Does photobiomodulation therapy combined to static magnetic field (PBMT-sMF) promote ergogenic effects even when the non-exercised leg is irradiated? A randomized, triple-blind, placebo-controlled trial.

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Abstract

Background The direct application of photobiomodulation therapy (PBMT) combined with a static magnetic field (sMF) (PBMT-sMF) to target tissues is shown to improve muscle performance and recovery. Studies have reported possible PBMT induced systemic effects. Notably, the extent of these effects on musculoskeletal performance and the optimal site of application remain unclear, although this information is clinically important because these factors directly affect the magnitude of the effect. We investigated the effects of PBMT-sMF on musculoskeletal performance and post-exercise recovery of muscles in exercised and non-exercised legs before the implementation of an exercise protocol.

Methods This randomized, triple-blind placebo-controlled study included 30 healthy men randomly assigned to the placebo, exercised-leg, and non-exercised leg groups. Active or placebo PBMT-sMF was applied to 6 sites of the quadriceps muscle of both legs. An eccentric exercise protocol was used to induce fatigue. The following parameters were analyzed to evaluate exercise performance: peak torque assessed by maximal voluntary contraction (MVC), delayed muscle pain assessed by the visual analogue scale (VAS), muscle injury assessed by serum creatine kinase (CK), and fatigue assessed by serum lactate levels. Evaluations were performed before implementation of the eccentric (baseline) exercise protocol, as well as immediately after and 1, 24, 48, and 72 hours upon protocol completion. The Bonferroni post-hoc ANOVA test was used considering the level of statistical significance p <0.05.

Results Compared to the placebo and systemic groups, irradiation with PBMT-SMF led to statistically significant improvement (p <0.05) with regard to all variables in the exercised-leg group. Results of irradiation in the non-exercised leg group were similar to those in the placebo group with regard to all variables.

Conclusion Our results support the current evidence that irradiation of all exercised
muscles produces ergogenic effects. PBMT-sMF improved performance and reduced muscle fatigue only when applied locally to muscles involved in physical activity.

INTRODUCTION

Photobiomodulation therapy (PBMT) refers to the application of electromagnetic radiation to biological tissues using low-power laser or light-emitting diodes [1, 2], which induces photochemical reactions in cells leading to a biomodulating therapeutic effect [3, 4, 5], without leading to ablative or thermal adverse reactions [4]. In recent years, this therapy has shown positive effects in the management of several musculoskeletal disorders and inflammatory conditions to promote pain relief and wound healing [6–12]. Recent studies have reported that PBMT increases muscle performance, reduces fatigue, and improves muscle recovery in athletes and physically active or sedentary individuals [13–23]. The mechanism of action of PBMT is attributed to the interaction of photons with cytochrome c-oxidase, a mitochondrial photoreceptor [24]. This interaction modulates mitochondrial activity leading to greater transfer of electrons and consequently mitochondrial respiratory chain activation, which increases mitochondrial adenosine triphosphate (ATP) production [24].

A static magnetic field (sMF) is also known to affect biological processes in the body. The SMF acts through the movement of electrically charged particles/electromagnetic waves on other body parts. [25, 26]. Wang et al. (2018) [25] reported that sMF increases ATP production and reduces oxidative stress [25, 26]. Recent studies have shown positive results with a combination of PBMT and sMF [15, 16, 17, 18, 19], which promotes antioxidant activity and potentiates the effects of PBMT in a few musculoskeletal conditions [25, 26]. PBMT-sMF has shown positive effects on muscle performance and post-exercise recovery in athletes and non-athletes. Additionally, such intervention is known to reduce dyspnea and fatigue in the lower limbs of patients with chronic
obstructive pulmonary disease [13, 23, 27, 28, 29, 30, 31]. Moreover, this therapy used in conjunction with different exercise programs has shown improved strength and aerobic fitness [18, 19].

Although these effects have been observed following the local application of PBMT directly to exercised muscles, a few pre-clinical studies suggest that PBMT application at sites distant from the target tissue may also produce positive effects, such as improvement in systemic diseases including hypertension and lupus [32, 33]. Batista et al. (2015) [34] investigated the systemic effects of PBMT on bone healing and repair and observed that compared with the control group, the experimental group did not show positive bone defect repair following application of PBMT at a site distant from the lesion [34]. Therefore, there is lack of consensus regarding the systemic effects of PBMT. To date, few clinical studies have investigated the systemic effects of PBMT-sMF on musculoskeletal performance and recovery, particularly with the use of biochemical markers to assess muscle injury. It is known that local application of PBMT-sMF to skeletal muscles produces positive effects [13, 15–23]; however, it is important to investigate whether these effects also occur systemically because it directly affects proportion of the magnitude of the effect in real-world clinical practice. Therefore, we used functional and biochemical markers to investigate the effects of PBMT-sMF on skeletal muscle performance and post-exercise recovery in the muscles of exercised- and non-exercised legs before the implementation of an exercise protocol to induce muscle fatigue.

METHODS

Study design

A randomized, triple-blind, placebo-controlled clinical trial approved by the research ethics committee was performed (protocol number 2100849) and registered with
Clinicaltrial.org. (protocol number NCT03695458). Samples were collected in the Phototherapy Laboratory and Innovative Technologies in Health, University Nove (UNINOVE).

Sample Characterization

Thirty sedentary males between 18 and 35 years old were recruited. All participants who agreed to participate in the study signed an informed consent form. The calculation of sample size with b value at 20% and 5% was based on the study by Antonialli et al. (2014) [15] who used PBMT-sMF to increase maximal voluntary contraction (MVC; our primary outcome) at 96 post-exercise (336.88 ± 27.92 N m) compared to baseline (286.63 ± 38.86 N m). Thus, resulting in 10 volunteers per group (30 volunteers in total).

Even though there were no dropouts in the study, the intention-to-treat analysis protocol, established a priori, was followed. The flowchart CONSORT (Consolidated Standards of Reporting Trials) shows lay out and all processes that were adopted to carry out the study (Fig. 1).

<<Figure 1>>

Inclusion and Exclusion Criteria

The study included male participants, aged between 18 and 35 years, with different skin tones and who performed up to 1 exercise session weekly. With no pre-existing musculoskeletal injuries in the hips or knees in the 2 months prior to the study and present at 100% of the collections.

Individuals who did not meet the above criteria were excluded from the study, such as those using food suplements, those who had chronic joint disease in the non-dominant lower limb, or who had musculoskeletal injury during the study.

Experimental Groups and Randomization
The 30 volunteers were randomly assigned to 3 experimental groups (n = 10 per group) according to the type of therapy and limb to be irradiated as described below.

**Placebo group:** anterior muscle irradiation with placebo PBMT-sMF, bilaterally in the lower limbs;
**Exercised leg group:** irradiation of the anterior thigh muscles with active PBMT-sMF in the lower limb exercised and irradiation with placebo PBMT-sMF of the non-exercised limb;
**Non-exercised leg group:** anterior thigh muscle irradiation with active PBMT-sMF of the non-exercised lower limb and placebo PBMT-sMF irradiation of the exercised limb thigh.

Randomization was performed 2 minutes after baseline evaluation by a researcher who was unaware of the groups correlation using randomization labels created random.org website, through the and a series of sealed, opaque, and numbered envelopes were used to ensure confidentiality and to determine to which experimental group each volunteer was be allocated.

The PBMT-sMF device was programmed by a researcher who did not participate in any of the stages of data collection and analysis. He was instructed not to disclose the programming until the study was completed. The device contained distinct programs, corresponding to active PBMT-sMF or placebo. The different programs looked the same regarding light, sound and application time, providing adequate researcher and volunteer blindness. In addition, the volunteers wore opaque glasses which assisted in the blinding.

The professional who evaluated MVC, CK, VAS, lactate and the one who applied the eccentric protocol was not the same one who irradiated the PBMT-sMF. All professionals involved in the collection as well as the analysis did not know the correspondence of the groups. Therefore, volunteers, experts and therapist were blinded, thus ensuring triple-blind design.

**Procedures**

**Analyze of Creatine Kinase (CK) Activity**

Blood samples were collected (5 ml by anterior cubital vein puncture) prior to stretching and warm-up (baseline) immediately after, 1, 24, 48 and 72 hours after the eccentric
exercise protocol. Fifteen minutes after collection the samples were centrifuged at 3000 rpm for 20 minutes and the supernatant serum was stored and kept at -80 °C until analysis.

Creatine kinase (CK) enzymatic activity as an indirect marker of muscle damage was analyzed by spectrophotometry using specific reagent kits (Labtest® - Brazil) following the manufacturer's instructions with the collected blood samples.

Blood Lactate Measurement

Blood samples were collected from the volunteers' fingertips prior to stretching and up (baseline), immediately after and 1 hour after the eccentric exercise protocol. After asepsis a puncture was performed with a disposable lancet, the first drop of blood was discarded to prevent contamination, then 25 µl were collected for biochemical analysis by the electroenzymatic method according to the instructions of the portable lactate analyzer manufacturer (Accutrend Lactate Plus Roche, Roche Diagnostics GmbH, Mannheim, Germany). The analyzer has a variation coefficient between 1.8 and 3.3% (intraclass correlation [ICC] r = 0.999), with good reliability for intra/inter-analyzers and between test strips. [35]

Evaluation of DOMS

Delayed onset muscle soreness (DOMS) of lower limb was evaluated by the Visual Analog Pain Scale (VAS), which consists of a 10 cm line. At the beginning of the line, number 0 corresponds to no pain and at the end, 10 corresponds to the worst possible pain. The volunteers were instructed to draw a line where their pain best fit at the moment. Assessments were performed prior to stretching and warm-up (basal), 1 min, 1, 24, 48 and 72 hours after the eccentric exercise protocol was performed.

Streching and Warm-Up
Before starting the isokinetic protocol, the volunteers performed 3 sets of 60 seconds of active stretching of the knee extensor muscles, bilaterally. Next, they walked for 5 minutes at 6 km/h on a treadmill as a general warm-up activity.

**Maximum Voluntary Contraction (MVC) Test**

The muscle strength assessment and execution of the eccentric exercise protocol for fatigue induction was performed using an isokinetic dynamometer (System 4 model, Biodex Medical Systems®, Inc., Shirley, NY, USA). Currently considered the gold standard method for assessing the maximum capacity for muscle strength generation and musculoskeletal performance [36, 37].

Immediately after stretching and warm-up exercises, the volunteers performed the maximum voluntary contraction test (MVC). They were positioned on the seat of the isokinetic dynamometer with a 100 ° angle between the trunk and the hip and secured to the dynamometer seat by belts. The non-dominant leg was positioned at 60 ° of knee flexion (0 ° corresponding to total knee extension) with the dynamometer axis parallel to the center of the knee joint.

The MVC test consisted of three isometric contractions of non-dominant lower knee extensors lasting 5 seconds with 30-second intervals between contractions. The highest peak torque value obtained in the three contractions was used for statistical analysis. The volunteers were instructed on how to perform the exercise prior to the initiation of the MVC protocol and during the test were verbally encouraged by a same researcher in all evaluations. The MVC test was performed previously (baseline), immediately after, 1, 24, 48 and 72 hours after the eccentric fatigue protocol.

**Photobiomodulation Therapy and static Magnetic Field – PBMT-sMF**

Active PBMT-sMF or active placebo was applied bilaterally, 2 minutes after baseline assessment (pre-exercise MVC test). The application technique used was direct skin
contact and light pressure, in 6 places on the knee extensor muscles (quadriceps): 2 lateral, 2 medial and 2 central (Fig. 2).

Clusters containing 12 diodes were used: 4 of 905 nm (0.3125 mW average power, 12.5 W peak power for each diode), 4 of 875 nm (17.5 mW average power for each diode) and 4 of 640 nm (15 mW average power for each diode) and a static magnetic field of (35 mT) (manufactured by Multi Radiance Medical®, Solon, OH, USA), the total dose was 180J per thigh. Given the extensive area of irradiation employed in the present work, the use of clusters was fundamental to optimize the therapy application. The choice of irradiation parameters and locations was made based on previous studies using the same equipment [15, 17, 18]. The complete description of the parameters is given in the table below (Table 1).
Table 1
Parameters for PBMT-sMF.

| Parameter                                               | Value                                  |
|---------------------------------------------------------|----------------------------------------|
| Number of lasers                                        | 4 Super-pulsed (infrared)              |
| Wavelength (nm)                                          | 905 (± 1)                              |
| Frequency (Hz)                                           | 250                                    |
| Peak power (W) - each                                    | 12.5                                   |
| Average mean optical output (mW) - each                  | 0.3125                                 |
| Power density (mW/cm²) - each                           | 0.71                                   |
| Energy density (J/cm²) - each                            | 0.162                                  |
| Dose (J) - each                                          | 0.07125                                |
| Spot size of laser (cm²) - each                          | 0.44                                   |
| Number of red LEDs                                       | 4 Red                                  |
| Wavelength of red LEDs (nm)                             | 640 (± 10)                             |
| Frequency (Hz)                                           | 2                                      |
| Average optical output (mW) - each                       | 15                                     |
| Power density (mW/cm²) - each                           | 16.66                                  |
| Energy density (J/cm²) - each                            | 3.8                                    |
| Dose (J) - each                                          | 3.42                                   |
| Spot size of red LED (cm²) - each                        | 0.9                                    |
| Number of infrared LEDs                                  | 4 Infrared                             |
| Wavelength of infrared LEDs (nm)                         | 875 (± 10)                             |
| Frequency (Hz)                                           | 16                                     |
| Average optical output (mW) - each                       | 17.5                                   |
| Power density (mW/cm²) - each                           | 19.44                                  |
| Energy density (J/cm²) - each                            | 4.43                                   |
| Dose (J) - each                                          | 3.99                                   |
| Spot Size of LED (cm²) - each                            | 0.9                                    |
| Static Magnetic Field (sMT)                              | 35                                     |
| Irradiation time per site (sec)                          | 228                                    |
| Total dose per site (J)                                  | 30                                     |
| Total dose applied in muscular group (J)                 | 180                                    |
| Aperture of device (cm²)                                 | 20                                     |
| Application mode                                         | Cluster probe held stationary in skin contact with a 90-degree angle and slight pressure |

<<Table 1>>

### Eccentric Exercise Protocol

Three minutes after the end of PBMT-sMF the volunteers performed the eccentric contractions protocol to induce fatigue. It consisted of 75 eccentric isokinetic contractions of the non-dominant lower limb knee extensor muscles (5 sets of 15 repetitions with 30 seconds interval between each set), with a velocity of 60° sec⁻¹ (both in the eccentric and concentric phase of the movement) and 60° range of motion (between 30° and 90° of knee flexion). At each contraction, the dynamometer automatically positions the knees (passively) at 30° and then flexes to 90°. The efficiency of this protocol has previously
been proven to induce muscle damage generated by exercise [15, 17, 20, 38].
The volunteers were instructed to resist the knee flexion movement imposed by the
dynamometer with maximum force and during the protocol were verbally encouraged by a single professional.

Statistical Analysis
The intention-to-treat analysis was followed a priori. The results were tested for normality by the Kolmogorov-Smirnov test and presented normal distribution, thus they were described as mean values with the respective standard deviations and confidence intervals. The Bonferroni post-hoc ANOVA test was used considering the level of statistical significance \( p < 0.05 \). Data were analyzed in their absolute values and in relation to their variation in percentage of values obtained in the pre-exercise (baseline) assessments. Data were graphically represented by their mean values and standard error of the mean.

RESULTS
Thirty male volunteers with mean age 26.83 years (± 6.02), height 175.67 cm (± 7.95) and body mass 73.03 kg (± 12.59) completed all procedures of the study. Data were analyzed and no statistically significant differences \( (p > 0.05) \) at baseline were observed between all experimental groups according to the MVC, CK, blood lactate and EVA variables. However, statistically significant differences \( (p > 0.05) \) were observed after the baseline between the placebo groups and the exercised leg in all variables. The group that received application on the exercised leg presented a statistically significant difference \( (p > 0.05) \) in relation to the placebo group in the MVC, lactate, CK activity and DOMS. The full description of these data in absolute values, expressed as mean, standard deviation and confidence interval are shown in table 2.

<<Table 2>>
Figure 3 shows a statistically significant lower (p <0.05) increase in CK enzymatic activity from 24 hours after the eccentric exercise protocol was performed between the exercised leg group (188.91% ± 69.64) compared to the placebo (378.27% ± 76.42) and non-exercised leg groups (328.90% ± 57.02). This difference was also observed 48 and 72 hours after the eccentric exercise protocol (Figure 3).

<<Figure 3>>

According to the blood lactate analysis, there was a statistically significant difference (p <0.05) right after the eccentric exercise protocol for the exercised leg group (133.85% ± 35.59) compared to the placebo (198; 98% ± 36.39) and non-exercised leg groups (222.21 ± 47.80%). No statistically significant differences were observed among the groups from one hour after the eccentric exercise protocol, these data are expressed in figure 4.

<<Figure 4>>

Visual Analog Scale evaluation demonstrates statistically lower significant DOMS (p<0.05) one hour after the eccentric exercise protocol between the exercised leg group (12.30 mm ± 14.30) and the placebo (37.40 mm ± 17.40) and non-exercised leg groups (42.40 mm ± 25.70). This difference was also observed 24, 48 and 72 hours after the eccentric exercise protocol (Figure 5).

<<Figure 5>>

Regarding the percentage variation of the MVC values, a statistically significant
improvement in the peak torque (p <0.05) was observed immediately after the eccentric exercise protocol in the exercised leg group (93.13 ± 16.44) compared to the placebo group (77.56% ± 5.62), as well as at all times evaluated. A statistically significant difference (p <0.05) was also observed 1h after the eccentric exercise protocol between the exercised leg group (87.87% ± 7.58) compared to the non-exercised leg group (73.46% ± 10.08). This difference remained 24, 48 and 72 hours after the eccentric exercise protocol, as shown in figure 6.

<<Figure 6>>

DISCUSSION

This study showed that PBMT-sMF did not increase performance or reduce strenuous exercise-induced fatigue when applied to sites distant from the exercised muscle. This finding supports the hypothesis that PBMT-sMF should be applied locally to muscles that will be exercised and concurs with the results reported by several previous studies [15, 18, 19], as well as the results of current systematic reviews and meta-analyses discussing this subject and the recently published guidelines [20, 39]. To summarize, the maximal voluntary contraction (MVC) in the exercised-leg group was similar to that reported by Antoniali et al. (2014)[15] who observed that application of local PBMT at an optimal dose (180 J/thigh) improves muscle performance. Moreover, we observed that compared to baseline levels, muscle strength in the exercised-leg group recovered completely within 48 hours after exercise. Muscle performance in the exercised-leg group at 72 hours was higher than that recorded at baseline evaluation, indicating better performance and more effective muscle recovery.

The eccentric exercise protocol effectively induced muscle fatigue, as confirmed by the
fact that the placebo group showed decreased performance (based on MVC assessment) after exercise and increased muscle injury and fatigue. These observations were verified by estimation of blood markers (creatine kinase [CK] and lactate) and muscle pain. The group that received local PBMT-sMF to the exercised leg showed lesser muscle injury and a minimal increase in serum CK levels within the first 24 hours after exercise. The group that received radiation to the exercised leg also showed reduced serum lactate levels immediately after exercise. Estimation of serum lactate shows good clinical applicability and is widely used for performance analysis in sport and exercise because measurement of this biochemical marker is easy and cost effective [40, 41].

Compared with the placebo and the non-exercised leg groups, the exercised-leg group showed a statistically significant reduction in strenuous exercise-induced pain [42, 43] at 1, 24, 48, and 72 hours after completion of the eccentric exercise protocol. These results are similar to those reported by previous studies [15, 18, 19, 39], suggesting that an increase in local microcirculation [1] effectively removes blood metabolites, reduces fatigue, and accelerates muscle recovery after exercise [40]. Clinically, attenuating the fatigue perception process is important for muscle recovery because it enables individuals to rapidly return to physical activities with lesser motor impairment [44].

Our results with regard to muscle strength and recovery from fatigue concur with those reported by Ferreira et al. (2018)\textsuperscript{45}, who observed that compared with placebo irradiation, PBMT irradiation of the exercised leg led to an 11.3\% improvement in functional performance. Notably, our results showed that compared with placebo irradiation, PBMT-sMF irradiation of the exercised leg led to a 20.5\% improvement in functional performance. In our view, the greater improvement observed in our study compared with that reported by Ferreira et al. (2018)\textsuperscript{45} is attributable to PBMT-sMF combination therapy.
Moreover, Ferreira et al. (2018)\textsuperscript{45} did not observe positive effects of this therapy on serum lactate levels, which is attributable to differences between the fatigue induction protocols implemented in these studies. Furthermore, the authors did not determine the serum CK level (which is an important biochemical marker of exercise-induced muscle injury \textsuperscript{21}).

Therefore, to determine the magnitude of the effects of PBMT on skeletal muscles and ensure consistent evaluation and robust results, we assessed serum CK levels, because this biochemical marker is commonly used in clinical practice to assess muscle recovery \textsuperscript{[46], [15, 17, 21]}. Some studies have reported a possible systemic effect of PBMT \textsuperscript{[47]}, suggesting that this effect may optimize the time of application and obviate the need to irradiate all muscle clusters involved in the exercise. This effect is attributed to the direct release of nitric oxide from hemoglobin and nitrosylated myoglobin \textsuperscript{[47]} causing vasodilatation, increased blood flow, and faster recovery of muscles throughout the body. However, it is also known that PBMT causes biological alterations at the cellular level secondary to interactions between photons and cytochrome c-oxidase, an enzyme present in the mitochondria, which is a cellular organelle that is not present in the blood. The duration of this interaction should be adequate to last until complete application of the ideal dose, thereby achieving the desired effect on the target tissue \textsuperscript{[1–4]}. It must be emphasized that PBMT can increase or decrease cellular activity, based on the therapeutic window and the applied dose \textsuperscript{[13–23]}. Moreover, at least 30 s are required for ergogenic effects on muscles, and the application should be performed in a stationary position \textsuperscript{[39]}.

We observed that PBMT-sMF did not produce effects when applied to sites distant from the irradiated area. This is an interesting finding that highlights an important aspect associated with the safety of this therapy and also the possibility of adverse effects, such as systemic effects caused by certain drugs \textsuperscript{[48]}. For example, some non-steroidal anti-
inflammatory drugs (NSAIDs) inhibit cyclo-oxygenase-2 (COX-2) activity, thereby reducing inflammation and pain [48]. However, the NSAID-induced systemic reduction in COX-2 activity may cause serious adverse effects [48], such as changes in gastric mucosal protection [49, 50] or a high risk of myocardial infarction [51].

The lack of effects in areas distant from the irradiated site leads to the conclusion that the interactions between PBMT-sMF and tissues occur only at the irradiated sites. This observation confirms that in addition to its aforementioned benefits, PBMT-sMF is a safe therapeutic alternative because it avoids systemic actions that could produce adverse effects in tissues distant from the application site. Therefore, our results highlight the relevance of PBMT-sMF in clinical practice and reiterate the importance of establishing an optimal approach for its application using the appropriate parameters. Moreover, our findings will guide therapists in the correct application of radiation to muscles involved in exercise to ensure improved performance and reduce fatigue [39]. In essence, partial irradiation or irradiation of muscles not involved in a specific activity is ineffective.

A limitation of the present study is that the possible effects of PBMT-sMF were not evaluated in tissues other than muscles in areas distant from the irradiated site. Therefore, further studies are needed to determine whether PBMT-sMF affects tissues other than muscles at sites distant from the irradiated area.

**CONCLUSION**

Our results show that PBMT-sMF irradiation can improve performance and reduce muscle fatigue only when applied locally to exercised muscles. No positive effects were observed in muscles distant from the irradiated site, indicating that muscles involved in physical activity should undergo local irradiation to achieve optimal ergogenic effects of PBMT-sMF therapy.
Abbreviations

ATP
adenosine triphosphate

COX2
oxygenated cycle 2

CK
Creatine Kinase

LLP
low power laser

LEDs
light emitting diode

MVC
Maximum Voluntary Contraction

NSAIDs
non-steroidal anti-inflammatory drugs

PBMT
Photobiomodulation Therapy

sMF
static magnetic field

VAS
visual analogic scale

Declarations

Ethics approval and consent to participate: The study followed the ethical guidelines of
and was approved by the Research Ethics Committee of Nove de Julho University (protocol
number 2100849).

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analyzed during the current
study are available from the corresponding author on reasonable request.

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Authors' contributions

CSMM, HLC, and ECPL-J contributed to the concept, design of the study, established the hypothesis, and wrote the original proposal. CSMM, HLC, AAV, JBA, PTCC and ECPL-J contributed significantly in creating the manuscript. AVV and PTCC performed critical revisions of the manuscript. CSMM, HLC, and ECPL-J wrote the final version of the manuscript. All authors read and approved the final version of the manuscript.

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table 2

Due to technical limitations Table 2 is available in the supplementary section

Figures
Figure 1

CONSORT flowchart.
Figure 2

Treatment sites at knee extensor muscles.
Figure 3
Percentage of change in CK activity. a indicates difference statistical significance of $p < 0.05$ of placebo; b indicates difference statistical significance of $p < 0.05$ of non-exercised leg.
Figure 4

Percentage of change in blood lactate. a indicates difference statistical significance of \( p < 0.05 \) of placebo; b indicates difference statistical significance of \( p < 0.05 \) of non-exercised leg.
- Evaluation of Delayed Onset Muscle Soreness (DOMS). a indicates difference statistical significance of $p < 0.05$ of placebo; b indicates difference statistical significance of $p < 0.05$ of non-exercised leg.
Percentage variation of the Maximum Voluntary Contraction (MVC). a indicates difference statistical significance of p < 0.05 of placebo; b indicates difference statistical significance of p < 0.05 of non-exercised leg.

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

CONSORT 2010 Checklist.doc
Table 2. Outcomes.docx