Therapeutic Potential of Low-Level Laser Photobiomodulation on Adjuvant Induced Temporomandibular Joint Arthritis in A Rat Model

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Abstract:

Objective: To test the efficacy of low-level laser therapy (LLLT) to alleviate temporomandibular TMJ induced arthritis in rats. **Materials and methods**: Rats were randomly allocated to three groups: group I (sham control), group II (arthritis) where animals were subjected to arthritis induction using complete Freund's adjuvant followed by LLLT (0 j/cm^2), and group III (LLLT) where animals were subjected to arthritis induction followed by LLLT (38 j/cm^2). One and three weeks after treatment onset, rats were euthanized and specimens were subjected to histological, histomorphometric, and histochemical analysis. **Results**: The sham group showed normal histology. The arthritic group showed severe changes with surface irregularity, loss of condylar zones, hypocellularity, and bone resorption. The articular disc was significantly increased in thickness (P=0.0005), while the condylar cartilage was atrophied (P<0.0001). LLLT treatment caused significant enhancement of disc (P<0.0001) and cartilage thickness (P=0.0001) compared to the arthritic group after three weeks. The histological structure of TMJs appeared more regularly arranged than the arthritis group with better results after three weeks, where articular surfaces showed uniform outlines, chondrocytes were well arranged, less osteochondral detachment was detected, and subchondral bone showed arranged trabeculae. However, LLLT did not restore complete normal architecture as compared to the sham group. **Conclusions**: LLLT can significantly promote the regeneration and healing of arthritic TMJs. However, longer follow-up periods or different laser doses may be recommended to restore normal tissue architecture.

Introduction:

The temporomandibular joint (TMJ) is a bilateral synovial joint responsible for dynamic movements in the human body. Temporomandibular joint disorders (TMDs) are the main cause of pain originating from non-dental etiologies in the orofacial area.[1] Patients suffer from a group of signs and symptoms affecting temporomandibular joints, the masticatory muscles, or both and are frequently accompanied by restriction of mandibular movements.[2]

Rheumatoid arthritis (RA) is one of the most common forms of inflammatory arthritis. It is a progressive inflammatory disease that affects the joints. A significant percentage of patients with RA have symptoms and signs of TMJ involvement.[3] It is believed that TMJ involvement occurs even before the onset of clinical symptoms. All the prerequisites for osteoclast differentiation and bone erosion are found in this subclinical preliminary phase.[4]

Patients usually complain of chronic pain that may also be accompanied by impairment in their general health, depression, or other psychological disabilities affecting the quality of life and the well-being of the patients.[5] Conventional treatment of arthritis involved non-steroidal anti-inflammatory drugs(NSAIDs), corticosteroids and immunosuppressives.[6] However, these treatments have many side effects and complications requiring the development of new treatment modalities.[7]

Low-level laser therapy (LLLT) was found to have analgesic and bio-modulative effects,[8] so it has been widely used in dental practice for the management of dentine hypersensitivity, soft tissue disorders, musculoskeletal pain, and for stimulating bone regeneration. It has also been applied as a conservative treatment modality enhancing function and reducing patients' complaints.[9] The use of LLLT in the management of TMD has been recommended due to its ability to relieve pain within minutes following application.[10] The photobiostimulatory effect of LLLT is attributed to the enhancement of cellular metabolism, synthesis of the collagenous matrix, chemotaxis, increasing microcirculation as well as blocking of pro-inflammatory mediators.[11]

However, so far, no laser therapy protocols have been standardized for TMD treatment with wide variation between different laser parameters including laser type, wavelength, duration, frequency, power, and density. This raised the need for more trials to determine the most effective laser regimen. So, this study was conducted to evaluate the regenerative effect of low-level laser therapy in a murine temporomandibular joint arthritis model histologically, histomorphometrically, and histochemically.

Materials and methods:

Animals: Experimental procedures were performed

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according to the protocol of the Mansoura University animal care and use committee (MU-ACUC) with code number: MU-ACUC (DENT.R.22.09.2). All the biosecurity and biosafety procedures for research work in laboratories were performed following the recommendations of Alderman et al.[12]

Sample size calculation was based on mean articular disc thickness among studied groups with control and arthritic groups retrieved from a previous study.[13] Using G*power [14] version 3.1.9.4 to calculate sample size, based on size effect of 3.84, two-tailed test, α error of 0.05, and 90% power, the total sample size was calculated to be at least 6 animals in each group.

Thirty-six male, pathogen-free, Sprague Dawley rats, weighing 250-300 gm were selected. Animals were kept in cages of six rats each in the medical experimental research center (MERC), Mansoura University, Mansoura, Egypt. They were kept in a light-controlled room with a 12h light-dark cycle, and 22 ºC temperature. Relative humidity of 65-70% was kept constant and animals received a commercial diet and water ad-libitum. Animals were acclimatized for two weeks before starting the experiment.

Study design: This was an experimental, randomized controlled study. Rats were divided, using a simple random sampling method, into three groups (n =12):

Group I (Sham control): rats received 50 µL PBS in TMJs and were subjected to diode laser application of 0 J/cm^2 where rats were anesthetized and only an aiming beam was applied over TMJs.The remaining rats were subjected to induction of RA in the left TMJs only in order not to affect the mastication efficiency and feeding of animals. One week after induction of RA, rats were further divided into two groups:

Group II (Arthritis): rats' TMJs were subjected to a diode laser application of 0 J/cm^2 every 48 hours for 7 days (4 sessions).

Group III (Arthritis + LLLT): rats' TMJs were subjected to a diode laser application of 38 J/cm^2 every 48 hours for 7 days (4 sessions).One week and three weeks after the beginning of LLLT treatment, six rats of each group were euthanized by an overdose of anesthesia using intraperitoneal injections of 500 mg/kg sodium pentobarbital.[15]

Induction of rheumatoid arthritis: Animals were anesthetized by inhaling halothane (4-5%) in a closed plastic container. The area over the TMJ was shaved and then wiped with Betadine. The postero-inferior border of the zygomatic arch was palpated, and an insulin syringe was inserted below this point and then advanced toward the condyle. RA was induced by a single intra-articular injection of 50 µL complete Freund's adjuvant (CFA) (Sigma Aldrich, St. Louis, Missouri, United States). The injection was done slowly over a time-span of 2 min.[13]

Low-Level Laser Therapy (LLLT): Each rat was anesthetized and TMJ was palpated 5-10 mm lateral to the outer eye canthus and by moving the mandible to precisely locate the joint. A diode laser was applied at a single point over the skin covering the TMJ with an angle of 90º after coverage of the surrounding area with an aluminum foil to avoid eye injury to the animal.

LLLT was performed using a Cheese II medical Diode laser device (Wuhan, China) with the following parameters: Active medium: InGaAs (semiconductor), wavelength 980 nm, tip diameter of 1 cm, continuous irradiation mode, power of 0.5 W, power density of 0.64 W/cm^2, energy of 30 J and energy density of 38 J/cm^2 for 60 sec, 0.5 cm above the skin. Irradiation was done every 48 hrs for 7 days (4 sessions).

Histological Examination: After animal euthanization, the TMJs were sampled and fixed in 10% neutral buffered formalin for 24 hours, and then decalcified, dehydrated, and embedded in paraffin blocks. Sections of 4–5 µm thickness were prepared for hematoxylin and eosin (H&E) and Masson trichrome staining.

Digital morphometric image analysis: Hematoxylin and eosin-stained slides were examined by a blinded examiner and photographed using ToupCam® digital camera (model no. XCAM1080PHA) mounted on a light microscope (Olympus®, CX22, Japan) with 0.5 photo adaptor, using 10x objective lens. Two slides from each specimen were examined. Images were analyzed on Intel® core I7®-based computer using Fiji ImageJ software (version 1.51r; NIH, Maryland, USA). Images were calibrated for micrometer measurements and the condylar cartilage and articular disc thickness was measured at three different sites in each slide at the anterior, middle, and posterior regions using the line tool, and the mean values were calculated. Data was then exported to an excel sheet for further statistical analysis.

Statistical analysis: Data was fed to the computer and analyzed using GraphPad Prism 8 (GraphPad Software). Quantitative data was described using mean ± standard deviation for normally distributed data after testing normality using the Shapiro-Wilk test. The significance of the obtained results was judged at the (0.05) level. Two Way ANOVA test was used to assess the combined effect of group and time factors on articular disc and condylar cartilage thickness with one-way ANOVA and Post Hoc Tukey test for pairwise comparison.

Results:

Hematoxylin and eosin stain: In the sham control group, the histological picture was similar at one and three weeks. The TMJs showed normal tissue arrangement with the condylar head showing regular thickness of fibrous and hyaline cartilage layers. The different zones of the condylar head were observed; fibrous, proliferative, and cartilage zones. The articular fibrous surface was regular with no obvious indentations or clefts. The condylar cartilage layer showed regular chondrocyte morphology, arrangement, and number. The cartilage-bone interface was normal, and the condylar head expressed regular bone trabeculae and marrow spaces. The articular disc was observed separating the joint cavity into upper and

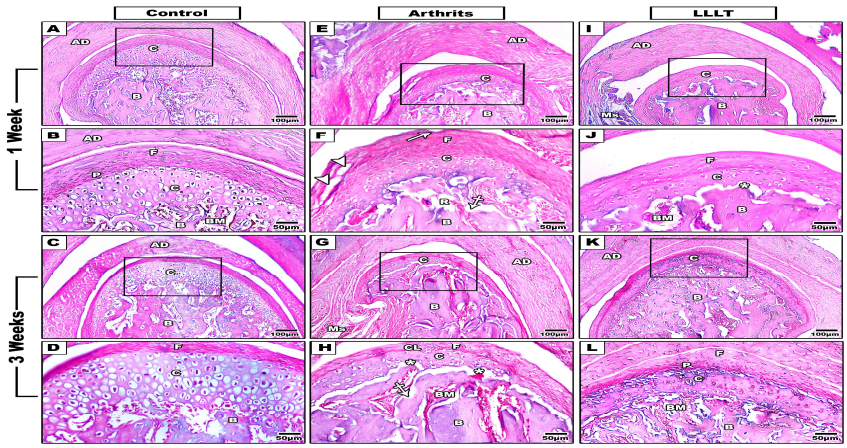

Figure 1: Hematoxylin and eosin staining of TMJ specimens of the control, arthritis and LLLT groups at 1 and 3 weeks after treatment. AD: articular disc, F: fibrous zone, P: proliferative zone, C: condylar cartilage, B: condylar bone, BM: marrow spaces, Ms: muscles, R: Bone resorption, Cl: Chondrocyte clusters, Asterisk indicates osteochondral interface detachment, Arrows indicate surface indentations, Arrowheads indicate horizontal clefts, Cross arrows indicate Howship's lacunae.

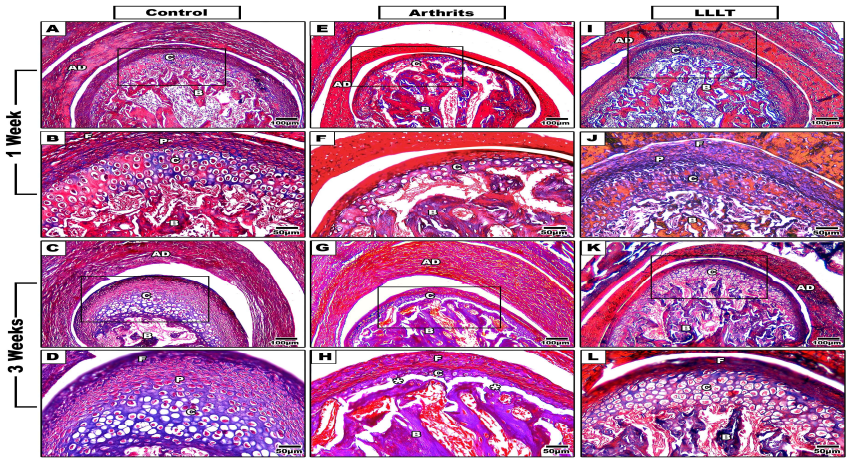

Figure 2: Masson trichrome staining of TMJ specimens of the control, arthritis and LLLT groups at 1 and 3 weeks after treatment. AD: articular disc, F: fibrous zone, P: proliferative zone, C: condylar cartilage, B: condylar bone, Asterisk indicates osteochondral interface detachment

lower compartments with a larger upper one. The disc showed a regular fibrous structure with some chondrocytes. The temporal bone was observed with a normal fibrous covering layer with some occasional chondrocytes as well, Figure 1 A-D.

The arthritis group showed several arthritic changes in one week where the condylar fibrous covering was irregular with surface indentations and horizontal clefts. The condylar cartilage zone thickness was reduced. Moreover, the cartilage chondrocytes were less in number, randomly arranged, and showed enlarged nuclei. The osteochondral interface was very irregular and undulated and cartilage was separated from the bone surface in some regions. Bone resorption with osteoclast howship's lacunae together with irregular arrangement of bone trabeculae and marrow spaces was observed. The articular disc was obviously thickened showing dense fibrotic tissue and the supradiscal space was much reduced, Figure 1 E, F.

After three weeks of intervention, TMJ arthritis was more pronounced. Clusters of chondrocytes were observed in the condylar fibrous layer. The condylar zones were less distinct with the proliferative zone almost diminished. The condylar cartilage showed more thinning, uneven thickness, and hypocellularity. The cartilage bony interface expressed a scalloping surface and was completely dissociated through the whole condylar outline. The articular disc was still thickened as well, Figure 1 G, H.

In the Diode laser-treated group after one week, the fibrous layer of the condyle showed a regular, even surface with a normal fibrous structure and some occasional chondrocytes. The condylar cartilage regained some thickness and structure uniformity. However, the osteochondral interface was still irregular with some areas of detachment. The subchondral bone showed more arranged,

densertrabeculae and the articular disc showed reduced thickness, Figure 1 I, J.

After three weeks of laser therapy, the condylar zones were restored with the proliferative zone increased in thickness. The cartilage zone appeared wider, with uniform, even thickness and higher cellularity in comparison to the arthritic group at the same period. The cartilage bone interface was more regular, and smooth with less areas of detachment. The condylar bone appeared well arranged with normal red bone marrow spaces and the articular disc showed much decreased thickness. However, the spaces of the upper and lower joint compartments were reduced. Moreover ,when compared to the sham control group, the condylar cartilage was less cellular and thinner and the chondrocytes were not as well arranged as in the sham group, Figure 1 K, L.

Masson trichrome stain: Sham control specimens showed normal architecture of TMJs with the articular disc showing thick, wavy, regular collagen bundles. The condylar head had a uniform, fibrous covering, and a thick uniform cartilage layer with increased staining around cell lacunae and decrease away from them. Densely colored subchondral bone trabeculae were observed, Figure 2 A-D.

In the arthritic group, trichrome staining of some specimens showed disarrangement of the collagen fibers in the articular disc and obvious thinning of the cartilage layer, especially after three weeks, Figure 2 E-H. The LLLT-treated TMJs revealed gradual enhancement through the three weeks of evaluation, with better arrangement of collagen fibers in the articular disc and fibrous layer together with restoration of thickness and uniformity of the proliferative as well as the cartilage zones, Figure 2 I-L.

Table 1: Two Way ANOVA analysis for the combined effect of intervention group and time factors on articular disk thickness

Source	F	P value
Intervention	8.454	0.0017*
Time	1.657	0.2103
Intervention Vs Time	2.806	0.0804

P: Probability, *: significance <0.05

Articular disc thickness: The two-way ANOVA showed a significant main effect of group factor (P = 0.0017), but an insignificant effect of time factor (P = 0.2103), and insignificant interaction between both factors (P = 0.0804), Table1.

Statistical analysis showed a significant increase in disc thickness in the arthritis group compared to the sham control group (P = 0.0005) and a significant thickness reduction in the laser-treated group compared to the arthritis group after three weeks of treatment onset (P < 0.0001). No significance was detected between the laser and control groups at weeks one (P = 0.5143) or three (P = 0.6388). Comparison of the two-time points showed significance only in the LLLT group with a significant decrease at week three, Table 2, Figure 3A.

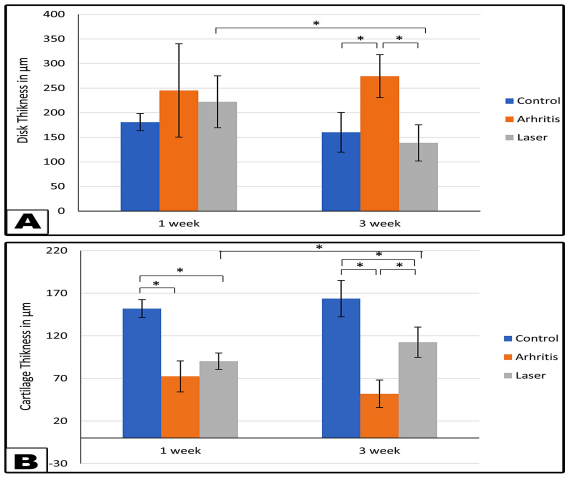

Figure 3: Bar graphs showing the statistical analysis for: A. Articular disc thickness, B. Condylar cartilage thickness. * Denotes statistical significance.

Condylar cartilage thickness: Two-way ANOVA revealed a significant main effect of group factor (P < 0.0001), while no significant effect of time factor (P = 0.4481) was detected, however, there was a significant interaction between time and group factors (P = 0.0182), Table 3.

Statistical analysis of the condylar cartilage thickness of different groups showed significant thickness reduction in the arthritic group at weeks one (P < 0.0001) and three (P < 0.0001) as compared to the control group. While the laser-treated group showed significantly higher thickness than the arthritis group after three weeks (P = 0.0001). However, after three weeks the cartilage dimensions in the laser group were still significantly less than the control group (P = 0.0007). Regarding the different time points, no significant difference was detected between weeks one and three except for the LLLT group, which showed a significant increase after three weeks, Table 4, Figure 3B.

Discussion:

One of the most common forms of inflammatory arthritis is rheumatoid arthritis. It is a progressive degenerative inflammatory disease targeting joints.[3] Rat adjuvant induced arthritis is a widely used experimental model of RA. Complete Freund's adjuvant consists of dead and dried mycobacterium tuberculosis in oil, which induces pathological RA that histologically resembles that developed in humans.[16] Several studies reported a beneficial effect of LLLT superior to that induced by other treatment modalities like occlusal splint application,[17] transcutaneous electrical neural stimulation,[18] or microcurrent electrical stimulation[19] in the treatment of TMD. In the present study, the inGaAs (semiconductor) diode laser,

Table 2: One way ANOVA followed by post-hoc Tukey test for pairwise comparison of mean disk thickness (µm) between different intervention groups for each time point, and T-test for comparison between the two time points for each group

	Control	Arthritis	Laser	P value	F
1 week	180.89±17.48	245.22±94.91	222.12±52.8	0.2385	1.58
Post-hoc		P1= 0.2183	P1= 0.5143 P2= 0.8062		
3 weeks	160.09±40.42	274.36±43.69	138.72±36.82	<0.0001*	19.54
Post-hoc		P1= 0.0005*	P1= 0.6388 P2< 0.0001*		
P value between 1&3 weeks by T-test	0.2742	0.5101	0.0099*		

P: Probability, *: significance <0.05, P1: significance vs control, P2: significance vs arthritis.

Table 3: Two Way ANOVA analysis for the combined effect of intervention group and time factors on condylar cartilage thickness

Source	F	P value
Intervention	88.26	<0.0001*
Time	0.595	0.4481
Intervention vs Time	4.759	0.0182*

P: Probability, *: significance <0.05

with a wavelength of 980-nm, energy density of 38J/cm^2. and output power of 0.5 Watt, significantly reduced the histological arthritic changes of the joints. The therapeutic potential of LLLT could be attributed to its inductive effect for the secretion of endogenous opioids, increasing vasodilatation, stimulating tissue regeneration, and reducing inflammation through the reduction of prostaglandin E2 and cyclo-oxygenase 2 (COX-2) release,[20] as well as decreasing reactive oxygen species (ROS) levels, mRNA expression of phospholipase A2 and TNF-α levels allowing LLLT to be a good alternative to NSAIDs avoiding any possible drugs' side effects.[21] In a study conducted by Karoussis et al.,[22] LLLT was found to enhance the expression of epidermal growth factor (EGF), transforming growth factor (TGF), and vascular endothelial growth factor (VEGF) as well as stimulating collagen production and cellular proliferation in human fibroblast cells.

In the current study, the arthritis group showed histological changes in the TMJ at one and three weeks post-treatment. Concomitantly, other studies observed deformation and resorption in the condylar cartilage and bone as well as articular disc thickening, destruction, and disarrangement of collagen fibers after three weeks of CFA-induced TMJ arthritis.[13, 23, 24]

Table 4: One way ANOVA followed by post-hoc Tukey test for pairwise comparison of mean cartilage thickness (µm) between different intervention groups for each time point, and T-test for comparison between the two time points for each group

	Control	Arthritis	Laser	P value	F
1 week	151.89±10.48	72.35±18.19	90.17±9.6	<0.0001*	58.84
Post-hoc		P1< 0.0001*	P1< 0.0001* P2= 0.0841		
3 weeks	163.65±21.43	51.89±16.14	112.51±17.79	<0.0001*	54.57
Post-hoc		P1< 0.0001*	P1= 0.0007* P2=0.0001*		
P value between 1&3 weeks by T-test	0.2534	0.0664	0.0221*		

P: Probability, *: significance <0.05,
P1: significance vs control,
P2: significance vs arthritis.

In a similar study, Memis et al. [25] observed irregular condylar surface, chondrocyte clusters, and thickened articular disc in mono-iodoacetate (MIA) induced osteoarthritis in rabbit TMJs. Moreover, our findings revealed atrophy of the proliferative zone of the

condyle which agrees with Ishizuka et al. [26] who reported a reduction in the superficial chondroprogenitor cell number in osteoarthritic mice models.

In the LLLT-treated group in the current study, histological deformity of the joint observed in the arthritis group was much alleviated with a significant reduction of the articular disc thickness as well as improved thickness and arrangement of condylar cartilage and subchondral bone. In agreement with our results, in a study that used LLLT (GaAlAs, 830-nm,

30mW, 3 J/cm^2) in induced arthritis of rat TMJs, less inflammatory cell infiltrate, enhanced TMJ histological and morphological picture, as well as better arrangement and compaction of the collagen fibers, were detected in the treated group.[23] Similarly, in an experimental histological study conducted by Alves et al.,[27] LLLT (780 nm, 22 mW, 7.7 J/cm^2) was able to modulate both early and late inflammatory progressive reactions of RA.

Lemos et al.[28] used GaAlAs LLLT at parameters of 830-nm, 30mW, and 5 J/cm^2 and reported a reduction of the proinflammatory cytokines; tumor necrosis factor (TNF-α), and interleukin (IL-1β) as well as more arrangement of the articular disc collagen fibers and increased proteoglycan content in the condylar cartilage. Other studies applied higher laser doses of 74 J/cm^2, 171 J/cm^2,[29] 51.02 J/cm^2,[30] 112 J/cm^2, 120 J/cm^2, and 225 J/cm^2 [31] and recorded positive results as well.

In our study, tissue specimens of the LLLT group after three weeks showed better histological regeneration than after one week. Similarly, in a CFA-induced rat TMJ arthritis model, LLLT (880nm, 100mW, 10 min, 0.8mm tip diameter) showed an insignificant difference between the control arthritic and LLLT group after three days, while after 7 days, significant change in the grading of cartilage defects, arthritis, number of cell layers and inflammatory cells were recorded.[8]

In the same context, Khozeimeh et al. [30] detected a significant reduction in tissue inflammation of LLLT-treated arthritic TMJs (810nm, 0.2 W, 51.02 J/cm^2) in the late phase more than the early phase of the experiment. Other studies suggested that LLLT for osteoarthritis should be continued for at least three weeks.[32] In contrast, clinical improvement of TMD symptoms was reported by patients [33] and proved in experimental animals [29] immediately after application. This may indicate that the biomodulatory effects of LLLT may require some time to develop histological enhancement. In agreement with the trichrome staining results of the arthritic group in this study, Ishizuka et al.[26] detected lower collagen levels and hypocellularity in osteoarthritic mice. On the other hand, in the laser-treated group, LLLT promoted collagen arrangement and condylar zone regeneration, which agrees with Al-Dubai et al.[34] who detected collagen and cartilage rearrangement after LLLT of arthritic guinea pig TMJs

According to the statistical analysis in the present study, the articular disc showed fibrosis and increased thickness in the arthritis group, while the condylar cartilage showed significant atrophy. However, LLLT significantly enhanced the healing and rearrangement of both the articular disc and cartilage when compared to the arthritic group. Similarly, in a study by Lemos et al.,[23] significant decrease in articular disc thickness anteriorly, posteriorly, and in the middle was reported after LLLT of rat arthritic TMJs.

On the other hand, in another study after LLLT, a significant decrease in the thickness of the middle part of the articular disc was detected; however, the articular cartilage thickness did not show a significant difference.[28] This may be attributed to the lower laser wavelength, power, and density implicated in that study when compared to the current study.

In comparison to the sham control group in the present study, the LLLT group did not show a significant difference in articular disc thickness after one or three weeks which affirmed the positive effect of laser treatment minimizing the fibrotic reaction associated with joint arthritis and restoring the disc to its normal dimensions. However, after three weeks, the condylar cartilage thickness of the LLLT group was not restored to the normal average dimensions of the control group. This may indicate that cartilage may require more time for complete regeneration and arrangement, whereas Huey et al.[35] reported that cartilage tissue has limited regeneration potential.

Corroborating our results in joints other than the TMJ, Alves et al. 2014 [36] demonstrated the significant ability of LLLT to enhance tissue repair and increase collagen fibers' organization in rats with induced arthritis in the knee joint. Moreover, the efficacy of LLLT in patients with RA suffering from carpal tunnel syndrome was evaluated by Ekim et al.[37] who reported a beneficial effect of laser therapy for pain relief and enhancing hand movement suggesting LLLT as a good therapy for RA patients with carpal
tunnel syndrome.
However, the study conducted by Emshoff et al.,[38] daCunha et al.[18], and Memis et al.[25] reported no statistically significant difference between LLLT and placebo groups. Another study conducted by Madani et el.[39] also reported that LLLT was not more effective than the placebo in pain reduction or mouth-opening improvement in TMJ osteoarthritis patients. Bjordal et al.[40] explained the variation in the results of LLLT

studies to the usage of different laser doses. The type of irradiation, wavelength, and the different parameters of exposure may affect the efficacy of laser treatment which could explain variation in results between different studies.

Some of the limitations of this study were a limited follow-up period of three weeks and the application of

only one laser protocol for treatment. Comparing different irradiation parameters could help establish the most effective protocols. In addition, more underlying cellular processes need to be investigated to further understand the mechanism of the therapeutic effect of LLLT. These limitations should be overcome in upcoming studies.

Conclusions:

LLLT showed promising results in the treatment of TMJ arthritis; however, more time or different laser parameters may be required to achieve complete tissue re-arrangement after injury, so further research is recommended to evaluate the efficacy of LLLT under various conditions and times and to establish the optimum parameters for different clinical applications.

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