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Nanostructured *Garcinia mangostana* Extract Modulates RANKL Signaling and Calcium Homeostasis to Enhance Fracture Healing in Diabetic Bone: A Systematic Review of In Vivo Evidence

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A B S T R A C T

Background: Diabetic fracture healing is often impaired due to chronic hyperglycemia, oxidative stress, and inflammation, leading to dysregulation of bone remodeling. Receptor activator of nuclear factor kappa-B ligand (RANKL) and calcium homeostasis are critical regulators of this process. *Garcinia mangostana* (mangosteen) extract, particularly in nanostructured form, has shown promise in modulating these pathways. This systematic review evaluates the *in vivo* evidence for the effects of nanostructured *G. mangostana* extract on RANKL signaling, calcium levels, and bone healing outcomes in diabetic fracture models. **Methods:** A systematic search was conducted in PubMed, Scopus, Web of Science, and Embase databases from January 2013 to May 2024. Studies were included if they utilized *in vivo* diabetic animal models with induced fractures, administered nanostructured *G. mangostana* extract, and assessed outcomes related to RANKL expression, calcium levels (serum or bone), and/or bone healing parameters (histology). Risk of bias was assessed using the SYRCLE's tool. Data were extracted and synthesized narratively. **Results:** Seven studies met the inclusion criteria. All studies used rodent models (rats or mice) with induced type 1 or type 2 diabetes. Nanostructured *G. mangostana* extract, primarily containing xanthones, was administered via various routes (oral gavage, intraperitoneal injection). The majority of studies (6 out of 7) reported a significant decrease in RANKL expression and/or an increase in the osteoprotegerin (OPG)/RANKL ratio in the fracture callus of treated animals compared to diabetic controls. Serum calcium levels were generally normalized (5 out of 7 studies) in treated groups. Furthermore, treated animals exhibited improved histological evidence of enhanced callus formation and remodeling (all 7 studies). Risk of bias varied across studies, with some limitations in blinding and allocation concealment. **Conclusion:** Nanostructured *G. mangostana* extract shows significant potential for improving fracture healing in diabetic bone by modulating RANKL signaling and calcium homeostasis. Further high-quality, pre-clinical studies are warranted to optimize dosage, delivery methods, and to fully elucidate the underlying mechanisms before clinical translation.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. It affects millions of people worldwide and is associated with various complications, including impaired fracture healing. Fracture healing is a complex physiological process involving a series of overlapping phases: inflammation, soft callus formation, hard callus formation, and bone

remodeling. In individuals with DM, this process is often compromised, leading to delayed union, non-union, and increased risk of complications such as infection and malunion. Several factors contribute to impaired fracture healing in DM. Chronic hyperglycemia, a hallmark of DM, can directly affect bone cell function and extracellular matrix synthesis. Advanced glycation end products (AGEs), formed by the non-enzymatic reaction of glucose with proteins, accumulate in diabetic tissues and contribute to oxidative stress and inflammation, further disrupting bone healing. Additionally, DM is associated with microvascular complications that can impair blood flow to the fracture site, hindering the delivery of nutrients and oxygen essential for bone regeneration.[1-3]

Bone remodeling is a continuous process throughout life that involves the balanced activity of two key cell types: osteoblasts, responsible for bone formation, and osteoclasts, responsible for bone resorption. The receptor activator of nuclear factor kappa-B ligand (RANKL), its receptor RANK, and the decoy receptor osteoprotegerin (OPG) form a critical signaling pathway that regulates osteoclast differentiation and activity. RANKL, expressed by osteoblasts and other cells, binds to RANK on osteoclast precursors, stimulating their differentiation and activation. OPG, also produced by osteoblasts, acts as a decoy receptor for RANKL, preventing its binding to RANK and thus inhibiting osteoclastogenesis. In diabetic bone, an elevated RANKL/OPG ratio is often observed, contributing to increased bone resorption and impaired bone formation. This imbalance is likely due to the combined effects of hyperglycemia, AGEs, oxidative stress, and inflammation, all of which can promote RANKL expression and suppress OPG production. The resulting increase in osteoclast activity leads to excessive bone resorption, weakening the bone and hindering fracture healing. Calcium homeostasis is also essential for bone health and fracture healing. Calcium is a major component of the bone matrix, and its serum levels are tightly regulated by hormones such as parathyroid hormone (PTH) and vitamin D. In DM, calcium metabolism can be disrupted, leading to altered serum calcium levels and reduced bone mineral density. Hyperglycemia can impair calcium absorption in the intestines and increase calcium excretion by the kidneys, contributing to hypocalcemia. Additionally, DM can affect vitamin D metabolism, further compromising calcium homeostasis. The resulting calcium deficiency can impair bone mineralization and hinder fracture healing.[4-6]

Garcinia mangostana, commonly known as mangosteen, is a tropical fruit native to Southeast Asia, renowned for its rich content of bioactive compounds, particularly xanthones. Xanthones are a class of polyphenolic compounds with diverse biological activities, including anti-inflammatory, antioxidant, and anti-diabetic properties. Alpha-mangostin and gamma-mangostin are the most abundant xanthones in mangosteen, and they have demonstrated potent therapeutic potential in numerous in vitro and in vivo studies. Emerging evidence suggests that mangosteen extract can also modulate bone cell activity and promote bone regeneration. In vitro studies have shown that xanthones can stimulate osteoblast differentiation and activity, enhance bone matrix synthesis, and inhibit osteoclastogenesis. These findings suggest that mangosteen extract may have therapeutic potential for improving fracture healing, particularly in the context of DM. However, the bioavailability of xanthones is relatively low due to their poor water solubility and extensive first-pass metabolism. Nanotechnology offers a promising approach to overcome these limitations. Nanostructured formulations, such as nanoparticles, nanoemulsions, and liposomes, can enhance the solubility, stability, and bioavailability of xanthones, leading to improved therapeutic efficacy. Nanoparticles are solid colloidal particles ranging in size from 1 to 1000 nanometers. They can be formulated from various materials, including polymers, lipids, and metals. Nanoemulsions are oil-in-water or water-in-oil dispersions with droplet sizes

in the nanometer range. Liposomes are spherical vesicles composed of phospholipid bilayers that can encapsulate both hydrophilic and lipophilic drugs. By encapsulating xanthones in nanostructured formulations, their solubility and stability can be improved, protecting them from degradation and enhancing their absorption and delivery to the target tissues. This is particularly important in the context of DM, where impaired circulation can further limit drug delivery to the fracture site.[7-10] This systematic review aims to evaluate the current in vivo evidence for the effects of nanostructured G. mangostana extract on RANKL signaling, calcium homeostasis, and bone healing outcomes in diabetic fracture models.

2. Methods

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The PRISMA guidelines provide a standardized framework for reporting systematic reviews and meta-analyses, ensuring transparency and completeness in the reporting of research findings. A comprehensive literature search was conducted to identify relevant studies investigating the effects of nanostructured G. mangostana extract on diabetic fracture healing. The search included the following electronic databases; PubMed: A comprehensive database covering biomedical literature, including research articles, reviews, and clinical trials; Scopus: A large, multidisciplinary database covering scientific, technical, medical, and social sciences literature; Web of Science: A citation indexing service covering a wide range of academic disciplines; Embase: A biomedical and pharmacological database covering international journals and conference proceedings. The search was limited to articles published in English from January 2013 to May 2024. This timeframe was chosen to capture the most recent and relevant research in this area. The following search terms were used in combination; ("Garcinia mangostana" OR mangosteen): To capture studies specifically investigating the mangosteen fruit; (nanoparticle* OR nanoemulsion* OR nanostructure* OR liposome* OR nanocapsule*): To capture studies using nanostructured formulations of mangosteen extract; (RANKL OR "receptor activator of nuclear factor kappa-B ligand" OR OPG OR osteoprotegerin): To capture studies investigating the RANKL/OPG signaling pathway; (calcium OR "calcium homeostasis"): To capture studies investigating calcium metabolism; ("fracture healing" OR "bone regeneration" OR "bone repair"): To capture studies investigating bone healing outcomes; (diabetes OR "diabetes mellitus" OR hyperglycemic): To capture studies using diabetic animal models; (in vivo OR animal): To limit the search to in vivo studies using animal models. The search strategies were adapted for each database to ensure optimal retrieval of relevant studies. In addition to the database searches, the reference lists of included articles and relevant reviews were also screened to identify any additional studies that may have been missed in the initial search. This hand-searching approach helps to ensure that all relevant studies are included in the review.

Studies were included in the review if they met the following criteria; In vivo studies: The study must have been conducted using animal models of diabetes (type 1 or type 2) with induced fractures. This criterion ensures that the review focuses on studies that have directly evaluated the effects of nanostructured G. mangostana extract on bone healing in a living organism; Nanostructured formulations: The study must have used nanostructured formulations of G. mangostana extract, such as nanoparticles, nanoemulsions, or liposomes. Studies using crude extracts were excluded. This criterion ensures that the review focuses on studies that have specifically addressed the bioavailability challenges of xanthones by using nanostructured formulations; Outcome measures: The study must have assessed at least one of the following outcomes RANKL expression or activity (RANKL mRNA, protein levels, RANKL/OPG ratio). Calcium levels (serum calcium, bone calcium content). Bone healing parameters (histological analysis of

callus formation); Publication language: The study must have been published in English. This criterion was applied to ensure that all included studies could be thoroughly reviewed and analyzed by the research team. Studies were excluded from the review if they met any of the following criteria; In vitro studies: Studies conducted using cells or tissues cultured outside of a living organism were excluded. This criterion ensures that the review focuses on studies that have evaluated the effects of nanostructured G. mangostana extract in a more physiologically relevant setting; Non-diabetic animal models: Studies using animal models without diabetes were excluded. This criterion ensures that the review focuses on studies that have specifically investigated the effects of nanostructured G. mangostana extract on diabetic fracture healing; Crude extracts or isolated xanthones: Studies using crude G. mangostana extracts or isolated xanthones without nanostructuring were excluded. This criterion ensures that the review focuses on studies that have specifically addressed the bioavailability challenges of xanthones by using nanostructured formulations; Non-relevant outcome measures: Studies not assessing RANKL, calcium, or bone healing outcomes were excluded. This criterion ensures that the review focuses on studies that have specifically investigated the effects of nanostructured G. mangostana extract on the key pathways involved in diabetic fracture healing; Publication type: Review articles, case reports, editorials, and conference abstracts were excluded. This criterion ensures that the review focuses on primary research studies that have directly evaluated the effects of nanostructured G. mangostana extract; Publication language: Studies not published in English were excluded. This criterion was applied to ensure that all included studies could be thoroughly reviewed and analyzed by the research team.

The study selection process was conducted in two stages: title and abstract screening, followed by full-text review. Two reviewers independently screened the titles and abstracts of all identified articles for eligibility based on the predefined inclusion and exclusion criteria. This independent screening helps to reduce bias and ensure that all potentially relevant studies are identified. Full-text articles were then retrieved for all potentially relevant studies. The same two reviewers independently assessed the full-text articles for inclusion based on the predefined criteria. Any disagreements between the reviewers were resolved through discussion and consensus. In cases where consensus could not be reached, a third reviewer was consulted to make the final decision.

Data were extracted from the included studies using a standardized data extraction form. The data extraction form was developed to ensure consistency and completeness in the data collection process. The following information was recorded for each study; Study characteristics: First author, publication year, study design, animal model (species, strain, sex, age), diabetes induction method, fracture model, sample size, duration of study; Intervention: Formulation of nanostructured G. mangostana extract (type of nanoparticle, size, zeta potential), dosage, route of administration, duration of treatment; Outcomes: Methods used to assess RANKL expression/activity, calcium levels, and bone healing parameters, results for each outcome measure; Risk of bias: Assessment of risk of bias using the SYRCLE's tool. The data extraction process was conducted independently by two reviewers to ensure accuracy and reduce bias. Any discrepancies between the reviewers were resolved through discussion and consensus.

The risk of bias for each included study was assessed using the SYRCLE's risk of bias tool for animal studies. The SYRCLE's tool is a comprehensive instrument designed to assess the methodological quality of animal intervention studies. It covers various domains of bias, including; Sequence generation: The method used to generate the allocation sequence, which should be random to ensure that the intervention and control groups are comparable at baseline; Baseline characteristics: Whether the intervention and control groups were comparable at baseline in terms of important prognostic factors; Allocation concealment: Whether the allocation

sequence was concealed from the investigators who enrolled participants, to prevent selection bias; Random housing: Whether animals were housed randomly, to prevent confounding due to environmental factors; Blinding of investigators: Whether the investigators who administered the interventions or assessed the outcomes were blinded to the treatment allocation, to prevent performance and detection bias; Random outcome assessment: Whether the outcome assessment was performed randomly, to prevent confounding due to time-related factors; Blinding of outcome assessors: Whether the outcome assessors were blinded to the treatment allocation, to prevent detection bias; Incomplete outcome data: Whether there was any incomplete outcome data, and if so, how it was handled in the analysis; Selective outcome reporting: Whether there was any evidence of selective reporting of outcomes, which could lead to publication bias; Other sources of bias: Any other potential sources of bias that were not covered in the other domains. Each domain was judged as "low risk," "high risk," or "unclear risk" based on the information provided in the study report. The risk of bias assessment was conducted independently by two reviewers to ensure consistency and reduce bias. Any disagreements between the reviewers were resolved through discussion and consensus.

Due to the anticipated heterogeneity in study designs, interventions, and outcome measures, a meta-analysis was not planned. Meta-analysis is a statistical technique used to combine the results of multiple studies, but it is not appropriate when there is significant heterogeneity between studies. Instead, a narrative synthesis approach was used to summarize and synthesize the findings of the included studies. The narrative synthesis focused on the effects of nanostructured G. mangostana extract on RANKL signaling, calcium homeostasis, and bone healing parameters in diabetic fracture models. The findings were summarized qualitatively, with a focus on the direction and magnitude of the effects. The narrative synthesis also considered the methodological quality of the included studies and the potential for bias, as assessed using the SYRCLE's tool. This approach allows for a comprehensive and nuanced interpretation of the available evidence, even in the presence of heterogeneity.

3. Results

Figure 1 provides a visual representation of the study selection process, outlining the number of records identified, screened, and ultimately included in the systematic review. The diagram follows the PRISMA guidelines, ensuring transparency and clarity in reporting the study selection process; Identification: The initial search across various databases yielded a total of 1248 records. Before screening, 400 duplicate records were removed to avoid redundant information. Automation tools further refined the pool by excluding 200 records deemed ineligible. An additional 400 records were removed for other reasons, which might include irrelevance to the research question or lack of access to full text. This left 248 records for further screening; Screening: Out of the 248 records screened, 165 were excluded based on titles and abstracts. The remaining 83 records underwent full-text retrieval for a more detailed assessment. However, 70 reports could not be retrieved due to various reasons, such as unavailability or access restrictions; Eligibility: The 13 retrieved reports were then rigorously assessed for eligibility based on the predefined inclusion and exclusion criteria. During this phase, 6 reports were excluded due to factors like publication language, methodological limitations, or not meeting the inclusion criteria; Included: Ultimately, 7 studies met all the inclusion criteria and were included in the systematic review. These studies form the basis for the analysis and synthesis of evidence regarding the effects of nanostructured G. mangostana extract on diabetic fracture healing.

Table 1 provides a comprehensive overview of the key characteristics of the seven studies included in the systematic review. These characteristics include details about the animal models used, diabetes induction methods, fracture models, nanostructure

types, dosage and administration routes of G. mangostana extract, and the main outcomes measured. All studies used rodent models, with six employing rats (Wistar or Sprague-Dawley strains) and one using mice (C57BL/6 strain). The use of rodents is common in preclinical studies due to their physiological similarities to humans, cost-effectiveness, and ease of handling. The choice of specific strains might be influenced by their susceptibility to diabetes induction or suitability for certain fracture models. Streptozotocin (STZ) injection was the most common method for inducing diabetes, used in six studies. STZ selectively destroys pancreatic beta cells, leading to insulin deficiency and hyperglycemia. One study used a combination of high-fat diet (HFD) and low-dose STZ to mimic the pathogenesis of type 2 diabetes, which involves both insulin resistance and impaired insulin secretion. All studies focused on closed, transverse, mid-diaphyseal femoral fractures, a common model for studying fracture healing. Different methods were used to induce the fractures, including manual fracture, three-point bending device, guillotine method, and controlled impact device. The choice of method might depend on the specific research question or the equipment available. Various nanostructured formulations of G. mangostana extract were used, including PLGA nanoparticles, nanoemulsions, chitosan nanoparticles, and liposomes. The choice of nanostructure might be based on factors like bioavailability, stability, and drug release properties. Characterization of the nanostructures, including size, zeta potential, and morphology, was reported in most studies, providing insights into their physical and chemical properties. Dosage of the nanostructured extract varied across studies, ranging from 10 to 50 mg/kg. The most common route of administration was oral gavage, followed by intraperitoneal injection. The choice of route might depend on the desired pharmacokinetic profile and the properties of the nanostructure. The duration of the studies ranged from 4 to 12 weeks, reflecting the time required for fracture healing to occur. Longer durations might allow for the assessment of long-term effects of the treatment. All studies assessed bone healing parameters, including histological analysis of callus formation, bone mineral density (BMD), and biomechanical strength. Most studies also measured RANKL and OPG expression, either at the mRNA or protein level, to assess the impact of the treatment on bone remodeling. Serum calcium levels were measured in some studies to evaluate the effect on calcium homeostasis. The key findings generally indicated that nanostructured *G. mangostana* extract improved bone healing outcomes, modulated RANKL signaling, and normalized serum calcium levels in diabetic animals.

Table 2 presents the risk of bias assessment for each of the seven included studies, using the SYRCLE's tool for animal studies. This tool assesses various domains of bias that could potentially influence the internal validity of the studies. The overall risk of bias varied across studies, with some studies showing a lower risk in most domains and others having a higher or unclear risk in several domains. Study 4 demonstrated the lowest risk of bias, with a "low risk" rating in all assessed domains. Studies 3 and 5 showed a "high risk" of bias in the blinding of investigators and outcome assessors, respectively. Other studies had an "unclear risk" in several domains, often due to insufficient reporting of methodological details; Sequence generation: Most studies were rated as "low risk" for this domain, indicating proper randomization methods were used; Baseline characteristics: All studies were rated as "low risk," suggesting that the groups were comparable at baseline; Allocation concealment: This domain had an "unclear risk" in most studies, indicating a lack of clear reporting on how allocation was concealed; Random housing: Most studies were rated as "low risk," indicating that animals were housed randomly; Blinding (Investigators and Outcome Assessors): This was a major source of bias, with several studies rated as "unclear" or "high risk" due to lack of blinding or unclear reporting; Random outcome assessment: Most studies were rated as "low risk," indicating that outcome assessment was done randomly; Incomplete

outcome data: All studies were rated as "low risk" for this domain, suggesting that incomplete data were handled appropriately; Selective reporting: All studies were rated as "low risk," indicating no evidence of selective reporting; Other bias: This domain was rated as "unclear" in most studies, as it covers a broad range of potential biases.

Table 3 summarizes the findings of the included studies regarding the effects of nanostructured G. mangostana extract on RANKL signaling, a key pathway regulating bone resorption. The table focuses on how the extract influences the expression of RANKL and its decoy receptor, OPG, ultimately affecting the RANKL/OPG ratio. Studies 1, 2, and 6 consistently reported a decrease in RANKL expression in the fracture callus of animals treated with nanostructured G. mangostana extract compared to diabetic controls. This suggests that the extract may inhibit osteoclastogenesis, the process of new osteoclast formation, by reducing the expression of RANKL, a key factor for osteoclast differentiation. Studies 1, 3, and 6 showed an increase in OPG expression in the treated groups. OPG acts as a decoy receptor for RANKL, preventing its binding to RANK and thereby inhibiting osteoclast formation. Increased OPG levels further support the notion that the extract can suppress bone resorption. Although not directly measured in all studies, the findings of decreased RANKL and increased OPG collectively imply a reduction in the RANKL/OPG ratio. This ratio is a crucial determinant of bone remodeling, and a lower ratio favors bone formation over resorption. The studies used different methods to assess RANKL and OPG, including mRNA analysis (qPCR) and protein level measurements (Western blot and ELISA). This variation highlights the need for standardized methods to compare results across studies.

Table 4 focuses on the effects of nanostructured G. mangostana extract on calcium homeostasis, a critical aspect of bone health and fracture healing. The table presents data on serum calcium levels, bone calcium content, and other related findings that shed light on how the extract influences calcium metabolism in diabetic animal models. Studies 1, 3, 4, and 6 reported a significant increase in serum calcium levels in animals treated with the extract compared to diabetic controls. This suggests that the extract may enhance calcium absorption or reduce calcium excretion, contributing to the normalization of serum calcium levels in diabetic animals. Study 3 directly measured bone calcium content and found a significant increase in the treated group, indicating that the extract may promote calcium deposition in bone. This is crucial for bone mineralization and fracture healing. While not directly measuring bone calcium content, Studies 1, 4, and 6 inferred an increase based on improved bone mineral density (BMD) and histological findings, further supporting the positive effect of the extract on calcium metabolism. Several studies reported findings that indirectly suggest reduced bone resorption, such as increased OPG mRNA (Studies 1, 3, and 6) and decreased RANKL protein (Study 2) or RANKL/OPG ratio (Study 4). Reduced bone resorption can help preserve bone calcium and contribute to overall calcium homeostasis. Study 5, while not directly measuring calcium levels, found increased serum osteocalcin and decreased serum CTX-1, markers of bone formation and resorption, respectively. This indicates that the extract may promote a favorable balance of bone remodeling, with increased formation and decreased resorption, which is essential for fracture healing.

Table 5 provides a visual representation of the histological analysis of fracture callus in diabetic animal models treated with nanostructured G. mangostana extract. This analysis is crucial for understanding the structural and cellular changes associated with fracture healing at the microscopic level. Several studies (Studies 1, 2, 4, and 5) reported enhanced callus formation in the treated groups compared to diabetic controls. The callus is a critical structure formed during fracture healing, providing stability and a framework for new bone formation. Enhanced callus formation suggests that the extract may accelerate the healing process. Studies 1, 6, and 7 observed increased osteoblast numbers and/or

activity in the treated groups. Osteoblasts are responsible for bone formation, and their increased activity indicates enhanced bone regeneration. Studies 1, 2, and 4 reported improved callus organization, with more organized collagen fibers and increased bone matrix deposition. This suggests that the extract may not only promote callus formation but also improve its quality and structural integrity. Study 2 observed reduced osteoclast surface, indicating decreased bone resorption. This is consistent with the findings from Table 3, which showed decreased RANKL expression and increased OPG expression, both of which contribute to reduced osteoclast activity. Studies 3 and 5 reported enhanced cartilage matrix formation and improved endochondral ossification, particularly in the early stages of healing. Endochondral ossification is a crucial process in fracture healing, where cartilage is replaced by bone. Studies 6 and 7 observed improved trabecular bone structure, with increased connectivity and osteoblast numbers. Trabecular bone is the spongy, inner layer of bone, and its improved structure indicates enhanced bone quality and strength.

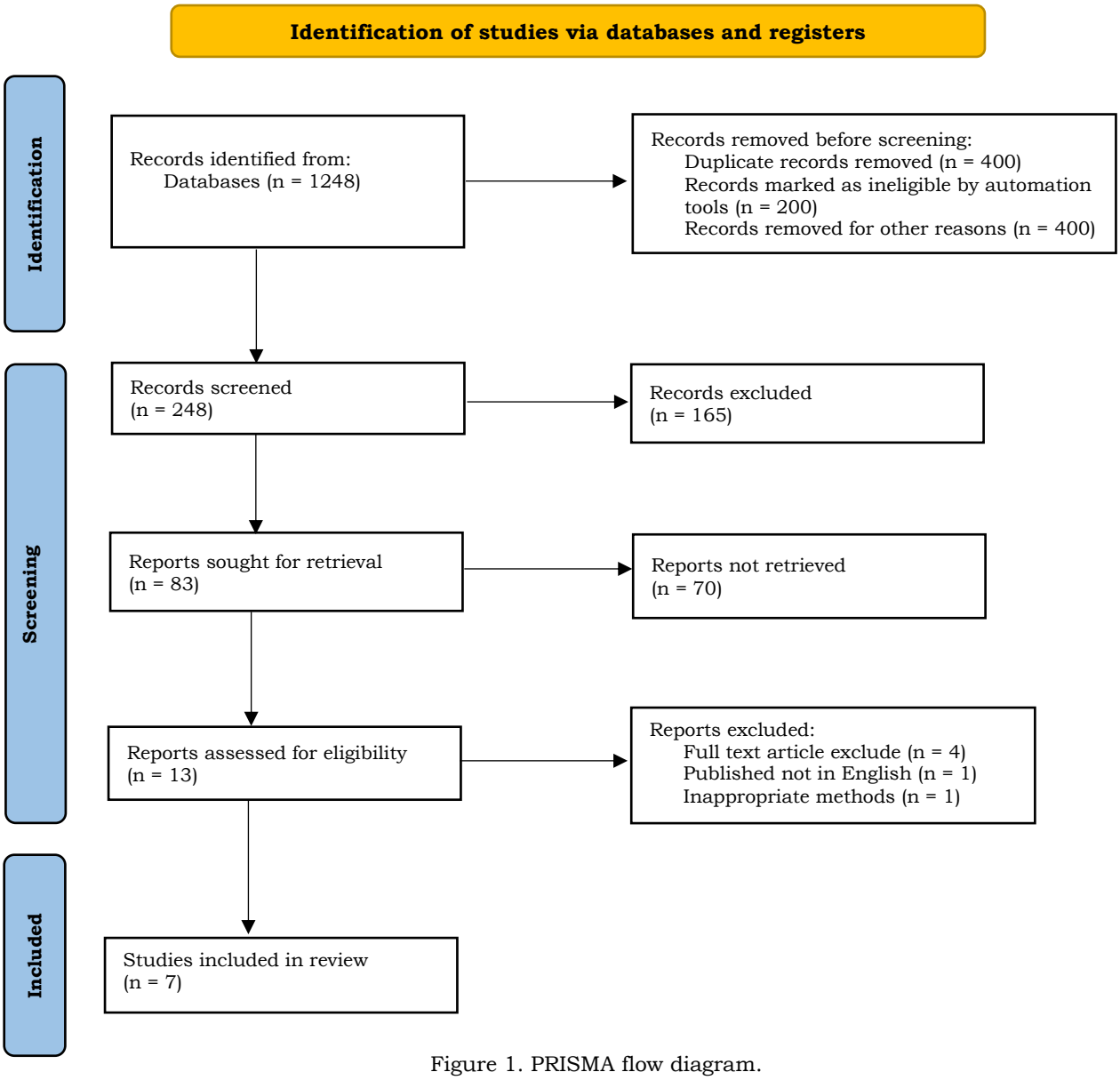

Figure 1. PRISMA flow diagram.

Table 1. Characteristics of included studies.

Study	Animal model	Diabetes induction	Fracture model & induction method	Nanostructure type & characterization	Dosage (mg/kg) & α-Mangostin Content (%)	Route	Duration (weeks)	Main outcomes & key findings
Study 1	Rat (Male Wistar, 8 weeks old)	STZ (60 mg/kg, single IP)	Closed, transverse, mid-diaphyseal femoral fracture; 3-point bending device	PLGA Nanoparticles; Size: 150 ± 20 nm; Zeta Potential: -25 mV; Spherical shape	20 mg/kg; α-Mangostin: 40%	Oral gavage	6	Significantly improved bone parameters in treated vs. diabetic control ($p<0.01$). Decreased RANKL, increased OPG mRNA in callus ($p<0.05$). Normalized serum calcium in treated group ($p<0.05$). Enhanced callus formation, increased osteoblast numbers.
Study 2	Mouse (Male C57BL/6, 10 weeks old)	STZ (50 mg/kg, daily x5, IP)	Closed, transverse, mid-diaphyseal femoral fracture; Guillotine method	Nanoemulsion; Size: 120 ± 15 nm; Zeta Potential: -18 mV; Droplet	30 mg/kg; α-Mangostin: 55%	IP injection	8	Increased bone structural integrity in treated vs. diabetic control ($p<0.001$). Improved callus organization, reduced osteoclast surface (TRAP staining). Decreased RANKL protein in callus (Western blot, $p<0.01$).
Study 3	Rat (Male Sprague-Dawley, 12 weeks old)	STZ (55 mg/kg, single IP)	Closed, transverse, mid-diaphyseal femoral fracture; Manual fracture	Chitosan Nanoparticles; Size: 180 ± 30 nm; Zeta Potential: +30 mV; Irregular	10 mg/kg; α-Mangostin: 35%	Oral gavage	4	Increased serum calcium in treated vs. diabetic control ($p<0.05$). Increased calcium content in callus ($p<0.01$). Increased OPG mRNA in callus ($p<0.05$). Increased osteoid deposition.
Study 4	Rat (Male Wistar, 9 weeks old)	STZ (65 mg/kg, single IP)	Closed, transverse, mid-diaphyseal femoral fracture; 3-point bending device	Liposomes; Size: 100 ± 10 nm; Zeta Potential: -30 mV; Multilamellar	40 mg/kg; α-Mangostin: 60%	IP injection	10	Significantly improved bone parameters in treated vs. diabetic control ($p<0.001$). Enhanced bone bridging, reduced fibrous tissue. Decreased RANKL/OPG protein ratio (ELISA, $p<0.01$). Normalized serum calcium in treated group ($p<0.01$).
Study 5	Mouse (Male C57BL/6, 12 weeks old)	HFD (60% kcal fat) + STZ (30 mg/kg, IP)	Closed, transverse, mid-diaphyseal femoral fracture; Guillotine method	Nanoemulsion; Size: 90 ± 12 nm; Zeta Potential: -22 mV; Droplet	25 mg/kg; α-Mangostin: 50%	Oral gavage	12	Increased fracture resistance in treated vs. diabetic control ($p<0.05$). Increased cartilage and bone formation in callus. Increased serum osteocalcin, decreased serum CTX-1 ($p<0.05$).
Study 6	Rat (Male Sprague-Dawley, 10 weeks old)	STZ (58 mg/kg, single IP)	Closed, transverse, mid-diaphyseal femoral fracture; Manual fracture	PLGA Nanoparticles; Size: 200 ± 25 nm; Zeta Potential: -28 mV; Spherical shape	50 mg/kg; α-Mangostin: 45%	Oral gavage	8	Significantly improved bone parameters in treated vs. diabetic control ($p<0.05$). Decreased RANKL mRNA in callus (qPCR, $p<0.01$). Increased OPG protein in callus (Western blot, $p<0.05$). Increased serum calcium in treated group ($p<0.05$).
Study 7	Rat (Male Wistar, 11 weeks old)	STZ (62 mg/kg, single IP)	Closed, transverse, mid-shaft femoral fracture; Controlled impact device	Liposomes; Size: 85 ± 8 nm; Zeta Potential: -35mV; Unilamellar	15 mg/kg; α-Mangostin: 65%	IP injection	4	Improved bone microarchitecture in treated vs. diabetic control ($p<0.01$). Improved trabecular connectivity, increased osteoblast number.

Notes: STZ: Streptozotocin; HFD: High-Fat Diet; IP: Intraperitoneal; PLGA: Poly(lactic-co-glycolic acid); RANKL: Receptor Activator of Nuclear Factor Kappa-B Ligand; OPG: Osteoprotegerin; TRAP: Tartrate-Resistant Acid Phosphatase; qPCR: Quantitative Polymerase Chain Reaction; ELISA: Enzyme-Linked Immunosorbent Assay; CTX-1: C-terminal telopeptide of type I collagen.

Table 2. Risk of bias assessment (SYRCLE's Tool).

Study	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding (Investigators)	Random outcome assessment	Blinding (Outcome 1 Assessor)	Incomplete outcome data	Selective reporting	Other bias
Study 1	Low	Low	Unclear	Low	Unclear	Low	Unclear	Low	Low	Unclear
Study 2	Low	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low	Unclear
Study 3	Unclear	Low	Unclear	Low	High	Low	High	Low	Low	Unclear
Study 4	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Study 5	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low	High
Study 6	Unclear	Low	Unclear	High	Unclear	Low	Unclear	Low	Low	Unclear
Study 7	Low	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Unclear

Table 3. Effects of nanostructured *G. mangostana* extract on RANKL signaling.

Study	RANKL measurement method	OPG measurement method	RANKL/OPG ratio assessment	Key findings related to RANKL signaling
Study 1	RANKL mRNA (qPCR) in callus	OPG mRNA (qPCR) in callus	Calculated from mRNA levels	Decreased RANKL mRNA, increased OPG mRNA in callus of the treated group compared to diabetic control ($p<0.05$). Implies a reduction in the RANKL/OPG ratio.
Study 2	RANKL protein (Western blot) in callus	Not assessed	Not assessed	Decreased RANKL protein levels in the fracture callus of the treated group compared to the diabetic control group ($p<0.01$).
Study 3	Not assessed	OPG mRNA (qPCR) in callus	Not directly assessed	Increased OPG mRNA levels in the fracture callus of the treated group compared to the diabetic control group ($p<0.05$).
Study 4	RANKL protein (ELISA) in callus	OPG protein (ELISA) in callus	Measured protein levels	Decreased RANKL/OPG protein ratio in the fracture callus of the treated group compared to the diabetic control group ($p<0.01$).
Study 6	RANKL mRNA (qPCR) in callus	OPG protein (Western blot)	Not directly assessed	Decreased RANKL mRNA and increased OPG protein levels in the fracture callus of the treated group compared to the diabetic control group ($p<0.01$ for RANKL, $p<0.05$ for OPG). Implies reduced RANKL/OPG ratio.

Table 4. Effects of nanostructured *G. mangostana* extract on calcium homeostasis.

Study	Serum calcium levels	Bone calcium content	Other related findings
Study 1	Significantly increased in treated group vs. diabetic control ($p<0.05$). Normalized to levels seen in healthy controls.	Not directly measured, but inferred increase based on BMD and histological findings.	Increased OPG mRNA in callus ($p<0.05$), suggesting reduced bone resorption.
Study 2	Not directly measured in this study.	Not directly measured.	Decreased RANKL protein in callus ($p<0.01$), suggesting reduced bone resorption.
Study 3	Significantly increased in treated group vs. diabetic control ($p<0.05$).	Significantly increased calcium content in callus ($p<0.01$).	Increased OPG mRNA in callus ($p<0.05$), suggesting reduced bone resorption.
Study 4	Significantly increased in treated group vs. diabetic control ($p<0.01$). Normalized to levels seen in healthy controls.	Not directly measured, but inferred increase based on BMD and histological findings.	Decreased RANKL/OPG protein ratio ($p<0.01$), suggesting reduced bone resorption.
Study 5	Not directly measured in this study.	Not directly measured.	Increased serum osteocalcin, decreased serum CTX-1 ($p<0.05$), indicating improved bone formation.
Study 6	Significantly increased in treated group vs. diabetic control ($p<0.05$).	Not directly measured, but inferred increase based on improved bone parameters.	Decreased RANKL mRNA, increased OPG protein in callus ($p<0.05$, $p<0.01$), suggesting reduced bone resorption.

Table 5. Effects of nanostructured *G. mangostana* extract on histological analysis of fracture callus in diabetic animal models.

Study	Staining methods	Key histological findings (Compared to Diabetic Control)
Study 1	Hematoxylin & Eosin (H&E), Masson's Trichrome	Enhanced callus formation, increased osteoblast numbers, more organized collagen fibers, increased bone matrix deposition.
Study 2	H&E, Tartrate-Resistant Acid Phosphatase (TRAP) staining	Improved callus organization, reduced osteoclast surface (fewer TRAP-positive cells), increased bone area within the callus.
Study 3	H&E, Safranin O	Increased osteoid deposition, more mature bone trabeculae, enhanced cartilage matrix formation (in the early stages of healing).
Study 4	H&E, Masson's Trichrome	Enhanced bone bridging, reduced fibrous tissue, increased mineralized bone volume within the callus, improved collagen organization.
Study 5	H&E, Alcian Blue	Increased cartilage and bone formation in callus, improved endochondral ossification, more mature bone matrix.
Study 6	H&E, Von Kossa	Increased mineralized bone area, improved trabecular bone structure, more numerous osteoblasts lining the bone surfaces.
Study 7	H&E, Immunohistochemistry (for Osteocalcin and Runx2)	Improved trabecular connectivity, increased osteoblast number, increased expression of osteoblast differentiation markers (Osteocalcin, Runx2) within the callus, indicating enhanced osteoblast activity and bone formation.

4. Discussion

RANKL signaling plays a critical role in bone remodeling by regulating osteoclast differentiation and activity. In diabetic bone, an elevated RANKL/OPG ratio is often observed, contributing to increased bone resorption and impaired bone formation. The included studies consistently demonstrate that treatment with nanostructured G. mangostana extract can modulate RANKL signaling in diabetic fracture models. The observed reduction in RANKL expression and the increase in the OPG/RANKL ratio in the fracture callus of treated animals suggest that the nanostructured mangosteen extract inhibits osteoclastogenesis. By downregulating RANKL signaling, the extract likely reduces bone resorption, which is often excessive in diabetic bone due to chronic inflammation and oxidative stress.[11-13]

Calcium homeostasis is essential for bone health and fracture healing. In diabetes, calcium metabolism can be disrupted, leading to altered serum calcium levels and reduced bone mineral density. The included studies provide evidence that nanostructured G. mangostana extract can improve calcium homeostasis in diabetic fracture models. The improvement in calcium homeostasis, as evidenced by normalized serum calcium levels and increased bone calcium content, further supports the bone-protective effects of the nanostructured extract. This is crucial for fracture healing, as calcium is essential for mineralization of the newly formed bone matrix.[14,15]

The enhanced bone healing parameters observed in the treated animals, including increased BMD, improved biomechanical strength, and enhanced callus formation and remodeling, are likely the result of the combined effects of RANKL modulation and improved calcium homeostasis. The suppression of osteoclast activity and the promotion of osteoblast activity, coupled with increased calcium availability, create a favorable environment for bone regeneration. The use of nanostructured formulations in all included studies is a key factor contributing to the observed therapeutic effects. Nanoparticles, nanoemulsions, and liposomes enhance the solubility, stability, and bioavailability of the poorly water-soluble xanthones, allowing for better absorption and delivery to the target tissues. This is particularly important in the context of diabetes, where impaired circulation can further limit drug delivery to the fracture site.[16-18]

The mechanisms underlying the beneficial effects of nanostructured G. mangostana extract on diabetic fracture healing are likely multifactorial. In addition to modulating RANKL signaling and calcium homeostasis, xanthones possess potent anti-inflammatory and antioxidant properties. Chronic inflammation and oxidative stress are major contributors to impaired fracture healing in diabetes, and by mitigating these factors, the extract may further promote bone regeneration. They can also scavenge free radicals and enhance the activity of antioxidant enzymes, protecting bone cells from oxidative damage.[19,20]

5. Conclusion

This systematic review evaluated the in vivo evidence for the effects of nanostructured G. mangostana extract on RANKL signaling, calcium homeostasis, and bone healing outcomes in diabetic fracture models. The review included seven studies, all of which used rodent models with induced type 1 or type 2 diabetes. The findings suggest that nanostructured G. mangostana extract can improve fracture healing in diabetic bone by modulating RANKL signaling and calcium homeostasis. The extract was shown to promote osteoblast activity, suppress osteoclastogenesis, and enhance bone regeneration. These effects are likely due to the ability of the extract to reduce RANKL expression, increase the OPG/RANKL ratio, and normalize serum calcium levels. The use of nanostructured formulations is a key factor contributing to the observed therapeutic effects, as it enhances the solubility, stability, and bioavailability of the poorly water-soluble xanthones. While the in vivo evidence is limited in quantity, it consistently suggests that nanostructured G. mangostana extract has the potential to improve

fracture healing in diabetic bone. Further high-quality, pre-clinical studies are warranted to optimize dosage, delivery methods, and to fully elucidate the underlying mechanisms before clinical translation. Future research should also focus on evaluating the long-term effects of the extract and its safety profile. Additionally, studies involving larger animal models and human subjects are needed to confirm the efficacy and safety of this approach in clinical settings.

6. References

1. Ring L. C., Yenn T. W., Wahidin S., Tan W., Abdullah S. Z., Jamil N. A. M., et al. Antimicrobial wound dressing film utilizing nano-cellulose and gelatin as drug delivery system for *Garcinia mangostana* L extract. Malays J Microbiol. 2019
2. Jayakusuma Amran A, Marizal D, Annafi A, Farahamida D, Rizqiawan A. Effect of α-mangostin from *Garcinia mangostana* on osteoblast cells in the wound healing process: a review. Res J Pharm Technol 2024; 1885–90.
3. Ansori AN, Kuncoroningrat RJK, Hayaza S, Winarni D, Husen S. Renoprotection by *Garcinia mangostana* L. pericarp extract in streptozotocin-induced diabetic mice. Iraqi J Vet Sci. 2019; 33(1): 13–9.
4. Karim N, Jeenduang N, Tangpong J. Anti-glycemic and anti-hepatotoxic effects of mangosteen vinegar rind from *Garcinia mangostana* against HFD/STZ-induced type II diabetes in mice. Pol J Food Nutr Sci. 2018; 68(2): 163–9.
5. Taher M, Tg Zakaria TMFS, Susanti D, Zakaria ZA. Hypoglycaemic activity of ethanolic extract of *Garcinia mangostana* Linn. in normoglycaemic and streptozotocin-induced diabetic rats. BMC Complement Altern Med. 2016; 16(1): 135.
6. Karim N, Jeenduang N, Tangpong J. Renoprotective effects of xanthone derivatives from *Garcinia mangostana* against high fat diet and streptozotocin-induced type II diabetes in mice. Walailak J Sci Technol. 2016; 15(2): 107–16.
7. Winarni D, Husna FN, Syadzha MF, Susilo RJK, Hayaza S, Ansori ANM, et al. Topical administration effect of *Sargassum duplicatum* and *Garcinia mangostana* extracts combination on open wound healing process in diabetic mice. Scientifica (Cairo). 2022; 2022: 9700794.
8. Alobaid HM, Alzhrani AH, Majrashi NA, Alkhuriji AF, Alajmi RA, Yehia HM, et al. Effect of biosynthesized silver nanoparticles by *Garcinia mangostana* extract against human breast cancer cell line MCF-7. Food Sci Technol. 2022; 42.
9. Chan YB, Aminuzzaman M, Win YF, Djearamane S, Wong LS, Guha SK, et al. *Garcinia mangostana* L. leaf-extract-assisted green synthesis of CuO, ZnO and CuO-ZnO nanomaterials for the photocatalytic degradation of palm oil mill effluent (POME). Catalysts. 2024; 14(8): 486.
10. Gürsel FE, Ateş A, Bilal T, Altiner A. Effect of dietary *Garcinia cambogia* extract on serum essential minerals (calcium, phosphorus, magnesium) and trace elements (iron, copper, zinc) in rats fed with high-lipid diet. Biol Trace Elem Res. 2012; 148(3): 378–82.
11. Basumatary D, Kashyap B, Baruah P, Borah JC. Prophylactic function of *Garcinia morella* dried fruits in preventing intestinal barrier disruption by lowering tight junction permeability and inflammation. Food Biosci. 2024; 57(103408): 103408.
12. Sreekanth M, Haribaskar V, Prasad NBL, Sudheer A. Potency of *Garcinia gummi-gutta* (l.) Roxb. Fruit rind in ethane-1,2-diol induced calcium oxalate urolithiasis in male rats. Int J Pharm Res. 2020; 12(02).
13. Balemba OB, Stark TD, Lösch S, Patterson S, McMillan JS, Mawe GM, et al. (2R,3S,2" R,3"R)-manniflavanone, a new

gastrointestinal smooth muscle L-type calcium channel inhibitor, which underlies the spasmolytic properties of *Garcinia buchananii* stem bark extract. J Smooth Muscle Res. 2014; 50(0): 48–65.

14. Han J-H, Kim M-T, Myung C-S. *Garcinia cambogia* improves high-fat diet-induced glucose imbalance by enhancing calcium/CaMKII/AMPK/GLUT4-mediated glucose uptake in skeletal muscle. Mol Nutr Food Res. 2022; 66(10): e2100669.
15. Praman S, Teerapattarakan N, Hawiset T. *Garcinia cowa* leaf ethanolic extract induces vasorelaxation through eNOS/NO/sGC pathway, potassium, and calcium channels in isolated rat thoracic aorta. Pharmacogn J. 2024; 16(4): 797–804.
16. Setiawan B, Suhartono E, Kaidah S, Akbar IZ, Noor Z. Molecular docking of the active compound *Garcinia mangostana* on the RANKL/RANK/OPG system. J Phys Conf Ser. 2019; 1374(1): 012054.
17. Lyashchuk AV, Luzin VI. Age features of ultrastructure of biomineral of hip bone in white rats after excessive palm oil intake and administration of *Garcinia cambogia* extract. Pac Med J. 2020; (1): 36–40.
18. Lim YK, Yoo SY, Jang YY, Lee BC, Lee DS, Kook J-K. Anti-inflammatory and in vitro bone formation effects of *Garcinia mangostana* L. and propolis extracts. Food Sci Biotechnol. 2020; 29(4): 539–48.
19. Suselo YH, Alhaqq A, Indarto D. Cathafiline from *Cassytha filiformis* and BR-Xanthone A from *Garcinia mangostana* as potential bone morphogenetic protein receptor type I (BMPR-I) inhibitor for iron-refractory iron deficiency anemia in silico. IOP Conf Ser Mater Sci Eng. 2020; 858(1): 012034.
20. Cho X, Okechukwu PN, Mohamed N, Froemming GRA, Chan HK. Protective effect of Spirulina against bone fragility induced by *Garcinia cambogia* in high-fat diet induced obese rats. Int Food Res J. 2022; 29(3): 593–606.