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High-Dose Melatonin in Tibial Fracture Healing: A Prospective Non-Randomized Controlled Pilot Study with 49-Day Follow-Up

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ADMINISTRATIVE INFORMATION

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Review Stage at time of this submission - Completed but not published.

Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 3 September 2025 and was last updated on 3 September 2025.

INTRODUCTION

Review question / Objective Potential effects of melatonin on bone healing.

Rationale This pilot study addresses critical translational gaps in melatonin research for bone healing by investigating high-dose melatonin (30 mg/day) in tibial fracture patients, where robust preclinical evidence showing melatonin's osteogenic, anti-inflammatory, and antioxidant properties contrasts sharply with sparse and inconsistent human studies limited to low doses (1-10 mg/day) that may be subtherapeutic due to melatonin's poor oral bioavailability (10-15%). The selected 30 mg/day dose represents a pharmacological strategy aimed at achieving supraphysiological serum levels based on preclinical efficacy data, while high-dose melatonin (≥30 mg/day) has never been systematically evaluated in orthopedic populations where polypharmacy and variable metabolic status may affect drug responses. As an exploratory pilot study conducted in the absence of prior clinical data on pharmacological melatonin doses in orthopedic fracture care, this investigation prioritizes feasibility, safety, and preliminary biological insights over definitive efficacy claims, focusing on protocol viability and intervention tolerability within Brazil's resource-constrained healthcare system (SUS) where cost-effective adjuvant therapeutic strategies are urgently needed to address the substantial burden of tibial fractures with their 12-19% delayed union rates and associated economic and social costs.

Condition being studied The condition being studied is tibial fracture healing, specifically focusing on the early bone healing process in patients with diaphyseal tibial shaft fractures.

Key details of the condition:

- Specific fracture type: Closed tibial shaft fractures classified as AO/OTA 42-A, 42-B, or 42-C
- Treatment method: Reamed intramedullary nailing (surgical fixation)
- Patient population: Adults aged 18-60 years with ASA physical status I or II
- Focus of study: Early bone healing and consolidation process during the first 49 days post-surgery

Context of the condition
- Tibial shaft fractures represent a significant orthopedic challenge with delayed union rates of 12-19% of cases
- These injuries often result from high-energy trauma and are associated with substantial soft tissue damage
- The study investigates whether high-dose melatonin (30 mg/day) can accelerate the natural bone healing process compared to standard postoperative care alone

The research specifically examines the biological and radiographic markers of bone healing, including serum alkaline phosphatase levels and Radiographic Union Score for Tibial fractures (RUST) scores, to determine if melatonin supplementation can improve fracture healing outcomes.
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METHODS

Search strategy Comprehensive literature review across multiple databases including PubMed, Embase, and Cochrane Library to identify relevant preclinical and clinical evidence supporting melatonin's role in bone healing. The search would employ a combination of MeSH terms and free-text keywords such as "melatonin," "bone healing," "fracture repair," "osteogenesis," "alkaline phosphatase," "tibial fractures," and "intramedullary nailing," using Boolean operators (AND, OR, NOT) to create targeted search strings. The strategy would prioritize systematic reviews and randomized controlled trials as the highest levels of evidence, while also including relevant preclinical studies that demonstrate melatonin's osteogenic mechanisms through MT1/MT2 receptor signaling, antioxidant properties, and modulation of bone turnover markers. Given the limited clinical data on high-dose melatonin in orthopedic applications, the search would extend to related fields including osteoporosis research, bone regeneration studies, and pharmacokinetic investigations to establish the theoretical framework and safety profile necessary for this pilot investigation.

Participant or population Adult patients (aged 18-60 years) with tibial shaft fractures (AO/OTA classification 42-A, 42-B, or 42-C) undergoing surgical treatment with intramedullary nailing. Participants should have ASA physical status I or II, representing healthy individuals or those with mild, well-controlled systemic disease.

Intervention Melatonin supplementation administered orally at various doses (ranging from 30 mg/day) for bone healing enhancement.

Comparator Standard postoperative care including analgesia, thromboprophylaxis, antibiotic prophylaxis, and rehabilitation protocols. Comparators may include placebo, no intervention, or other bone healing adjuvants such as bisphosphonates, teriparatide, or platelet-rich plasma.

Study designs to be included Randomized controlled trials (RCTs), non-randomized controlled trials, prospective and retrospective cohort studies, case-control studies, and pilot studies investigating melatonin's effects on bone fracture healing in human subjects.

Eligibility criteria Inclusion: Studies involving human subjects with long bone fractures, melatonin as intervention, bone healing outcomes reported, English language publications. Exclusion: Pathological fractures, animal studies, case reports with <5 participants, studies without clear outcome measures, duplicate publications.

Information sources Electronic databases: PubMed/MEDLINE, Embase, Cochrane Central Register of Controlled Trials, Web of Science, and Scopus. Additional sources include clinical trial registries (ClinicalTrials.gov, WHO ICTRP), reference lists of included studies, conference abstracts, and contact with experts in the field for unpublished data.

Main outcome(s) Primary outcomes include biochemical markers of bone formation (alkaline phosphatase levels, osteocalcin, P1NP) measured at 3-6 weeks post-intervention, and radiographic healing assessment using standardized scores (RUST, mRUST) evaluated at 6-24 weeks post-surgery. Time to radiographic union and functional recovery measures will also be assessed.

Additional outcome(s) Secondary outcomes include adverse events, treatment adherence rates, sleep quality measures (PSQI, ESS), pain scores, time to weight-bearing, infection rates, delayed union or nonunion rates, and patient-reported outcome measures. Long-term functional outcomes and quality of life measures at 6-12 months follow-up.

Data management Study records will be managed using reference management software (EndNote/Zotero). Data extraction will be performed using standardized forms in Microsoft Excel or RevMan 5. Two reviewers will independently screen titles, abstracts, and full-text articles.

Quality assessment / Risk of bias analysis This study was reported in accordance with multiple international guidelines. The TREND (Transparent Reporting of Evaluations with Nonrandomized Designs) check-list structured the reporting of participant flow, intervention allocation, and outcome assessment (Supplementary Table S1). The TIDieR (Template for Intervention De-scription and Replication) checklist was employed to detail the intervention protocol, ensuring replicability in future trials (Supplementary Table S2). Additionally, key elements from the STROBE statement were integrated to support robust observational reporting (Supplementary Table S3), and the CONSORT 2025 (Consolidated Stand-ards of Reporting Trials) extension for non-randomised trials was used to enhance clarity in describing eligibility, flow, and results (Supplementary Table S4). Further supporting materials include the eligibility criteria used during patient selection (Sup-plementary Table S5) and the pharmaceutical technical dossier for the compounded melatonin (Supplementary Table S6). Quality assessment will use the Cochrane Risk of Bias tool (RoB 2) for randomized trials and ROBINS-I for non-randomized studies. Assessment domains include randomization process, deviations from intended interventions, missing outcome data, measurement of outcomes, and selection of reported results. GRADE approach will assess evidence certainty.

Strategy of data synthesis This pilot study employed a comprehensive quantitative data synthesis approach combining descriptive and inferential statistical methods. Primary outcome measures (serum alkaline phosphatase levels and RUST scores) were analyzed using two-way repeated measures ANOVA to assess intervention effects across three time points (baseline, 21 days, 49 days). Inter-rater reliability for RUST assessments was evaluated through Bland-Altman analysis. Secondary outcomes including adherence rates, adverse events, and sleep quality metrics were synthesized using descriptive statistics. Data synthesis utilized STATISTICA 7.0 for statistical computations and GraphPad Prism 5.0 for visualization, with 95% confidence intervals calculated for all primary measures. Effect sizes were computed using partial eta squared for ANOVA results, providing comprehensive evidence synthesis for this exploratory investigation.

Subgroup analysis Fracture type (closed vs. open).

Sensitivity analysis Sensitivity analyses will exclude studies with high risk of bias, include only randomized controlled trials, exclude studies with significant missing data, and test the impact of different statistical models (fixed vs. random effects). Analysis excluding outlier studies and including only studies with clearly defined outcome measures will be conducted.

Language restriction No language restrictions will be applied initially, but studies in languages other

than English will require translation for full assessment.

Country(ies) involved Brazil.

Other relevant information The dissemination strategy for this high-dose melatonin pilot study targets multiple stakeholders through academic publication in orthopedic journals, presentations at international conferences, and engagement with practice guideline committees.

Keywords tibial fracture; bone healing; melatonin; high-dose; intramedullary nailing; fracture union; RUST score; alkaline phosphatase; pilot study; clinical safety.

Contributions of each author

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