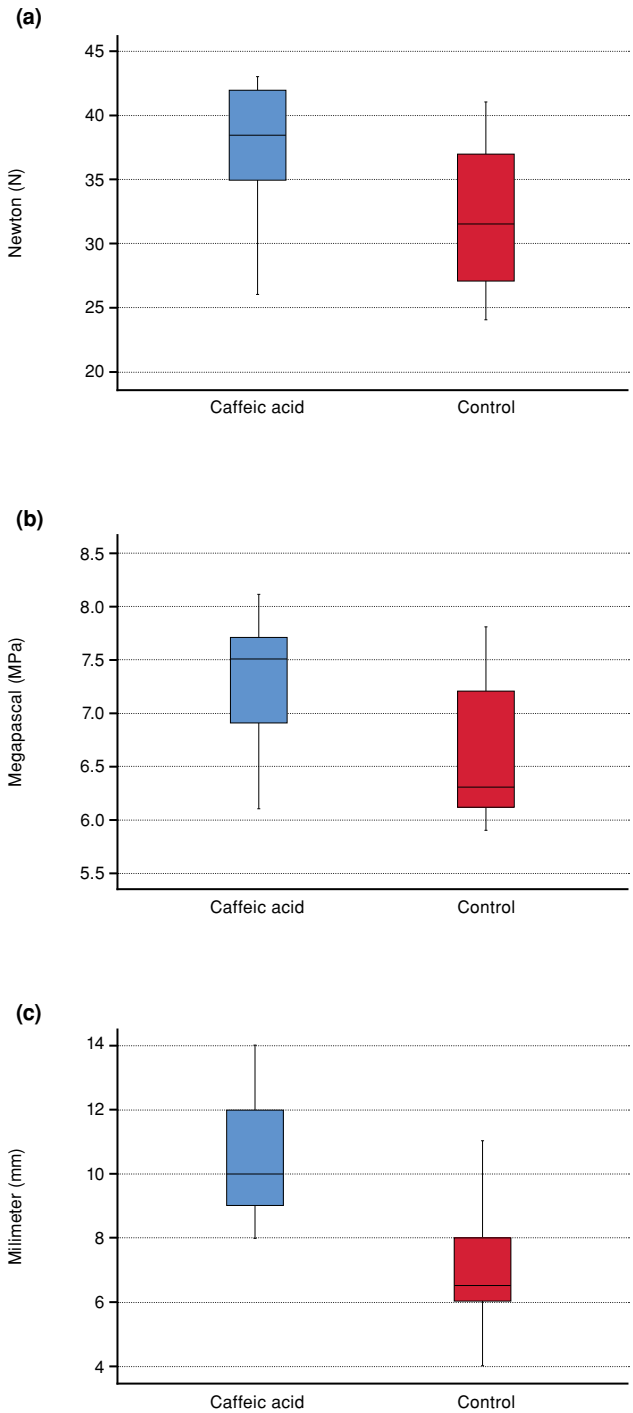

FIGURE 7. The comparison of biomechanical parameters in both groups **(a)** failure load (N), **(b)** displacement (mm), **(c)** ultimate stress (MPa).

showing secondary antioxidant activity. A number of recent studies have been conducted on the effects of the antioxidant property of the caffeic acid on musculoskeletal tissue. In a meta-analysis examining the effects of the caffeic acid on bone tissue, Ekeuku et al.,[17] reported that this compound prevented bone loss by reducing osteoclastogenesis activity with its antioxidant properties. It was shown that these phenolic compounds prevented bone loss with their antioxidant activities, particularly against the oxidative stress caused by estrogen deficiency after ovariectomy. On the other hand, in another study by Zych et al.[18] on rats, the effects of different phenolic acids (ferulic acid, caffeic acid, P-coumaric acid, and chlorogenic acid) on bone tissue were examined and they reported that caffeic acid weakened the biomechanical properties of the bone. Studies examining the effects of the caffeic acid on the musculoskeletal system have mostly focused on bone tissue and its effect on bone tissue has not been clarified yet. Studies on its effects on the tendons are very limited, and in our literature review, there is no study examining its effect on tendon healing.[19-24]

In the literature, there are studies synthesizing caffeic acid derivatives and evaluating their effectiveness in different areas.[25,26] Mia and Bank[19] reported that the caffeic acid phenethyl ester, formed after the catalysis of caffeic acid with phenethyl alcohol, inhibited collagen synthesis in a study on rats. They reported that collagen inhibition occurred with the disruption of the collagen formation pathway after myofibroblast activation of transforming growth factor beta 1 (TGFβ1). Larki et al.[20] reported that, in rats with pulmonary fibrosis model, a decrease in type 1 collagen synthesis occurred in the group treated with caffeic acid phenethyl ester, compared to the control group. The authors concluded that caffeic acid phenethyl ester decreased type I collagen concentration by modulating interferon-gamma (IFN-γ) levels. Despite these studies reporting that caffeic acid phenethyl ester inhibited collagen synthesis, we found that the caffeic acid increased tendon healing. This can be attributed to the fact that caffeic acid phenethyl ester is a different antioxidant and may have different bioactivities, although it is derived from caffeic acid. Probably in the future, further studies investigating the effect of caffeic acid phenethyl ester on tendon healing or comparing its effectiveness with caffeic acid can be conducted.

Desteli et al.[21] evaluated the effect of propolis, which contains many antioxidants such as caffeic acid, caffeic acid phenethyl ester and quercetin, on tendon healing in rats with an Achilles tendon injury model. They reported that despite the capillary increase in the healing tissue, they did not observe a significant difference compared to the control group. González-Masís et al.[22] adsorbed propolis

into scaffolds consisting of collagen extracted as nanoparticles from rat tails. They showed that propolis increased the denaturation temperature and tensile strength of collagen. Although there are other components in propolis other than caffeic acid, the increase in the tensile strength of the collagen structure is consistent with the increase in the strength required for the rupture of the healing tendons by caffeic acid in our study. Several studies have been conducted on the Dipsaci Radix (DR), another herbal product that also contains caffeic acid, and is widely used in traditional Chinese medicine. Chan et al.[23] reported that the DR did not cause a significant change in the rats with patellar tendon injury models, but further studies are still needed. In another study, Lv et al.[24] evaluated the effect of the DR on knee osteoarthritis and reported that it could be an important prophylactic agent in preventing osteoarthritis. They attributed this prophylactic feature of the DR to the fact that the caffeic acid it contains provides protection against interleukin (IL)-1β-induced inflammatory responses and cartilage deterioration in joint chondrocytes. The effects of natural agents such as propolis and DR, which contain antioxidants such as caffeic acid and are widely used in Far Eastern medicine, on the musculoskeletal system have been the subject of recent research. In this sense, our study includes findings that caffeic acid can be used as an auxiliary agent in tendon healing.

Nonetheless, there are some limitations to this study. First, there is no study available in the literature to compare our study, as no animal studies have previously been conducted on the effect of the caffeic acid on tendon healing. Second, we were unable to perform immunohistochemical analysis to evaluate collagens in our study.

In conclusion, we found that the caffeic acid contributed positively to tendon healing histopathologically and biomechanically in rats with an Achilles tendon injury model. We believe that it is promising that an antioxidant such as caffeic acid, which is common in nature, helps the healing of a tissue such as a tendon, which has low blood supply and, thus, a weak regeneration potential. However, the limited number of the animal studies on this subject indicates the need for further studies.

Ethics Committee Approval: The study protocol was approved by the Selçuk University Experimental Medicine Application and Research Center Animal Experiments Ethics Committee (date: 27.01.2023, no: 2020/58). Throughout our study, the principle of the Declaration of Helsinki was adhered to and care was taken to ensure that the animals did not feel pain.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Conceptualization, methodology, software, data curation, writing, original draft: A.Y., N.M., M.K.; Methodology, project administration: A.Y., F.D.; Histopathological analysis: M.Ç.

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REFERENCES

1. Kuşcu B, Bilal Ö, Doğar F, Topak D, Gürbüz K, Dere Kİ, et al. Effects of L-carnitine on healing of Achilles tendon in rats. Jt Dis Relat Surg 2023;34:84-91. doi: 10.52312/jdrs.2023.853.
2. Tuncer K, Demir M, Şenocak E, Mendil AS, Gezer A, Pür B, et al. The effects of Tendoflex® (polytendon complex) and Hypericum perforatum (St. John's wort oil) on repaired Achilles tendon healing in rats. Jt Dis Relat Surg 2021;32:676-87. doi: 10.52312/jdrs.2021.10.
3. Eren Y, Adanır O, Dinçel YM, Genç E, Arslan YZ, Çağlar A. Effects of low molecular weight heparin and rivaroxaban on rat Achilles tendon healing. Eklem Hastalik Cerrahisi 2018;29:13-9. doi: 10.5606/ehc.2018.54577.
4. Türközü T, Güven N, Altindağ F, Tokyay A, Gökalp MA, Ismailov U, et al. Can pirfenidone prevent tendon adhesions? An experimental study in rats. Jt Dis Relat Surg 2023;34:396-404. doi: 10.52312/jdrs.2023.1012.
5. Sarıkaya B, Yumuşak N, Yigin A, Sipahioğlu S, Yavuz Ü, Altay MA. Comparison of the effects of human recombinant epidermal growth factor and platelet-rich plasma on healing of rabbit patellar tendon. Eklem Hastalik Cerrahisi 2017;28:92-9. doi: 10.5606/ehc.2017.55396.
6. Fu SC, Hui CW, Li LC, Cheuk YC, Qin L, Gao J, et al. Total flavones of Hippophae rhamnoides promotes early restoration of ultimate stress of healing patellar tendon in a rat model. Med Eng Phys 2005;27:313-21. doi: 10.1016/j.medengphy.2004.12.011.
7. Kot B, Wicha J, Piechota M, Wolska K, Gruzewska A. Antibiofilm activity of trans-cinnamaldehyde, p-coumaric, and ferulic acids on uropathogenic Escherichia coli. Turk J Med Sci 2015;45:919-24. doi: 10.3906/sag-1406-112.
8. Rastogi N, Domadia P, Shetty S, Dasgupta D. Screening of natural phenolic compounds for potential to inhibit bacterial cell division protein FtsZ. Indian J Exp Biol 2008;46:783-7.
9. Scherer R, Godoy H. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. Food Chemistry 2009;112:654-8. doi: 10.1016/j.foodchem.2008.06.026.
10. Matejczyk M, Świsłocka R, Golonko A, Lewandowski W, Hawrylik E. Cytotoxic, genotoxic and antimicrobial activity of caffeic and rosmarinic acids and their lithium, sodium and potassium salts as potential anticancer compounds. Adv Med Sci 2018;63:14-21. doi: 10.1016/j.advms.2017.07.003.
11. Min J, Shen H, Xi W, Wang Q, Yin L, Zhang Y, et al. Synergistic anticancer activity of combined use of caffeic acid with paclitaxel enhances apoptosis of non-small-cell

lung cancer H1299 cells in vivo and in vitro. Cell Physiol Biochem 2018;48:1433-42. doi: 10.1159/000492253.
12. Zhang Z, Wang D, Qiao S, Wu X, Cao S, Wang L, et al. Metabolic and microbial signatures in rat hepatocellular carcinoma treated with caffeic acid and chlorogenic acid. Sci Rep 2017;7:4508. doi: 10.1038/s41598-017-04888-y.
13. Mehrotra A, Shanbhag R, Chamallamudi MR, Singh VP, Mudgal J. Ameliorative effect of caffeic acid against inflammatory pain in rodents. Eur J Pharmacol 2011;666:80-6. doi: 10.1016/j.ejphar.2011.05.039.
14. Pavlíková N. Caffeic acid and diseases-mechanisms of action. Int J Mol Sci 2022;24:588. doi: 10.3390/ijms24010588.
15. Bozkurt O, Bağır M, Mirioğlu A, Tekin M, Biçer ÖS, Özkan C, et al. The histological effect of tranexamic acid on tendon-to-bone healing histologically in rats. Jt Dis Relat Surg 2021;32:688-97. doi: 10.52312/jdrs.2021.42.
16. Onat KA, Kürkçü MS, Çöl B. Fenolik bileşiklerden Sinnamik Asit, Kafeik Asit ve p-kumarik Asit'in bazı biyolojik aktiviteleri. J Ins Sci Tech 2021;11:2587-98.
17. Ekeuku SO, Pang KL, Chin KY. Effects of caffeic acid and its derivatives on bone: A systematic review. Drug Des Devel Ther 2021;15:259-75. doi: 10.2147/DDDT.S287280.
18. Zych M, Folwarczna J, Pytlik M, Sliwiński L, Gołden MA, Burczyk J, et al. Administration of caffeic acid worsened bone mechanical properties in female rats. Planta Med 2010;76:407-11. doi: 10.1055/s-0029-1240603.
19. Mia MM, Bank RA. The pro-fibrotic properties of transforming growth factor on human fibroblasts are counteracted by caffeic acid by inhibiting myofibroblast formation and collagen synthesis. Cell Tissue Res 2016;363:775-89. doi: 10.1007/s00441-015-2285-6.
20. Larki A, Hemmati AA, Arzi A, Borujerdnia MG, Esmaeilzadeh S, Zad Karami MR. Regulatory effect of caffeic acid phenethyl ester on type I collagen and interferon-gamma in bleomycin-induced pulmonary fibrosis in rat. Res Pharm Sci 2013;8:243-52.
21. Desteli EE, Erdogan M, Imren Y, Onger ME. Propolis has no effect on tendon healing: An experimental study. Med-Science 2017;6:442-6 doi: 10.5455/medscience.2017.06.8591.
22. González-Masís J, Cubero-Sesin JM, Corrales-Ureña YR, González-Camacho S, Mora-Ugalde N, Baizán-Rojas M, et al. Increased fibroblast metabolic activity of collagen scaffolds via the addition of propolis nanoparticles. Materials (Basel) 2020;13:3118. doi: 10.3390/ma13143118.
23. Chan KM, Fu SC, Hui WC, Chan LS, Rui YF, Qin L, et al. Radix Dipsaci does not improve tendon healing in a rat model of patellar tendon donor site injury. Orthop Surg 2010;2:187-93. doi: 10.1111/j.1757-7861.2010.00085.x.
24. Lv Y, Wu H, Hong Z, Wei F, Zhao M, Tang R, et al. Exploring active ingredients of anti-osteoarthritis in raw and wine-processed Dipsaci Radix based on spectrum-effect relationship combined with chemometrics. J Ethnopharmacol 2023;309:116281. doi: 10.1016/j.jep.2023.116281.
25. Xia CN, Li HB, Liu F, Hu WX. Synthesis of trans-caffeate analogues and their bioactivities against HIV-1 integrase and cancer cell lines. Bioorg Med Chem Lett 2008;18:6553-7. doi: 10.1016/j.bmcl.2008.10.046.
26. da Cunha FM, Duma D, Assreuy J, Buzzi FC, Niero R, Campos MM, et al. Caffeic acid derivatives: In vitro and in vivo anti-inflammatory properties. Free Radic Res 2004;38:1241-53. doi: 10.1080/10715760400016139.