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OSTEOGENIC POTENTIAL OF *MORINGA OLEIFERA* – A SYSTEMATIC REVIEW OF *IN-VIVO* STUDIES

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ABSTRACT: Background: Alveolar bone defects are common in dentistry. Bone tissue repair involves a complex inflammatory process, regulated by osteoprogenitor cells from the periosteum and endosteum, which are vital for new bone matrix formation. Recently, plant extracts have gained attention as potential treatments for bone lesions. *Moringa oleifera* exhibits various medicinal properties, largely due to its flavonoid content, which supports osteoblastic proliferation. These compounds enhance osteogenic differentiation and aid in bone defect healing. This review aims to assess the effectiveness of *Moringa oleifera* in promoting bone regeneration in *in-vivo* models. **Methodology:** A comprehensive electronic literature search was conducted using Medline/PubMed database based on PICO analysis. From an initial pool of 40 papers, 6 *in-vivo* studies using *Moringa oleifera*-based biomaterials met the inclusion criteria. **Results:** After removing duplicates and irrelevant abstracts, 9 studies remained. Four were excluded for not meeting inclusion criteria. One additional paper was identified through hand searching, totaling 6 included studies. Animal models used were *Cavia cobaya*, rats, and rabbits. *Moringa oleifera* was applied in various forms: leaf extract combined with DFDBBX, beta-tricalcium phosphate, marine collagen, hydrogel, and PRF. Bone regeneration was primarily assessed using micro CT and histological analysis. **Conclusion:** *Moringa oleifera* promotes bone repair by enhancing osteogenesis, increasing calcification, and supporting bone callus formation and mineralization. These effects are likely due to its anti-inflammatory and antioxidant properties.

INTRODUCTION: Bone remodelling is a highly coordinated process involving a series of cellular events, including inflammation, cell proliferation, and matrix remodelling, regulated by osteogenesis and angiogenesis [1]. The skeletal system possesses a remarkable ability to regenerate, allowing it to maintain both structure and function. However, certain clinical conditions require accelerated bone formation to optimize healing outcomes [2].

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To address this, researchers have been investigating the inflammatory mechanisms that govern bone repair, aiming to identify novel therapeutic targets for treating bone defects [3, 4]. In this regard, natural compounds, biomaterials, and their derivatives have emerged as promising alternatives, offering potential benefits such as reduced side effects, lower costs, and enhanced healing efficiency [5].

Plants have long been a vital source of nutraceuticals, offering health benefits, disease prevention, and therapeutic applications, while also playing a significant role in modern pharmaceutical research. Approximately 75% of the global population [6] relies on plant-derived medicines, which remain the primary treatment approach in many developing countries [7].

Research indicates that certain bioactive compounds extracted from natural sources exhibit potent anti-inflammatory, antioxidant, and regenerative properties, supporting their effectiveness in treating various diseases [8]. However, the precise mechanisms by which these natural compounds influence inflammation and bone healing remain inadequately understood. Existing evidence is limited, fragmented, and based on isolated studies, leading to inconsistencies in the literature. Despite this uncertainty, it is believed that plant extracts contribute to bone repair by enhancing antioxidant defenses, reducing tissue inflammation, promoting vascularization, and stimulating the proliferative activity of bone cells.

Moringa oleifera is one of the most extensively cultivated species within the Moringaceae family. It originates from regions of Southeast Asia, Africa, and the Americas. In these areas, its leaves, flowers, and young pods are commonly consumed as vegetables [9]. The plant is renowned for its rich nutritional and phytochemical composition, serving as an excellent source of proteins, vitamins, and bioactive compounds. It contains antioxidants, flavonoids, saponins, alkaloids, tannins, and phenolic compounds, along with essential minerals such as calcium, phosphorus, magnesium, potassium, sodium, sulphur, zinc, copper, manganese, iron, and selenium [10]. Every part of *Moringa oleifera* possesses medicinal properties, demonstrating a wide range of pharmacological activities, including antihyperglycemic, antidyslipidemic, antioxidant, antihypertensive, immunomodulatory, chemoprotective, radio-protective, diuretic, anti-inflammatory, antipyretic, antiepileptic, antitumor, antiulcer, antispasmodic, antibacterial, and antifungal effects [11]. This extensive therapeutic potential is likely attributed to its unique composition of bioactive compounds, such as rhamnosyloxy benzyl isothiocyanate and its derivatives, niaziminins, niazinins, β-sitosterol, niacin, phenolic acids, glucosinolates, flavonoids, gallic acid, coumarin, and caffeic acid.

Notably, the leaves and other plant parts contain a distinctive combination of zeatin, quercetin, β-sitosterol, caffeoylquinic acid, and kaempferol [12, 13]. Among these, β-sitosterol and quercetin have been shown to promote cell proliferation and regeneration [14]. Therefore, this systematic review aims to evaluate the osteogenic potential of *Moringa oleifera* in facilitating bone regeneration.

MATERIAL AND METHODS: The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [15] offered a precise process that was followed in selecting the systematic review's methods and inclusion criteria **Fig. 1.**

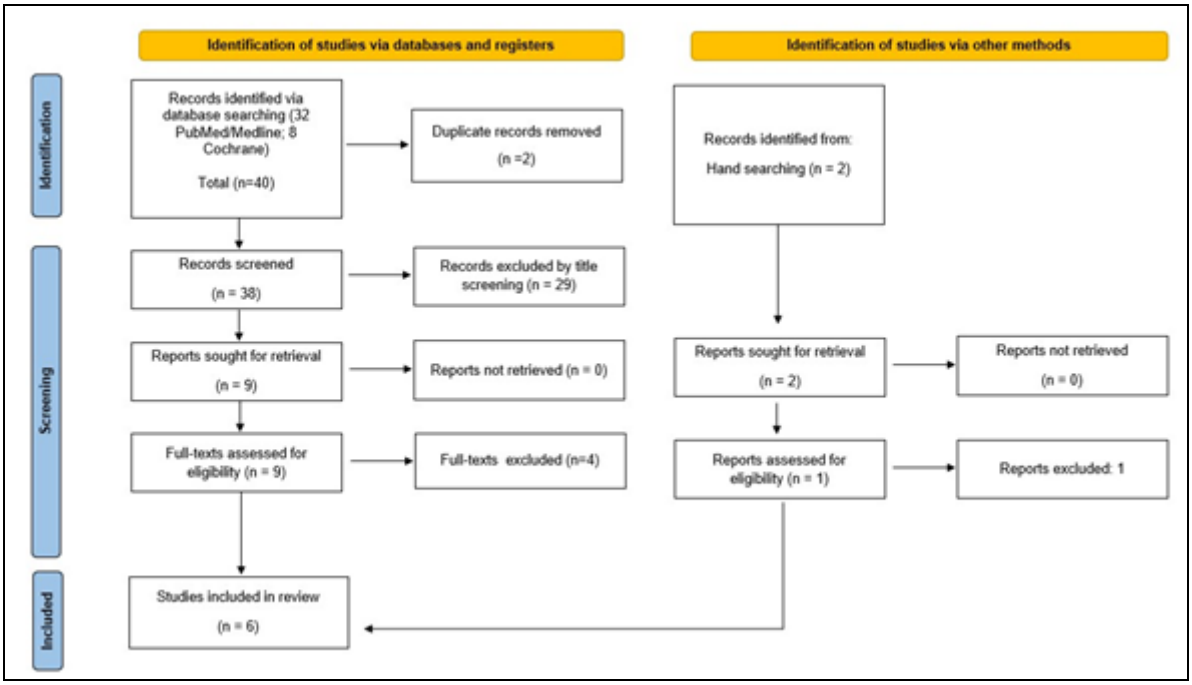

FIG. 1: PRISMA FLOWCHART

Research Question: Is *Moringa oleifera* effective in promoting bone regeneration?

Population, Intervention, Comparison and Outcome (PICO) Analysis: The PICO analysis [16] was used as follows: population/problem (P) - osseous defects OR bone defects OR intrabony defects in animal models, intervention (I) – *Moringa oleifera*, comparison (C) – control OR vacant OR unfilled bone defects, and outcome (O) - bone regeneration OR bone fill.

Inclusion and Exclusion Criteria: This review included only research studies, excluding conference papers, abstracts, pilot studies, reviews, communications, letters to the editor, and editorials. Additionally, studies published in languages other than English were not considered. Regarding study relevance, only *in-vivo* research involving *Moringa oleifera* was selected, without restrictions on animal species or bone defect modelling techniques. Any *Moringa oleifera*-based biomaterial, such as hydrogels, scaffolds, etc were included. There were no limitations on the types of biomaterials or chemical compounds combined with *Moringa oleifera*.

Search Strategy & Selection of Studies: In February 2025, an extensive electronic literature review was conducted on Medline/PubMed database to search for the article published in English language, without any limitations on the year of search using the terms "bone defects", "*Moringa oleifera*", "bone fill", "bone regeneration". Additionally, a manual search was conducted to find other suitable studies by examining the references of the included studies and other published reviews.

The selection process began with the removal of duplicate articles, followed by screening the titles and abstracts of the remaining studies to determine their eligibility. After applying the inclusion and exclusion criteria, full-text assessments were conducted on the qualifying articles. Reviewer RG carried out the article selection process based on the predefined criteria, while reviewer RC re-evaluated the selection strategy and provided input in cases of uncertainty regarding article inclusion or exclusion. Any disagreements were resolved through a consensus-based approach, and the level of agreement among the reviewers was measured using Kappa statistics.

Data Extraction: Data was extracted by RG from each study under the following headings - author, year, study design, aim, methodology, results and conclusion.

Risk of Bias (ROB) Assessment: For animal studies, the SYRCLE's RoB tool an adaptation of the Cochrane ROB tool was utilized to address biases specific to animal intervention research [17]. This tool assessed multiple domains, including selection bias (covering sequence generation, baseline characteristics, and allocation concealment), performance bias (evaluating random housing and blinding methods), detection bias (considering randomization and blinding in outcome assessment), attrition bias (examining incomplete outcome data), reporting bias (analyzing selective outcome reporting), and other potential sources of bias. To facilitate this evaluation, signal questions were used, with responses categorized as "Yes" for a low risk of bias, "No" for a high risk of bias, and "Unclear" when insufficient information was available to determine the bias risk accurately.

RESULTS:

Literature Search: Initially, the literature search resulted in 40 papers. Thirty-eight papers remained after excluding duplicate articles, out of which 29 papers were further excluded following the screening the abstracts, resulting in 9 studies for retrieval. Four studies did not fulfil the inclusion criteria, hence were excluded **Fig. 1**. The hand search resulted in 1 additional paper, and finally, 6 articles were included in this review. The reviewers had a high level of agreement (k= 0.867).

Study Description: The characteristics of the included studies are tabulated in **Table 1.** The animals used in *in-vivo* studies included *Cavia cobaya*, [18, 19] rats [20, 21] and rabbits [22, 23]. The bone defect models in *Cavia cobaya* included mandibular incisor sockets [18, 19], in rats it was the femoral bone [20, 21], in rabbits one study used buccal bone defects [22] and other study used mandibular edentulous area [23] as bone defect model. The various forms in which MO was used in bone defects were, *Moringa* leaf extract and DFDBBX,

[18, 19]combination of MO and marine collagen,[20, 21] moringa hydrogel and PRF, [22] MO plus beta tricalcium phosphate [23]. Assessment of bone regeneration in almost all studies was done by microCT and histological analysis.

TABLE 1: CHARACTERISTICS OF INCLUDED STUDIES

Sl. no.	Author/ Year	Study design	Aim	Animal model/ age/gender/ weight	Methodology	Results	Conclusion
1	Rostiny et al. 2016[18]	*In-vivo*	To evaluate the effect of combined *Moringa oleivera* leaf extract and demineralized freeze-dried bovine bone xenograft (DFDBBX) towards the formation of osteoblasts and osteoclasts in the tooth extraction sockets of *Cavia cobaya*	28 *Cavia cobayas*, weighing 300-350 grams, aged 3-3.5 months were divided into four groups	The combination of *Moringa oleifera* leaf extract and DFDBBX was inducted intothe sockets of lower incisor tooth with certain dose in each group, ointment 1 containing PEG (a mixture of PEG 400 and PEG 4000) for control group, ointment 2 containing *Moringa oleifera* leaf extract and DFDBBX and PEG (at active substance concentration of 0.5%) for group 1, ointment 3 containing *Moringa oleifera* leaf extract and DFDBBX and PEG (at active substance concentration of1%) for group 2, and Ointment 4 containing *Moringa oleifera* leaf extract and DFDBBX and PEG (at active substance concentration of 2%) for group 3 and assessed on 28th day histologically.	Highest and lowest mean number of osteoblast and osteoclast cells respectively was in the group treated with the active substance concentration of 2% (group 3), followed by group 2, group 1.	The combination of *Moringa oleifera* leaf extract and DFDBBX at 2% concentration canincrease the number of osteoblasts and decrease osteoclasts in the healing of tooth extraction sockets of *Cavia cobaya*.
2	Kresnoadi U et al. 2019[19]	*In-vivo*	To analyze the role of the combination of *Moringa* leaf extract and DFDBBX induced in socket preservation when generating TGF-β1 and osteocalcin expressions in alveolar bone of *Cavia cobaya*	56 *Cavia cobaya* (weighed 300–350g and aged3–3.5 months.)	The left mandibular incisors of *Cavia cobaya* were extracted and divided intofour groups subjected to different socket preservation treatments. The first group treated with polyethylene glycol, the second group with DFDBBX, the third group with *Moringa* leaf extract, and the fourth groupwith a combination of DFDBBX and *Moringa* leaf extract. The *C. cobaya* were examined on days 7 and 30, after which the specimens were	Significant difference was found in TGF-β1 and osteocalcin expressions between the groups ($P < 0.05$). The highest mean amount of TGF-β1 and osteocalcin was found in the fourth group on both days 7 and 30.	The combination of osteoconduction and osteoinduction properties of *Moringa* leaf extract and DFDBBX generated significant amounts of TGF-β1 and osteocalcin expressions. Therefore, the combination of these two materials leads to a greater chance of successful post-extraction socket

					sacrificed and examined using an immunohistochemical technique.		preservation by accelerating alveolar bone regeneration.
3	Al-Azzawi AS & Al-Ghaban NMH 2021[20]	*In-vivo*	To evaluate the differences between the healing process of MO, Marine collagen (MC) and their combination (MO & MC) on bone defects.	20 albino rats, aged 4-5 months, weighing 350-450 gms.	Both femurs of rats were prepared by drilling intrabony defects (2mm diameter, 3mm depth). In 10 rats, right defects consider control while left defects treated with MO, in other 10 rats right defect treated with MC while left defects treated with MM. Rats were sacrificed after 2 and 4 weeks and evaluated for bone healing by histological and histomorphometric analysis.	Histological result revealed acceleration of bone healing in combination group (MO&MC) than other groups.	Combination of MO with MC improve bone healing and increase osteogenic capacity.
4	Al-Azzawi AS & Al-Ghaban NMH 2022[21]	*In-vivo*	Differences in healing process in bones were evaluated in this study among bone defects that heal normally (control group) and (experimental groups) using Marine collagen (MC), *Moringa oleifera* extract (MO) and combination of (MO &MC).	20 albino rats, aged 4-5 months, weighing 350-450 gms	Bone defects were created in both femurs of rats by drilling. In ten rats, the right bone defects left to heal normally, and left MO added to the bone defects, in other ten rats MC added to the right bone defects, left defects treated with MO & MC. Rats were scarified after 2 and 4 weeks. Immunohistochemical analysis of bone marrow stromal cells, osteocytes, osteoblasts and osteoclasts done using Procollagen type I N-terminal Propeptide (PINP) at 2nd and 4th week.	Stronger positive expression of PINP was seen in bone defects treated with (MO&MC) than other groups at 2 weeks and moderate expression seen at 4 weeks duration.	Treatment of bone defects with MO and MC showed increased osteogenic capacity by increase in the immunore activity of PINP.
5	El Soudany KS et al. 2023[22]	*In-vivo*	To evaluate the effect of *Moringa oleifera* (MO) gel and platelet-rich fibrin (PRF) in the treatment of intrabony defects in rabbits.	8 adult male rabbits weighing 3.5–4 kgs	16 buccal bone defects were divided into 2 groups; group (1) treated with moringa hydrogel and PRF (right site), group (2) treatedwith PRF (left site). Computed tomography (CT) radiography and histological examination were assessedat baseline, 14 and 28 days. The defects were induced in the form of one osseous wall defect between the1st and the 2nd molars.	CT radiograph results showed there was a significant increase in bone density at 28 days ingroup 1 than in group 2 (843.13 ± 97.82 to 713.0 ± 51.09). The histological result revealed the defect area on the (PRF + Moringa) was	Radiographical examination, histological and healing scores confirmed the superiority of Moringa + PRF mix results in an increase in bone fill and density in induced periodontal intrabony defects regeneration in rabbits.

						almost filled completely by newly formed bone with few spots of retarded calcification. While (PRF) showed complete filling of the defect area by more fibrous tissue. The healingscore showed a significant elevation of bone defect healing score in (PRF + Moringa group) when comparedto (PRF group) at both times of evaluation.	
6	Elsadek NA et al. 2024[23]	*In-vivo*	To histologically and histomorphometrically evaluate the efficiency of *Moringa oleifera* leafextract as an osteopromotive biomaterial.	24 adult male New Zealand rabbits, aged 6–7 months and weighing 2.5–3.5 kgs.	Critical-sized bone defects were created in the edentulous areas of the mandibles of rabbits. The defects of the control group were filled with Beta-tricalcium phosphate, while the defects of the test group were filled with Beta-tricalcium phosphate combined with *Moringa oleifera* leaf extract. The results were evaluated histologically and histomorphometrically after 4 and 8 weeks.	Significant increase in the surface area of bone and the number of osteoblasts in test groups compared to those in the control groups. In test group, the trabeculae were thicker and more inter-communicated. Considerable blood supply was also seen between intervening connective tissue.	The formed trabeculae are thicker and more inter-communicate. Considerable blood supplyis also seen between intervening connective tissue.

Quality Assessment: The risk of bias (RoB) assessment for *in-vivo* studies was performed using SYRCLE's RoB tool, as illustrated in **Fig. 2**. The analysis revealed a low RoB in the selection bias domain. Similarly, the reporting bias domain showed a low RoB, attributed to comprehensive documentation of both primary and secondary outcomes, as well as well-structured methodology and results sections across all studies. However, in other domains, such as random housing and blinding, more than 70% of the studies had an unclear RoB. This was mainly due to insufficient reporting rather than a lack of methodological rigor in housing conditions or outcome assessment timing.

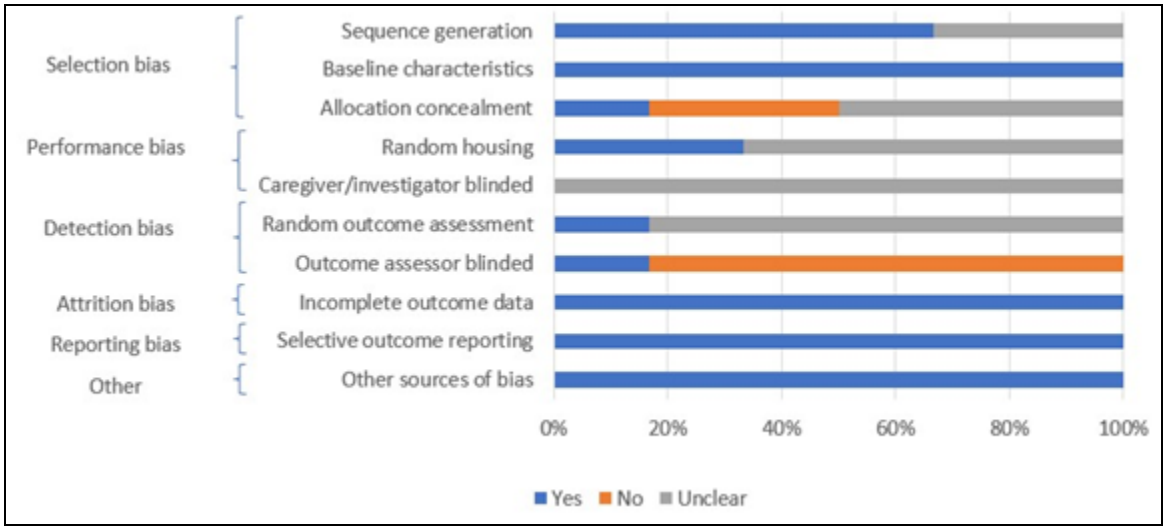

FIG. 2: RESULT OF SYRCLE'S ROB ASSESSMENT

DISCUSSION: In this systematic review we aimed to evaluate the efficacy of *Moringa oleifera* (MO) extracts and their derivatives on bone repair in animal models. Despite the significant variability among the included studies, the overall findings suggest that MO extracts were effective in promoting bone healing. Treatment with MO extract led to the release of markers and anti-inflammatory mediators, accelerating the bone repair process. Additionally, histopathological and radiological assessments revealed key indicators of bone remodeling, such as new bone formation, callus development, increased cell proliferation, and enhanced osteogenesis. These findings suggest that certain bioactive compounds within the extracts may stimulate the proliferation of specific cell types, likely due to their interactions with cellular components involved in the healing process.

The studies included in this review utilized rats, rabbits, and *Cavia cobaya* as experimental models. While the results from these animal models cannot be directly extrapolated to humans, [24] they offer valuable insights into the biology and pathophysiology of bone lesions and are essential for research [25]. The main advantage of using these animals lies in the reduced costs, as more animals can be housed in a smaller space, and their shorter reproductive cycles. These factors make it possible to generate a sufficient number of animals for large study groups in a relatively short period, facilitating robust statistical analyses [26]. Other parameters that showed significant variation included the type and size of bone defects, as well as the treatment duration. Radiological assessments were useful for evaluating bone fill, while immunohistochemical and histopathological analyses were crucial for interpreting and confirming the cellular effects of phytotherapeutic compounds in tissue repair [27]. These analyses helped identify the role of osteogenic cells in bone callus formation, cell organization, and the release of immunomarkers [28, 29]. The synthesis of proinflammatory mediators, observed in both spongy and compact bone regions through immunological and histopathological analyses, was higher in the MO treatment groups compared to the control groups, indicating the effectiveness of the extracts in promoting bone repair. This suggests that certain components of the extract, or their synergistic effects, may enhance the synthesis of specific mediators and stimulate the proliferation of certain cell types, thereby accelerating bone matrix synthesis and bone callus formation [30].

Moringa leaf extract is abundant in flavonoids, saponins, alkaloids, and tannins. Flavonoids, particularly kaempferol, quercetin, and others, are effective in managing various inflammatory conditions [31] due to their ability to inhibit the cyclooxygenase-2 (COX-2) enzyme, which plays a role in producing inflammatory mediators derived from arachidonic acid. These flavonoids also block the release of histamine from mast cells and basophils, exerting anti-inflammatory effects. As a result, the activity of COX-2 reduces the production of pro-inflammatory mediators such as IL-1, IL-6, and TNF-α [32]. Kaempferol and quercetin also directly inhibit TNF-α activity and

RANKL expression [33]. Both flavonoids are capable of binding to atoms or acting as scavengers for free radicals, preventing the excessive formation of reactive oxygen species (ROS). Additionally, quercetin can inhibit osteoclast differentiation and activation, as well as induce osteoclast apoptosis [34, 35].

Moringa leaf extract possesses indirect osteoinductive properties due to its unique composition. A study by Zhang *et al.* demonstrated that flavonoids can stimulate osteoblast proliferation and differentiation [36]. This finding is further supported by Patel's research, which showed that flavonoid compounds from *Moringa* leaf extract aid in osteoblast differentiation, promoting bone formation [37]. Additionally, saponins found in *Moringa* leaf extract enhance osteogenic activity, encouraging the proliferation and differentiation of osteoblasts [38]. Tannins, present in the extract, have been shown to inhibit osteoclast differentiation, thus supporting the formation of new bone [33]. Consequently, studies that combined *Moringa* extract with bone substitutes such as DFDBBX [18, 19] and β-TCP [23] reported improved bone regeneration compared to controls, owing to the osteoinductive properties of *Moringa* and the osteoconductive properties of the bone substitutes, which act as scaffolds.

M.I. Khan *et al.*[39] conducted a study to elucidate the dose dependent effects of *Moringa oleifera* leaf extract on the growth of the human osteoblast-like osteosarcoma SaOS-2 cell line and primary osteoblast cells. β-sitosterol was found to be biologically active components against G-protein coupled receptors (GPCR) and acting as ion channel modulator, nuclear receptor, protease inhibitor and enzyme inhibitor. Quercetin and kaempferol, on the other hand, showed active kinase and other enzyme inhibitors and act as active components against nuclear receptor. Authors concluded that MO worked as anti-osteosarcoma agent at high concentration via modulating ROS, chromatin condensation, and cell cycle arrest. Low concentrations, on the other hand, stimulated proliferation, differentiation, and mineralization and induced expression of BMP2 and Runx2 (runt-related transcription factor 2) osteogenic genes. Thus, hermetic like biphasic concentration-dependent response of MO leaves might be crucial element in bone pathogenesis. Moreover, *Moringa* leaf extract contains various phytochemicals, particularly phytoestrogens, which have beneficial effects on bone health. Phytoestrogens are plant-derived compounds that mimic the bioactivity of estrogen due to their structural similarities with estradiol (17-beta-estradiol), one of the naturally occurring forms of estrogen in the body. These phytoestrogens are primarily classified as flavonoids within the isoflavonoid group. They positively influence bone health by stimulating osteoblast activity through estrogen-mediated mechanisms [40, 41].

P.R. Pachimalla *et al.*[42] formulated a hydrophilic gel containing Acemannan and *Moringa oleifera* to be used in conjunction with dental implant placement, aiming to create a hydrophilic surface for the implant, enhance blood-implant contact, and improve bone-implant integration in rabbits. The results demonstrated significant new bone formation in the tibia, suggesting that the hydrogel's components promoted bone growth by increasing osteoblastic activity. As noted in studies by Das *et al.*[43] and Patel *et al.*[37] there were no signs of degenerative changes, necrosis, fibrosis, or inflammation at the site of the new bone-implant interface.

One limitation of this review is the potential publication bias within the included articles. Several experimental aspects were overlooked, such as the absence of randomization, which highlights the need for improved experimental designs and stricter adherence to guidelines in reporting animal studies to ensure reliable scientific evidence. Furthermore, the incomplete phytochemical characterization of *Moringa oleifera* extracts makes it challenging to pinpoint the specific compounds responsible for the observed positive effects. Lastly, the methodologies and evaluation parameters across the studies were highly varied, with different measures reported in each study, contributing to a lack of consistency.

CONCLUSION:

The following conclusions can be drawn:

1. *Moringa* extracts promote bone repair by enhancing osteogenesis, increasing calcification, and facilitating the formation and

mineralization of bone callus, there by accelerating the process of new bone formation.

2. These effects are likely linked to the anti-inflammatory and antioxidant properties of the extracts.

3. However, more detailed methodological descriptions are necessary to facilitate better comparison across studies and ensure the reproducibility of future research.

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