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ORIGINAL PAPER

Effect of curcumin on tendon healing: an experimental study in a rat model of Achilles tendon injury

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Abstract

Purpose This in vivo study aims to investigate the effects of curcumin which is recently developed for tendon healing using a rat Achilles tendon injury model.

Materials and methods Eighteen male Wistar albino rats weighing 300–400 g were used in this study. Under anesthesia, Achilles tendon injuries were created and repaired surgically. Nine rats of the study group received curcumin (suspended in saline at a dose of 200 mg/kg orally) and eight rats of the control group received only saline solution by oral gavage for a period of 28 days. Animals were euthanized on the 28th post-operative day, and all the Achilles tendons were removed and transferred immediately for biomechanic and histological analysis.

Results Macroscopically, all the tendons were fully healed. Total mean Bonar score was higher in the control group. When the parameters of Bonar score were analysed separately, tenocyte morphology, collagen, and ground substance scores were statistically lower than the control group ($p = 0.03$, 0.041, 0.049, respectively). Vascularity parameter did not show any statistical difference ($p > 0.05$). Of the nine biomechanical parameters, five of them (failure load, cross-sectional area, length, ultimate stress, strain) showed better results which were also statistically significant ($p = 0.046$, 0.027, 0.011, 0.021, 0.002, respectively). When the remaining four parameters were examined, the study group also had better results, but this difference was not statistically significant.

Conclusion Curcumin had better results for total tendon healing not only histologically but also biomechanically. Curcumin could be an additional agent in the management of surgically repaired tendon injuries.

Keywords Achilles tendon · Curcumin · Tendon injuries · Rat · Antioxidant

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Introduction

Tendons are important for normal joint motion and stability. Injuries of the tendons are among the most common injuries of the body [1]. Tendon injuries are responsible for serious health care costs, which generally brings about restricted activity and individual morbidity [2]. Tendon healing process consists of three complex phases which are inflammatory, proliferative, and remodeling. Many factors have been studied for better tendon healing process such as stem cells, growth factors, and haemostatic agents [3–5]. But, there is not a perfect agent for shortening the healing process and lowering the complications such as adhesions and poor mechanical properties. Still healing process of tendon injuries is a significant challenge for orthopaedic and plastic surgeons.

Curcumin, a naturally occurring compound extracted from turmeric (*Curcuma longa*), shows strong antioxidant and anti-inflammatory activities. More than 7000 articles have discussed the molecular basis of curcumin's attributed antioxidant, anti-inflammatory, and anti-infectious activities of curcumin in the last two decades [6–10]. Curcumin has a variety of pharmacologic properties that contribute to the reduction of tissue damage and promotion of tissue repair. There are papers about efficiency and tissue injuries of dermis, nerve fibres, burns by activating cell migration, and wound healing [11–13]. It has been suggested that decreasing the inflammatory process could have a positive effect on the tissue quality of injured tendon and also antioxidant therapy could have a positive effect on tendon healing process [2, 14, 15].

Until now, there is not any study about oral curcumin's effect on total tendon injury model. There are only two studies about curcumin's effect on tendon healing: one of them used a partial tendon injury model (using tendon window defect [2]) and the second study used a local curcumin-loaded nanomicelles for preventing adhesions [6].

In this experimental study, we aimed to investigate the effects of oral curcumin on tendon healing using a total rat Achilles tendon injury model.

Materials and methods

Eighteen male Wistar albino rats weighing 300–400 g were used in this study. The experimental design and all procedures were approved by Kobay A.S Animal Research and Ethics Committee (protocol number 2017/140). Animals for this study were purchased from the Laboratory of Experimental Animals, Kobay AŞ, Ankara, Turkey.

A well-known rat Achilles tendon injury model was used for this study [1, 16–18]. Surgical procedures were conducted under general anaesthesia with intraperitoneal injection of ketamine 50 mg/kg (Ketalar; Eczacıbaşı, İstanbul, Turkey) and xylazine hydrochloride 10 mg/kg (Rompun; Bayer, Leverkusen, Germany). Bilaterally, the lower limbs were shaved and disinfected using povidone-iodine, and surgery was performed under sterile conditions. Posterior midline incisions were made to expose the Achilles tendon. Bilateral Achilles and plantaris tendons were exposed and tenotomized with no. 15 scalpels at approximately 5 mm proximal to the insertion to the calcaneus. All tendons repaired with 4/0 polypropylene suture (Propilen, Doğsan, Trabzon, Turkey) using modified Kessler-type technique. During the repair, only one suture was used to hold the cut edges of the tendons. There was no gap between the tendon ends after repair in the lesion side. The surgical sites were closed with 3/0 monofilament interrupted skin sutures only (Propilen, Doğsan, Trabzon, Turkey). No bandage or casting was used after the surgeries. All rats were allowed to mobilize freely and were fed with standard laboratory food and tap water. Rats were divided randomly into two groups.

Eight rats were assigned for the control group and nine rats were assigned for the study group. Nine rats of study group received curcumin (suspended in saline at a dose of 200 mg/kg orally) and eight rats of control group received only saline solution by oral gavage for a period of 28 days. The dosage of curcumin was based on previous similar studies [19]. Rats were euthanized with high-dose ketamine hydrochloride 28 days following the tenotomy procedures. All the Achilles tendons were removed and transferred immediately for biomechanic and histological analysis.

Histological analysis

Left Achilles tendons were used for histological analysis. A buffered formalin solution was used for fixation and the tendons were embedded in paraffin. Five longitudinal serial sections, each 5-mm thick, were taken from the injury site of each tendon. Haematoxylin and eosin and Alcian blue staining were performed. Bonar's histological grading scale was used for scoring the samples. This classification includes the tenocyte morphology, collogen bundle characteristics, neovascularization, and the presence or absence of ground substance parameters (Table 1) [1]. According to this scale, higher scores indicate poorer tendon healing. Histological analyses were performed by an experienced pathologist who was blinded to study.

Biomechanical analysis

Right Achilles tendons of the rats were used for biomechanical analysis. Biomechanical testings were performed using an Elista TST 2500 material testing machine (Elista, Istanbul, Turkey). The Achilles tendons were mounted with clamps on the testing device. These clamps gripped each tendon specimen at both the musculotendinous junction and calcaneus. A distraction force was applied at 3 mm/minutes. Nine parameters (load to failure, cross-sectional area at failure, displacement at failure, maximum energy, total energy to failure, length at failure, stiffness, ultimate stress, and strain at failure) were recorded for each specimen.

Statistical analysis

Chi-square test was used for histological result analyses to evaluate the differences of the two groups. And for the analysis of biomechanical results, Student's T test was used. All the analyses were performed by using IBM SPSS version 20.0 software (IBM Corp., Armonk, NY, USA). A p value < 0.05 was considered to be statistically significant.

Results

Histological scores are presented in Table 2. Macroscopically, all the tendons were fully healed. Total mean Bonar score was

Table 1 Bonar's histological grading scale

Variables	Grade 0	Grade 1	Grade 2	Grade 3
Tenocytes	Inconspicuous elongated spindle shaped nuclei with no obvious cytoplasm upon light microscopy	Increased roundness: nucleus becomes more ovoid to round in shape without conspicuous cytoplasm	Increased roundness and size: the nucleus is round, slightly enlarged and a small amount of cytoplasm is visible	Nucleus is round, large with abundant cytoplasm and lacuna formation (chondroid change)
Ground substance	No stainable ground substance	Stainable mucin between fibers but bundles still discrete	Stainable mucin between fibers with loss of clear demarcation of bundles	Abundant mucin throughout with inconspicuous collagen staining
Collagen	Collagen arranged in tightly cohesive well-demarcated bundles with a smooth dense bright homogeneous polarization pattern with normal crimping	Diminished fibers polarization: separation of individual fibers with maintenance of demarcated bundles	Bundle changes: separation of fibers with loss of demarcation of bundles giving rise to expansion of the tissue overall and clear loss of normal polarization pattern	Marked separation of fibers with complete loss of architecture
Vascularity	Inconspicuous blood vessels coursing between bundles	Occasional cluster of capillaries, less than one per 10 high power fields	1–2 clusters of capillaries per 10 high power fields	Greater than two clusters per 10 high power fields

higher in the control group. When the parameters of Bonar score analyzed separately, tenocyte morphology, collogen, and ground substance scores were statistically lower than the control group ($p = 0.03$, $p = 0.041$, 0.049) (Fig. 1). Vascularity parameter did not show any statistical difference ($p > 0.05$).

Biomechanical results are presented in Table 3. Of the nine biomechanical parameters, five of them showed better results which were also statistically significant. In the remaining four parameters, study group also had better results, but this difference was not statistically significant ($p > 0.05$). The comparison graph of load and tension curves are presented in Fig. 2.

Discussion

The purpose of this study was to investigate the effects of oral curcumin on tendon healing process. Since curcumin is a well-known antioxidant, anti-inflammatory, microbial agent, its effect on total tendon rupture model has not been studied in English literature.

Tendon injuries constitute an important part of orthopaedic and plastic surgeons' daily work. Although the treatment methods of tendon injuries have been improved over the past decades, clinical results of surgery of tendon injuries are still one of the major problems [2]. The recovery and regeneration of tendon injury has become a significant challenge in orthopaedics. So, a more in-depth investigation of new strategies for promoting tendon regeneration may provide opportunities for such patients [2].

The effects of different agents were studied on tendon healing [1, 3–5]. According to previous studies, curcumin has the potential for elevation of synthesis of collogen, promoting angiogenesis, decreasing reactive oxygen radicals, and promoting healing process [12, 20, 21].

Previous studies suggested that nonsteroidal anti-inflammatory drugs impair the tendon healing process, but they also have a positive effect on preventing adhesions [6, 22, 23]. Free radicals and oxidative reaction products result in tissue damage and play a major role in tissue injury. Oxidative stress can cause damage leading to tendon tissue degeneration

Table 2 Histologic scores according to Bonar's scoring system

Parameter	Quartiles						Mean scores		
	Q1 control (8)	Q1 curcumin (9)	Q2 (median) control (8)	Q2 (median) curcumin (9)	Q3 control (8)	Q3 curcumin (9)	Control (8)	Curcumin (9)	*p* values
Tenocytes	1	1	2	1	2.5	1	1.87	1.00	*0.033*
Ground substance	1	1	1	1	2.5	2	1.75	1.11	*0.049*
Collagen	1	1	2	1	3	1	2.00	0.80	*0.041*
Vascularity	1	1	1	1	1	2	1.37	1.33	0.203
Total score	4	4	6	4	9	6	6.77	4.1	*0.021*

Italic items are statistically significant

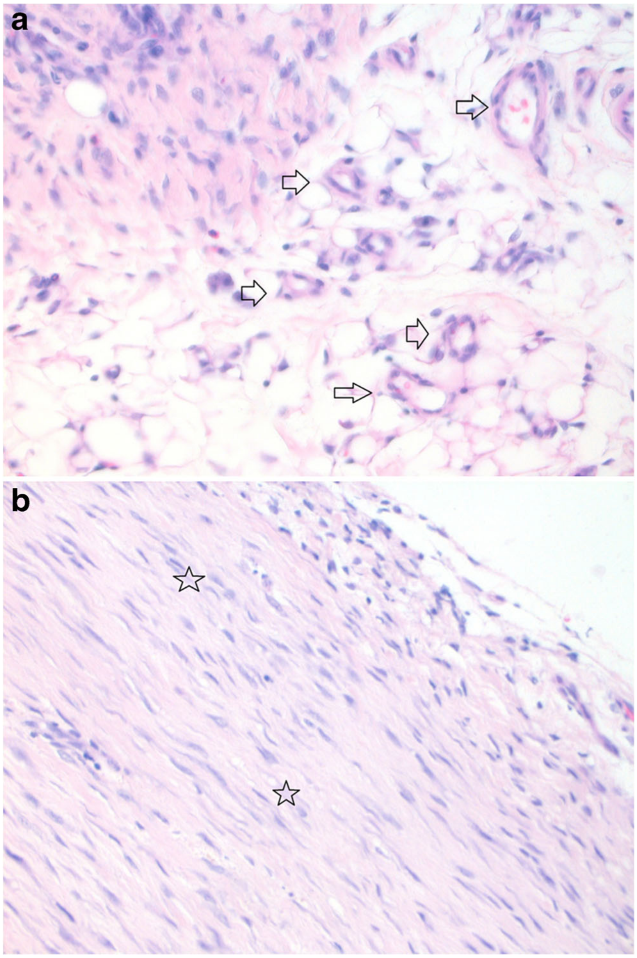

Fig. 1 **a** Histologic image of the curcumin group with H&E stain, 40 × 10 magnification. Arrows showing neovascularization. **b** Histologic image of the control group with H&E stain, 40 × 10 magnification. Stars showing organized collogen fibers

[24]. It has been shown that tendon injury process benefit from antioxidant agents [15].

Table 3 Biomechanic results

Parameter	Mean scores		
	Control (8)	Curcumin (9)	*p* values
Failure load (N)	35.03	41.88	*0.046*
Cross section area (mm^2)	6.20	5.33	*0.027*
Displacement (mm)	6.67	7.90	0.167
Maximum energy (J)	87.00	97.88	0.139
Total energy (J)	168.00	196.77	0.093
Length (mm)	9.86	10.96	*0.011*
Stiffness (N/mm)	3.50	3.76	0.277
Ultimate stress (MPa)	5.73	7.97	*0.021*
Strain (%)	68.45	49.16	*0.002*

Italic items are statistically significant

Curcumin's positive effect on partial tendon healing reported in a recent study. They have shown that curcumin has a key role in healing and development of ligaments by regulating collogen I and III [2]. The same study also suggests that curcumin improved tendon healing via well-organized collogen fibres. Indeed curcumin application results in more rapid wound closure and greater collogen deposition in healing wounds [25].

In our study, we created a total Achilles tendon injury model and searched for the effects of oral curcumin. The histological results are compatible with previous similar studies [2, 6]. We looked for four parameters and three of them were superior than the control groups. Only vascularity parameter did not show any statistical significance. We think that curcumin's antineoplastic and antioxidant effects could be responsible for this result.

We also searched for the biomechanical results. We investigate the tendon parameters by a testing machine which gives the outcomes until failure of the tendons. Nine parameters investigated and five of them showed significantly superior results in the study group. All the specimen failures were at the repair site. This location is similar to human tendon re-ruptures.

Among the biomechanical parameters, the most important ones (load to failure, displacement) were better in the study group. Load to failure parameter is an important factor for investigating the tendon healing process. According to our results, curcumin enhances the load parameter. This is important for preventing from re-ruptures. At these days, early motion is popular for regaining range of motion and avoiding from peritendinous adhesions. In a previous study, it was shown that curcumin has positive effect on preventing of peritendinous adhesions [6]. Enhanced load parameter can be useful for early forced physical therapy. On the other hand, displacement parameter results were better in the study group compared to the control group. Displacement is one of the important characteristics of the tendons. High displacement results mean that repaired tendon has longer distance for failure during loading.

There are also some limitations of our study. First, there is a lack of hydroxyproline tests and immunohistochemical evaluation which can be an important examination for evaluating the histological results. Secondly, the number of the rats used could be higher for more adequate results.

Clinical use of antioxidant and anti-inflammatory agents are popular for better healing process of soft tissue injuries. In human clinical trials, curcumin has been found to be safe at gram doses. Curcumin's safety and efficacy have already been proven by clinical trials [7, 8]. Curcumin has been used in Asian countries to treat a variety of diseases due to its antioxidant, anti-inflammatory, immunomodulatory, and antimicrobial properties [6–13] for a long time. Also, it has been published that curcumin may assist cutaneous wound healing even under complicated conditions, as observed for irradiated

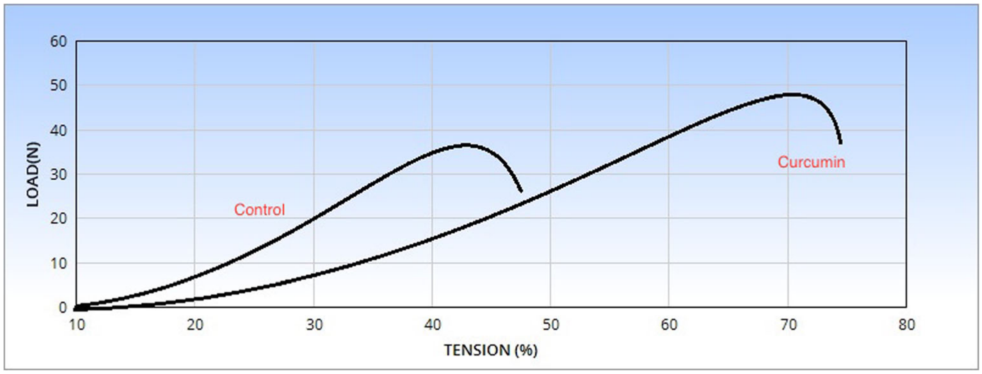

Fig. 2 Comparison of load-tension curves until breaking point due to biomechanical test results

wounds [25], burn wounds [11], and diabetic wounds [19, 25]. In our experimental study, we detect that curcumin use improved the tendon healing according to biomechanical and histological evaluation. We think that as it has positive effects on wound healing and also, it may be used to promote the tendon healing process.

In conclusion according to our results, curcumin had better results for total tendon healing not only histologically but also biomechanically. Curcumin could be an additional agent in the management of surgically repaired tendon injuries. Further studies including more rats and immunohistochemical analysis are needed to investigate the effects of curcumin.

Statement of welfare of animals All applicable international, national, and institutional guidelines for the care and use of animals were followed for this study. All procedures performed in this study were in accordance with ethical standards of the institution (Kobay Lab, Ankara, Turkey) where this study was conducted.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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