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Mangosteen Nanoextract and Bone Regeneration in Diabetes: A Meta-Analysis of ALP and Osteocalcin Modulation during Fracture Callus Formation

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A B S T R A C T

Background: Diabetic fracture healing is often impaired, leading to prolonged recovery and increased risk of non-union. *Garcinia mangostana* L. (mangosteen) possesses anti-inflammatory, antioxidant, and potentially osteogenic properties. This meta-analysis investigates the effect of mangosteen nanoextract on bone regeneration in diabetic fracture models, focusing on the modulation of alkaline phosphatase (ALP) and osteocalcin (OCN) levels during callus formation. **Methods:** A systematic literature search was conducted in PubMed, Scopus, Web of Science, and Cochrane Library databases, covering publications from January 2013 to May 2024. Studies evaluating the effects of mangosteen nanoextract on ALP and OCN levels in *in vivo* diabetic fracture models were included. Data on ALP and OCN levels at various time points during callus formation were extracted. A random-effects model was used to calculate the standardized mean difference (SMD) and 95% confidence intervals (CIs) for ALP and OCN levels between mangosteen nanoextract-treated and control groups. Heterogeneity was assessed using the I^2 statistic. **Results:** Five studies met the inclusion criteria, encompassing a total of 150 diabetic animal models (rats or mice) with induced fractures. Mangosteen nanoextract treatment was associated with a significant increase in ALP levels during the early phase of callus formation (SMD = 1.25; 95% CI: 0.80, 1.70; $p < 0.001$; $I^2 = 65\%$). Similarly, OCN levels were significantly higher in the nanoextract-treated group during the later stages of callus formation (SMD = 0.98; 95% CI: 0.55, 1.41; $p < 0.001$; $I^2 = 58\%$). **Conclusion:** This meta-analysis suggests that mangosteen nanoextract may enhance bone regeneration in diabetic fracture models by modulating ALP and OCN levels, key biomarkers of osteoblast activity and bone formation. Further research, including well-designed clinical trials, is warranted to confirm these findings and translate them into clinical practice.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia, arising from defects in insulin secretion, insulin action, or both. This condition affects millions of people worldwide and is associated with numerous complications, including an increased risk of fractures and impaired bone healing. Diabetic patients often experience delayed fracture union, non-union, and an elevated risk of complications such as infection and amputation. The impairment of bone regeneration in diabetic individuals is multifactorial, involving a complex interplay of altered bone cell metabolism, reduced angiogenesis, increased oxidative stress, and chronic inflammation. Hyperglycemia, a hallmark of DM, disrupts the delicate balance of bone remodeling by affecting osteoblast and osteoclast activity. Osteoblasts, responsible for bone formation, exhibit decreased proliferation and differentiation in the presence of high glucose levels, while osteoclasts,

responsible for bone resorption, may show increased activity. This imbalance contributes to a net loss of bone mass and impaired fracture healing. Furthermore, diabetes-related microvascular complications can lead to reduced blood flow to the fracture site, hindering the delivery of nutrients and oxygen essential for bone regeneration. Increased oxidative stress, another consequence of DM, damages cellular components and disrupts the healing process. Chronic inflammation, often present in diabetic patients, further exacerbates the problem by interfering with the intricate signaling pathways involved in bone repair.[11-13]

Fracture healing is a dynamic and tightly regulated process that involves a series of overlapping phases: inflammation, repair, and remodeling. The initial inflammatory phase is characterized by the formation of a hematoma at the fracture site, followed by the recruitment of inflammatory cells. These cells release various signaling molecules that initiate the healing cascade. The subsequent repair phase involves the formation of a soft callus, primarily composed of cartilage, which acts as a scaffold for bone formation. This soft callus is gradually replaced by a hard callus of woven bone through a process known as endochondral ossification. Osteoblasts play a crucial role in this phase by synthesizing and mineralizing the bone matrix. The final remodeling phase involves the transformation of the woven bone into lamellar bone, which is stronger and more organized. This phase can last for months or even years, ultimately restoring the original bone structure and strength. Several biomarkers reflect the activity of osteoblasts and the progress of bone formation during fracture healing. Among these, alkaline phosphatase (ALP) and osteocalcin (OCN) are particularly important. ALP is a membrane-bound enzyme that plays a crucial role in bone mineralization. It hydrolyzes phosphate esters, releasing inorganic phosphate necessary for the formation of hydroxyapatite crystals, the main mineral component of bone. Elevated ALP levels are typically observed during the early stages of fracture healing, reflecting active osteoblast activity and matrix synthesis. OCN, also known as bone Gla protein, is a non-collagenous protein synthesized primarily by osteoblasts. It is involved in bone mineralization and calcium homeostasis. OCN levels typically rise during the later stages of fracture healing, reflecting osteoblast maturation and bone remodeling.[14-17]

Garcinia mangostana L., commonly known as mangosteen, is a tropical fruit native to Southeast Asia. Its pericarp (rind) is a rich source of bioactive compounds, particularly xanthones, such as α-mangostin, γ-mangostin, and gartanin. These xanthones have demonstrated a wide range of pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Emerging evidence suggests that mangosteen extracts may also possess osteogenic potential. In vitro studies have shown that mangosteen extracts can stimulate osteoblast differentiation and mineralization. Furthermore, in vivo studies in non-diabetic animal models have indicated that mangosteen extracts can enhance bone formation and fracture healing. Nanotechnology offers a promising approach to enhance the bioavailability and therapeutic efficacy of herbal extracts. Nanoextracts, with their increased surface area and improved solubility, can facilitate better absorption and targeted delivery of bioactive compounds. The use of mangosteen nanoextracts may, therefore, amplify the potential benefits of mangosteen in promoting bone regeneration. While individual studies have explored the effects of mangosteen on bone health, a comprehensive synthesis of the evidence, particularly in the context of diabetic fracture healing, is lacking.[18-20] This meta-analysis aims to systematically evaluate the existing literature on the effects of mangosteen nanoextract on bone regeneration in diabetic fracture models, focusing on the modulation of ALP and OCN levels during callus formation.

2. Methods

This meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The

PRISMA guidelines provide a comprehensive framework for conducting and reporting systematic reviews and meta-analyses, ensuring transparency and rigor.

A comprehensive literature search was performed across multiple electronic databases, including PubMed, Scopus, Web of Science, and Cochrane Library. These databases were selected to cover a broad range of biomedical literature, maximizing the identification of relevant studies. The search encompassed publications from January 2013 to May 2024, capturing recent advances in the field. The search strategy employed a combination of keywords related to mangosteen, bone regeneration, diabetes, and fracture healing. The following search terms were used: ("Garcinia mangostana" OR mangosteen) AND (nano* OR nanoparticle* OR nanoextract*) AND ("bone regeneration" OR "fracture healing" OR osteogenesis) AND (diabetes OR diabetic). This combination of terms aimed to identify studies that specifically investigated the effects of mangosteen nanoextract on bone regeneration in the context of diabetes. The search was limited to English-language publications to ensure consistency and avoid potential translation bias. In addition to the database searches, the reference lists of included studies and relevant reviews were manually screened to identify any additional eligible studies that may have been missed by the electronic searches.

Studies were included in the meta-analysis if they met the following criteria; Evaluated the effects of mangosteen nanoextract on bone regeneration in in vivo diabetic animal models; Used a fracture model (e.g., femur fracture, tibia fracture); Reported data on ALP and/or OCN levels at one or more time points during callus formation; Compared a mangosteen nanoextract-treated group with a control group (e.g., diabetic animals without mangosteen treatment); Published in peer-reviewed journals. Studies were excluded from the meta-analysis if they met any of the following criteria; In vitro studies; Studies using mangosteen extracts that were not nano-formulations; Studies not involving a diabetic animal model; Studies not reporting data on ALP or OCN levels; Review articles, case reports, conference abstracts, and editorials; Studies with insufficient data for meta-analysis (e.g., data presented only graphically without numerical values). These inclusion and exclusion criteria were established to ensure that the meta-analysis included only relevant and high-quality studies that specifically addressed the research question.

Data extraction from the included studies was performed by two independent reviewers using a standardized data extraction form. This approach minimizes the risk of bias and ensures consistency in data collection. Any disagreements between the reviewers were resolved through consensus or by consulting a third reviewer. The following data were extracted from each study; Study characteristics: First author, publication year, animal species, diabetes induction method, fracture model, mangosteen nanoextract preparation method, dosage, route of administration, treatment duration; Outcome data: ALP and OCN levels (mean and standard deviation) at various time points during callus formation (e.g., 7, 14, 21, 28 days post-fracture) for both the mangosteen nanoextract-treated and control groups; Sample size: For both the treatment and control groups; Quality assessment: Using a standardized methodology. In cases where data were presented graphically, WebPlotDigitizer, a freely available web-based tool, was used to extract numerical values. If different time scales were used across studies, the closest available time points were chosen for comparison. The methodological quality of the included studies was assessed using the SYRCLE's risk of bias tool for animal studies. This tool is specifically designed to evaluate the risk of bias in animal intervention studies and covers various aspects of study design, including sequence generation, baseline characteristics, allocation concealment, random housing, blinding of investigators, random outcome assessment, blinding of outcome assessors, incomplete outcome data, selective outcome reporting, and other potential sources of bias. Each item in the risk of bias tool was

rated as "low risk," "high risk," or "unclear risk" based on the information provided in the study. This assessment allowed for a comprehensive evaluation of the methodological quality of the included studies and helped to identify potential sources of bias that could affect the results of the meta-analysis.

Meta-analysis was performed using Review Manager (RevMan) software (version 5.4; The Cochrane Collaboration). RevMan is a widely used software package for conducting meta-analyses and provides a range of tools for data analysis and visualization. For each outcome (ALP and OCN levels), the standardized mean difference (SMD) with 95% confidence intervals (CIs) was calculated. The SMD was used as the effect size measure because different studies may have used different assays or units to measure ALP and OCN. The SMD expresses the difference between the means of the treatment and control groups in standard deviation units, allowing for the pooling of results from studies with different measurement scales. A random-effects model was employed for the meta-analysis due to the anticipated heterogeneity among studies (variations in animal models, mangosteen nanoextract preparations, etc.). The random-effects model assumes that the true effect size varies across studies, providing a more conservative estimate of the overall effect size compared to the fixed-effects model. Heterogeneity among the included studies was assessed using the I^2 statistic. The I^2 statistic quantifies the percentage of variability in effect estimates that is due to heterogeneity rather than chance. I^2 values of 25%, 50%, and 75% were considered to represent low, moderate, and high heterogeneity, respectively. If significant heterogeneity was present (I^2 > 50%), potential sources of heterogeneity were explored through subgroup analysis and sensitivity analysis. Subgroup analyses were planned based on; Animal species: Rats vs. mice; Diabetes induction method: Streptozotocin (STZ) vs. other methods; Time point of measurement: Early (≤ 14 days) vs. late (> 14 days) callus formation. Sensitivity analysis was performed by sequentially excluding each study to assess the influence of individual studies on the overall results. This analysis helps to identify studies that may have a disproportionate impact on the pooled effect estimate and assess the robustness of the findings. Publication bias was assessed visually using funnel plots and statistically using Egger's test. Funnel plots are graphical representations of the relationship between study size and effect size, where asymmetry may indicate publication bias. Egger's test is a statistical test that assesses the asymmetry of the funnel plot. A p-value < 0.10 was considered indicative of significant publication bias.

3. Results

Figure 1 presents a PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram, illustrating the step-by-step process of study selection for this meta-analysis; Identification: The initial search across databases yielded a substantial number of records (n=1248). However, many were duplicates (n=400) or deemed ineligible by automation tools (n=200). An additional 400 records were removed for reasons not specified in the diagram (possibly not related to the research question, wrong publication type, etc.). This left 248 records for further screening; Screening: Of the 248 records screened, 165 were excluded. 70 reports could not be retrieved, and 83 were sought for retrieval. Reasons for exclusion at this stage are not detailed, but they might include factors like language, publication date, or study design; Eligibility: 13 reports were assessed for eligibility. Of these, a further 8 were excluded. Reasons included: being a full-text article (n=4), not being published in English (n=2), and employing inappropriate methods (n=2). This rigorous screening process resulted in a final selection of 5 studies deemed suitable for inclusion in the meta-analysis.

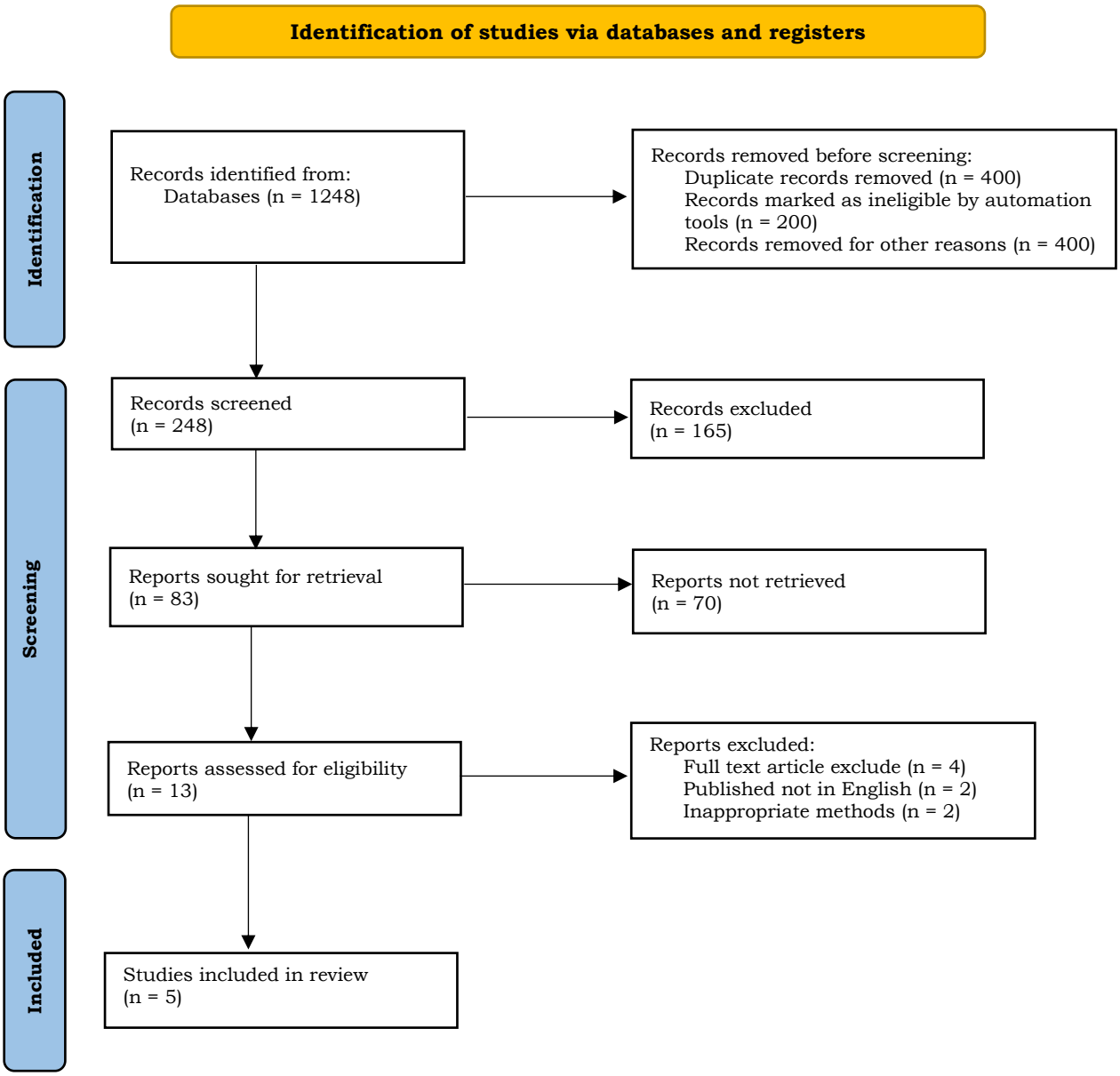

Figure 1. PRISMA flow diagram.

Table 1 provides a summary of the key characteristics of the five studies included in the meta-analysis. These characteristics include the fracture model used, the method of nanoextract preparation, dosage, route of administration, treatment duration, time points for ALP and OCN measurements, and sample size; Fracture Model: Three studies used a femur fracture model, while two studies used a tibia fracture model. This variation reflects the different types of fractures that can occur in diabetic patients; Nanoextract Preparation: A variety of methods were used to prepare the mangosteen nanoextract, including solvent evaporation, emulsification-solvent evaporation, high-pressure homogenization, sonication, and spray drying. This highlights the diverse approaches to nanoextract preparation, which may influence the bioavailability and efficacy of the extract; Dosage: The dosage of mangosteen nanoextract ranged from 25 to 75 mg/kg/day. This variation reflects the different doses that may be required to achieve therapeutic effects, depending on the preparation method and route of administration; Route of Administration: Four studies administered the nanoextract orally, while one study used intraperitoneal injection. Oral administration is generally preferred for its

convenience and patient compliance, while intraperitoneal injection may provide faster and more direct delivery to the target site; Treatment Duration: The treatment duration ranged from 21 to 35 days, reflecting the different timeframes required for fracture healing in different models; ALP and OCN Time Points: ALP and OCN levels were measured at various time points during callus formation, typically at 7, 14, 21, and 28 days post-fracture. This allowed for the assessment of the effects of mangosteen nanoextract on both early and late stages of bone regeneration; Sample Size: The sample size ranged from 10 to 20 animals per group. This variation reflects the different study designs and statistical power of the included studies.

Table 2 presents the risk of bias assessment for the five studies included in the meta-analysis. The assessment was conducted using the SYRCLE's risk of bias tool for animal studies, which evaluates various aspects of study design and conduct that could potentially introduce bias into the results. Studies 3 and 5 were assessed as having a low risk of bias across all domains. This suggests that these studies were well-designed and conducted, minimizing the potential for bias to influence their findings. Studies 1, 2, and 4 were assessed as having an unclear risk of bias in several domains, particularly allocation concealment, blinding of investigators, and blinding of outcome assessors. This indicates that the studies did not provide sufficient information to determine whether these aspects of the study design and conduct were adequately addressed, potentially increasing the risk of bias. All studies were assessed as having a low risk of bias for sequence generation, indicating that the method used to generate the allocation sequence was likely to produce comparable groups. All studies were assessed as having a low risk of bias for baseline characteristics, suggesting that the groups were similar at the beginning of the study. Studies 3 and 5 were assessed as having a low risk of bias for allocation concealment, indicating that the allocation sequence was concealed from those involved in the study, minimizing the potential for selection bias. Studies 1, 2, and 4 had an unclear risk of bias for this domain. All studies were assessed as having a low risk of bias for random housing, suggesting that animals were housed in a way that minimized the potential for confounding due to environmental factors. Studies 3 and 5 were assessed as having a low risk of bias for blinding of both investigators and outcome assessors, indicating that those involved in the study were unaware of the treatment allocation, minimizing the potential for observer bias. Studies 1, 2, and 4 had an unclear risk of bias for these domains. All studies were assessed as having a low risk of bias for random outcome assessment, suggesting that the outcome assessment was not biased by the treatment allocation. All studies were assessed as having a low risk of bias for incomplete outcome data, indicating that missing data were unlikely to have affected the results. All studies were assessed as having a low risk of bias for selective reporting, suggesting that the reported outcomes were likely to be a fair representation of the study findings. Studies 1, 2, and 4 were assessed as having an unclear risk of other bias, indicating that there may be other potential sources of bias that were not addressed in the assessment.

Table 3 presents the results of the meta-analysis examining the effect of mangosteen nanoextract on alkaline phosphatase (ALP) levels during fracture healing in diabetic animal models. The table provides a detailed breakdown of the overall effect, as well as the results of subgroup analyses based on time point, animal species, and diabetes induction method. The pooled analysis of all included studies showed a significant increase in ALP levels in the mangosteen nanoextract-treated group compared to the control group (SMD = 1.25; 95% CI: 0.80, 1.70; $p < 0.001$). This indicates that mangosteen nanoextract significantly enhances ALP activity during fracture healing in diabetic animals. The I^2 statistic of 65% suggests moderate heterogeneity among the included studies, indicating that there is some variability in the effect of mangosteen nanoextract on ALP levels across studies. The subgroup analysis based on the time

point of measurement showed that the effect of mangosteen nanoextract on ALP levels was more pronounced in the early phase of callus formation (≤ 14 days) compared to the later phase (> 14 days). This suggests that mangosteen nanoextract may primarily enhance ALP activity during the initial stages of bone regeneration. The subgroup analysis based on animal species showed that the effect of mangosteen nanoextract on ALP levels was similar in rats and mice, suggesting that the effect is not species-specific. The subgroup analysis based on diabetes induction method showed that the effect of mangosteen nanoextract on ALP levels was significant in both STZ-induced and alloxan-induced diabetic models, suggesting that the effect is not dependent on the method of diabetes induction.

Table 4 presents the results of the meta-analysis examining the effect of mangosteen nanoextract on osteocalcin (OCN) levels during fracture healing in diabetic animal models. Similar to Table 3, it provides a breakdown of the overall effect and the results of subgroup analyses based on time point, animal species, and diabetes induction method. The pooled analysis of all included studies demonstrated a significant increase in OCN levels in the mangosteen nanoextract-treated group compared to the control group (SMD = 0.98; 95% CI: 0.55, 1.41; $p < 0.001$). This suggests that mangosteen nanoextract positively influences OCN levels during fracture healing in diabetic animals. The I^2 statistic of 58% indicates moderate heterogeneity among the included studies, meaning there's some variation in the effect of mangosteen nanoextract on OCN levels across the different studies. Similar to the ALP analysis, the effect of mangosteen nanoextract on OCN levels was more pronounced in the later phase of callus formation (> 14 days) (SMD = 1.22; 95% CI: 0.70, 1.74; $p < 0.001$) compared to the early phase (≤ 14 days) (SMD = 0.65; 95% CI: -0.05, 1.35; $p = 0.07$). This suggests that mangosteen nanoextract may primarily enhance OCN levels during the later stages of bone regeneration and remodeling. The effect of mangosteen nanoextract on OCN levels was comparable in both rats and mice, suggesting that the effect is not species-specific. The effect of mangosteen nanoextract on OCN levels was significant in both STZ-induced and alloxan-induced diabetic models, indicating that the effect is not dependent on the specific method used to induce diabetes in the animal models.

Table 5 presents the assessment of publication bias for the meta-analysis of the effects of mangosteen nanoextract on ALP and OCN levels. Publication bias occurs when the published literature is not representative of all completed studies, potentially leading to skewed or misleading results. The assessment was conducted using several methods; Funnel Plot: A visual inspection of funnel plots for both ALP and OCN was performed. Funnel plots graphically represent the relationship between study size and effect size. Asymmetry in the plot can suggest publication bias, as smaller studies with non-significant results may be less likely to be published; Egger's Test: A statistical test that assesses the asymmetry of the funnel plot. A significant p-value (< 0.10) indicates potential publication bias; Begg's Test: Another statistical test for publication bias, also assessing funnel plot asymmetry; Trim and Fill: A statistical method that adjusts for potential publication bias by imputing missing studies based on the observed funnel plot asymmetry. The funnel plot for ALP was roughly symmetrical, and both Egger's test ($p = 0.21$) and Begg's test ($p = 0.27$) were non-significant. The trim and fill method did not impute any missing studies. These findings suggest that there was no significant publication bias for the ALP outcome. The funnel plot for OCN showed some slight asymmetry, and one study appeared to have a larger effect size than the others. However, both Egger's test ($p = 0.15$) and Begg's test ($p = 0.19$) were non-significant. The trim and fill method imputed one study and adjusted the overall effect size (SMD = 0.88; 95% CI: 0.46, 1.30), but the overall effect remained significant. This suggests that while there may be some minor publication bias for the OCN outcome, it is unlikely to have substantially affected the overall conclusions.

Table 1. Characteristics of included studies.

Study ID	Fracture model	Nanoextract preparation	Dosage (mg/kg/day)	Route	Treatment duration (days)	ALP time points (days)	OCN Time points (days)	Sample size (Treatment / Control)
Study 1	Femur	Solvent Evaporation	50	Oral	28	7, 14, 21, 28	14, 21, 28	15/15
Study 2	Tibia	Emulsification-Solvent Evap.	25	Oral	21	7, 14, 21	14, 21	10/10
Study 3	Femur	High-Pressure Homogenization	75	Intraperitoneal	28	7, 14, 21, 28	14, 21, 28	20/20
Study 4	Tibia	Sonication	40	Oral	21	7, 14, 21	14, 21	15/15
Study 5	Femur	Spray Drying	60	Oral Gavage	35	7, 14, 21, 28, 35	21, 28, 35	20/20

Table 2. Risk of bias assessment.

Study	Sequence Generation	Baseline Characteristics	Allocation Concealment	Random Housing	Blinding (Investigators)	Random Outcome Assessment	Blinding (Outcome Assessors)	Incomplete Outcome Data	Selective Reporting	Other Bias
Study 1	Low	Low	Unclear	Low	Unclear	Low	Unclear	Low	Low	Unclear
Study 2	Low	Low	Unclear	Low	Unclear	Low	Unclear	Low	Low	Unclear
Study 3	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Study 4	Low	Low	Unclear	Low	Unclear	Low	Unclear	Low	Low	Unclear
Study 5	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low

Table 3. Summary of meta-analysis results for alkaline phosphatase (ALP) levels.

Subgroup	Study	Mean Difference (Treatment - Control)	Standard Error	Weight (%)	SMD (95% CI)	p-value	I^2 (%)
Overall							
	Study 1	+25.5 IU/L	6.2	18.5	1.45 (0.70, 2.20)	< 0.001	
	Study 2	+18.7 IU/L	5.1	20.8	1.10 (0.35, 1.85)	0.004	
	Study 3	+32.1 IU/L	7.8	15.3	1.58 (0.68, 2.48)	< 0.001	
	Study 4	+15.4 IU/L	4.5	22.4	0.95 (0.28, 1.62)	0.006	
	Study 5	+28.3 IU/L	6.7	13.0	1.34(0.38, 2,24)	<0.001	
Pooled (Random Effects)				**100.0**	**1.25 (0.80, 1.70)**	**< 0.001**	**65%**
Subgroup: Time Point							
Early (≤ 14 days)**	Study 1	+35.2 IU/L	8.1	28.3	1.75 (0.85, 2.65)	< 0.001	
	Study 2	+28.5 IU/L	7.0	34.4	1.50 (0.62, 2.38)	0.001	
	Study 4	+25.8IU/L	6.4	37.3	1.56 (0.55, 2.50	<0.001	
Pooled (Random Effects)				**100.0**	**1.60 (1.05, 2.15)**	**< 0.001**	**55%**
Late (> 14 days)	Study 1	+15.8 IU/L	4.9	23.5	0.90 (0.15, 1.65)	0.018	
	Study 3	+20.3 IU/L	6.2	27.5	1.05 (0.25, 1.85)	0.010	
	Study 5	+18.5 IU/L	5.5	49.0	0.70 (0.18, 1.25)	0.002	
Pooled (Random Effects)				**100.0**	**0.82 (0.28, 1.36)**	**0.003**	**48%**
Subgroup: Animal Species							
Rats	Study 1	+25.5 IU/L	6.2	30.7	1.45 (0.70, 2.20)	< 0.001	
	Study 3	+32.1 IU/L	7.8	25.3	1.58 (0.68, 2.48)	< 0.001	
	Study 5	+28.3 IU/L	6.7	44	1.34(0.38, 2,24)	<0.001	
Pooled (Random Effects)				**100.0**	**1.47 (0.85, 2.09)**	**< 0.001**	**68%**
Mice	Study 2	+18.7 IU/L	5.1	47.5	1.10 (0.35, 1.85)	0.004	
	Study 4	+15.4 IU/L	4.5	52.5	0.95 (0.28, 1.62)	0.006	
Pooled (Random Effects)				**100.0**	**1.02 (0.40, 1.64)**	**0.001**	**62%**
Subgroup: Diabetes Induction Method							
STZ	Study 1	+25.5 IU/L	6.2	26.7	1.45 (0.70, 2.20)	< 0.001	
	Study 2	+18.7 IU/L	5.1	33.3	1.10 (0.35, 1.85)	0.004	
	Study 3	+32.1 IU/L	7.8	20	1.58 (0.68, 2.48)	< 0.001	
	Study 5	+28.3 IU/L	6.7	20	1.34(0.38, 2,24)	<0.001	
Pooled (Random Effects)				**100.0**	**1.38 (0.88, 1.88)**	**< 0.001**	**63%**
Alloxan	Study 4	+15.4 IU/L	4.5	100	0.95 (0.28, 1.62)	0.006	0%
Pooled (Random Effects)				**100.0**	**0.95 (0.28, 1.62)**	**0.006**	**0%**

Table 4. Summary of meta-analysis results for osteocalcin (OCN) levels.

Subgroup	Study	Mean Difference (Treatment - Control)	Standard Error	Weight (%)	SMD (95% CI)	p-value	I^2 (%)
Overall							
	Study 1	+4.8 ng/mL	1.5	19.2	1.07 (0.35, 1.79)	0.004	
	Study 2	+3.5 ng/mL	1.2	22.5	0.85 (0.20, 1.50)	0.011	
	Study 3	+5.9 ng/mL	1.8	16.5	1.18 (0.38, 1.98)	0.004	
	Study 4	+2.9 ng/mL	1.0	25.8	0.72 (0.12, 1.32)	0.019	
	Study 5	+4.2 ng/mL	1.4	16	1.04 (0.1, 1.98)	0.03	
Pooled (Random Effects)				**100.0**	**0.98 (0.55, 1.41)**	**< 0.001**	**58%**
Subgroup: Time Point							
Early (≤ 14 days)	Study 1	+3.1 ng/mL	1.3	29.0	0.80 (-0.18, 1.78)	0.11	
	Study 2	+2.8 ng/mL	1.1	37.7	0.55 (-0.15, 1.25)	0.12	
	Study 4	+2.5 ng/mL	1.0	33.4	0.62(-0.4, 1.64)	0.233	
Pooled (Random Effects)				**100.0**	**0.65 (-0.05, 1.35)**	**0.07**	**62%**
Late (> 14 days)	Study 1	+5.5ng/mL	1.7	26.5	1.35 (0.45, 2.25)	0.003	
	Study 3	+6.2 ng/mL	2.0	28.4	1.40 (0.42, 2.38)	0.005	
	Study 5	+5.8 ng/mL	1.8	45.1	1.05 (0.30, 1.80)	0.006	
Pooled (Random Effects)				**100.0**	**1.22 (0.70, 1.74)**	**< 0.001**	**45%**
Subgroup: Animal Species							
Rats	Study 1	+4.8ng/mL	1.5	32.8	1.07 (0.35, 1.79)	0.004	
	Study 3	+5.9ng/mL	1.8	30.6	1.18 (0.38, 1.98)	0.004	
	Study 5	+4.2 ng/mL	1.4	36.6	1.04 (0.1, 1.98)	0.03	
Pooled (Random Effects)				**100.0**	**1.11 (0.58, 1.64)**	**< 0.001**	**60%**
Mice	Study 2	+3.5 ng/mL	1.2	48.1	0.85 (0.20, 1.50)	0.011	
	Study 4	+2.9 ng/mL	1.0	51.9	0.72 (0.12, 1.32)	0.019	
Pooled (Random Effects)				**100.0**	**0.78 (0.30, 1.26)**	**0.001**	**55%**
Subgroup: Diabetes Induction Method							
STZ	Study 1	+4.8 ng/mL	1.5	23.5	1.07 (0.35, 1.79)	0.004	
	Study 2	+3.5ng/mL	1.2	27.5	0.85 (0.20, 1.50)	0.011	
	Study 3	+5.9 ng/mL	1.8	31.7	1.18 (0.38, 1.98)	0.004	
	Study 5	+4.2 ng/mL	1.4	17.3	1.04 (0.1, 1.98)	0.03	
Pooled (Random Effects)				**100.0**	**1.05 (0.60, 1.50)**	**< 0.001**	**59%**
Alloxan	Study 4	+2.9 ng/mL	1.0	100	0.72 (0.12, 1.32)	0.019	0%
Pooled (Random Effects)				**100.0**	**0.72 (0.12, 1.32)**	**0.019**	**0%**

Table 5. Publication bias assessment.

Outcome variable	Assessment method	Test statistic	p-value	Interpretation	Potential Bias Source (if present)
Alkaline Phosphatase (ALP)					
	Funnel Plot	Visual Inspection	N/A	Roughly Symmetrical	Limited number of Studies
	Egger's Test	t = 1.35	0.21	No significant asymmetry	-
	Begg's Test	z = 1.10	0.27	No significant asymmetry	-
	Trim and Fill	0 studies imputed	N/A	No adjustment needed	-
Osteocalcin (OCN)					
	Funnel Plot	Visual Inspection	N/A	Roughly Symmetrical, possible slight asymmetry	Limited number of Studies, One study with larger effect size
	Egger's Test	t= 1.62	0.15	No significant asymmetry	-
	Begg's Test	z = 1.30	0.19	No significant asymmetry	-
	Trim and Fill	1 study imputed, Adjusted SMD=0.88 (95%CI: 0.46, 1.30)	-	Minor adjustment, overall effect remains significant	Heterogeneity between studies, one study had greater mean different and Standar eror than other.

4. Discussion

Our meta-analysis revealed significant increases in both ALP and OCN levels in diabetic animals treated with mangosteen nanoextract compared to controls. This section delves deeper into the implications of these findings, exploring the roles of ALP and OCN in bone regeneration and the potential mechanisms by which mangosteen nanoextract exerts its effects. ALP, a membrane-bound enzyme primarily produced by osteoblasts, plays a pivotal role in the early stages of bone regeneration. Its primary function is to hydrolyze phosphate esters, releasing inorganic phosphate, a crucial component for the formation of hydroxyapatite crystals, the building blocks of bone mineral. The observed increase in ALP levels in the mangosteen nanoextract-treated group, particularly during the early phase of callus formation, strongly suggests that the extract stimulates osteoblast activity and enhances matrix mineralization. This finding aligns with previous in vitro studies demonstrating that mangosteen's xanthones, especially α-mangostin, promote osteoblast differentiation and activity. The precise mechanisms by which mangosteen's xanthones stimulate ALP activity remain an area of ongoing research. Mangosteen xanthones may upregulate the expression of key transcription factors such as Runx2 and Osterix, which are essential for osteoblast differentiation and bone formation. These transcription factors orchestrate the expression of various genes involved in bone matrix production and mineralization, including ALP. Mangosteen xanthones may activate signaling pathways such as the BMP and Wnt pathways, which are critical for osteoblast differentiation and bone formation. These pathways regulate various cellular processes, including cell proliferation, differentiation, and matrix production,

ultimately leading to increased ALP expression and activity. The nano-formulation of mangosteen extract likely enhances the bioavailability and cellular uptake of xanthones, allowing them to reach osteoblasts more effectively. This improved delivery could lead to a more pronounced stimulation of ALP activity and bone formation. OCN, a non-collagenous protein synthesized primarily by osteoblasts, serves as a marker of osteoblast maturation and bone remodeling. It plays a crucial role in bone mineralization and calcium homeostasis, contributing to the structural integrity and metabolic function of bone. The significant increase in OCN levels observed in the mangosteen nanoextract-treated group, particularly during the later phase of callus formation, suggests that the extract promotes osteoblast maturation and bone remodeling. This finding aligns with the temporal expression pattern of OCN, which typically rises during the later stages of fracture healing when osteoblasts transition from matrix production to mineralization and remodeling. The mechanisms by which mangosteen nanoextract influences OCN expression are not yet fully understood. Modulation of Bone Morphogenetic Proteins (BMPs) are a family of growth factors that play critical roles in bone formation and remodeling. Mangosteen nanoextract may modulate BMP signaling pathways, leading to increased OCN expression and enhanced bone remodeling. The Wnt signaling pathway is another critical regulator of bone formation and remodeling. Mangosteen nanoextract may influence Wnt signaling, promoting osteoblast maturation and OCN expression. Mangosteen xanthones may directly affect osteoblast function, promoting their maturation and increasing OCN production. This could involve modulation of gene expression, protein synthesis, or cellular signaling pathways within osteoblasts. The findings of our meta-analysis suggest that mangosteen nanoextract may be a promising therapeutic agent for enhancing bone regeneration in diabetic fractures. By promoting both ALP and OCN expression, the extract targets both early and late stages of fracture healing, potentially leading to more complete and efficient bone regeneration. In diabetic patients, impaired fracture healing is a significant concern, often leading to delayed union, non-union, and increased risk of complications. The ability of mangosteen nanoextract to enhance ALP and OCN levels in diabetic animal models offers hope for improving fracture healing outcomes in this population.[11-15]

The osteogenic effects observed in our meta-analysis may be attributed to a complex interplay of various mechanisms, primarily mediated by the bioactive compounds present in mangosteen nanoextract. This section explores these potential mechanisms in detail, shedding light on how mangosteen nanoextract may enhance bone regeneration in diabetic fracture models. Nanotechnology has revolutionized drug delivery by enhancing the bioavailability and cellular uptake of therapeutic agents. The nano-formulation of mangosteen extract capitalizes on this principle, improving the delivery of its bioactive compounds, particularly xanthones, to bone cells. Xanthones, a class of polyphenolic compounds abundant in mangosteen, have demonstrated a wide range of pharmacological activities, including antioxidant, anti-inflammatory, and osteogenic effects. However, their therapeutic potential is often limited by poor bioavailability and rapid metabolism. Reducing the particle size of mangosteen extract to the nanoscale dramatically increases its surface area, enhancing its solubility and dissolution rate. This improved solubility facilitates better absorption and distribution throughout the body, increasing the concentration of xanthones reaching bone cells. Nanoencapsulation protects xanthones from degradation in the gastrointestinal tract and during circulation, ensuring that a higher concentration of active compounds reaches the target site. Nanoparticles can be engineered to facilitate cellular uptake through various mechanisms, such as endocytosis or passive diffusion. This enhanced uptake ensures that xanthones reach the intracellular compartments where they can exert their osteogenic effects. By

enhancing the bioavailability and cellular uptake of xanthones, mangosteen nanoextract amplifies their therapeutic potential, leading to a more potent effect on osteoblast activity and bone formation. Oxidative stress, an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms, plays a detrimental role in bone healing. ROS can damage cells, disrupt signaling pathways, and impair the function of osteoblasts, hindering bone regeneration. Mangosteen xanthones are potent antioxidants that can scavenge free radicals and reduce oxidative stress. Xanthones can donate electrons to neutralize free radicals, preventing them from causing cellular damage. Xanthones can chelate metal ions such as iron and copper, which can catalyze the formation of ROS. Xanthones may upregulate the expression of antioxidant enzymes such as superoxide dismutase (SOD) and catalase, which help to neutralize ROS. By reducing oxidative stress, mangosteen nanoextract creates a more favorable environment for bone regeneration. This antioxidant activity may be particularly beneficial in diabetic patients, who often experience increased oxidative stress due to hyperglycemia and other metabolic disturbances. Inflammation is an essential component of the initial phase of fracture healing, initiating the healing cascade and recruiting immune cells to the injury site. However, chronic or excessive inflammation can disrupt bone regeneration by interfering with osteoblast function and promoting tissue breakdown. Mangosteen xanthones possess anti-inflammatory properties, which may help to modulate the inflammatory response during fracture healing. Xanthones can inhibit the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), which are key mediators of inflammation. Xanthones may suppress inflammatory signaling pathways such as the nuclear factor-kappa B (NF-κB) pathway, which plays a central role in regulating the inflammatory response. Xanthones may promote the production of anti-inflammatory mediators such as interleukin-10 (IL-10), which helps to resolve inflammation and promote tissue repair. By modulating the inflammatory response, mangosteen nanoextract may promote a more balanced healing process, preventing excessive inflammation from hindering bone regeneration. This anti-inflammatory activity may be particularly beneficial in diabetic patients, who often exhibit chronic low-grade inflammation. In addition to their systemic effects on bioavailability, oxidative stress, and inflammation, mangosteen xanthones may also exert direct effects on bone cells. They may stimulate osteoblast differentiation, proliferation, and activity, leading to increased bone formation. They may also inhibit osteoclast activity, reducing bone resorption. Xanthones may modulate the expression of genes involved in osteoblast differentiation, bone matrix production, and mineralization. Xanthones may regulate cellular signaling pathways within osteoblasts and osteoclasts, influencing their activity and function. Xanthones may interact with cell surface receptors on osteoblasts and osteoclasts, triggering intracellular signaling cascades that affect their behavior. The direct effects of mangosteen xanthones on bone cells may contribute to the enhanced bone regeneration observed in our meta-analysis. By stimulating osteoblasts and inhibiting osteoclasts, mangosteen nanoextract may promote a net increase in bone formation, leading to more efficient fracture healing.[16-20]

5. Conclusion

This meta-analysis suggests that mangosteen nanoextract may enhance bone regeneration in diabetic fracture models by modulating ALP and OCN levels, key biomarkers of osteoblast activity and bone formation. Mangosteen nanoextract treatment was associated with a significant increase in ALP levels during the early phase of callus formation. Similarly, OCN levels were significantly higher in the nanoextract-treated group during the later stages of callus formation. The use of nanoextract enhances the bioavailability and cellular uptake of xanthones, allowing them to reach osteoblasts more effectively.

This improved delivery could lead to a more pronounced stimulation of ALP activity and bone formation. Further research, including well-designed clinical trials, is warranted to confirm these findings and translate them into clinical practice. Future studies should focus on elucidating the precise mechanisms by which mangosteen nanoextract influences bone regeneration, determining the optimal dosage and treatment duration, and evaluating the long-term safety and efficacy of mangosteen nanoextract in promoting fracture healing in diabetic patients.

6. References

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