



## **Original Article**

# The effects of low-level laser therapy in extensor tendon injuries between zones 5 and 8 of the hand: A double-blind, randomized, placebo-controlled study

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#### ABSTRACT

Objectives: This study aims to examine whether low-level laser therapy (LLLT), when combined with conventional therapy, contributes to the rehabilitation of patients with zone 5 to 8 extensor tendon injuries.

Patients and methods: A total of 55 patients (44 males, 11 females; mean age: 34.2±11.3 years; range, 18 to 55 years) with hand extensor tendon injury were included in the double-blind randomized controlled study between April 30, 2020, and December 30, 2020. Controlled active motion protocol was applied to all patients. In addition, LLLT was applied to one group and sham laser to another group for 10 sessions. Patients were evaluated at baseline and four and eight weeks after intervention. Visual Analog Scale for pain, Quick Disabilities of the Arm, Shoulder, and Hand questionnaire for upper extremity symptoms and functions, and nine-hole peg test for hand dexterity were used. The range of motion of the hand and metacarpophalangeal joint circumference were measured. Grip strength was evaluated only at the eighth postoperative week.

Results: In both groups, significant improvement was observed in all evaluation parameters, except for wrist range of motion at the eighth postoperative week measurement (p<0.05). When the clinical outcomes were compared between the groups, no significant difference was observed in all clinical parameters both in the fourth and eighth week measurements after intervention (p>0.05).

Conclusion: In our study, no additional contribution of LLLT to the rehabilitation of extensor tendon injuries between zones 5 and 8 was

Keywords: Hand injuries, low-level light therapy, physical therapy modalities, rehabilitation, tendon injuries.

Traumatic extensor tendon injuries are frequently encountered in clinical practice due to the superficial location of tendons on the dorsum of the hand. The fact that it generally affects the young, male, and worker population and causes loss of workforce makes early repair and rehabilitation important.[1]

Good results can be obtained with appropriate splinting and exercise programs in the rehabilitation of extensor tendon injuries. Today, it has been shown that the early active mobilization protocol provides better functional results in the acute period. [2]

Low-level laser therapy (LLLT) is used in both wound healing and tendon healing treatment

with its effects such as increasing adenosine triphosphate production by mitochondrial respiratory stimulation, increasing collagen and nucleic acid synthesis, stimulation of granulation tissue formation, and modulation of pain and inflammation.[3,4]

The effectiveness of LLLT in flexor tendon injuries has been demonstrated; [5,6] however, no study examined LLLT in extensor tendon injuries.<sup>[5]</sup> This study aims to examine whether LLLT, when combined with conventional therapy, contributes to the rehabilitation of patients with zone 5 to 8 extensor tendon injuries.

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Received: February 13, 2024 Accepted: November 05, 2024 Published online: August 22, 2025

Cite this article as: Erdoğan F, Başkan B, Yurdakul FG, Bodur H. The effects of low-level laser therapy in extensor tendon injuries between zones 5 and 8 of the hand: A double-blind, randomized, placebocontrolled study. Turk J Phys Med Rehab 2025;71(3):333-341. doi: 10.5606/tftrd.2025.14790.



## PATIENTS AND METHODS

This study was planned as a double-blind, placebo-controlled, randomized, two-armed prospective study. Eighty-nine patients who applied to the Physical Medicine and Rehabilitation Clinic or the Traumatic Hand Rehabilitation Clinic of the Ankara City Hospital between April 30, 2020, and December 30, 2020, due to hand extensor tendon injury were evaluated. Fifty-five patients (44 males, 11 females; mean age: 34.2±11.3 years; range, 18 to 55 years) who met the study criteria and agreed to participate were included in the study. The inclusion criteria were as follows: (i) age between 18 and 65 years; (ii) primary repair due to extensor tendon injury between zones 5 and 8; (iii) application to the outpatient clinic in the first postoperative week. The exclusion criteria were as follows: (i) concomitant fracture, flexor tendon, vascular, or nerve injury; (ii) actively using steroids or nonsteroidal anti-inflammatory drugs; (iii) systemic infection, wound infection, or malignancy; (iv) pregnancy or breastfeeding; (v) cognitive dysfunction. Written informed consent was obtained from each patient. The study protocol was approved by the Ankara City Hospital Ethics Committee (Date: 02.04.2020; No: E1-20-415). The study was conducted in accordance with the principles of the Declaration of Helsinki.

The patients were randomly assigned to a laser + controlled active motion (CAM) therapy group (n=27) or a sham laser + CAM therapy group (n=28). Randomization was performed using computer-generated random numbers by an independent researcher.

The first evaluation of the patients was made at the end of the first postoperative week. A patient evaluation form was filled in, including demographic information, comorbidities, type and zone of injury, dominant and injured hand, smoking status, and information about the operation. A splint that holds the wrist in 40° extension, blocks the metacarpophalangeal (MCP) joint in 30° flexion, and keeps the interphalangeal (IP) joints in neutral position was applied to the patients. Controlled active motion exercises were started according to the early active mobilization protocol.[7] The patients were told that they should do the exercises in the splint and never remove it. In addition to the exercises, the retrograde massage technique was shown for edema control and recommended to be done every 2 h for 10 min.

Low-level laser therapy was applied to Group 1, and sham laser therapy was applied to Group 2 at the end of the second postoperative week, for a total of 10 weekdays throughout the treatment period of two weeks. Ilux 1064 laser device (Mectronic Medicale SRL, Bergamo, Italy) was used for the treatment. Both groups continued exercise therapy following the CAM program. After the sutures were removed, scar massage techniques were shown to the patients and added to their treatment.

In both treatment groups, the probe was placed to completely cover the injury site. Laser therapy was applied to Group 1 with the probe at 25 mm from the skin, a wavelength of 1064 nm, a power of 100 mW, a duration of 300 sec, an energy density of 7.5 J/cm², and accumulated energy delivered from all sessions of 300 J. In group 2, the screen of the laser device was always active, but the energy was selected as 0 J and the power as 0 mW. In addition, the laser light and the sound created by the device when the pedal was pressed were active for 300 sec. During both treatments, the patient and the therapist used protective glasses.

Patients in both groups were followed weekly until the completion of the eighth postoperative week following the CAM program. The exercise content of the CAM program is shown in Table 1.<sup>[7]</sup>

### Clinical assessment

Patients were evaluated by a different investigator who was not involved in the randomization and laser therapy stages before treatment (second week), at the end of the treatment (fourth week), and after completion of the eighth postoperative week. Visual Analog Scale (VAS) for pain,<sup>[8]</sup> Quick Disabilities of the Arm, Shoulder, and Hand (QuickDASH) questionnaire for upper extremity symptoms and functions,<sup>[9]</sup> and nine-hole peg (NHP) test for hand dexterity were used.<sup>[10]</sup> The range of motion (ROM) of the hand was measured with a goniometer,<sup>[11]</sup> the edema in the hand was measured at the level of the MCP joint with a tape measure<sup>[12]</sup> using a standardized protocol, and the injured side was compared with the noninjured side.

In our study, the values obtained from finger ROM measurement were classified according to the system defined by Kleinert and Verdan, in which total active motion (TAM) was evaluated. Total active motion is the sum of angles formed by MCP, proximal IP, and distal IP joints in maximum active flexion minus the total extension lag at each joint. The obtained degrees of the injured and noninjured

TABLE 1 Controlled active motion program							
Week	Exercise						
Postoperative first 4 weeks	IPJ1 hook and actively extend Place and hold MCPJs² into hyperextension keeping IPJs relaxed						
End of the 4 <sup>th</sup> postoperative week	Continue previous exercises Wrist tenodesis						
End of the 5 <sup>th</sup> postoperative week	Continue previous exercises Tendon gliding						
End of the 6 <sup>th</sup> postoperative week	Continue previous exercises Composite passive flexion Strengthening						
IPJ: Interphalangeal joint; MCPJ: Metacarpophalangeal joint.							

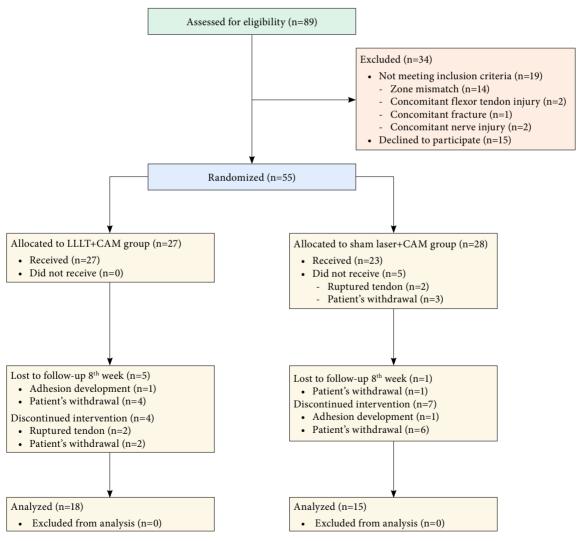


Figure 1. Flowchart of patient inclusion.

LLLT: Low-level laser therapy; CAM: Controlled active motion.

sides are compared and classified according to the percentage of the noninjured side. The classification is as follows: excellent=TAM is normal; good=TAM is greater than 75% of the normal side; fair=TAM is greater than 50% of the normal side; poor=TAM is less than 50% of the normal side.<sup>[7,13]</sup> Flexion and extension ROMs of the wrist were measured, and the obtained degrees were added.

When the eighth postoperative week was completed, a measurement with a Jamar dynamometer (Sammons Preston, Inc., Bolingbrook, IL, USA) was added to evaluate the grip strength.<sup>[14]</sup>

## Sample size determination

G\*Power version 3.1.9.4 (Heinrich-Heine Universität Düsseldorf, Düsseldorf, Germany)

				TABLI						
		Demographic data of patients and information about injury								
	A	All patients (n=55)			Group 1 (n=27)			Group 2 (n=28)		
Variables	n	%	Mean±SD	n	%	Mean±SD	n	%	Mean±SD	p
Age (year)			34.2±11.3			32.4±11.2			35.9±11.3	0.285
Sex										0.502
Male	44	80		21	47.7		23	52.3		
Female	11	20		6	54.5		5	45.5		
Education level										0.214
Illiterate	2	3.6		1	50		1	50		
Primary school	28	50.9		15	53.6		13	46.4		
High school	16	29.1		6	37.5		10	62.5		
Graduate	8	14.5		4	50		4	50		
Postgraduate	1	1.8		1	100		0	0		
Smoking status										0.813
Smoker	29	52.7		15	51.7		14	48.3		0.013
Non-smoker	26	47.3		12	46.2		14	53.8		
	20	17.5		12	10.2		17	33.0		
Occupation										0.583
Worker	33	60		14	42.4		19	57.6		
Public servant	13	23.6		7	53.8		6	46.2		
Student	4	7.3		3	75		1	25		
Unemployed	5	9.1		3	60		2	40		
Injured hand										0.891
Dominant	28	50.9		14	50		14	50		
Non-dominant	27	49.1		13	48.1		14	51.9		
Injured zone										0.951
Zone 5	27	49.1		13	48.1		14	51.9		****
Zone 6	13	23.6		6	46.1		7	53.9		
Zone 7	8	14.5		4	50		4	50		
Zone 8	7	12.7		4	57.1		3	42.9		
True of initiation										0.469
Type of injury	21	20.2		11	F2 2		10	477		0.468
Glass Metal	21	38.2 20		11	52.3		10 6	47.7		
Knife	11 8	14.5		5 3	45.5 37.5		5	54.5 62.5		
Spiral stone	3	5.5		2	66.7		1	33.3		
Saw	5 5	9.1		0	00.7		5	100		
Marble stone	5 4	7.3		3	75		5 1	25		
Sheet metal	2	3.6		2	100		0	0		
Bear bite	1	1.8		1	100		0	0		
	1	1.0		1	100		U	U		
Operation site										0.686
Emergency	44	80		21	47.7		23	52.3		
Operating room	11	20		6	54.5		5	45.5		
Accident type										0.512
Industrial accident	20	36.4		9	45		11	55		
Home accident	35	63.6		18	51.4		17	48.6		

was used to calculate the adequate sample size for repeated measures with two groups. To obtain a power of 0.80 [alpha (type 1 error) was 0.05 and beta (type 2 error) was 0.20], the appropriate total sample size was 34.

## Statistical analysis

Data were analyzed using IBM SPSS version 23.0 software (IBM Corp., Armonk, NY, USA). Whether the numerical data were normally distributed or not was examined by the Shapiro-Wilk test. General descriptive statistics were summarized as mean, median, standard deviation, minimum, and maximum values for continuous variables, and as frequency and percentages (%) for categorical variables. The chi-square test or Fisher exact test was used to examine the distribution of discrete variables between groups. Continuous variables in two different treatment groups were compared with one-way analysis of variance or the Kruskal-Wallis test. Post hoc tests (Bonferroni) were used to understand which group differed from the other. Measurement results before and after the treatment were evaluated with analysis of variance in repeated measurements. The results were evaluated at the 95% confidence interval, and the significance level was set at p<0.05.

## **RESULTS**

The flowchart of the patients included in the study is shown in Figure 1. Fifty-five patients included in the study were divided into two groups according to age and injury zone by covariant focused randomization/minimization method. There was no significant difference between the groups in terms of demographic data and injury information (p>0.05; Table 2). In terms of pretreatment clinical features, there was no significant difference between the two groups (p>0.05; Table 3).

At fourth and eighth postoperative weeks, TAM grade, MCP joint circumference, NHP test completion time, and QuickDASH score parameters improved significantly in both groups (p<0.05). The VAS score showed significant improvement at each measurement in Group 1 (p<0.05). Conversely, in Group 2, it did not show significant improvement at the eighth week measurement (p=0.066). In both groups, no significant improvement was observed in wrist ROM in any measurement (p>0.05; Table 4).

When the clinical outcomes were compared between the groups, no significant difference was observed in all clinical parameters (TAM degree, wrist ROM, MCP joint circumference, NHP test

TABLE 3  Pretreatment clinical features of the patients										
	All patients (n=55)				Group 1 (n=27)			Group 2 (n=28)		
Variables	n	n % Mean±SD		n	%	Mean±SD	n	%	Mean±SD	p
TAM (degree) Injured side Non-injured side			270.11±16.97 152.60±41.88			273.20±14.05 157.68±41.65			266.25±19.72 146.25±42.36	0.358 0.837
TAM classification Excellent	0	0		0	0		0	0		0.841
Good Fair	6 26	13.3 57.8		4 14	66.7 53.8		2 12	33.3 46.2		
Poor	13	28.9		7	53.8		6	46.2		
Wrist ROM (degree) Injured side Non-injured side			142.14±14.39 67.86±30.39			148.33±2.88 80.00±32.78			137.50±18.48 58.75±29.54	0.280 0.289
MCP Joint circumference (mm) Injured side Non-injured side			209.27±15.55 214.27±16.11			209.85±16.30 216.00±16.60			208.61±15.00 212.30±15.68	
NHP test (sec)			52.52±44.28			53.37±41.19			51.55±48.47	0.548
QuickDASH score			50.47±18.47			52.23±16.88			48.47±20.32	0.574
VAS score			2.73±2.31			2.77±2.56			2.70±2.05	0.879

SD: Standard deviation; TAM: Total active motion; ROM: Range of motion; MCP: Metacarpophalangeal; NHP: Nine Hole Peg; QuickDASH: Quick disabilities of arm, shoulder and hand questionnaire; VAS: Visual Analog Scale.

	TABl Intragroup comparison		ions				
	0 1 1	Group		Group 2			
Variables		Mean±SD	<i>p</i> *	Mean±SD	<i>p</i> *		
	Baseline (2 <sup>nd</sup> week)	161.37±43.31	-	139.17±44.91	-		
TAM (degree)	4th postoperative week	207.19±40.74	< 0.001	194.58±35.70	< 0.001		
-	8 <sup>th</sup> postoperative week	238.13±34.19	< 0.001	235.00±36.49	< 0.001		
. **	Comparison between groups	Baseline-4th	week	0.454			
p**		Baseline-8th	week	0.378			
	Baseline (2nd week)	82.50±45.96	_	55.00±35.00	_		
Wrist ROM (degree)	4 <sup>th</sup> postoperative week	97.50±38.89	0.205	70.00±43.58	0.188		
	8 <sup>th</sup> postoperative week	110.0±28.28	0.272	85.00±37.74	0.122		
<i>p</i> **		Baseline-4th	week	0.388			
	Comparison between groups	Baseline-8 <sup>th</sup>	week	0.480			
MCP Joint circumference (mm)	Baseline (2nd week)	212.18±17.85	_	208.86±17.60	_		
	4 <sup>th</sup> postoperative week	210.24±16.43	0.002	206.79±17.47	0.008		
	8 <sup>th</sup> postoperative week	207.53±17.45	< 0.001	205.86±16.90	0.002		
p**		Baseline-4 <sup>th</sup>	week	0.651 0.694			
	Comparison between groups	Baseline-8th	week				
NHP test (sec)	Baseline (2 <sup>nd</sup> week)	54.22±48.36	-	61.83±59.58	-		
	4 <sup>th</sup> postoperative week	31.46±24.75	0.043	29.13±8.24	0.044		
	8 <sup>th</sup> postoperative week	21.61±3.33	0.011	$20.80\pm4.41$	0.019		
		Baseline-4 <sup>th</sup>	week	0.974 0.737			
p**	Comparison between groups	Baseline-8th	week				
	Baseline (2 <sup>nd</sup> week)	2.47±2.32	_	2.86±2.03	_		
VAS score	4 <sup>th</sup> postoperative week	1.24±1.52	0.014	1.64±1.64	0.006		
	8 <sup>th</sup> postoperative week	$0.88\pm2.11$	0.030	1.57±1.91	0.066		
<i>p</i> **		Baseline-4 <sup>th</sup>	week	0.833 0.379			
	Comparison between groups	Baseline-8th					
	Baseline (2nd week)	51.17±13.89	_	57.42±13.62	-		
QuickDASH score	4th postoperative week	32.32±18.77	< 0.001	34.70±17.51	< 0.00		
	8 <sup>th</sup> postoperative week	12.52±12.30	< 0.001	13.75±11.06	< 0.00		
. ***		Baseline-4th	week	0.666			
p**	Comparison between groups	Baseline-8 <sup>th</sup>	week	0.354	0.354		

SD: Standard deviation; TAM: Total active motion; ROM: Range of motion; MCP: Metacarpophalangeal; NHP: Nine Hole Peg; VAS: Visual Analog Scale; QuickDASH: Quick disabilities of arm, shoulder, and hand questionnaire; p\*: Intragroup comparison of clinical evaluations; p\*\*: Comparison of clinical evaluations between groups.

completion time, VAS score, and QuickDASH score) both in the fourth and eighth week measurements (p>0.05; Table 4).

Table 5 demonstrates the classification of TAM of the patients in all measurements. It was observed that the majority of the patients in both groups were classified as "fair" at the postoperative fourth week, and as "good" at the postoperative eighth week. There was no statistically significant difference between the groups in terms of classification distributions in both measurements (p>0.05).

In the grip strength measurements performed at the eighth postoperative week, the noninjured side showed a mean of 32.68±9.26 kg in Group 1 and  $33.41\pm8.44$  kg in Group 2, and there was no significant difference between the groups (p=0.667). The injured side showed a mean of  $19.77\pm9.79$  kg in Group 1 and  $19.88\pm8.88$  kg in Group 2, and there was no significant difference between the groups (p=0.968).

## **DISCUSSION**

The effects of LLLT on tendon healing have been studied on the flexor tendon injuries of the hand and Achilles tendons of animals. In this study, we showed the effects of laser therapy on extensor tendon injuries. To our knowledge, this is the first study on this subject in the literature.

	assification of TAM of the patients in all measurements						
TAM classification	All patients		Gro	oup 1	Gro		
	n	%	n	%	n	%	p
2 <sup>nd</sup> postoperative week	45		25		20		0.84
Excellent	0	0	0	0	0	0	
Good	6	13.3	4	66.7	2	33.3	
Fair	26	57.8	14	53.8	12	46.2	
Poor	13	28.9	7	53.8	6	46.2	
4 <sup>th</sup> postoperative week	35		20		15		0.12
Excellent	2	5.7	2	9.5	0	0	
Good	14	40.0	6	28.6	8	57.1	
Fair	18	51.4	13	61.9	5	35.7	
Poor	1	2.9	0	0	1	7.1	
8 <sup>th</sup> postoperative week	28		16		12		0.85
Excellent	6	21.4	4	25.0	2	16.7	
Good	18	64.3	10	62.5	8	66.7	
Fair	4	14.3	2	12.5	2	16.7	
Poor	0	0	0	0	0	0	

Low-level laser therapy positively affects tendon healing by ensuring proper alignment and organization of collagens and accelerating the transition from the inflammatory phase to the proliferative phase. The irregular arrangement of collagen fibers due to decreased type 1 and increased type 3 collagen levels makes tendons more prone to rupture. De Jesus et al. [3] examined the effect of LLLT on tissue repair in rats with partial Achilles tendon injury and showed that after laser therapy in the affected tendon, type 1 collagen levels increased, and type 3 collagen levels decreased.

Low-level laser therapy facilitates wound healing by its mitochondrial effects and by stimulating fibroblast proliferation, angiogenesis, and reducing inflammation.[15] Houreld and Abrahamse[16] examined the effects of LLLT on fibroblast cells and wound healing. It has been shown that better results are obtained with 632.8 nm (visible) than 1064 nm (infrared) light for wound healing. Avcı et al.[17] stated that light with a wavelength of 390 to 600 nm affects superficial tissues and 600 to 1100 nm affects deeper tissues. In his review of the effects of photobiomodulation on wound healing, Kuffler<sup>[15]</sup> stated that monolayer cell cultures were used for in vitro experiments and, therefore, laser light does not need much depth. However, Kuffler reported that more depth may be needed for in vivo treatment of a wound. In our study, we chose 1064 nm as the wavelength, as it affects deeper tissues.

In a systematic review by Bjordal et al.,[18] it was stated that 1060 nm lasers should have a power density of 15-105 mW/cm<sup>2</sup> and an energy density of 0.5-15 J/cm<sup>2</sup> to be effective in the finger region. In another systematic review by Bjordal et al., [19] a power density of 5-171 mW/cm<sup>2</sup> and an energy density of 0.7-19 J/cm<sup>2</sup> were suggested for anti-inflammatory effect. Therefore, 25 mW/cm<sup>2</sup> power and 7.5 J/cm<sup>2</sup> energy density were performed as treatment doses in our study. Although there are different recommendations regarding sessions and irradiation times, World Association for Photobiomodulation Therapy stated that the irradiation time should be 20 to 300 sec for 780 to 860 nm GaAlAs lasers and 30 to 600 seconds for 904 nm GaAs lasers. World Association for Photobiomodulation Therapy also recommended daily treatment for two weeks or treatment every other day for three to four weeks.[20,21] In our study, the irradiation time was determined as 300 sec, and daily treatment was applied for two weeks.

We could not find a significant difference between the two groups in terms of TAM grades. Similarly, in a study by Özkan et al., [6] in which the effects of LLLT on the rehabilitation of flexor tendon injuries were examined, no significant difference was observed between the treatment and placebo groups in terms of TAM grades. Conversely, in a study by Poorpezeshk et al., [5] a significant improvement was observed in the laser

therapy group compared to the control group in terms of total passive ROM.

Edema is an expected response that occurs during the inflammatory phase of wound healing (0 to 5 days); however, its control reduces the inflammatory response and prevents the development of fibrosis. [22] In our study, there was no significant difference between the two groups in terms of MCP joint circumference. There are studies in the literature showing the effectiveness of LLLT in the management of postoperative edema.[6,23] Laser therapy was started on the eighth postoperative day by Ozkan et al., [6] and on the first postoperative day by Gasperini et al. [23] In our study, laser therapy was started on the 15th postoperative day. The absence of significant difference between the two groups was attributed to the application of laser therapy after the inflammatory phase of wound healing was completed.

The pain encountered after injuries was mostly associated with the inflammatory response, and it was observed that the pain decreased as the tissue healed. [19,24] Bjordal et al. [19] showed that LLLT modulates the inflammatory process and reduces acute pain in the short term. They suggested that an energy density of 7.5 J/cm² be used for the first 72 h for optimal effect in acute pain management and continuing with lower doses such as 2 J/cm² in the following days to stimulate tissue repair. In our study, an energy density of 7.5 J/cm² was used, and there was no significant difference between the two groups in terms of VAS scores. These results were associated with the inability to apply laser therapy in the acute phase.

In our study, no significant difference was found between the two groups in the grip strength measurements performed at the postoperative eighth week. Similarly, in the study of Ozkan et al., [6] no significant difference was observed between the groups in the grip strength measurements made at the postoperative 12<sup>th</sup> week.

This study had some limitations. First, isolated extensor tendon injuries were included in the study, and those with concomitant injuries were excluded from the study. In addition, patients with injuries in the proximal zones (zones 5 to 8) were included in the study. Therefore, the obtained data cannot be generalized to patients with concomitant injuries or injuries in the distal zones (zones 1 to 4). The follow-up of the patients was terminated in the eighth postoperative week. Therefore, a clear idea about the

long-term effects of LLLT could not be obtained. The dropout rate was high due to the young, male, worker patient population and the COVID-19 (coronavirus disease 2019) pandemic.

In conclusion, no additional contribution of LLLT to the rehabilitation of extensor tendon injuries between zones 5 to 8 was observed. The significant improvement observed in both groups in all the parameters examined, once again demonstrated the importance of early active mobilization protocol and appropriate splint use in rehabilitation. The recommendation of different laser therapy doses for different parameters in tendon injury rehabilitation in the literature makes it difficult to choose the appropriate laser therapy doses. The combined use of different wavelengths and energy densities can be beneficial for better results. To determine the efficacy and appropriate doses of laser therapy in extensor tendon rehabilitation, new studies with different doses are needed on larger populations.

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

**Author Contributions:** Idea/concept: F.E., B.B.; Control/supervision: F.G.Y., B.B., H.B.; Data collection and processing: F.E., F.G.Y., B.B.; Analysis and interpretation: F.E., F.G.Y.; Design, writing the article, critical review, references and materials: F.E., B.B., F.G.Y., H.B. All authors have read and approved the final version of the manuscript.

**Conflict of Interest:** The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

**Funding:** The authors received no financial support for the research and/or authorship of this article.

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