Examining the Acute Effects of Low-Level Laser Therapy (LLLT) on Pain Threshold and Pain Tolerance: A Randomized Controlled Trial

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Research Problem, Aim, and Significance

Introduction

Pain is defined by the International Association for the Study of Pain (IASP, 2020) as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage." Pain can be classified into several types: nociceptive pain, neuropathic pain, and nociplastic pain. Nociceptive pain results from actual or potential damage to non-neural tissue and is due to the activation of nociceptors. It is typically acute and arises from injury, inflammation, or mechanical stimuli. Neuropathic pain, on the other hand, is caused by damage or disease affecting the somatosensory nervous system. Nociplastic pain involves altered nociception despite no clear evidence of tissue damage or nerve injury.

Acute nociceptive pain is one of the most common presentations in musculoskeletal conditions and plays a crucial protective role by alerting the body to tissue damage and prompting healing responses.(Woolf, 2010) However, when inadequately controlled, it may induce central sensitization and predispose patients to chronic pain syndromes. To address acute pain non-pharmacologically, physiotherapists typically employ a multimodal approach: Transcutaneous Electrical Nerve Stimulation (TENS), which harnesses sensory-level stimulation to activate spinal inhibitory circuits and dampen pain signals (Khadilkar et al., 2020); therapeutic ultrasound (US), where mechanical vibration and mild thermal effects accelerate inflammatory resolution and collagen synthesis (Uddin et al., 2021); cryotherapy and thermotherapy, which modulate local tissue temperature to reduce nociceptor excitability and optimize perfusion (Jinnah et al., 2019; Chen et al., 2024); and manual therapy—including joint mobilization, soft-tissue techniques, and muscle-energy methods—that elicits both mechanical and neurophysiological analgesia, improving range of motion and function (Serrano-García et al., 2025).

More recently, laser therapy has emerged as a powerful adjunct for acute pain relief. Laser therapy refers to the medical use of specific light wavelengths to elicit therapeutic effects in tissues. Broadly, there are two main types used in Pysiotherapy: High-intensity laser therapy (HILT) and Low-Level Laser Therapy (LLLT). HILT operates at higher power outputs (typically >500 mW) and can penetrate deeper tissues, producing photothermal effects. In contrast, LLLT (also termed photobiomodulation or cold laser

therapy) uses lower power levels and longer exposure times, primarily inducing photochemical and photobiological changes without significant heating . LLLT is favored for its safety and gentle stimulation of tissue repair in a wide range of all conditions.

LLLT, involves the application of red or near-infrared light (typically 600–1100 nm) at low intensities to avoid thermal effects. It has been widely adopted in clinical practice due to its non-invasive nature, minimal side effects, and effectiveness in reducing pain, promoting wound healing, and modulating inflammation (Lang-Illievich et al., 2020; Lawrence, 2024).

The mechanisms underlying LLLT are generally thought to involve the absorption of photons by cellular chromophores, most notably cytochrome c oxidase in the mitochondria, which in turn enhances mitochondrial respiration and ATP production, modulation of reactive oxygen species, and the induction of transcription factors that promote cell survival and tissue repair (Arslan et al., 2018; Lawrence, 2024). These cellular effects may also extend to modulation of the autonomic nervous system, where LLLT has been shown to promote parasympathetic activity and reduce sympathetic dominance, potentially contributing to its overall analgesic and anti-inflammatory outcomes (Chow et al., 2011; Laakso & Burke, 2019).

In clinical research, it is well established that participants' expectations can influence perceived outcomes, a phenomenon known as the placebo effect. The placebo effect refers to improvements in symptoms resulting from an individual's belief that they are receiving an active treatment, rather than from the treatment itself (Niazi, 2024). In pain research, placebo responses can elicit real neurophysiological changes, including activation of endogenous opioid and dopamine pathways, modulation of pain-related brain regions, and alterations in peripheral nociceptive processing (Blythe et al., 2023). These effects can significantly influence subjective measures of pain, such as pain threshold and tolerance, and may confound the assessment of an intervention's true efficacy. Therefore, to differentiate the specific effects of LLLT from expectancy-driven improvements, it is essential to include a sham (placebo) control group, in which participants undergo a procedure identical to the active treatment but without therapeutic laser emission. This design strengthens the internal validity of the study by controlling for placebo effects and allowing for accurate evaluation of LLLT's immediate analgesic properties.

These effects are all relevant to pain: reduced inflammation and faster tissue repair can indirectly alleviate pain, and direct neural effects can alter pain signaling. Indeed, a body of evidence indicates that LLLT has clinically significant analgesic properties. Studies in various conditions such as (orthopedic injuries, rheumatologic diseases, and neuropathic pain syndromes) have shown that LLLT helps reduce pain. For instance, photobiomodulation has been successfully used to relieve musculoskeletal pain (such as neck and knee pain) and to accelerate recovery in sports injuries, often

leading to improvements in patients' pain ratings and functional outcomes (Hamblin, 2018). The appeal of LLLT in physiotherapy is further enhanced by its safety profile; it is generally well tolerated with minimal side effects, making it a useful adjunct or alternative to pharmacological analgesics in certain scenarios (Hamblin, 2018; Awatani et al., 2021).

Despite the growing use of LLLT for pain management, there remains a notable gap in the research literature regarding its immediate effects on pain perception in healthy, pain-free individuals. Much of the existing evidence for LLLT's analgesic efficacy comes from clinical studies on patients with chronic pain or from experiments measuring outcomes like visual analog scale (VAS) pain ratings, often after a series of treatment sessions (Hamblin, 2018; Awatani et al., 2021). These studies have established that LLLT can reduce pain over the course of days or weeks for various conditions. However, it is still unclear whether a single, brief application of LLLT can produce a meaningful change in an individual's pain threshold or pain tolerance in the immediate term. Pain threshold refers to the lowest intensity at which a stimulus is perceived as painful, while pain tolerance is the maximum intensity a person can endure. These measures are critical in evaluating the efficacy of analgesic interventions (Russo et al., 2018; Cimpean & David, 2019).Assessing both is important because they provide objective, complementary indices - threshold indexing sensory detection and tolerance indexing affective–motivational endurance - and both are well suited to detect immediate, single-session hypoalgesic effects and to inform possible peripheral or central mechanisms (Rolke et al., 2006).

In our study, we focus primarily on nociceptive pain, as the pain stimulus and measurement in this protocol target the activation of peripheral nociceptors through external stimulation in healthy individuals. Thus, understanding changes in nociceptive threshold and tolerance is central to evaluating the immediate analgesic effects of LLLT.`

Aim:

To assess the immediate effect of a single session of Low-Level Laser Therapy (LLLT) versus sham-control on pain threshold and pain tolerance in healthy adults.

Hypotheses:

Hypothese 1:

H0: There is no significant change in pain threshold and pain tolerance between the LLLT group and the sham-control group immediately after intervention.

H1: There is significant change in pain threshold and pain tolerance between the LLLT group and the sham-control group immediately after intervention.

Hypothese 2:

H0: There is no significant pre-to-post change in pain threshold and pain tolerance within the LLLT group.

H1: There is significant pre-to-post change in pain threshold and pain tolerance within the LLLT group.

Hypothese 3:

H0: There is no significant pre-to-post change in pain threshold and pain tolerance within the sham-control group.

H1: There is significant pre-to-post change in pain threshold and pain tolerance within the sham-control group.

Method

Methodology

Study Design;
This is a randomized, sham-controlled trial with a parallel-group, two-arm, superiority design, using a 1:1 allocation ratio.

Study site and population

The universe of this study will consist of healthy adult individuals who are currently enrolled at, or employed by, the European University of Lefke (EUL), Faculty of Health Sciences, located in the Turkish Republic of Northern Cyprus (TRNC). These individuals will represent a non-clinical population suitable for evaluating the acute effects of LLLT on experimentally induced pain responses, specifically pain threshold and pain tolerance levels.

The accessible population will include volunteers from the Department of Physiotherapy and Rehabilitation, as well as other departments within the university who meet the inclusion criteria.

All participants of the study will be informed about the research, and their verbal and written consent will be obtained (Appendix 1 and 2). Healthy adult volunteers will be recruited. Data collection will take place in the department's Physiotherapy and Rehablitation units.

After obtaining ethical approval, the study will start in October 2025, and participant recruitment and data collection will run from October 2025 to January 2026.

Randomization and blinding technique

All eligible participants are randomly assigned to one of two groups - (LLLT) group or the Sham-control group in a 1:1 ratio using computer-generated randomization. The allocation sequence is sealed in opaque envelopes. This study will be conducted using a single-blind design. Participants will be blinded to the group allocation. The participants will not be informed whether they are receiving active LLLT or sham laser treatment. To maintain blinding integrity, only the researcher administering the interventions will have access to the randomization codes, which will be concealed until the completion of data analysis.

Inclusion Criteria

- Age between 18 and 35 years.
- Healthy individuals without any chronic medical conditions.
- Free from both chronic and acute pain at the time of participation.
- All genders will be included to ensure broader generalizability of results.

Exclusion Criteria

- Menstruation at the time of testing, to minimize variability in pain perception related to hormonal fluctuations.
- Sensory deficits, such as failure to pass the sharp–dull discrimination test, to confirm intact somatosensory function.
- Pregnancy, due to safety considerations and physiological changes affecting pain perception.
- History of Epilepsy.
- Presence of cardiovascular conditions, including cardiac pacemakers.
- Acute hand injuries (e.g., fractures or recent trauma).
- Diagnosed neurological disorders.
- Use of analgesic or non-steroidal anti-inflammatory drugs (NSAIDs) within the previous 48 hours, as these may influence autonomic responses and alter pain perception.
- Any contraindications to the application of LLLT or electrical stimulation (e.g., epilepsy, malignancy, light sensitivity).

Sample Size and Sampling Technique

The study population will include healthy adults aged 18–35 years from the European University of Lefke, Faculty of Health Sciences. Participants will be selected through voluntary recruitment and screened according to predefined inclusion and exclusion criteria. The sample size was estimated using G*Power (v3.1.9.4 for Mac) for a 2 × 2 mixed repeated-measures ANOVA (within-factor = Time [pre, post]; between-factor = Group [LLLT, Sham]). Assuming a moderate effect size ($f = 0.25$), $\alpha = 0.05$, power $(1 – \beta) = 0.80$, and correlation among repeated measures $r = 0.5$, the required total sample was calculated as 52 participants (26 per group). This size provides adequate power to detect significant Group × Time interactions in pain-threshold and pain-tolerance measures, consistent with similar LLLT studies in healthy volunteers (Chow et al., 2009; Dundar et al., 2015; Alfredo et al., 2009).

Research Variables:

Dependent Variables:

Dependent variables are: Pain Threshold and Pain Tolerance.

Independent Variables:

Independent variables are: Age, Gender, Height, Weight, Body Mass Index (BMI), Dominant Hand, and Occupation.

These variables will be measured pre- and post-intervention to evaluate the effects of LLLT.

Data Collection Tools:

Demographic Information Form (see Appendix 3,4)

This form gathers essential participant details such as age, gender, dominant hand, occupation, anthropometric measurements (height, weight, BMI), history of pain, and any contraindications for laser or electrical stimulation.

Pain Assessment (see Appendix 5)

Pain threshold and tolerance will be assessed via tetanic faradic stimulation with gradual current increase in current intensity. Participants will provide verbal feedback to indicate initial pain perception and their maximum tolerance level. Throughout the procedure, The researcher will actively monitor the procedure and ask participants about their sensations during the electrical stimulation to ensure accurate reporting. Electrode placement and stimulation parameters will follow a standardized protocol to ensure consistency.

Pain Assessment Protocol:

- Before starting the assessment, a **sharp/dull sensory test** will be performed on the dominant hand to confirm intact cutaneous sensation and to ensure that participants are able to provide reliable feedback. Participants who demonstrate impaired sensory perception will not proceed further.
- Pain threshold and tolerance will then be evaluated **before and after** LLLT(or shamLLLT) using **tetanic faradic stimulation** delivered by a Enraf **Nonius IONOPULSIS 4192** electrotherapy unit (Doğuluer, Gurses, Demir, & Ozyilmaz, 2003).

Electrode placement:

- **Cathode:** will be placed on the palmar surface of the third finger of the dominant hand. As an active electrode pen electrode will be used.
- **Anode :** will be positioned over the medail epicondyle of the same arm. As an passive electrode disposable electrode (15*5cm) will be used.
- This placement is chosen to follow the pathway of the **median and radial nerves**, allowing for consistent stimulation and reliable assessment of pain perception (Jiang, Muceli, Graimann, & Farina, 2011).
- A new set of self-adhesive electrodes will be applied for each participant to maintain hygiene and standardization.

Stimulation parameters:

Current type: tetanic faradic current

Pulse duration: 1 ms

Interpulse interval: 20 ms

Intensity: will be increased gradually at a rate of 0.1 mA/sec

Stimulation time will be continuously monitored by the researcher.

Assessment procedure:

Participants will be instructed to provide verbal feedback at two points during stimulation:

Pain threshold: Point at which sensation becomes painful.

Pain tolerance: Maximum tolerable level of pain.

The researcher will supervise the procedure throughout, monitor participant responses, and ask about the sensations experienced to ensure accuracy and reliability of the data collected.

Interventions

Participants will be randomly assigned two groups. LLLT group will receive laser treatment, sham-control group will have same prosedures without the activation of the LLLT.

LLLT group

LLLT will be applied using a 904 nm wavelength laser device. The parameters and treatment procedure are as follows:

Device: Chattanooga model no. 422.

Frequency: 1000 Hz

Dosage: 0.9 J/cm²

Application time: 6 minutes total (2 minutes per point)

Number of points: 3 points

Treatment area: Medail epicondyle region, targeting the **ulnar and median nerve pathways** for standardized application.

During all applications, both the participant and the researcher will wear **protective laser safety glasses** to ensure safety and prevent accidental exposure. A consistent protocol will be followed for all participants to ensure treatment reliability and reproducibility.

Sham LLLT protocol: Participants in the sham group will receive the same device placement and application duration as the active group, but the laser output will be switched off. and participants will wear the same safety goggles. This sham procedure maintains blinding while delivering no therapeutic laser energy.

Sham-control group

Participants in the sham laser treatment group will undergo the same treatment protocol as the LLLT group; however, the laser device will be deactivated or set to emit no therapeutic dose. This procedure ensures that participants experience similar interactions and procedures without receiving active treatment, thereby maintaining blinding and controlling placebo effects.

Post-treatment Pain Reassessment:

Both groups will receive the **same faradic stimulation protocol** will be repeated after the intervention (active or sham) to reassess pain threshold and tolerance.

Statistical Analysis

Data will first be screened for accuracy, missing values, and outliers. The Shapiro–Wilk test and Levene's test will be used to examine the assumptions of normality and homogeneity of variances, respectively. Descriptive statistics will be presented as mean ± standard deviation or median (min–max) for continuous variables, and as frequency (percentage) for categorical variables. The main analysis will be conducted using a 2 × 2 mixed repeated-measures ANOVA, with Group (LLLT vs Sham) as the between-subjects factor and Time (Pre vs Post) as the within-subjects factor. The Group × Time interaction will be the primary focus, indicating whether treatment-related changes in pain threshold and pain tolerance differ between the two groups. When significant effects are observed, Bonferroni-adjusted pairwise comparisons will be performed to determine the direction of the differences. If the assumptions of normality or homogeneity of variances are violated, a nonparametric alternative will be applied. Specifically, the Aligned Rank Transform (ART) ANOVA procedure will be used, as it allows factorial analyses and interaction testing under nonparametric conditions. In cases where the ART model is not applicable, separate Wilcoxon signed-rank tests (for within-group comparisons) and Mann–Whitney U tests (for between-group comparisons) will be performed. Effect sizes will be reported as partial eta squared (η^2_p) for parametric analyses and as r for nonparametric tests. A p-value < 0.05 will be considered statistically significant in all tests. All statistical analyses will be performed using IBM SPSS Statistics (version 26 for Mac) and R (v4.3 for Mac).

Appendices

Appendix 1: Informed Consent(English)

INFORMED CONSENT FORM

You have been invited to take part in the study titled "**Examining of the acute effects of Low-Level Laser Therapy (LLLT) on pain threshold and tolerance: a randomized controlled trial**" This study is conducted for research purposes and participation is voluntary. After you have been fully informed about the study and your questions have been answered, you will be asked to sign this form if you wish to participate. This research is under the responsibility of Asst. Prof. Dr. Beraat ALPTUG from the Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, European University of Lefke.

The aim of this study is to examine the immediate effects of Low-Level Laser Therapy (LLLT) on pain threshold and pain tolerance. Within the scope of our study, you will undergo assessment of pain perception through tetanic faradic stimulation before and after the application of LLLT. LLLT will be applied to the medail epicondyle region for a few minutes, following a standardized protocol by a physiotherapist.

The researchers will use your personal information to conduct the research and statistical analysis, but your identity will be kept confidential. At the end of the study, you have the right to request information

about your own results. The study results may be published in the medical literature at the end of the study, but your identity will not be disclosed. There will be no conflict of interest, such as the participation of individuals in the study in a physiotherapy program, which will provide financial support to any of the researchers at the end of the study.

I have read the information that must be given to the volunteer before the research above. I have been given written explanations about these. Under these conditions, I agree to participate in the research with my own consent, without any pressure or coercion.

Name-Surname:

Date:

Signature:

Phone Number:

The researcher who made the written statements:

Name-Surname:

Phone Number:

Signature:

The person who gave the witness:

Name-Surname:

Phone Number:

Signature:

Appendix 3: Demographic Information Form

- Age: _____
- Gender: ☐ Male ☐ Female ☐other
- Dominant Hand: ☐ Right ☐ Left
- Occupation: _____
- Height : _____
- Weight: _____
- Body Mass Index (BMI):_____
- Do you have any history of acute or chronic pain?

  ☐ No ☐ Yes If yes, please specify: _____
- Do you have any known contraindications for laser therapy or electrical stimulation?

  ☐ No ☐ Yes If yes, please provide details (e.g., pacemaker, pregnancy, epilepsy):

  _____

Appendix 5: Evaluation Record Table

Test Phase	**Value (mA)**	**Test Phase**	**Value (mA)**
BTh (Before Threshold)		ATh (After Threshold)	
BTt (Before Tolerance)		ATt (After Tolerance)	

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