# Effects of photobiomodulation in pain and articular degeneration in mice arthritis model

*Efeitos da fotobiomodulação na dor e degeneração articular em modelo de artrite em camundongos*

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## ABSTRACT

**BACKGROUND AND OBJECTIVES**: Rheumatoid arthritis is a set of symmetrical inflammatory processes that affects the joints. Currently, drug therapy is the first choice for the treatment of the disease, however it can cause serious side effects. In this context, photobiomodulation appears as a potential resource for treatment. Therefore, the aim of the study was to analyze the effects of low intensity laser treatment on pain and joint degeneration in the arthritis model in mice.

**METHODS**: 24 mice were randomly divided into 4 groups with 6 animals each: SG, ZG, LG and DG. Zymosan was used to induce arthritis. The hyperalgesia was measured using the Von frey test. For the histological analysis, talocrural region samples were collected. Low intensity AsGa Laser equipment was used for the treatment, operating in 904 ηm wavelength, F:1000 Hz, W:50 mW and dose of 1 J/cm$^2$.

**RESULTS**: There was a significant difference ($p<0.05$) in all times of evaluation in hyperalgesia for the SG group compared with the ZG group, presenting an altered pain threshold. In the histological evaluation, the application of the laser (1 J/cm$^2$) to the arthritic joint reduced the analyzed scores (1.20 ± 0.20) and showed a statistical difference when compared to the ZG group (LG=1.20 ± 0.20; ZG=3.80 ± 0.20, $p<0.05$).

**CONCLUSION**: The low intensity laser with the parameters described in the study was effective in reducing pain and promoting a protective effect on cartilage.


**Keywords**: Arthritis, Inflammation, Low-level light therapy.


## RESUMO

**JUSTIFICATIVA E OBJETIVOS:** A artrite reumatoide é um conjunto de processos inflamatórios simétricos que afeta as articulações. Atualmente a terapia farmacológica é a primeira escolha para o tratamento da doença, contudo, pode causar efeitos adversos graves que podem ser evitados com técnicas não farmacológicas, como a fotobiomodulação. O objetivo deste estudo foi analisar os efeitos do tratamento com laser de baixa intensidade na dor e degeneração articular em modelo de artrite em camundongos.

**MÉTODOS**: 24 camundongos foram divididos aleatoriamente em 4 grupos com 6 animais cada: GS, GZ, GL E GD. Para a indução da artrite foi utilizado zymosan. A hiperalgesia foi avaliada utilizando o teste de Von Frey. Para análise histológica, amostras da região talocrural foram coletadas. Para o tratamento foi utilizado o equipamento Laser AsGa de baixa intensidade operando em comprimento de onda de 904 ηm, F: 1000 Hz, W: 50 mW e dose de 1 J/cm$^2$.

**RESULTADOS**: Houve diferença estatística ($p<0,05$) em todos os momentos na avaliação da hiperalgesia para o grupo GS quando comparado com o grupo GZ, apresentando limiar de dor alterado. Na avaliação histológica, a aplicação do laser (1 J/cm$^2$) na articulação com artrite reduziu os escores analisados (1,20 ± 0,20) e apresentou diferença estatística quando comparado ao grupo GZ (GL=1,20 ± 0,20; GZ=3,80±0,20, $p<0,05$).

**CONCLUSÃO**: O laser de baixa intensidade com os parâmetros descritos no estudo foi eficaz, diminuindo o quadro álgico e promovendo efeito protetor na cartilagem.


**Descritores**: Artrite, Inflamação, Terapia com luz de baixa intensidade.

## INTRODUCTION

Rheumatoid arthritis (RA) is a self-immune inflammatory disease that features chronic degeneration of synovial joints. When this condition remains untreated, it causes progressive joint destruction, bone and cartilage loss, functional deficiency and systemic complications[1]. It affects about 1% of the world's adult population and the ratio of the disease development is of three women to every two men. There can be variation among population groups, northern Europe (29 cases/100.000), North America (38 cases/100.000), and southern Europe (16.5 cases/100.000). The occurrence of RA is observed in all ethnic groups, with the first symptoms manifesting between 40 and 60 years of age, with prevalence increasing with age[2,3].

RA therapy has been focused on slowing down the degenerative progression of the disease by administering nonsteroidal anti-

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-inflammatory drugs (NSAIDs), corticosteroids, disease course modifying drugs (DMARDs), immunosuppressive, biological and synthetic drugs, used alone or in combination[4,5]. Although effective in treating RA, these drugs can cause serious adverse effects, such as gastrointestinal ulcers, cardiovascular problems, nephrotoxicity, lung toxicity, and cirrhosis[6,7].

Therefore, it becomes important to identify other treatment strategies with anti-inflammatory effect. In this context, low intensity laser therapy (Light Amplification by Stimulated Emission of Radiation) can be considered a promising non-pharmacological alternative in the treatment of RA[8]. Photobiomodulation is used in the treatment of several musculoskeletal and inflammatory conditions, such as arthritis, tendinopathies, sore throat, low back pain, muscle fatigue, and periodontitis, with the ability to stimulate healing, modulate the inflammatory process, and achieve pain relief effects[8,9].

There is no consensus in the literature about the most effective laser parameters for RA treatment and, therefore, no standardization in the protocols used. The objective of this study was to investigate the effects of low intensity laser on pain and joint degeneration in an experimental model of induced arthritis.

## METHODS

24 male Swiss mice weighing between 25 and 30g were used. They were provided by the Animal Facility of the Federal University of Piauí - Campus Ministro Petrônio Portella. They were housed under controlled temperature of 25 ± 2°C with a 12/12 h light/dark cycle with food ad libitum and water. The tolerable sampling error of 0.25 and a standard deviation in power of 0.4 were considered, thus defining the sample size used according to the formula: n = (1.96 x 0.4/ 0.25) 2 = 6 animals per group.

**Induction of arthritis**

For the induction of arthritis, 180 µg of zymosan (Sigma Chemical Company, St. Louis, MO, USA) was dissolved in 10 µL of sterile saline solution, administered in the region of the talocrural joint (right) of the mice. To promote euthanasia, the 4% isoflurane anesthetic agent was used via inhalation with subsequent cervical dislocation, as previously approved by the animal experiment committee.

**Experimental groups**

Animals were randomly divided into 4 groups:

Saline group (SG, n=6): received saline only, 10 µL of sterile saline (50 µL at 0.9%/joint).

Zymosan group (ZG, n=6): submitted to arthritis induction with injection of 180 µg of zymosan dissolved in 10 µL of sterile saline solution (50 µL at 0.9%/joint) in the right talocrural region and untreated.

Dexamethasone group (DG, n=6): animals that received intraperitoneal dexamethasone (4 mg/kg). Thirty minutes after the dexamethasone induction the animals were submitted to arthritis induction with injection of 180 µg of zymosan dissolved in 10 µL of sterile saline (50 µL at 0.9%/article) in the right talocrural region.

Laser group (LG, n=6): animals submitted to arthritis induction with injection of 180 µg of zymosan dissolved in 10µL of sterile saline solution (50 µL at 0.9%/articulation) in the right talocrural region and treated with the 1 J/cm² laser.

**Laser treatment**

In this study, the low intensity AsGa Laser equipment (Gallium Arsenide, KLD Biosystems Equipments Electronics Ltda., Brazil; Model LLT 0107) was used, operating at a wavelength of 904 ηm for irradiation on the animals' joints. Laser parameters: frequency of 1000 Hz, power of 50 mW, irradiation time of 12 s, irradiated area of 0.01 cm², corresponding to the dose of 1 J/cm². The total energy of the treatment was 0.96 J at the dose of 1 J/cm². The laser optical power was calibrated using a Newport multifunction optical meter (model 1835-C).

**Pain assessment by the Von Frey test**

The Von Frey Digital filament equipment (Insight®) was used to assess hyperalgesia. The test was performed with the animal manually restrained and the filament applied to the talocrural joint of the right hind limb. The polypropylene tip of the filament was applied perpendicularly to the location, with a gradual increase in pressure and, as soon as the animal withdrew the limb, the test was interrupted, and the withdrawal threshold was recorded. The pressure values (in grams) were collected, and the mean and standard deviation of the values were used for analysis. After 1 hour of habituation, 5 evaluations were made, starting from the 4th hour from the moment of zymosan application until the 5th hour, with 15 minutes intervals between evaluations; after the 5th hour all groups were euthanized.

**Histomorphological analysis**

The euthanasia for histological analysis was performed 101 hours after the induction of arthritis, the zymosan-induced inflammation peaked between days 1 and 7. After euthanasia, the tissues from the talocrural region were collected, cut into sections, and fixed in 10% buffered formaldehyde. After dehydration in increasing concentrations of alcohol and immersion in xylol, the specimens were embedded in paraffin. The 6 µm thick sections were stained with hematoxylin and eosin, followed by the mounting of the slides for visualization under a light microscope (NOVA®). The scores used for cartilage evaluation were based on the study by Chen et al.[10], score zero = normal synovium; 1 = hypertrophy of the synovial membrane and cellular infiltrates; 2 = pannus and cartilage erosion; 3 = large cartilage erosion and subchondral bone; and 4 = loss of joint integrity and ankylosis.

All experiments were performed according to the Guide for the Care and Use of Laboratory Animals (National Institute of Health, Bethesda, MD, USA) and were approved by the Research Ethics Committee of the Federal University of Piauí under protocol n° 406/17.

**Statistical analysis**

The results were expressed as mean ± S.E.M. Statistical significance of differences between groups was determined by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test. For histopathological scores, the Kruskal-Wallis test followed by Dunn's test were performed. Differences were considered significant when $p<0.05$. All analyses were performed using GraphPad Prism version 8.

## RESULTS

The administration of zymosan caused a significant reduction (p<0.05) in the animal's pain withdrawal threshold (Figure 1). When groups were compared, none of the groups presented values returning to the baseline, LG when compared to ZG presented a significant increase in the withdrawal threshold in all times of assessment.

There was a significant difference in the values found for SG when compared to ZG, presenting altered pain threshold, and showing the effectiveness of arthritis induction. The DG when compared to LG showed no statistical difference, but when compared to the ZG showed a statistical difference at all moments of the evaluation (Table 1). This can also be seen in Figure 1, in which the ZG showed a significant reduction in pain threshold (p<0.05), while the LG and DG groups increased the pain threshold when compared to the ZG group.

The administration of zymosan into the joint caused a significant (p<0.05) increase in scores in the ZG group (3.80 ± 0.20) compared to the group that received only saline solution (0.80 ± 0.20). The application of laser (1 J/cm$^2$) to the joint with arthritis reduced the analyzed scores (1.20 ± 0.20) and showed a statistical difference when compared to ZG (3.80 ± 0.20). The DG group when compared to LG showed no statistical difference, but when compared to ZG it showed a statistical difference (Figure 2).

The ZG showed changes in cartilage structure, decreased joint space, surface discontinuity and loss of continuity with adjacent bone (Figure 3b) compared to the SG (Figure 3a). The LG showed a protective effect, preserving the cartilage surface (Figure 3c), reproducing positive results similar to DG (Figure 3d).

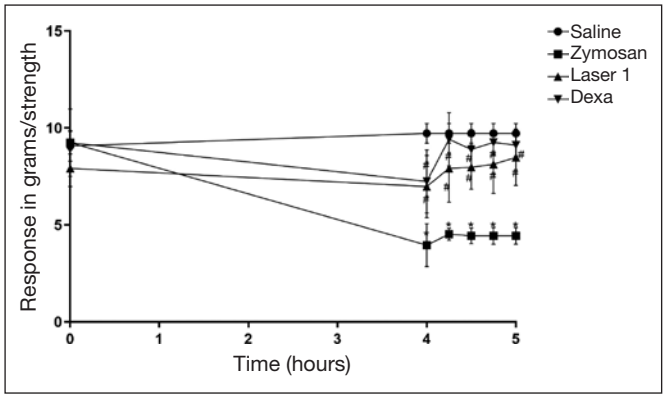


**Figure 1**. Assessment of zymosan-induced hyperalgesia. The animals were treated with laser at a dose of 1 J/cm$^2$ after the zymosan administration in the joint. The evaluation was performed between the fourth and fifth hour with 15-minute intervals. Results were compared with the negative CG; *(p <0.05) statistical difference when compared to SG, #(p <0.05) statistical difference when compared to ZG.

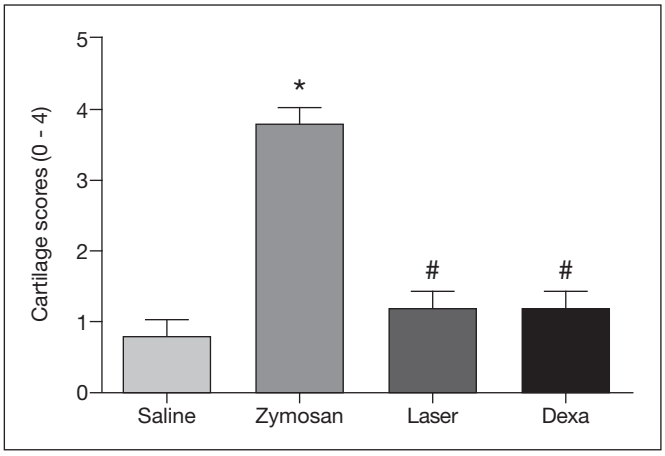


**Figure 2**. Cartilage scores
The scores were measured after the induction of arthritis. The LG presented statistical difference (p<.05) when compared to the ZG. When DG and LG were compared, there was no statistical difference.
* p<0.05 statistical difference when compared to SG and # p<0.05 statistical difference when compared to ZG.

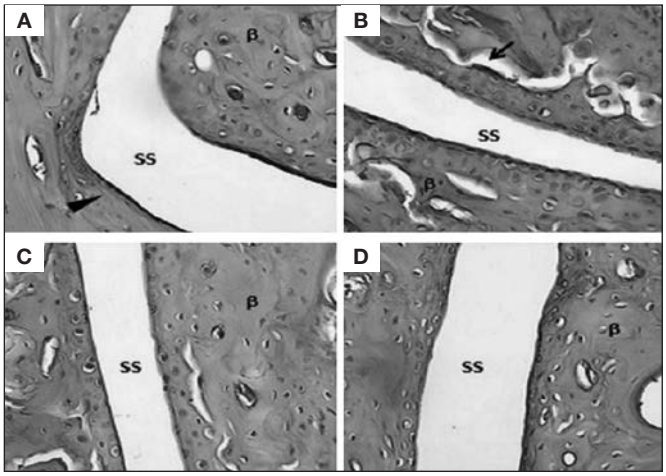


**Figure 3**. Joint region of the different groups
SS = synovial space; ß = bone.
A) SG, demonstrating normal cartilaginous tissue through the arrow and synovial space (SS); B) ZG loss of continuity (arrow) with adjacent bone (ß) and decreased synovial space; treatment with Laser 1 in C) reduced the histological changes caused by zymosan, with preservation of cartilaginous surface and continuity with adjacent bone (ß); D) Treatment with dexamethasone. Laser treatment improved the cartilage score to the dose of 1 (p>0.05) compared to ZG. All photomicrographs are at original magnification of 600x and stained with hematoxylin and eosin.

**Table 1.** Values observed for the limb withdrawal test for the different groups

| | AS 1 | AS 2 | AS 3 | AS 4 | AS 5 |
|---|---|---|---|---|---|
| SG | 9.72 ± 0.22 | 9.72 ± 0.22 | 9.72 ± 0.22 | 9.72 ± 0.22 | 9.72 ± 0.22 |
| ZG | 3.96 ± 0.45* | 4.52 ± 0.12* | 4.44 ± 0.16* | 4.44 ± 0.17* | 4.44 ± 0.17* |
| LG | 6.98 ± 0.65# | 7.92 ± 0.71# | 7.98 ± 0.46# | 8.12 ± 0.61# | 8.48 ± 0.59# |
| DG | 7.24 ± 0.66# | 9.42 ± 0.55# | 8.90 ± 0.41# | 9.26 ± 0.25# | 9.12 ± 0.23# |

*(p<0.05) statistical difference when compared to SG # (p<0.05) statistical difference when compared to ZG. SG = saline group; ZG = zymosan group; LG = laser group; DG = dexamethasone group, at the different assessment times (AS). Data presented as mean and standard deviation.

## DISCUSSION

This study showed that the use of the 1 J/cm$^2$ laser reduced hyperalgesia when compared to the ZG (Figure 1 and Table 1). The result corroborates the study[11], which evaluated arthritis in the rat temporomandibular joint. The results demonstrated the action of the low intensity laser, reducing hyperalgesia, evaluated by the Von Frey test.

The study[12] described that the wave lengths of 670 nm and 830 nm produced alteration in the hypernociception in the knees of mice when evaluated with local pressure, and 670 nm presented early reduction. When the pressure was done in the distal region (plantar region), only 830 nm was able to decrease the hypernociception. Study[13] observed that red (630 ± 10 nm) and infrared (850 ± 10 nm) LEDs were effective in reducing the painful scenario. In the mentioned studies there was a reduction in pain, which corroborates the findings of the present study, because treatment with the 1 J/cm$^2$ laser showed significant improvement ($p < 0.05$) in pain (Figure 1 and Table 1).

In the sections stained with hematoxylin, the group treated with the 1 J/cm$^2$ laser showed lower cartilage scores compared to the ZG, showing a positive effect on joint degeneration (Figure 2). This result corroborates the data from the study[14], which described the positive effect of laser therapy, presenting less severe aspects of joint degradation.

Study[15] on an arthritis model using fluence of 830 ηm showed the ability to reverse tissue damage and, consequently, the potential to reverse cartilage degeneration. Other studies using wavelengths between 660 and 780 ηm have described reversing effects on cartilage degradation, preservation of the joint capsule, and reduction of edema[16-18]. The results of the present study were similar, as laser treatment showed a protective effect on cartilage after zymosan injury, with preservation of the cartilaginous surface and continuity with adjacent bone (Figure 3).

## CONCLUSION

The results of the present study showed that the 904 ηm wavelength and 1 J/cm$^2$ dose were effective in reducing pain and presented a protective effect on the morphology of the joint area, highlighting the clinical potential of the laser for the treatment of arthritis. Nevertheless, long-term studies are needed in order to investigate its effects.

## AUTHORS' CONTRIBUTIONS

**José Carlos Rapozo Mazulo-Neto**
Statistical analysis, Data collection, Conceptualization, Research, Methodology, Writing - Preparation of the original, Writing – Review and Editing, Software, Validation, Visualization

**Luzeni Garcez Souza**
Conceptualization, Methodology, Writing - Preparation of the original

**Flavia Rafaella dos Santos Mazulo**
Conceptualization, Methodology, Writing - Preparation of the original

**Marcelo de Carvalho Filgueiras**
Funding Acquisition, Project Management, Supervision, Visualization

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