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Morphometric aspects of the articular cartilage of rats treated with low-level laser therapy and exercise in a rheumatoid arthritis model

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ABSTRACT

Introduction: Rheumatoid arthritis (RA) is classified as an autoimmune, chronic disease affecting diarthrodial joints and periarticular structures. **Objective:** To evaluate whether low-intensity laser treatment (LLLT) and/or exercise reduce the deleterious effects of tissue in a rheumatoid arthritis model. **Methods:** 128 rats were divided into two inflammatory periods: acute (7 days) and chronic (28 days) and subdivided into control, injury and treatment. The protocol with Freund's Complete Adjuvant was used in two inoculations, one intradermal and one intraarticular in the tibiofemoral joint, the control animals received saline solution. For treatment, LLLT 660 nm, 5 J/cm² was used in the sensitized joint and climbing exercise in stairways with an overload of 100 grams. After the experimental period, the animals were euthanized and the joints were prepared for morphometric analysis of the total thickness, superficial, deep, and cellular density of the articular cartilage. Generalized Linear Models with Sidak post-test were chosen. **Results:** The control group was found to be different from the lesion group with greater joint cartilage thickness, and the animals treated with exercise alone increased the joint cartilage compared to the control group. **Conclusion:** The animals treated with laser association and exercise showed improvement in the morphometric aspects of the articular cartilage.

Keywords: cartilage; exercise; laser therapy; rheumatoid arthritis.

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INTRODUCTION

Rheumatoid arthritis (RA) is classified as an autoimmune, chronic disease affecting diarthrodial joints and periarticular structures[1,2]. The progression of structural damage in the RA is associated with joint deformity and cartilage destruction, which culminates in physical disability and deficits in the quality of life of affected individuals[3]. Tissue changes are related to cell metabolism. Synovial fibroblasts respond markedly to

cellular invasion associated with increased chondrocyte catabolism and synovial osteoclastogenesis, destroying joint tissue[4].

Pharmacological treatment is indicated for RA remission; however, it becomes a pertinent control of disease activity, prevention and control of joint damage, loss of function, and reduction of symptoms such as pain and joint stiffness[2]. Among the modalities that can be used, low-level laser therapy (LLLT) presents beneficial effects in terms of the relief of the stiffness and pain symptoms, besides the modulation of the inflammatory profile[5-7].

Another therapeutic modality is physical exercise, used for the treatment and prevention of joint degeneration, and reduction of the inflammatory process, due to the modulation action of joint cartilage proteolysis[8-10]. The relationship between exercise and cartilage results from the mechanism of mechanocellular transduction, and the discharge of weight responds to chondrocytes, as well as an increase in proteoglycans after exercise[11].

Neves et al. inferred that the association of LLLT and exercise modulates the inflammatory process, decreasing cell infiltration in synovial fluid and promoting the improvement of peripheral function in an experimental RA model[6]. But little is known about quantitative changes in joint cartilage and whether treatments used alone or in association modulate the degenerative joint aspects of the disease.

Given the above, the objective of the study is to evaluate whether treatment with low-level laser therapy and/or exercise reduces the deleterious effects of tissue in a model of Freund Complete Adjuvant induced rheumatoid arthritis in rats.

METHODS

This was a randomized experimental study of 128 male Wistar rats, 15 weeks old, weighing 250±19 g. The study was approved by the Ethics Committee on Animal Use of the State University of Western Paraná.

Animals

The animals were randomly randomized into two inflammatory periods: acute, evaluated at 7 days, and chronic at 28 days. Each group with 64 animals in each, is subdivided into 8 groups: GC: control group, LG: lesion group, CLaG: laser control group, CEG: exercise control group, CLaEG: laser control group, and exercise, LEG: exercise lesion group, LLaG: laser lesion group and LLaEG: laser lesion and exercise group.

Experimental rheumatoid arthritis induction model

As a protocol for rheumatoid arthritis induction in an experimental model as described by[6]. For the experiment, the animals of the injury group were submitted to two inoculations of a substance called Freund Complete Adjuvant (FCA) containing Mycobacterium butyricum (0.5 mg/ml, Difco), and the animals of the control groups were injected with two isotonic sodium chloride solutions (0.9%, Aster) so that they were exposed to the same physical stress as the needle insertion.

Initially, the animals of LG, LEG, LLaG, and LLaEG were inoculated by intradermal injection at the base of the tail containing 50 µl of FCA, and the animals of CG, CLaG, CEG, and CLaEG the isotonic solution. The administration area of the substance was trichotomized with the aid of a 1 ml syringe and a 13x4.5 mm needle. The needle was inserted approximately 1 cm into the base of the tail subcutaneously. The first inoculation was considered the systemic contact of the animal with the bacteria.

The second inoculation was performed in the tibiofemoral joint of the right pelvic limb seven days after the intradermal injection. The same concentrations and solutions of the intradermal injection were used in this region. The animals were manually contained, the anterior area of the knee was trichotomized, and with a 1 ml syringe and 13x4.5 mm needle the application was conducted[6].

Treatment protocol

For the treatment of CEG, CLaEG, LEG, and LLaEG animals, the animals were submitted to climbing resistance exercises on stairs. A vertical wooden staircase was used, with 67 iron steps, 1.18 m high, 20.5 cm wide, and 60º inclination. At the top of the staircase, a box, 20x20 cm high and wide, was positioned to rest the animals between the series, with an interval of 60 seconds[12].

The treatment protocol for the animals in the acute group was 4 series of 5 climbs on the ladder, with an overload of 100 grams coupled to the tail, starting 24 hours after intra-articular application for a total of 4 days of treatment. The chronic group was initially submitted to 4 series of 5 climbs, with the same overload, with increases in the second week of treatment to 4 series of 7 climbs, and the last week, to 4 series of 10 climbs totaling 14 days of treatment.

The animals of the group's CLaG, CLaEG, LLaG, and LLaEG, were submitted to treatment with LLLT in the knee region of the sensitized right pelvic limb. The points for application were: anterior patella, medial face at the tibiofemoral joint, lateral face at the tibiofibular joint, and posterior at the popliteal region. The equipment was gauged for its power, and the parameters of use were point technique, the wavelength of 660 nm, power of 30 mW, spot area: 0.06 cm^2, energy density: 5 J/cm^2 per point, time per point: 10 seconds, total energy per point: 0.003 J. The animals of the acute inflammatory period underwent 4 applications and chronic 14 applications.

Morphometric analysis

The animals of the acute inflammatory period, 7 days after intra-articular injection, and the chronic group 28 days, were

submitted to euthanasia, previously anesthetized with an intraperitoneal injection of ketamine hydrochloride (Ketalar, Brazil) (95 mg/kg) and xylazine (Xilazin, Brazil) (12 mg/kg). The animal's state of consciousness was verified (observed by the absence of motor response to tail clamping and interdigital folds), and the right knee joints (pelvic limb contralateral to that sensitized to intra-articular injection) were dissected, reduced in the transverse section of tibia and femur and fixed in Metacarn (70% Methanol, 20% Chloroform, 10% glacial acetic acid) for 48 hours, after which the pieces were fixed in 70% alcohol for 15 days.

The joints were washed for 24 hours in running water and the material was decalcified in 5% trichloroacetic acid for 7 days, followed by a routine histological procedure for inclusion in paraffin. After embrocation, the material was sectioned in the sagittal plane in Olympus CUT 4055 microtome, 7 µm thick, and mounted on glass slides. For staining, a hematoxylin and eosin protocol was used. The slides were analyzed under a light microscope and photomicrographed under Olympus DP71 (USA).

Morphometry obtained photomicrographs of the cartilage of the femoral and tibial joint of the right pelvic limb. Three images were analyzed in standard points for the femur and tibia: P1 (anterior region of the joint cartilage near the patella), P2 (midpoint between anterior and posterior), and P3 (a posterior region near the popliteal fossa). The images were obtained at 40x magnification and analyzed using the Image-Pro Plus 6.0 calibrated program for articular cartilage measurements. The morphometric parameters verified in Figure 1 were analyzed in the following regions: the total thickness of cartilage obtained from the surface to the subchondral bone at the midpoint of the image, surface area that corresponds to the thickness between the surface and the tidal mark, and the deep area, the thickness obtained from the tidal mark to the subchondral bone. In the same image, the number of chondrocytes was checked using a 300x300 µm standardization

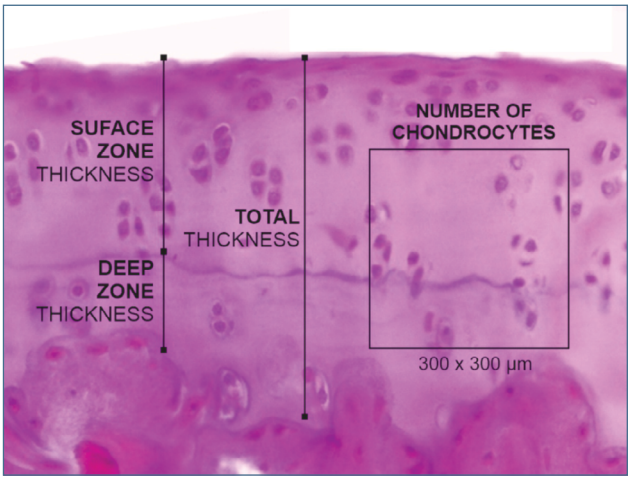

Figure 1: Analysis of articular cartilage. Illustration of measurements performed on the knee cartilage of Wistar rats.

for the cell count in the articular cartilage at each point mentioned above. In addition to the individual thicknesses, the mean P1-P3 was checked.

Statistical analysis

The data were analyzed using the statistical package SPSS 2.0, for morphometric analysis of total thickness, surface area, deep, area and number of chondrocytes, and $\overline{X}$ P1-P3 of the cited variables, opting for the Generalized Linear Models (GLM) to determine the difference between the means obtained from each group evaluated comparing them. And after Sidak test for comparison between these means. In all cases, the accepted significance level was $p<0.05$. The sample size calculation was performed in the program G*Power 3.1.9.7, with effect size 0.5; alpha -0.05; power 0.96; 16 groups, and a sample size of 128.

RESULTS

In the morphometric analysis of the total thickness of the joint cartilage in the acute inflammatory period, it was found that there was no significant difference between the groups in P1, P2, and P3 in the femur and tibia. In the $\overline{X}$ P1-P3 in the femur, there was a significant difference between LG and all groups denoting lower thickness, still, LEG, LLaG, and LLaEG resembled the GC with higher total cartilage thickness.

In the thickness of the joint cartilage surface area in the acute inflammatory period, there was no significant difference for femoral bone and tibia in P1, P2, and P3, yet, there was a significant difference in $\overline{X}$ P1-P3, in the femur, CG was different from LG with higher surface area thickness. At $\overline{X}$ P1-P3 in the tibia, LEG was different from CLaG, CEG, and CLaEG with higher surface zone thickness. Furthermore, the animals of LLaG and LLaEG showed statistical similarity with GC (Table 1).

In the analysis of the total thickness of the cartilage in the chronic inflammatory period, in P1, tibia and femur showed a difference between GC and LG, and LEG, LLaG, and LLaEG resembled GC with greater thickness of joint cartilage. In the P2 femur, GC showed a significant difference when compared to LG, and statistical similarity between LEG and LLaEG and GC was observed both in the femur and tibia. In the P3 region, LG showed less cartilage thickness when compared to LEG. At $\overline{X}$ P1-P3 in femur and tibia, LG was different from all groups with thinner joint cartilage (Table 2).

In the analysis of the thickness in the deep area of the joint cartilage, there was no significant difference in P1, P2, and P3 in the femur and tibia. When analyzed at $\overline{X}$ P1-P3, it was observed that the femur LEG was different from CG, LG, CLaG, CEG, and CLaEG with lower thickness in the deep zone.

The total number of chondrocytes showed no significant difference in P1, P2, and P3 in the femur and tibia. At $\overline{X}$ P1-P3, there

was a significant difference for the femur, CG was different from LG with a higher number of cells, yet LEG, LLaG, and LLaEG were similar to CG (Table 3).

In the thickness of the surface area of the articular cartilage, in P1 in the femur, LG was different from LEG, with less thickness of the surface area. In the tibia, LG was different from all other groups with lower thickness, and the groups undergoing isolated or combined therapy resembled CG. In P3, femur, and tibia, LEG was different from LG and similar to CG with a thicker surface area. Furthermore, LLaG in the tibia was different from LG and similar to CG. In $\overline{X}$ P1-P3, the femur LEG was different from LG and similar to CG. In the tibia, CG was different from LG and similar to the other groups with higher cartilage thickness (Table 4).

In the analysis of thickness in the deep zone, in P1 of LG femur, it was different from LEG with lower thickness. In the tibia CG showed a significant difference compared to LG, still, LLaG and LLaEG showed statistical similarity with CG with higher cartilage thickness. In P2, femur, LG showed a significant difference when compared to CLaG, in the tibia, CG was different from LG, still, LEG and LLaEG were similar to CG. In $\overline{X}$ P1-P3 in CG femurs showed a significant difference when compared to CLaG with lower thickness in the deep cartilage zone (Table 4).

Table 1: Morphometric data of the total thickness, surface area, Deep zone, and chondrocyte count of the joint cartilage in the femur, acute inflammatory period.

Thickness	Femur P1	Femur P2	Femur P3	Femur $\overline{X}$ P1-P3
CG	167±17	166±23	144±21	149±6 ^{A}
LG	158±39	141±12	129±18	123±7 ^{B}
CLaG	132±7	148±26	139±18	140±5 ^{A}
CEG	156±15	147±20	135±18	146±6 ^{A}
CLaEG	143±18	140±18	140±18	141±5 ^{A}
LEG	125±23	152±16	169±12	148±6 ^{A}
LLaG	150±16	136±6	134±19	140±5 ^{A}
LLaEG	150±20	148±13	140±18	146±6 ^{A}
Surface Área	**Femur P1**	**Femur P2**	**Femur P3**	**Femur $\overline{X}$ P1-P3**
CG	86±15	85±11	72±12	81±7 ^{AC}
LG	60±44	50±15	51±13	59±10 ^{B}
CLaG	61±9	63±11	59±36	61±5 ^{A}
CEG	65±14	65±13	65±2	65±5 ^{A}
CLaEG	61±10	63±36	59±22	61±5 ^{A}
LEG	78±11	96±3	118±10	97±8 ^{BC}
LLaG	76±31	59±13	67±30	67±5 ^{A}
LLaEG	85±32	63±8	67±22	72±6 ^{A}
Deep Zone	**Femur P1**	**Femur P2**	**Femur P3**	**Femur $\overline{X}$ P1-P3**
CG	74±16	80±11	52±14	68±4 ^{A}
LG	60±13	60±10	59±14	60±3 ^{A}
CLaG	53±11	63±15	53±8	56±3 ^{A}
CEG	62±10	66±14	63±5	64±4 ^{A}
CLaEG	64±4	55±8	50±11	56±3 ^{A}
LEG	42±11	52±6	50±9	48±3 ^{B}
LLaG	56±12	61±10	47±19	55±3 ^{AB}
LLaEG	50±4	67±9	58±6	58±3 ^{AB}
Chondrocyte Count	**Femur P1**	**Femur P2**	**Femur P3**	**Femur $\overline{X}$ P1-P3**
CG	35±3	44±14	35±7	38±2 ^{AC}
LG	37±7	40±9	37±5	31±3 ^{BD}
CLaG	35±4	36±6	28±5	33±2 ^{AC}
CEG	55±7	48±5	31±2	45±3 ^{CD}
CLaEG	41±9	34±5	36±7	37±2 ^{AC}
LEG	36±3	32±2	40±10	36±2 ^{AC}
LLaG	30±3	36±9	37±19	35±2 ^{AC}
LLaEG	34±5	36±9	34±8	35±2 ^{AC}

P: dot, X: mean. Data expressed as mean and standard deviation. Similar letters show similarity within the group. Morphometric analysis of femoral in three areas (P1, P2, and P3), and $\overline{X}$ P1-P3. A significant difference between the groups in femur $\overline{X}$ P1-P3 ($p<0.001$).

The total number of chondrocytes in P1 femur CG was different from LG with a higher chondrocyte number, still, LEG and LLaEG were different from LG and similar to CG. In P2 femur LG was different from LLaEG with a lower chondrocyte amount. In $\overline{X}$ P1-P3 for the femur, CG was different from LG with a higher cell number and similar to LEG, LLaG, and LLaEG (Table 4).

DISCUSSION

In the present study, it was found that the experimental model of CFA-induced rheumatoid arthritis alters the thickness and cell density of the articular cartilage of the femur and tibia of Wistar rats. The treatment modalities adopted in this study were effective in restoring the morphometric aspects analyzed.

Induction of rheumatoid arthritis in animals simulates the inflammatory signs of the disease in humans, including joint edema, cell infiltration, hypersensitivity, and histopathological changes[13]. In this study, it is noted that the animals of LG showed morphometric changes with lower cartilage thickness and cell density when compared to CG.

In the progression of inflammatory disease, cytokines present in the synovial fluid migrate to the cartilaginous tissue, promoting changes and adjacent tissue degradation[13]. Increased vascular

Table 2: Morphometric data of the total thickness, surface area, Deep zone, and chondrocyte count of the joint cartilage in the femur, chronic inflammatory period.

Thickness	Femur P1	Femur P2	Femur P3	Femur $\overline{X}$ P1-P3
CG	137±21 ^{A}	140±15 ^{A}	138±27	145±5 ^{A}
LG	113±9 ^{B}	125±16 ^{B}	136±23	115±4 ^{B}
CLaG	145±18 ^{A}	150±17 ^{A}	139±21	148±6 ^{A}
CEG	139±15 ^{A}	145±26 ^{A}	144±19	145±5 ^{A}
CLaEG	145±12 ^{A}	131±27 ^{AB}	133±17	143±5 ^{A}
LEG	141±29 ^{A}	149±6 ^{A}	146±3	148±6 ^{A}
LLaG	144±22 ^{A}	143±17 ^{AB}	136±26	136±5 ^{A}
LLaEG	146±23 ^{A}	137±3 ^{A}	134±11	142±5 ^{A}
Surface Area	**Femur P1**	**Femur P2**	**Femur P3**	**Femur $\overline{X}$ P1-P3**
CG	69±13 ^{ABCD}	63±14 ^{AB}	61±16 ^{AB}	68±4 ^{ACD}
LG	59±15 ^{D}	59±19 ^{AB}	48±9 ^{B}	51±3 ^{BC}
CLaG	73±13 ^{BC}	77±21 ^{AB}	66±9 ^{AB}	77±5 ^{ACD}
CEG	57±15 ^{ABCD}	65±9 ^{A}	63±7 ^{AB}	62±4 ^{C}
CLaEG	71±15 ^{ABD}	64±10 ^{A}	60±7 ^{AB}	64±4 ^{AC}
LEG	84±17 ^{C}	90±13 ^{AB}	90±10 ^{A}	88±5 ^{D}
LLaG	73±5 ^{ABCD}	88±7 ^{B}	80±36 ^{AB}	82±5 ^{AD}
LLaEG	80±11 ^{ABCD}	61±10 ^{A}	56±12 ^{AB}	73±4 ^{ACD}
Deep zone	**Femur P1**	**Femur P2**	**Femur P3**	**Femur $\overline{X}$ P1-P3**
CG	46±6 ^{AB}	63±7 ^{AC}	55±12	57±4 ^{AB}
LG	41±4 ^{B}	54±10 ^{C}	50±13	46±4 ^{A}
CLaG	53±6 ^{AB}	69±14 ^{A}	64±10	65±4 ^{B}
CEG	49±11 ^{AB}	63±9 ^{ABC}	57±15	55±4 ^{AB}
CLaEG	54±13 ^{AB}	57±13 ^{ABC}	49±6	60±4 ^{AB}
LEG	56±12 ^{A}	60±4 ^{ABC}	49±4	56±4 ^{AB}
LLaG	49±12 ^{AB}	48±3 ^{BC}	50±16	48±4 ^{AB}
LLaEG	47±10 ^{AB}	59±3 ^{ABC}	43±6	51±4 ^{AB}
Chondrocyte Count	**Femur P1**	**Femur P2**	**Femur P3**	**Femur $\overline{X}$ P1-P3**
CG	34±6 ^{A}	34±3 ^{AB}	30±6 ^{A}	34±2 ^{AD}
LG	24±6 ^{B}	30±5 ^{B}	30±8 ^{A}	28±2 ^{B}
CLaG	37±4 ^{A}	35±6 ^{AB}	32±13 ^{A}	35±2 ^{AD}
CEG	38±5 ^{A}	40±7 ^{AB}	40±2 ^{A}	34±2 ^{ABD}
CLaEG	31±8 ^{A}	34±11 ^{AB}	33±7 ^{B}	38±3 ^{AD}
LEG	37±7 ^{A}	34±1 ^{AB}	39±7 ^{CD}	38±3 ^{AD}
LLaG	29±4 ^{AB}	32±7 ^{AB}	34±3 ^{AD}	31±2 ^{AB}
LLaEG	46±10 ^{A}	43±4 ^{A}	33±4 ^{AD}	38±3 ^{D}

Data expressed as mean and standard deviation. Similar letters show similarity within the group. Morphometric analysis of femoral bone in three areas (P1, P2, and P3), and $\overline{X}$ P1-P3. Significant difference between the groups in femur P1 (p=0.002), P2 and $\overline{X}$ P1-P3 (p<0.001), tibia P1 (p<0.001), P2 (p=0.02), P3 and $\overline{X}$ P1-P3 (p<0.001).

permeability and influx of immune system cells, including mononucleated cells, favoring tissue damage[14].

Smith postulates that LLLT is effective in stimulating tissue repair, analgesia, and reducing vascular permeability, and its mechanism of action is related to the interaction of the radiation emitted by the equipment with cytochrome c oxidase, located in the mitochondria, resulting in increased cell metabolism[15]. Also, in the present study, the LLLT applied individually or associated with exercise favored tissue repair, with an increase in total thickness and cell density in animals of the acute and chronic inflammatory period, already for the analysis of the superficial and deep zone, it was verified that the LLaG presented better effects in the increase of the superficial and deep zone thickness in the chronic group.

LLLT has anti-inflammatory and analgesic effects, influencing tissue maintenance, thus favoring repair. The wavelength of 660 nm promotes resorption on the joint surface and maintenance of the capsule, in addition, stimulates the metabolism of collagen[16]. To Trawitzki et al.[17], the 660 nm wavelength laser reduces clinical signs of joint discomfort and inflammation. Beneficial results are also found in the expression of vascular endothelial growth factors in animals[18]. In addition, LLLT has differentiated effects on tissues according to use parameters such as dose, wavelength, continuous, and pulsed mode, in addition to the area of application[19].

Table 3: Morphometric data of the total thickness, surface area, Deep zone, and chondrocyte count of the joint cartilage in the tibia, acute inflammatory period.

Thickness	Tibia P1	Tibia P2	Tibia P3	Tibia $\bar{X}$ P1-P3
CG	147±16	150±15	145±24	148±6
LG	146±24	147±12	160±16	147±6
CLaG	144±13	142±25	132±9	140±6
CEG	145±16	147±22	149±2	148±6
CLaEG	141±15	144±9	140±2	142±6
LEG	158±28	154±14	156±7	156±6
LLaG	145±10	156±1	134±16	145±6
LLaEG	139±11	154±6	130±4	140±6
Surface Área	**Tibia P1**	**Tibia P2**	**Tibia P3**	**Tibia $\bar{X}$ P1-P3**
CG	82±4	80±15	73±34	80±6 ^{ABCD}
LG	101±30	68±13	98±15	88±7 ^{ABD}
CLaG	59±14	59±24	57±15	59±5 ^{C}
CEG	66±15	66±30	78±1	68±5 ^{BC}
CLaEG	71±13	64±15	63±21	66±5 ^{ABC}
LEG	97±31	104±6	105±13	102±8 ^{D}
LLaG	88±13	71±13	67±9	73±6 ^{ABCD}
LLaEG	90±11	76±16	69±18	72±6 ^{ABCD}
Deep Zone	**Tibia P1**	**Tibia P2**	**Tibia P3**	**Tibia $\bar{X}$ P1-P3**
CG	51±4	71±11	62±10	64±5
LG	60±10	51±9	59±14	51±5
CLaG	50±11	65±11	57±6	58±4
CEG	56±4	62±14	64±6	62±4
CLaEG	55±13	64±4	57±6	59±4
LEG	56±11	52±9	47±3	55±4
LLaG	58±11	67±7	53±8	56±4
LLaEG	61±7	71±3	55±10	62±4
Chondrocyte Count	**Tibia P1**	**Tibia P2**	**Tibia P3**	**Tibia $\bar{X}$ P1-P3**
CG	36±4	38±6	32±4	39±2 ^{ABDE}
LG	44±17	44±10	53±5	37±3 ^{B}
CLaG	35±5	32±5	31±13	33±2 ^{ADE}
CEG	36±6	44±5	42±1	42±3 ^{ABDE}
CLaEG	40±10	36±13	28±5	36±2 ^{ABDE}
LEG	37±7	33±8	31±2	34±2 ^{DE}
LLaG	41±12	39±5	31±6	37±2 ^{ABDE}
LLaEG	36±6	39±3	31±6	34±2 ^{E}

Data expressed as mean and standard deviation. Similar letters show similarity within the group. Morphometric analysis of tibia in three areas (P1, P2, and P3), and $\bar{X}$ P1-P3. A significant difference between the groups in femur $\bar{X}$ P1-P3 (p=0.001).

In this study, it is noted that the continuous wavelength of 660 nm applied to the knee joint was effective in promoting tissue remodeling.

An increase in joint cartilage thickness was observed in the LEG about the CG in the acute and chronic inflammatory period. According to Roos & Dahlberg[20], the relationship between exercise and cartilage thickness is the result of the mechanocellular transduction mechanism, and chondrocytes consequently respond to weight discharge by increasing the proteoglycan content after exercise. In addition, it was found that the cell density of LEG and LLaEG animals was higher when compared to LG.

Physical exercise is one of the main tools for joint maintenance in individuals with RA[21] proved that exercise, aerobic or muscle strength, shows significant improvement in micro and macrovascular endothelial function in patients with RA. The practice of physical exercise helps in the maintenance of movement, thus promoting a reduction in joint stiffness due to improved intra-articular flow through the synovial fluid, stimulating the nutrition of cartilaginous tissue and regeneration of this structure[11].

Exercise therapy is recommended for RA patients as a reeducation of life habits, in addition, LLLT is recommended as an association in the modulation of inflammatory events[22]. The association of LLLT and exercise has positive effects on the modulation of

Table 4: Morphometric data of the total thickness, surface area, Deep zone, and chondrocyte count of the joint cartilage in the tibia, chronic inflammatory period.

Thickness	Tibia P1	Tibia P2	Tibia P3	Tibia $\bar{X}$ P1-P3
CG	150±13 ^{A}	148±12 ^{A}	130±16 ^{AB}	143±4 ^{A}
LG	116±6 ^{B}	130±9 ^{BC}	110±9 ^{B}	116±3 ^{B}
CLaG	143±10 ^{A}	137±15 ^{AC}	134±6 ^{A}	137±4 ^{A}
CEG	148±11 ^{A}	149±9 ^{A}	145±1 ^{A}	148±4 ^{A}
CLaEG	140±10 ^{A}	144±7 ^{A}	134±18 ^{A}	138±4 ^{A}
LEG	143±14 ^{A}	145±2 ^{A}	137±3 ^{A}	141±4 ^{A}
LLaG	144±9 ^{A}	145±24 ^{A}	129±11 ^{AB}	139±4 ^{A}
LLaEG	147±9 ^{A}	132±3 ^{B}	125±7 ^{AB}	130±3 ^{AB}
Surface Area	**Tibia P1**	**Tibia P2**	**Tibia P3**	**Tibia $\bar{X}$ P1-P3**
CG	76±13 ^{A}	72±13	69±11 ^{A}	72±3 ^{A}
LG	50±7 ^{B}	56±13	48±7 ^{BC}	52±2 ^{B}
CLaG	72±14 ^{A}	56±11	57±6 ^{AC}	58±3 ^{AB}
CEG	82±11 ^{A}	64±7	63±1 ^{A}	68±3 ^{A}
CLaEG	79±11 ^{A}	67±6	63±14 ^{AC}	72±3 ^{A}
LEG	64±6 ^{A}	76±1	69±8 ^{A}	71±3 ^{A}
LLaG	67±5 ^{A}	65±19	67±8 ^{A}	66±3 ^{A}
LLaEG	67±5 ^{A}	64±8	56±8 ^{AC}	59±3 ^{AB}
Deep zone	**Tibia P1**	**Tibia P2**	**Tibia P3**	**Tibia $\bar{X}$ P1-P3**
CG	55±11 ^{A}	71±4 ^{A}	55±12 ^{A}	58±3 ^{A}
LG	40±4 ^{B}	48±11 ^{B}	40±4 ^{B}	41±2 ^{B}
CLaG	52±6 ^{AB}	48±12 ^{BC}	62±12 ^{A}	57±3 ^{A}
CEG	63±11 ^{A}	64±5 ^{A}	61±1 ^{A}	62±3 ^{A}
CLaEG	62±7 ^{A}	54±7 ^{B}	61±8 ^{A}	59±3 ^{A}
LEG	49±7 ^{AB}	64±1 ^{A}	56±6 ^{A}	54±3 ^{A}
LLaG	54±9 ^{A}	63±11 ^{AC}	57±3 ^{A}	58±3 ^{A}
LLaEG	62±11 ^{A}	57±6 ^{A}	61±8 ^{A}	62±3 ^{A}
Chondrocyte count	**Tibia P1**	**Tibia P2**	**Tibia P3**	**Tibia $\bar{X}$ P1-P3**
CG	37±3 ^{AB}	39±8	31±7 ^{A}	36±2 ^{AC}
LG	33±13 ^{A}	31±10	28±4 ^{A}	29±1 ^{B}
CLaG	36±4 ^{AB}	31±6	30±2 ^{A}	33±2 ^{AB}
CEG	47±9 ^{B}	36±5	42±1 ^{B}	41±2 ^{C}
CLaEG	36±6 ^{AB}	32±4	33±2 ^{AB}	33±2 ^{AB}
LEG	34±5 ^{A}	36±2	32±5 ^{AB}	35±2 ^{ABC}
LLaG	38±4 ^{AB}	40±2	33±2 ^{AB}	36±2 ^{AC}
LLaEG	42±2 ^{AB}	35±5	30±3 ^{A}	35±2 ^{ABC}

Data expressed as mean and standard deviation. Similar letters show similarity within the group. Morphometric analysis of tibia in three areas (P1, P2, and P3), and $\bar{X}$ P1-P3. A significant difference between the groups in femur and tibia $\bar{X}$ P1-P3 ($p<0.001$).

the inflammatory process, reduction in leukocyte migration, and maintenance of functionality[7]. In the present study, it was found that the association of therapies resulted in an improvement in the morphometric parameters analyzed, stimulating the recovery of joint cartilage and cell density.

The present study verified the morphometric aspects of joint cartilage in an experimental rheumatoid arthritis model of rats treated with LLLT, and stair climbing exercise and the association of both results suggest that the combination of therapies has better effects on joint cartilage remodeling. The study is limited to evaluating the effects of RA in male rats. In addition, it is suggested that new studies evaluate exercise variables such as intensity, dose, and frequency so that effective protocols can be established.

It is concluded that LLLT treatment, exercise, and the association of therapies reduce the deleterious effects of tissue in a CFA-induced rheumatoid arthritis model.

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