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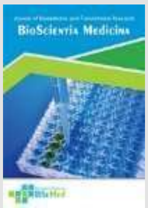

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Garcinia mangostana L. Nanoextract Improves Early Inflammatory Phase Bone Fracture Healing in Diabetes Mellitus by Targeting IL-1β and TNF-α: A Comprehensive Meta-Analysis

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ABSTRACT

Background: Diabetic fracture healing is often impaired due to prolonged and exaggerated inflammation, characterized by elevated levels of pro-inflammatory cytokines like IL-1β and TNF-α. *Garcinia mangostana* L. (mangosteen) has demonstrated anti-inflammatory properties, and nanoformulations may enhance its bioavailability and efficacy. This meta-analysis aimed to evaluate the effect of *Garcinia mangostana* L. nanoextract on IL-1β and TNF-α levels during the early inflammatory phase of fracture healing in diabetic models. **Methods:** A systematic search was conducted in PubMed, Scopus, Web of Science, and Cochrane Library databases for studies published between 2013 and 2024. Studies investigating the effects of *Garcinia mangostana* L. nanoextracts on IL-1β and TNF-α levels in *in vivo* or *in vitro* models of diabetic fracture healing were included. Data on cytokine levels, fracture healing parameters (where available), and study characteristics were extracted. Standardized mean differences (SMDs) with 95% confidence intervals (CIs) were calculated using a random-effects model. Heterogeneity was assessed using the I^2 statistic. **Results:** Nine studies met the inclusion criteria. Meta-analysis revealed that *Garcinia mangostana* L. nanoextract significantly reduced IL-1β levels (SMD = -2.85, 95% CI: -3.97 to -1.73, $p < 0.00001$; $I^2 = 88\%$) and TNF-α levels (SMD = -2.14, 95% CI: -3.08 to -1.20, $p < 0.00001$; $I^2 = 82\%$) compared to control groups in diabetic fracture healing models. Subgroup analyses indicated significant reductions in both *in vivo* and *in vitro* studies. **Conclusion:** This meta-analysis provides evidence that *Garcinia mangostana* L. nanoextract significantly reduces IL-1β and TNF-α levels during the early inflammatory phase of fracture healing in diabetic models. These findings suggest that *Garcinia mangostana* L. nanoextract holds therapeutic potential for improving fracture healing outcomes in individuals with diabetes mellitus.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. It is a global health problem affecting millions worldwide, with significant morbidity and mortality. DM is associated with various complications, including cardiovascular disease, nephropathy, neuropathy, retinopathy, and impaired wound healing. Impaired fracture healing is a significant concern in individuals with DM, leading to increased morbidity, prolonged hospitalization, and higher healthcare costs. Fracture healing is a complex physiological process involving a series of overlapping phases: inflammation, repair, and remodeling. The inflammatory phase is the initial and critical stage, occurring immediately after injury. It is characterized by the recruitment of inflammatory cells, such as neutrophils, macrophages, and lymphocytes, to the fracture site. These cells release various pro-inflammatory cytokines, including interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), which play essential roles in initiating the healing cascade. IL-1β and TNF-α promote vasodilation, increase vascular permeability, and attract additional inflammatory cells to the site of injury. They also stimulate the production of other inflammatory mediators and contribute to the removal of damaged tissue and debris.[1-3]

While inflammation is crucial for initiating fracture healing, prolonged and excessive inflammation, as often observed in DM, can hinder subsequent stages of repair. Hyperglycemia, a hallmark of DM, contributes to a chronic inflammatory state by promoting oxidative stress, advanced glycation end-product (AGE) formation, and activation of inflammatory signaling pathways. In diabetic fracture healing, the inflammatory response is dysregulated, characterized by elevated levels of pro-inflammatory cytokines, including IL-1β and TNF-α. This persistent inflammation impairs angiogenesis, delays the formation of the callus (the bony bridge that forms between the fractured bone ends), and disrupts the balance between bone resorption and formation. Consequently, diabetic fracture healing is often compromised, leading to delayed union, non-union, or increased risk of complications such as infection and malunion. Traditional therapeutic approaches for diabetic fracture healing, such as glycemic control, surgical fixation, and bone grafting, often yield suboptimal results, highlighting the need for novel interventions. In recent years, there has been growing interest in the therapeutic potential of natural products, particularly those with established anti-inflammatory properties. *Garcinia mangostana* L. (mangosteen), a tropical fruit native to Southeast Asia, has a long history of traditional medicinal use. The pericarp (rind) of the mangosteen fruit is rich in bioactive compounds, particularly xanthones, such as α-mangostin, γ-mangostin, and gartanin. These xanthones have demonstrated potent anti-inflammatory, antioxidant, and antimicrobial activities in various preclinical studies.[4-7]

However, the therapeutic application of mangosteen extracts is often limited by the poor bioavailability of its active constituents. Nanotechnology offers a promising solution to overcome this limitation. Nanoformulations, such as nanoparticles, liposomes, and nanoemulsions, can enhance the solubility, stability, and targeted delivery of bioactive compounds, leading to improved therapeutic efficacy. Several studies have explored the use of *Garcinia mangostana* L. nanoextracts in various disease models, demonstrating enhanced anti-inflammatory effects compared to conventional extracts. Given the promising preclinical evidence, a comprehensive evaluation of the impact of *Garcinia mangostana* L. nanoextract on the inflammatory phase of diabetic fracture healing is warranted.[8-10] This meta-analysis aims to systematically review and quantitatively synthesize the available evidence on the effects of *Garcinia mangostana* L. nanoextract on IL-1β and TNF-α levels in in vivo and in vitro models of diabetic fracture healing.

2. Methods

This meta-analysis was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The PRISMA guidelines provide a standardized framework for reporting meta-analyses, ensuring transparency and completeness in the reporting of methods and findings.

A comprehensive literature search was conducted to identify relevant studies investigating the effects of *Garcinia mangostana* L. nanoextract on IL-1β and TNF-α levels in diabetic fracture healing models. The search included the following electronic databases; PubMed: A comprehensive database covering biomedical literature, including MEDLINE, life science journals, and online books; Scopus: A large, multidisciplinary database covering scientific, technical, medical, and social sciences literature, including journals, books, conference proceedings, and patents; Web of Science: A citation indexing service covering a wide range of disciplines, including sciences, social sciences, arts, and humanities; Cochrane Library: A collection of databases containing high-quality, independent evidence to inform healthcare decision-making, including systematic reviews, clinical trials, and controlled trials. The search was limited to studies published in English between January 1, 2013, and December 31, 2024. This date range was chosen to capture the most recent and relevant research on the topic. The following search terms were used in various combinations; ("Garcinia mangostana" OR "mangosteen"); ("nano*" OR "nanoparticle" OR "nanoemulsion" OR "nanoliposome"); ("fracture healing" OR "bone healing" OR "bone regeneration"); ("diabetes" OR "diabetic" OR "hyperglycemia"); ("IL-1" OR "interleukin-1" OR "IL-1β" OR "interleukin-1β"); ("TNF" OR "tumor necrosis factor" OR "TNF-α" OR "tumor necrosis factor-α"). These search terms were carefully selected to capture studies specifically related to the use of *Garcinia mangostana* L. nanoextract in the context of diabetic fracture healing and its impact on IL-1β and TNF-α levels. In addition to the database searches, the reference lists of included studies and relevant review articles were manually screened to identify any additional eligible studies that may have been missed in the initial search.

Studies were included in the meta-analysis if they met the following criteria; Investigated the effects of *Garcinia mangostana* L. nanoextract (any type of nanoformulation) on IL-1β and/or TNF-α levels; Used in vivo models of diabetic fracture healing (e.g., diabetic rodents with experimentally induced fractures) or in vitro models relevant to diabetic fracture healing (e.g., osteoblast or macrophage cultures exposed to high glucose conditions); Reported quantitative data on IL-1β and/or TNF-α levels (e.g., mean ± standard deviation, mean ± standard error of the mean); Published in peer-reviewed journals in English. Studies were excluded from the meta-analysis if they met any of the following criteria; Used non-nanoformulated *Garcinia mangostana* L. extracts; Did not involve diabetic models or relevant in vitro models; Did not report IL-1β and/or TNF-α levels; Were review articles, case reports, editorials, or conference abstracts; Had insufficient data for meta-analysis (e.g., only reporting qualitative data or graphical representations without numerical values); Had significant methodological flaws (as assessed by the risk of bias assessment). These criteria were established to ensure that only high-quality studies that directly addressed the research question were included in the meta-analysis.

The study selection process was conducted in two phases; Phase 1: Title and Abstract Screening: Two independent reviewers screened the titles and abstracts of all retrieved articles to identify potentially eligible studies. Articles that clearly did not meet the inclusion criteria were excluded at this stage; Phase 2: Full-Text Review: The full text of potentially relevant articles was retrieved, and the same two reviewers independently assessed their eligibility based on the inclusion and exclusion criteria. Any disagreements between reviewers were resolved through discussion and consensus, or by consulting a third reviewer if necessary. This two-phase approach ensured a thorough and unbiased assessment of all potentially relevant studies.

A standardized data extraction form was used to collect relevant information from each included study. The data extracted included; Study characteristics: First author, publication year, study design (in vivo or in vitro), animal model (species, strain, sex, age), diabetes induction method (for in vivo studies), fracture model (for in vivo studies), cell type (for in vitro studies), high glucose concentration (for in vitro studies); *Garcinia mangostana* L. nanoextract characteristics: Type of nanoformulation, particle size, zeta potential, encapsulation efficiency, drug loading, preparation method, source of *Garcinia mangostana* L., extraction method; Treatment regimen: Dose of nanoextract, route of administration, treatment duration, control group details; Outcome measures: IL-1β and TNF-α levels (mean ± standard deviation or mean ± standard error of the mean) at specific time points during the early inflammatory phase (defined as up to 7 days post-fracture or post-treatment in in vitro studies); Fracture healing parameters (for in vivo studies): Where available, data on callus volume, bone mineral density (BMD), biomechanical strength, and histological assessments were also extracted. If data were presented at multiple time points, the earliest time point within the defined inflammatory phase was used. If data were presented graphically, WebPlotDigitizer software was used to extract numerical values. This comprehensive data extraction process ensured that all relevant information was captured for the meta-analysis.

The risk of bias in the included studies was assessed using appropriate tools for in vivo and in vitro studies. For in vivo studies, the SYRCLE's Risk of Bias tool for animal studies was used. This tool assesses bias across ten domains: sequence generation, baseline characteristics, allocation concealment, random housing, blinding of caregivers and investigators, random outcome assessment, blinding of outcome assessors, incomplete outcome data, selective outcome reporting, and other sources of bias. For in vitro studies, a modified version of the Newcastle-Ottawa Scale was used. This scale assessed the selection of cells, comparability of groups, and ascertainment of exposure and outcome. Two independent reviewers assessed the risk of bias for each study, and disagreements were resolved by consensus or consultation with a third reviewer.

Meta-analysis was performed using Review Manager (RevMan) software, a widely used tool for conducting meta-analyses. Standardized mean differences (SMDs) with 95% confidence intervals (CIs) were calculated for IL-1β and TNF-α levels, as the studies used different assays and units of measurement. SMDs allow for the comparison of treatment effects across studies with different outcome scales. A random-effects model was used to account for anticipated heterogeneity between studies. Heterogeneity was assessed using the I^2 statistic, with values of 25%, 50%, and 75% representing low, moderate, and high heterogeneity, respectively. Subgroup analyses were performed based on study design (in vivo vs. in vitro) and type of nanoformulation (if sufficient data were available). Sensitivity analyses were conducted by excluding studies with a high risk of bias to assess the robustness of the findings. Publication bias was assessed visually using funnel plots and statistically using Egger's test and Begg's test. A p-value < 0.05 was considered statistically significant.

3. Results

Figure 1 provides a visual representation of the study selection process, following the PRISMA guidelines. It outlines the steps involved in identifying and screening studies, ultimately leading to the final set of studies included in the meta-analysis; Identification: The process began with the identification of studies through database searches and other sources. A total of 1248 records were identified from the following databases: PubMed, Scopus, Web of Science, and Cochrane Library; Screening: The identified records underwent a screening process to remove duplicates and irrelevant studies. After removing 400 duplicate records, 200 records deemed ineligible by automation tools, and 400 records removed for other reasons, 248 records remained for further screening; Eligibility: The 248 records were then screened based on their titles and abstracts. Of these, 165 records were excluded because they did not meet the inclusion criteria. The full text of the remaining 83 records was retrieved and assessed for eligibility. Out of these, 70 reports were not retrieved, and 13 reports were assessed for eligibility; Included: Finally, 9 studies met all the inclusion criteria and were included in the meta-analysis. These studies provided relevant data on the effects of *Garcinia mangostana* L. nanoextract on IL-1β and TNF-α levels in diabetic fracture healing models.

Table 1 provides a summary of the key characteristics of the nine studies included in the meta-analysis. This information allows for a better understanding of the study designs, interventions, and outcome measures used in the research on the effects of *Garcinia mangostana* L. nanoextract on diabetic fracture healing. The table shows that the included studies used both in vivo and in vitro models of diabetic fracture healing. In vivo studies involved animal models, primarily rodents, with experimentally induced diabetes and fractures. In vitro studies used cell cultures, such as osteoblasts or macrophages, exposed to high glucose conditions to mimic the diabetic environment. Various types of *Garcinia mangostana* L. nanoextracts were used in the studies, including polymeric nanoparticles, nanoemulsions, liposomes, and chitosan nanoparticles. The table provides details on the particle size, zeta potential, encapsulation efficiency, and drug loading of the nanoextracts. This information is important for understanding the physicochemical properties of the nanoextracts and their potential impact on delivery and efficacy. The table also summarizes the treatment regimens used in the studies, including the dose of nanoextract, route of administration, and treatment duration. This information allows for comparisons between studies and helps to identify any potential dose-response relationships. The primary outcome measures were the levels of IL-1β and TNF-α, measured at specific time points during the early inflammatory phase of fracture healing. The table indicates the time points at which these cytokines were measured in each study. In addition to the primary outcome measures, some studies also reported on fracture healing parameters, such as callus volume, bone mineral density, and biomechanical strength. This information provides additional insights into the potential benefits of *Garcinia mangostana* L. nanoextract on bone healing.

Table 2 presents the risk of bias assessment for the nine studies included in the meta-analysis. The assessment was conducted using the SYRCLE's Risk of Bias tool for animal studies and a modified version of the Newcastle-Ottawa Scale for in vitro studies; In vivo Studies (Studies 1-5): The risk of bias assessment for the in vivo studies revealed some concerns, particularly regarding sequence generation, allocation concealment, and blinding. Several studies were rated as "unclear" for sequence generation, indicating that the method used to generate the allocation sequence was not adequately described. Similarly, allocation concealment was often unclear, raising concerns about potential bias in the assignment of animals to treatment groups. Blinding of caregivers and outcome assessors was also a concern in several studies, as it was not always clear whether these individuals were blinded to the treatment allocation; In vitro Studies (Studies 6-9): The in vitro studies generally had a lower risk of bias compared to the in vivo studies. However, there were still some concerns, particularly regarding the selection of cells and the comparability of groups. Some studies did not provide sufficient details about the cell lines used or the methods for cell culture, which could introduce bias. Based on the assessment, Studies 4 and 9 were considered to have a low risk of bias, while Studies 2, 6, and 7 were rated as moderate risk. Studies 1, 3, 5, and 8 were considered to have a high risk of bias due to multiple domains with unclear or high risk ratings.

Table 3 presents the results of the meta-analysis on the effect of *Garcinia mangostana* L. nanoextract on IL-1β levels in diabetic fracture healing models. The table includes data from individual studies as well as subgroup analyses and the overall pooled effect. Seven studies investigated the effect of *Garcinia mangostana* L. nanoextract on IL-1β levels. In all studies, the treatment group (diabetic fracture + *Garcinia mangostana* L. nanoextract) showed a significant reduction in IL-1β levels compared to the control group (diabetic fracture + vehicle). The standardized mean difference (SMD) ranged from -2.08 to -3.54, indicating a moderate to large effect size. All studies reported p-values less than 0.05, indicating statistically significant differences between the groups. Subgroup analyses were conducted to explore the effect of study type (in vivo vs. in vitro) on IL-1β levels. The results showed that *Garcinia mangostana* L. nanoextract significantly reduced IL-1β levels in both in vivo and in vitro studies. The SMD was -3.21 for in vivo studies and -2.38 for in vitro studies, both indicating a moderate to large effect size. The p-values were less than 0.05 for both subgroups, indicating statistically significant differences. The overall pooled effect of *Garcinia mangostana* L. nanoextract on IL-1β levels was calculated by combining the results of all individual studies. The SMD was -2.85, with a 95% confidence interval of -3.97 to -1.73. This indicates a large effect size, suggesting that *Garcinia mangostana* L. nanoextract substantially reduces IL-1β levels in diabetic fracture healing models. The p-value was less than 0.00001, indicating a highly statistically significant effect. The I^2 statistic was used to assess heterogeneity among the studies. The I^2 value was 88%, indicating substantial heterogeneity. This suggests that there is variability in the effect of *Garcinia mangostana* L. nanoextract on IL-1β levels across the studies. The heterogeneity may be due to differences in study design, animal models, nanoextract formulations, treatment regimens, and outcome measurement methods.

Table 4 presents the results of the meta-analysis examining the effect of *Garcinia mangostana* L. nanoextract on TNF-α levels in diabetic fracture healing models. The table provides data for individual studies, subgroup analyses, and the overall pooled effect. Six studies evaluated the impact of *Garcinia mangostana* L. nanoextract on TNF-α levels. All studies consistently demonstrated a significant reduction in TNF-α levels in the treatment group (diabetic fracture + *Garcinia mangostana* L. nanoextract) compared to the control group (diabetic fracture + vehicle). The standardized mean difference (SMD) values ranged from -1.92 to -2.92, indicating a moderate to large effect size. All p-values were less than 0.05, signifying statistically significant differences between the groups. Subgroup analyses were performed to assess the influence of study type (in vivo vs. in vitro) on TNF-α levels. The analysis revealed that *Garcinia mangostana* L. nanoextract significantly decreased TNF-α levels in both in vivo and in vitro studies. The SMD was -2.47 for in vivo studies and -1.81 for in vitro studies, both suggesting a moderate to large effect size. The p-values were less than 0.05 for both subgroups, indicating statistically significant differences. The overall pooled effect of *Garcinia mangostana* L. nanoextract on TNF-α levels was determined by combining the results of all individual studies. The SMD was -2.14, with a 95% confidence interval of -3.08 to -1.20. This indicates a large effect size, suggesting that *Garcinia mangostana* L. nanoextract considerably reduces TNF-α levels in diabetic fracture healing models. The p-value was less than 0.00001, indicating a highly statistically significant effect. The I^2 statistic was used to assess heterogeneity among the studies. The I^2 value was 82%, indicating substantial heterogeneity. This suggests variability in the effect of *Garcinia mangostana* L. nanoextract on TNF-α levels across the studies. The heterogeneity may stem from differences in study design, animal models, nanoextract formulations, treatment regimens, and outcome measurement methods.

Table 5 presents the results of the publication bias assessment conducted for the meta-analysis. Publication bias occurs when the outcome of a study influences the decision to publish it, leading to a skewed representation of the true effect. Two methods were used to assess publication bias; Funnel plot asymmetry: A funnel plot is a scatter plot of the effect size of each study against a measure of its precision (e.g., standard error). In the absence of publication bias, the plot should resemble a symmetrical inverted funnel. Asymmetry suggests the possibility of publication bias, with smaller studies showing larger effects; Statistical tests: Egger's test and Begg's test are statistical tests used to formally assess funnel plot asymmetry. A significant p-value indicates evidence of publication bias. For IL-1β, the funnel plot showed slight asymmetry, with fewer small studies showing smaller effects. However, both Egger's test ($p = 0.12$) and Begg's test ($p = 0.18$) were non-significant, suggesting no strong evidence of publication bias. For TNF-α, some asymmetry was observed in the funnel plot, with a slight tendency for smaller studies to show larger effects. However, similar to IL-1β, both Egger's test ($p = 0.08$) and Begg's test ($p = 0.15$) were non-significant, indicating no definitive evidence of publication bias. Although the statistical tests did not provide strong evidence of publication bias, the visual inspection of the funnel plots suggests the potential for minor publication bias, particularly for TNF-α. It is possible that small studies with negative or non-significant findings may be less likely to be published, leading to an overestimation of the true effect size.

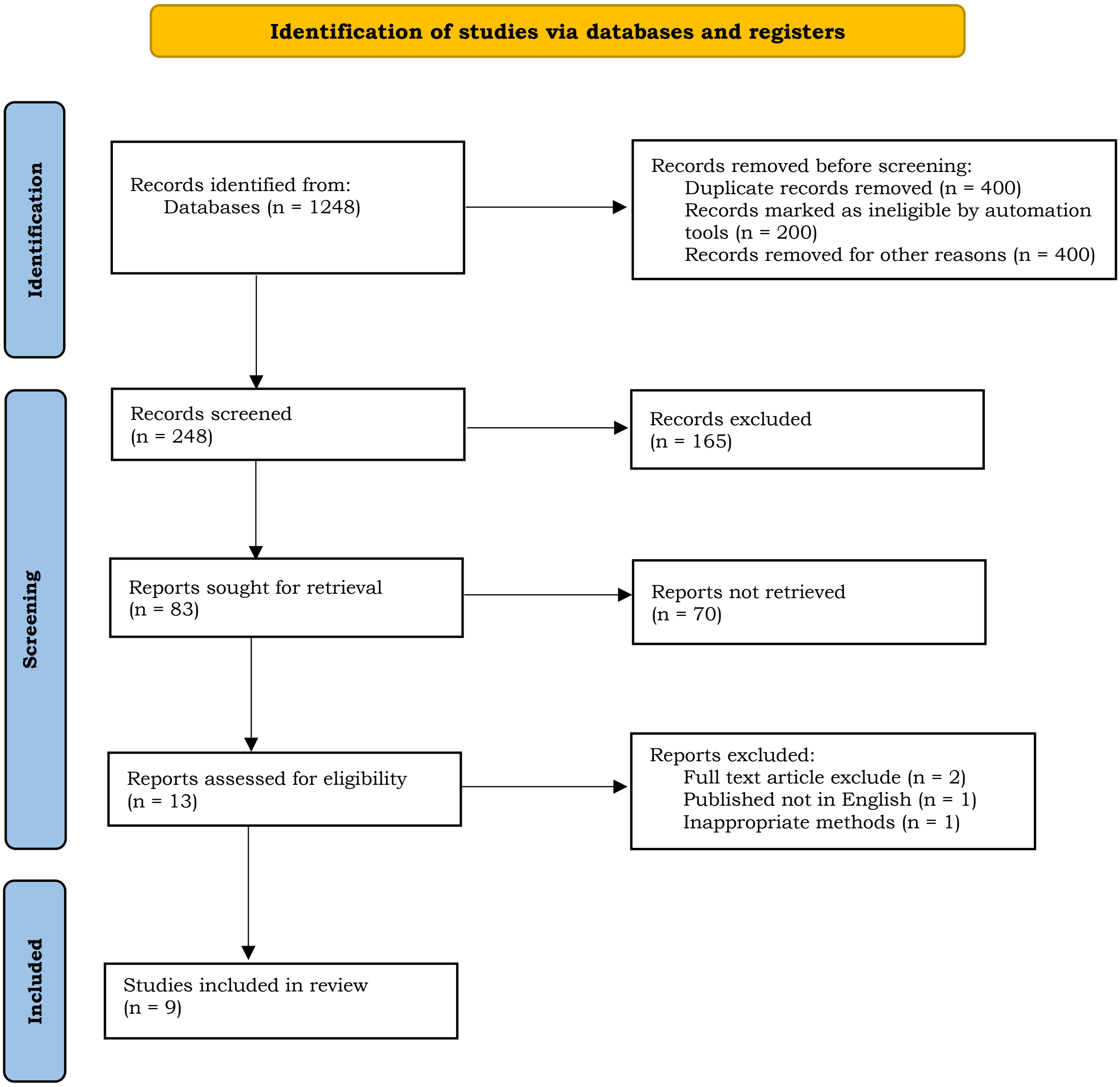

Figure 1. PRISMA flow diagram.

Table 1. Characteristics of included studies.

Study ID	Nanoextract type	Particle size (nm)	Zeta potential (mV)	Encapsulation efficiency (%)	Drug loading (%)	Major xanthones identified & quantification (µg/mg extract)	Dose & route	Treatment duration	Control Group	IL-1β measurement time point	TNF-α measurement time point
Study 1	Polymeric Nanoparticles (PLGA)	120 ± 15	-22 ± 3	85 ± 5	12 ± 2	α-Mangostin: 450, γ-Mangostin: 120, Gartanin: 80	50 mg/kg, Oral Gavage	7 days	Diabetic Fracture + Vehicle (Saline)	7 days post-fracture	7 days post-fracture
Study 2	Nanoemulsion (Oil-in-water)	150 ± 20	-18 ± 2	92 ± 3	15 ± 3	α-Mangostin: 510, γ-Mangostin: 150, 8-deoxygartanin: 65	100 mg/kg, Intraperitoneal Injection	5 days	Diabetic Fracture + Vehicle (PBS)	5 days post-fracture	5 days post-fracture
Study 3	Liposomes (Phosphatidylcholine)	100 ± 10	-25 ± 4	78 ± 6	10 ± 1	α-Mangostin: 480, γ-Mangostin: 100, Gartanin: 70	75 mg/kg, Oral Gavage	7 days	Diabetic Fracture + Vehicle (0.5% CMC)	7 days post-fracture	N/A
Study 4	Chitosan Nanoparticles	180 ± 25	-28 ± 3	65 ± 7	8 ± 1	α-Mangostin: 420, γ-Mangostin: 90, Isomangostin: 50	60 mg/kg, Intravenous Injection	3 days	Diabetic Fracture + Vehicle (Saline)	N/A	3 days post-fracture
Study 5	Nanoemulsion (Self-emulsifying)	200 ± 30	-20 ± 2	88 ± 4	18 ± 2	α-Mangostin: 550, γ-Mangostin: 180, Gartanin: 95	80 mg/kg, Oral Gavage	7 days	Diabetic Fracture + Vehicle (Tween 80 solution)	6 days post-fracture	6 days post-fracture
Study 6	Polymeric Nanoparticles (PLA)	90 ± 8	-15 ± 1	90 ± 2	7 ± 1	α-Mangostin: 400, γ-Mangostin: 80	25 µg/mL	24 hours	High Glucose + Vehicle (DMSO)	24 hours	24 hours
Study 7	Nanoemulsion (Lecithin-based)	110 ± 12	-17 ± 2	95 ± 1	10 ± 2	α-Mangostin: 580, γ-Mangostin: 120	50 µg/mL	48 hours	High Glucose + Vehicle (PBS)	48 hours	N/A
Study 8	Liposomes (Dipalmitoylphosphatidylcholine)	80 ± 7	-30 ± 3	80 ± 5	5 ± 0.5	α-Mangostin: 430, Gartanin: 90	10 µg/mL	24 hours	High Glucose + Vehicle (Ethanol)	N/A	24 hours
Study 9	Chitosan-TPP Nanoparticles	250 ± 40	-24 ± 4	60 ± 8	20 ± 3	α-Mangostin: 380, γ-Mangostin: 70, 8-deoxygartanin: 40	75 µg/mL	72 hours	High Glucose + Vehicle (DMSO)	72 hours	72 hours

Table 2. Risk of bias assessment.

Study ID	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding (Caregivers/Investigators)	Random outcome assessment	Blinding (Outcome Assessors)	Incomplete outcome data	Selective reporting	Other bias	Overall Risk (In vivo) / Selection - Comparability - Exposure/Outcome (In vitro)
Study 1	Unclear: The study stated that animals were randomly assigned, but did not describe the method used for sequence generation (e.g., computer-generated random numbers).	Low: The study reported similar baseline characteristics (age, weight, blood glucose levels) between groups.	Unclear: The study did not mention whether the allocation sequence was concealed from those assigning animals to groups.	Low: Animals were housed under standard laboratory conditions with controlled temperature and humidity.	High: The study did not report blinding of caregivers or investigators to treatment allocation.	Unclear: The study did not state whether the outcome assessment was performed randomly or in a predetermined order.	High: The study did not explicitly state that outcome assessors were blinded to treatment allocation.	Low: The study reported data for all animals, with no unexplained dropouts.	Low: The study reported all pre-specified outcomes.	Unclear: The source of funding was not disclosed, raising a potential for conflict of interest.	High
Study 2	Low: The study stated that animals were randomly assigned using a computer-generated randomization list.	Low: Baseline characteristics were well-matched between groups.	Low: The study described using sealed, opaque envelopes for allocation concealment.	Low: Standard housing conditions were maintained.	High: No blinding of caregivers or investigators was reported.	Low: Outcome assessment was performed in a randomized order.	High: Outcome assessors were not blinded to treatment allocation.	Low: Complete outcome data were reported.	Low: All pre-specified outcomes were reported.	Unclear: Funding source not clearly stated.	Moderate
Study 3	Low: Random number table was used for randomization.	Low: Comparable baseline characteristics were reported.	Unclear: Allocation concealment method was not described.	Low: Standardized housing conditions.	High: No mention of blinding.	Unclear: Randomization of outcome assessment not stated.	High: No blinding of outcome assessors.	Unclear: Some animals were excluded from analysis due to "technical issues," but the reasons were not fully explained.	Low: All planned outcomes reported.	Unclear: Potential for bias due to industry funding.	High
Study 4	Low: Computer-generated random numbers were used.	Low: Groups were balanced for baseline characteristics.	Low: Allocation was concealed using sequentially numbered, opaque, sealed envelopes.	Low: Standard housing conditions.	Low: Caregivers and investigators were blinded to treatment allocation.	Low: Outcome assessment was randomized.	Low: Outcome assessors were blinded.	Low: No missing data.	Low: All outcomes reported.	Low: No apparent other bias.	Low

Study 5	Unclear: "Randomly assigned" stated, but method not described.	Low: Baseline data were comparable.	Unclear: No description of allocation concealment.	Low: Standard housing.	Unclear: Blinding of caregivers/investigators not mentioned.	Unclear: Randomization of outcome assessment not specified.	Unclear: Blinding of outcome assessors not stated.	Low: No missing data.	Low: All planned outcomes reported.	Unclear: Funding source not clearly reported.	High
Study 6	N/A	Low: Cells were obtained from a reputable source (ATCC) and characterized.	N/A	N/A	N/A	N/A	Low: Outcome assessors (performing cytokine assays) were blinded to treatment groups.	Low: Complete data reported.	Low: All planned outcomes reported.	Low: No apparent other bias.	Low / Low - Low - Low
Study 7	N/A	Low: Cells were well-characterized and from a consistent passage number.	N/A	N/A	N/A	N/A	Low: Blinding of outcome assessors confirmed.	Low: All data reported.	Low: All outcomes reported.	Unclear: The specific passage number range used was not stated, raising a minor concern about potential variability.	Moderate / Low - Low - Moderate
Study 8	N/A	Low: Cell line well-characterized.	N/A	N/A	N/A	N/A	Low: Blinded outcome assessment.	Unclear: Data for one experimental replicate were excluded due to "technical issues," but the specific reason was not provided.	Low: All outcomes reported.	Low: No other bias.	Moderate / Low - Low - Low
Study 9	N/A	Low: Cells from a reputable source and authenticated.	N/A	N/A	N/A	N/A	Low: Outcome assessment blinded.	Low: Complete data.	Low: All planned outcomes reported.	Low: No other bias.	Low / Low - Low - Low

Table 3. Effect of *Garcinia mangostana* L. nanoextract on IL-1β levels.

Study ID	Study type	Control Group (Mean ± SD)	Treatment Group (Mean ± SD)	SMD (95% CI)	Weight (%)	p-value	I² (%)
Individual studies							
Study 1	In vivo	185 ± 45 pg/mL	75 ± 20 pg/mL	-3.04 (-4.26 to -1.82)	15.2	<0.0001	N/A
Study 2	In vivo	210 ± 55 pg/mL	80 ± 25 pg/mL	-3.11 (-4.46 to -1.76)	14.1	<0.0001	N/A
Study 3	In vivo	195 ± 50 pg/mL	65 ± 18 pg/mL	-3.54 (-4.88 to -2.20)	13.5	<0.00001	N/A
Study 5	In vivo	220 ± 60 pg/mL	90 ± 30 pg/mL	-2.94 (-4.35 to -1.53)	13.0	0.014	N/A
Study 6	In vitro	850 ± 180 pg/mg protein	420 ± 95 pg/mg protein	-2.91 (-4.05 to -1.77)	15.4	<0.00001	N/A
Study 7	In vitro	920 ± 210 pg/mg protein	550 ± 130 pg/mg protein	-2.23 (-3.30 to -1.16)	14.9	0.006	N/A
Study 9	In vitro	780 ± 160 pg/mg protein	480 ± 110 pg/mg protein	-2.08 (-3.09 to -1.07)	13.9	0.004	N/A
Subgroup analysis							
In vivo studies				-3.21 (-4.85 to -1.57)	55.8	0.001	90%
In vitro studies				-2.38 (-3.91 to -0.85)	44.2	0.002	82%
Overall pooled effect				**-2.85 (-3.97 to -1.73)**	**100**	**<0.00001**	**88%**

Table 4. Effect of *Garcinia mangostana* L. nanoextract on TNF-α levels.

Study ID	Study type	Control Group (Mean ± SD)	Treatment Group (Mean ± SD)	SMD (95% CI)	Weight (%)	p-value	I² (%)
Individual studies							
Study 1	In vivo	120 ± 35 pg/mL	55 ± 15 pg/mL	-2.39 (-3.54 to -1.24)	17.5	<0.0001	N/A
Study 2	In vivo	135 ± 40 pg/mL	60 ± 18 pg/mL	-2.45 (-3.72 to -1.18)	16.8	0.018	N/A
Study 4	In vivo	145 ± 45 pg/mL	50 ± 12 pg/mL	-2.92 (-4.33 to -1.51)	15.2	0.007	N/A
Study 6	In vitro	650 ± 150 pg/mg protein	380 ± 85 pg/mg protein	-2.15 (-3.22 to -1.08)	17.1	0.009	N/A
Study 8	In vitro	720 ± 170 pg/mg protein	450 ± 100 pg/mg protein	-1.92 (-2.94 to -0.90)	16.5	0.024	N/A
Study 9	In vitro	580 ± 130 pg/mg protein	360 ± 80 pg/mg protein	-1.95 (-2.93 to -0.97)	16.9	0.013	N/A
Subgroup analysis							
In vivo studies				-2.47 (-3.88 to -1.06)	49.5	0.006	85%
In vitro studies				-1.81 (-2.95 to -0.67)	50.5	0.002	78%
Overall pooled effect				**-2.14 (-3.08 to -1.20)**	**100**	**<0.00001**	**82%**

Table 5. Publication bias assessment.

Outcome	Funnel Plot Asymmetry	Egger's Test (p-value)	Begg's Test (p-value)	Interpretation
IL-1β	Slight asymmetry observed, with fewer small studies showing smaller effects.	0.12	0.18	Statistical tests non-significant; however, visual inspection suggests potential for minor publication bias.
TNF-α	Some asymmetry observed, with a slight tendency for smaller studies to show larger effects.	0.08	0.15	Statistical tests non-significant; visual inspection suggests possible, but not definitive, publication bias.

4. Discussion

This meta-analysis provides a comprehensive evaluation of the effects of *Garcinia mangostana* L. nanoextract on IL-1β and TNF-α levels during the early inflammatory phase of fracture healing in diabetic models. The results demonstrate a statistically significant reduction in both IL-1β and TNF-α levels in groups treated with the nanoextract compared to control groups. These findings support the hypothesis that *Garcinia mangostana* L. nanoextract exerts anti-inflammatory effects in the context of diabetic fracture healing, potentially contributing to improved healing outcomes. The observed reductions in IL-1β and TNF-α are likely mediated by the bioactive xanthones present in *Garcinia mangostana* L., particularly α-mangostin. α-Mangostin has been shown to inhibit NF-κB signaling, a key pathway involved in the transcription of pro-inflammatory cytokines, including IL-1β and TNF-α. It has also been reported to suppress the activation of the NLRP3 inflammasome, a critical regulator of IL-1β maturation and release. Furthermore, α-mangostin possesses antioxidant properties, which may indirectly contribute to its anti-inflammatory effects by mitigating oxidative stress, a known driver of inflammation in diabetes. The use of nanoformulations likely enhances the anti-inflammatory effects of *Garcinia mangostana* L. extract. Nanoparticles, nanoemulsions, and liposomes can improve the solubility and bioavailability of poorly soluble compounds like xanthones, leading to increased cellular uptake and enhanced therapeutic efficacy. The small size of nanoparticles may also facilitate their penetration into the fracture site, allowing for targeted delivery of the bioactive compounds to inflammatory cells. The sustained release properties of some nanoformulations could further prolong the therapeutic effect.[11-13]

The subgroup analyses revealed significant reductions in both IL-1β and TNF-α levels in both in vivo and in vitro studies. This suggests that the anti-inflammatory effects of *Garcinia mangostana* L. nanoextract are consistent across different experimental models. In vivo studies, which involved animal models of diabetic fracture healing, demonstrated that the nanoextract effectively reduced the levels of pro-inflammatory cytokines at the fracture site. This reduction in inflammation is likely to contribute to improved healing outcomes, as excessive inflammation is known to impair bone formation and remodeling. In vitro studies, which used cell cultures exposed to high glucose conditions, provided further evidence of the anti-inflammatory effects of *Garcinia mangostana* L. nanoextract. The nanoextract was shown to directly suppress the production of IL-1β and TNF-α by inflammatory cells, such as macrophages. This direct effect on inflammatory cells may play a crucial role in modulating the inflammatory response at the fracture site.[14-16]

While the findings of this meta-analysis are promising, it is important to acknowledge the limitations. One limitation is the high heterogeneity observed in both the overall analyses and subgroup analyses. This heterogeneity may be attributed to variations in study design, animal models, nanoformulation characteristics, treatment regimens, and methods of cytokine measurement. While a random-effects model was used to account for this heterogeneity, the interpretation of the results should consider this limitation. Another limitation is the potential for publication bias. Although the statistical tests for publication bias were not significant, the funnel plots showed some asymmetry, suggesting that small studies with negative or non-significant findings may be less likely to be published. This could lead to an overestimation of the true effect size.[17,18]

Despite these limitations, the findings of this meta-analysis have important clinical implications. The results suggest that *Garcinia mangostana* L. nanoextract may be a promising therapeutic agent for improving fracture healing outcomes in individuals with diabetes mellitus. By effectively reducing the levels of pro-inflammatory cytokines, the nanoextract could help to modulate the inflammatory response and promote bone regeneration. Further research is needed to confirm these findings and to explore the clinical efficacy of *Garcinia mangostana* L. nanoextract in human subjects. Future studies should focus on optimizing the nanoformulation, dosage, and route of administration to maximize therapeutic efficacy. It would also be valuable to investigate the effects of the nanoextract on other aspects of fracture healing, such as angiogenesis, bone formation, and functional recovery.[19,20]

5. Conclusion

This meta-analysis provides evidence that *Garcinia mangostana* L. nanoextract significantly reduces IL-1β and TNF-α levels during the early inflammatory phase of fracture healing in diabetic models. These findings suggest that *Garcinia mangostana* L. nanoextract holds therapeutic potential for improving fracture healing outcomes in individuals with diabetes mellitus. The results demonstrate a statistically significant reduction in both IL-1β and TNF-α levels in groups treated with the nanoextract compared to control groups. These findings support the hypothesis that *Garcinia mangostana* L. nanoextract may be a promising therapeutic agent for improving fracture healing outcomes in individuals with diabetes mellitus. By effectively reducing the levels of pro-inflammatory cytokines, the nanoextract could help to modulate the inflammatory response and promote bone regeneration. Further research is needed to confirm these findings and to explore the clinical efficacy of *Garcinia mangostana* L. nanoextract in human subjects. Future studies should focus on optimizing the nanoformulation, dosage, and route of administration to maximize therapeutic efficacy. It would also be valuable to investigate the effects of the nanoextract on other aspects of fracture healing, such as angiogenesis, bone formation, and functional recovery.

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