# Effect of photobiomodulation therapy (660 nm and 830 nm) on carrageenan-induced edema and pain behavior in mice

*Efeito da terapia por fotobiomodulação (660 nm e 830 nm) no comportamento da dor e edema induzidos por carragenina em camundongos*

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## ABSTRACT

**BACKGROUND AND OBJECTIVES**: Photobiomodulation (PBM) is an important therapeutic tool for inflammatory process modulation. In this study, the anti-inflammatory and analgesic effect of two different energies and two different wavelengths (660 nm and 830 nm) were investigate and compared through the model of carrageenan-induced paw edema in mice.
**METHODS**: Male Swiss mice, 36 animals (n=6 animals/group) were divided into six groups: Group 1 (saline-control), Group 2 (carrageenan), Group 3 (carrageenan + laser 660 nm, 5.88 J), Group 4 (carrageenan + laser 660 nm, 2.94 J), Group 5 (carrageenan + laser 830 nm, 5.88 J), and Group 6 (carrageenan + laser 830 nm, 2.94 J). PBM was applied 1h after the carrageenan injection which induced paw edema and hyperalgesia, which were measured by means of a plethysmometer and by flicker test using a water bath at 38ºC (±0.5ºC), respectively. Left paws of mice injected with carrageenan exhibited local edema that persisted for up to 6h after its administration. All animals were evaluated before, 1, 2, 3, 4, and 6 h after the injection of carrageenan.

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**HIGHLIGHTS**
• The present study demonstrated that PBM was effective in minimizing pain and edema in the acute inflammatory process in an experimental model.
• The 830 nm wavelength (5.88 J energy) was more effective in improving edema in the acute inflammatory process in an experimental model.
• In pain analysis, the energy of 2.94 J was more effective regardless of the wavelength used in the acute inflammatory process in an experimental model.

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**RESULTS**: PBM, specially the 830 nm wavelength with 2.94 J of energy, reduced the paw edema induced by carrageenan. In addition, the 660 nm wavelengths (5.88 J / 2.94 J) and 830 nm (2.94 J) inhibited thermal hyperalgesia induced by carrageenan after 4 h of paw injection.
**CONCLUSION**: There was evidence that the PBM 830 nm (2.94 J) produced a more pronounced anti-inflammatory effect, while the 660 nm (5.88 J / 2.94 J) energy laser was more effective to inhibit the hyperalgesia response induced by the carrageenan injection.
**Keywords**: Edema, Hyperalgesia, Inflammation, Low level laser therapy, Pain.

## RESUMO

**JUSTIFICATIVA E OBJETIVOS:** A fotobiomodulação (FBM) é uma importante ferramenta terapêutica para modulação dos processos inflamatórios. Neste estudo, investigou-se o efeito anti-inflamatório e analgésico de duas energias e dois comprimentos de onda diferentes (660 nm e 830 nm) através do modelo de edema de pata induzido por carragenina em camundongos.
**MÉTODOS**: Trinta e seis camundongos *Swiss* machos (n=6 animais/grupo) foram divididos em seis grupos: Grupo 1 (controle salino), Grupo 2 (carragenina), Grupo 3 (carragenina + laser 660 nm, 5,88 J), Grupo 4 (carragenina + laser 660 nm, 2,94 J), Grupo 5 (carragenina + laser 830 nm, 5,88 J) e Grupo 6 (carragenina + laser 830 nm, 2,94 J). A FBM foi aplicada 1h após a injeção de carragenina que induziu o edema de pata e a hiperalgesia térmica, os quais foram medidos por meio de um pletismômetro e pelo *flicker test* em banho-maria a 38ºC±0,5ºC, respectivamente. As patas esquerdas injetadas com carragenina apresentaram edema local que persistiu por até 6h após sua administração. Todos os animais foram avaliados antes, 1, 2, 3, 4, e 6 horas após a injeção de carragenina.
**RESULTADOS**: A FBM, principalmente o comprimento de onda 830 nm com 2,94 J de energia, reduziu o edema de pata induzido pela carragenina. Além disso, o comprimento de onda 660 nm (5,88 J / 2,94 J) e o 830 nm (2,94 J) inibiram a hiperalgesia térmica induzida pela carragenina após 4h da injeção na pata.
**CONCLUSÃO**: Evidenciou-se que a FBM 830 nm (2,94 J) produziu efeito anti-inflamatório mais pronunciado, enquanto

o laser de 660 nm (5,88 J / 2,94 J) de energia foi mais eficaz para reduzir a resposta de hiperalgesia induzida pela injeção de carragenina.

**Descritores**: Dor, Terapia a Laser de baixa intensidade, Edema, Hiperalgesia, Inflamação, Carragenina.

# INTRODUCTION

Inflammation is an initial physiological phenomenon unleashed during the loss of homeostasis due to lesions and infections. This phenomenon initiates by innate immune response that recognizes the damaged and pathogens cells. The acknowledgment of pathogenic microorganisms is essential to initiate immune responses, such as inflammatory processes mediated by pattern recognition receptors (PRRs), which identify molecular structures that are broadly shared by pathogenic agents, known as pathogen-associated molecular patterns (PAMPs)[1-3].

The induction of the inflammatory process produces edema on mice paw. In the inflammation, there is a loss of balance between hydrostatic and osmotic pressure, with output of proteins from the blood vessels, and increase of extracellular osmotic pressure, causing the outflow of fluids from the vessels to the interstice, thus, contributing to edema formation[4]. Thereby, there are a variety of stimulus to induce migration of polymorphonuclear cells and consequent inflammatory acute processes on mice paw, such as the carrageenan, which is used to induce inflammation in animals; this stimuli act as an algogenic substance in animal paws causing inflammatory response with consequent cellular migration and hyperalgesia[3-7].

The injection of carrageenan provokes the inflammatory process and consequent edema formation, increasing tissue volume, and exacerbating the sensibility to thermal and mechanical stimulus[8-11]. There are different mediators involved on the inflammation, such as histamine, serotonin (5-HT), and bradykinin (BK), which are the first detectable mediators during the initial phase on carrageenan-induced paw inflammation. Prostaglandins (PG), produced through cyclooxygenase (COX), are involved in the increase of vascular permeability, and are detectable mainly during the late phase of the inflammatory process. Moreover, local and/or systemic inflammation is associated with up regulation of pro-inflammatory cytokines, such as TNF, IL-1, and IL-6[12-15].

In experimental studies, the use of carrageenan establishes a simple animal model for evaluation of pain and edema on the site of the acute inflammation, with slight lesion or damage to the inflamed tissue. This experimental model provides variation of different therapeutic and pharmacological resources, with the objective of minimizing the inflammatory process[6,7,13-18].

Electrophysical resources, as the photobiomodulation therapy (PBMT), are being largely used in research on different experimental models, such as tendons, peripheral nerve, cutaneous tissue, bones, and muscles[19-24]. Moreover, they are being applied for the treatment of acute inflammatory processes, and ensuing resolution of the acute edema[5,6,25-30]. The protective effects of PBM are associated with different actions, such as: i) increase of local micro-circulation and stimulation of angiogenesis; ii) vasodilation; iii) down regulation of pro-inflammatory mediators, like PGE2; iv) modulation of innate and adaptive immune cells; v) antioxidant effects; and vi) healing and tissue repair[31].

The authors[32-34] describe that the modulation of pain and edema can be based on the mechanisms of action of PBM in the inflammatory process, decreasing nerve conduction (afferent impulses) and action on proinflammatory cytokines, causing pain improvement and edema caused by the inflammatory process.

Study[35] used PBMT for the reduction of TNF-α in acute lung lesion. Authors observed that laser reduced expression of TNF-α. In the bibliography review manuscript conducted in another study[36], laser modulates the inflammatory process in a dose-dependent way. The authors[5,6] conducted two researches comparing 660 nm and 684 nm lasers in the inflammatory process induced by carrageenan injection on mice paw, showing the improvement of edema and reduction of cytokines levels.

Most articles use visible PBM for the treatment of the acute inflammatory process[5,25,37-41], and few articles use near infrared and infrared PBM[42-44]. The 808 nm PBM in experimental models of acute inflammatory process in the temporomandibular joint of rats was described in a recent research[43], which observed the ability to modulate the inflammatory process by reducing pro-inflammatory cytokine levels. Authors[45] demonstrated the action of PBM on mRNA in a model of paw edema in rats and the research carried out by the study[42] used 830 nm PBM in acute edema of the paw of rats. The authors observed a modulating effect of PBM in the inflammatory process of the evaluated animals. In another study[24], the authors described the action of PBM on edema and pain in a model of complex reaction type 1 pain syndrome. The authors demonstrated improvement in the variables evaluated in the study.

The use of PBM to modulate the acute inflammatory process has been studied, with greater use of the visible wavelength, but there are still several questions that need to be answered such as: investigating different doses to provide better results in this research model and carry out further studies with near-infrared and infrared wavelengths[32]. Therefore, the present study was designed to investigate and compare the effect of two different wavelength and two energies of the PBMT during acute inflammatory process on mice paw.

# METHODS

Thirty-six male Swiss mice (30-40 g) were used in the pre-clinical experiments. Mice were obtained from Federal University of Santa Catarina, in Florianópolis, and kept at the Araranguá Campus during experimental protocols. The mice were kept in groups of four to six animals per cage, maintained under controlled temperature (22 ± 2°C) and humidity (60-80%), with a 12 h light/dark cycle (lights on at 7:00 am) and were given free access to food (*Puro Trato*®, Santo Augusto, Rio Grande do Sul, Brazil) and water. All procedures used in the present study followed the "Principles of laboratory animal care" (NIH publication nº 85-23) and were approved by the Animal Ethics Committee of UFSC (CEUA-UFSC, protocol number PP00956). The

ARRIVE (Animal Research: Reporting of In Vivo Experiments) checklist was used.

The group size was calculated based on previous experiments performed in the laboratory, thus, a convenience sample with 36 animals was used, a minimum number necessary to demonstrate consistent data.

Interventions were performed by researcher 1 and evaluations were performed by researcher 2, blinded to the group, both trained before the beginning of data collection. Data analysis and processing were performed by researcher 3, also blindly.

### Experimental groups

The study was performed with 36 mice randomly divided into six groups through a draw by sealed envelope, with 6 animals in each, named as Group 1: saline-control; Group 2: carrageenan; Group 3: carrageenan + laser 660 nm, with 5.88 J of energy; Group 4: carrageenan + laser 660 nm, with 2.94 J of energy; Group 5: carrageenan + Laser 830 nm, with 5.88 J of energy; and Group 6: carrageenan + Laser 830 nm, with 2.94 J of energy. All procedures were performed on the plantar region of the mice's left hind paw, and PBM was performed 1 hour after carrageenan administration. All animals from PBM underwent punctual technique with contact on the plantar region of the left hind paw. The choice for the effective dose of the laser was based on pilot experiments (data not shown) or on previous data described in the literature[5,36]. In the animals of Group 1 (control), only saline substance was administered in the plantar region of the left hind paw.

### Edema induction

To induce acute the inflammatory process, animals received an injection of λ-carrageenan (2.5%) in 50 µL of saline solution (0.9% NaCl) on the subcutaneous regions of the plantar surface of the left hind paw[46].

### Edema and hyperalgesia evaluation

Animals were acclimatized for 30 min before behavioral testing, and to determine the basal edema and thermal thresholds all the groups were evaluated before inflammation induction. Acute edema on animal's left hind paw was induced by intra--plantar carrageenan injection, was analyzed by plethysmography and demonstrates volume referent to edema on the animal's left hind paw.

Edema was measured via plethysmography (Model 7150™, Ugo Basile, Varese, Italy) before, 1, 2, 3, 4, and 6 h after the injection of carrageenan. Data were expressed in milliliters (mL) of water. With the immersion of the animal's paw in the plethysmometer, the movement of water occurs, thus, the millimeters of water displaced are quantified.

Thermal hyperalgesia evaluation was performed by flicker test using a water bath at 38ºC (± 0.5ºC) and it was quantified in seconds. In the thermal hyperalgesia test, the quantification of seconds started with the immersion of the animal's paw in the water and ended when the animal moved its paw in an attempt to get it out of the water. Data collection occurred at the same intervals cited to measure edema.

### Photobiomodulation

The equipment Gallium Aluminum Arsenide (GaAlAs, Ibramed Medical Equipment™, Amparo, São Paulo, Brazil) diode laser, emitting 830 nm of wavelength, was used in this study, likewise the Aluminum, Gallium, Indium, and Phosphorus (AlGaInP, Ibramed Medical Equipment™, Amparo, São Paulo, Brazil) diode laser, transmitting 660 nm of wavelength, both with continuous bundle. Table 1 shows the description of parameters utilized.

**Table 1.** Photobiomodulation parameters used in this study.

| Laser parameters | Energy (J) | Irradiation time (s) | Bean area (cm²) | Potency (mW) |
|---|---|---|---|---|
| Group 3 (660 nm) | 5.88 | 196 | 0.06 | 30 |
| Group 4 (660 nm) | 2.94 | 98 | 0.06 | 30 |
| Group 5 (830 nm) | 5.88 | 196 | 0.11 | 30 |
| Group 6 (830 nm) | 2.94 | 98 | 0.11 | 30 |

J = Joule; s = seconds; cm² = square centimeters; mW = miliwatts.

The dose of 5.88 J and the use of the wavelength of 660 nm was based on the study[40]. For comparison, another wavelength of 830 nm was used, and another energy, 2.94 J, was used with the same power in the two probes, as described in table 1.

### Statistical analysis

Data from the groups was analyzed using GraphPad Prism 6 (GraphPad Software, Inc. La Jolla, CA, USA). Before the analysis of each group, the normality on the data distribution was observed using the Shapiro-Wilk test. Results are representative of one or two independent experiments. A statistical comparison of data was performed by one- and two-way ANOVA followed by the Bonferroni post hoc test, depending on the experimental protocol. Value $p < 0.05$ and 0.001 were considered significant.

## RESULTS

### Effect of photobiomodulation on paw edema induced by carrageenan

Paw edema induced by carrageenan was compared with the saline control group, left paws of mice injected with carrageenan exhibited local edema (Figure 1a) that persisted for up to 6 h after its administration. Relevantly, the application of PBM, particularly the 830 nm laser with 2.94 J of energy, reduced the paw edema induced by carrageenan (Figure 1a), with inhibition of 60.1% in $\Sigma\ 1 - 6$ h (F (5.28) = 60.98), after carrageenan injection when compared to control group, based on the area under the curve (AUC) (Figure 1b). However, treatment with 660 nm and 830 nm laser (5.88 J of energy) failed to inhibit edema induced by the carrageenan injection (Figure 1).

Animals were treated with 660 nm (5.88 J or 2.94 J of energy) or 830 nm (5.88 J or 2.94 J of energy) laser, 1 h after injection of carrageenan (2.5%, 50 µL, i.pl.) (a). Area under the curve (AUC) between $\Sigma 1 - 6$ h after carrageenan injection (b). The re-

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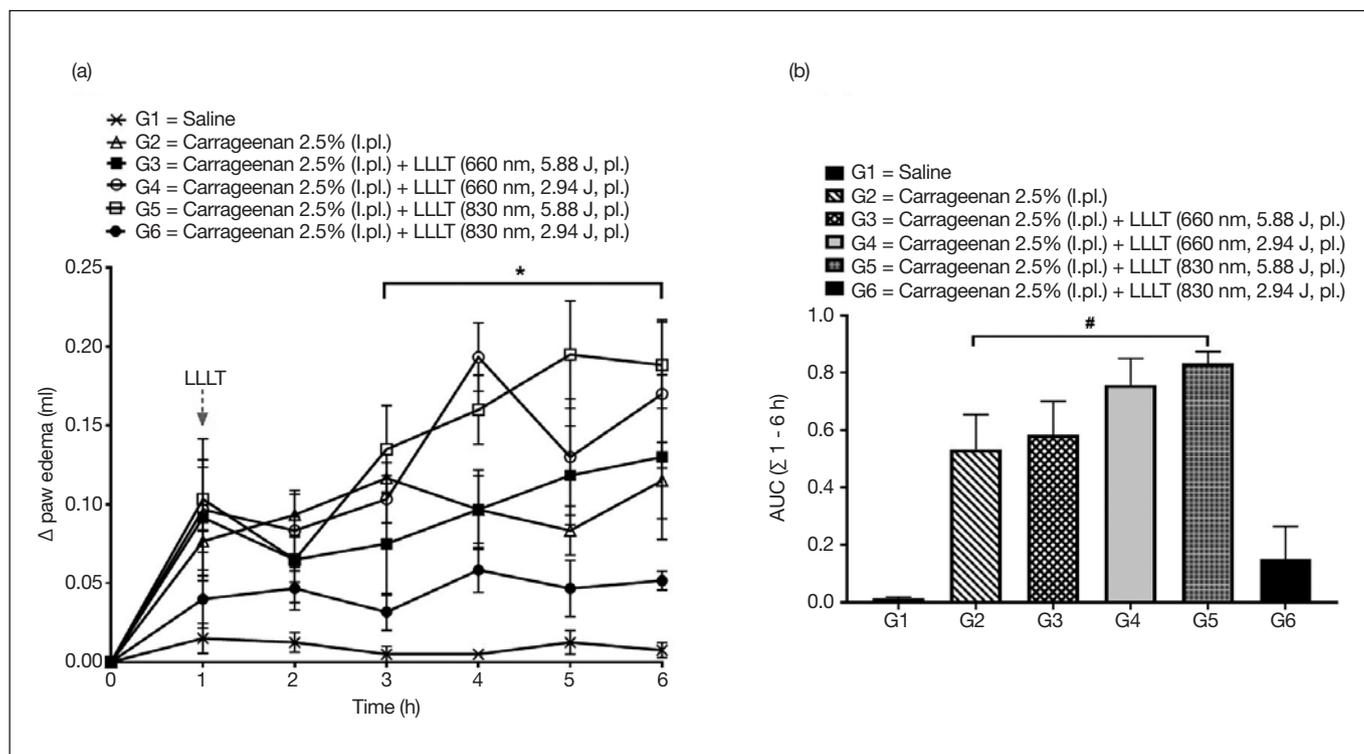Neves LM, Kuriki HU and Dutra RC



**Figure 1**. Effect of photobiomodulation on paw edema induced by carrageenan in mice

I.pl. = intrapleural; LLLT = low level laser therapy; pl. = pleural; * = p<0.0001 versus the control group except when compared with Group 6; # = p<0.050 versus the saline and G6 groups. (two-way ANOVA followed by Bonferroni post hoc test).
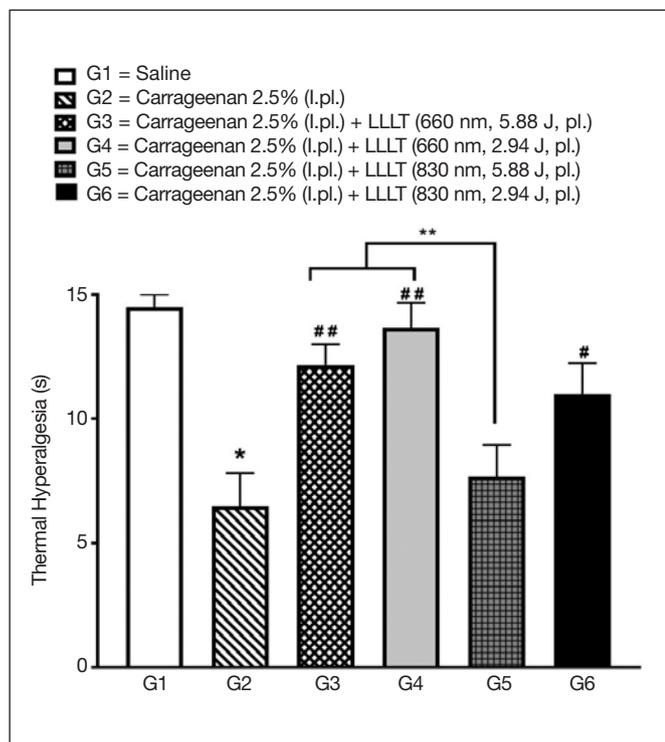


**Figure 2**. Effect of photobiomodulation on thermal hyperalgesia induced by carrageenan in mice

I.pl. = intrapleural; LLLT = low level laser therapy; pl. = pleural; * = p<0.050 versus the control group; # = p<0.050; # # = p<0.002 versus the carrageenan group; ** = p<0.0002 vs. the 830 nm PBM (5.88 J) group (one-way ANOVA followed by Bonferroni post hoc test).

sults are expressed as mean ± Standard Error of the Mean (SEM), n = 6 animals per group.

**Effect of photobiomodulation on thermal hyperalgesia induced by carrageenan**

As expected, the carrageenan application on the plantar surface of the hind paw of the animals increased paw withdrawal latency to heat stimulus when compared to control group (Figure 2). The results depicted in figure 2 shows that the 660 nm PBM (5.88 J or 2.94 J of energy) and 830 nm PBM (2.94 J of energy) inhibited thermal hyperalgesia induced by carrageenan after 4 h of paw injection (F (5.26) = 7.59, figure 2). Adversely, treatment with 830 nm laser (5.88 J of energy) was not effective (Figure 2). Importantly, animals subjected to 660 nm PBM (5.88 J or 2.94 J of energy) exhibited significant improvement in thermal hyperalgesia when compared with those subjected to 830 nm PBM with 5.88 J of energy (Figure 2).

Animals were treated with 660 nm (5.88 J or 2.94 J of energy) or 830 nm (5.88 J or 2.94 J of energy) laser, 1h after injection of carrageenan (2.5%, 50 µL, i.pl.). Thermal hyperalgesia was assessed through water bath at 38ºC ± 0.5ºC – was evaluated 4 h after carrageenan injection. The results are expressed as mean ± SEM (n = 6 animals per group).

## DISCUSSION

In agreement with the anti-inflammatory and analgesic effects that have been previously observed in the present work, PBM

inhibited inflammation and pain during acute inflammatory response[47]. Additionally, the study compared the effects of two different wavelengths of PBM laser in the resolution of acute inflammatory process on mice paw.

Different studies in the literature use animal models as tools for better understanding of the inflammatory responses, such as paw edema[5,6,46], pulmonary lesion[29,35], osteoarthritis models[48,49], skin lesion models[23,50] and inflammatory skeletal muscle disorder models[30]. Hence, to study the role of PBM in the inflammatory process, the carrageenan-induced paw edema in mice was used, which induces inflammatory responses, including edema formation, neutrophil infiltration, and the development of hyperalgesia.

Moreover, carrageenan injection into the rodent paw induces edema that develops in the first 6h up to 24h. This inflammatory response is mainly mediated by histamine, and by an increasing synthesis of PGE2, following peripheral release of nitric oxide (NO), TNF, IFN-y, and IL-1, which have been shown to induce iNOS in a variety of cells[5,7,47].

Thus, the authors[51,52] suggest that there may be a differentiated action in the activation of metabolic cascades involving the action of different red and near infrared wavelengths, causing some changes such as: modulate the levels of pro and anti-inflammatory cytokines, modulatory action on peripheral nerve stimuli, in addition to causing differentiation, migration and cell proliferation. In the present study, different manifestations can be observed when the wavelengths of 660 nm and 830 nm are evaluated. In the assessment of edema, PBM near infrared with lower energy obtained better results, however, when observing the red PBM, the best results were in relation to hyperalgesia.

Although low level PBM is used to treat inflammatory process on animals, a consensus between the parameters and wavelength utilized was still not observed. The present study compares two wavelengths (660 nm and 830 nm), visible and infrared, respectively, both with continuous bundle, with energy of 5.88 J in accordance to the author[5] and 2.94 J according to the author[53] which showed that anti-inflammatory effects of the laser can be reached within a therapeutic window of 0.6 to 9.6 J of total energy emitted[26-30].

The study[24] demonstrated that laser (650 nm) inhibited inflammatory response through release of adrenal hormones. According to the study[27], 780 nm laser with dose of 0.4 J decreased the inflammatory process, down regulated TNF level, however no alteration in IL-6 expression was observed. Also of interest are the results from the same group showing that the laser blocked IL-6 and TNF levels during a model of lesion by incision in mice's skin[26].

Authors[5,6] showed that the peak of edema is at the second hour after induction and observed the improvement of the volume of the animals' paw 4h after edema induction and 3h after laser application with 660 nm and 685 nm of wavelength. Likewise, they observed the decrease of TNF with application of PBM. Authors[42] observed that the peak of edema was established at the third hour after the induction, and observed edema reduction after 6h, corroborating the study[45], which detected the peak of edema 4h after carrageenan injection, and the study[35], which reported up regulation of TNF level four hours after edema induction.

The studies[5,6] showed that 5.88 J of energy with visible laser inhibited edema in the hind left paw of the animals. Furthermore, the same report did not show significant difference between wavelength with 5.88 J of energy. By analyzing Group 6 (laser of 830 nm, with 2.94 J of energy), it's possible to conclude that this treatment had higher efficacy for edema when compared to other groups, corroborating previous reports[25,47,42], which used energy emission of 2.04 J, 0.96 J, and 1.0 J respectively, obtaining improvement in the animals' paw edema.

Authors[47] refer that PBM enhances the pain induced in the animal's paw. These authors utilized wavelength of 632.8 nm and accomplished positive results, increasing pain threshold, matching the present study's data that show improvement in thermal hyperalgesia of the animals treated with PBM, which was statistically different from the other groups. In the systematic review[36], the therapeutic window for pain relief is 5.0 J to 19.5 J, corroborating Groups 3 and 5 of the present study. However, Group 4, with energy inferior to the observed in the review[36], also achieved positive results, minimizing animal's hyperalgesia. Accordingly, it can be inferred that improvement of edema and hyperalgesia in animals can be explained by the PBM action of the laser in the inflammatory process[25].

The limitations found in the present study were the lack of analysis of inflammatory and anti-inflammatory mediators, which could better answer the effects related to the hyperalgesia improvement with a major energy and the edema improvement with lower energy. Thereby, new studies should be performed to better elucidate this question.

The present study highlights the effects of PBM and brings important data on the use of the 830 nm wavelength, improving edema volume, and 660 nm wavelength, with better results for thermal hyperalgesia. Regarding the existing gap, the present study brings an important point about the use of an energy of 2.94 J, obtaining better results when compared to twice that energy and with the same power in both devices.

Altogether, the data suggest that PBM was efficient to improve edema and hyperalgesia in mice. Moreover, 660 nm laser with 2.94 J of energy was more effective to inhibit hyperalgesia response, while 830 nm laser with 2.94 J of energy was more effective in preventing edema formation in mice paws. Thus, it's important to emphasize that, for future studies, the energy of 2.94 J can be used in other experimental models and with different parameters of PBM, such as: the use of 904 nm wavelengths and also different powers.

Despite increased efforts by the scientific community, the available therapies to treat these conditions have partially effective actions and numerous side effects, which have profound implications upon the patients' quality of life.

## CONCLUSION

The fact that PBM was effective on carrageenan-induced edema and pain behavior in mice, even if in an animal model, is espe-

cially relevant for the following treatment protocols that could be indicated to prevent and treat edema and inflammatory pain disorders. Finally, the data confirms and extends previous literature results on the anti-inflammatory and analgesic effect of PBM and suggests that PBM might be clinically important and complementary to the treatment of inflammatory diseases.

## AUTHORS' CONTRIBUTIONS

**Alexandre Marcio Marcolino**
Statistical analysis, Project Management, Supervision

**Ketlyn Germann Hendler**
Data collection, Methodology, Writing - Preparation of the original

**Rafael Inacio Barbosa**
Statistical analysis, Research, Writing - Review and Editing

**Lais Mara Siqueira das Neves**
Data Collection, Research, Writing - Preparation of the original, Writing - Review and Editing

**Heloyse Uliam Kuriki**
Data Collection, Conceptualization, Methodology

**Rafael Cypriano Dutra**
Funding acquisition, Resource Management, Methodology, Writing - Review and Editing

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