RESEARCH PAPER

Molecular Evaluation of Osteoprotegerin (OPG) mRNA Expression in Osteoblast Cells Treated with Moringa oleifera: An Integrated In Vitro and In Silico Analysis

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ABSTRACT
Background: Bone remodeling is regulated by the balance between osteoblast and osteoclast activity, primarily controlled through the osteoprotegerin (OPG)/RANKL signaling pathway. Natural phytochemicals have gained attention as potential modulators of bone metabolism with fewer adverse effects. This study evaluated the effect of Moringa oleifera extract on OPG mRNA expression in osteoblast-like cells and supported the findings using in silico molecular docking analysis.
Methods: SaOS2 osteoblast-like cells were divided into three groups: untreated control, Moringa oleifera extract 2.5 µg/mL, and 25 µg/mL. Following 24-hour treatment, RNA was isolated and quantitative real-time PCR was performed. Relative OPG mRNA expression was calculated using the 2ΔΔCt method. Statistical analysis was performed using Krushkal wallis test and dunn post hoc tests. Molecular docking was conducted using mouse RANKL (PDB ID: 1S55) against selected phytochemicals including quercetin, kaempferol, and chlorogenic acid.
Results: A significant dose-dependent increase in OPG mRNA expression was observed following treatment. Docking analysis showed stable ligand–protein interactions, with kaempferol and CID 1794427 demonstrating the strongest binding affinity (−8.2 kcal/mol). Key interactions involved residues THR260, ASN262, SER267, TRP263, and HIS270.
Conclusion: Moringa oleifera may promote a bone-protective environment by enhancing OPG expression and interacting with RANKL-associated targets, supporting its potential application in bone regeneration and future dental therapeutics..
Keywords: Bone remodeling, OPG/RANKL signaling pathway, osteoblast differentiation, phytochemicals, and molecular docking (SDG 3: Good Health and Well-being and SDG 9: Industry, Innovation and Infrastructure)..
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INTRODUCTION

Bone remodeling is a continuous and tightly regulated biological process that maintains skeletal strength and structural integrity through a delicate balance between bone-forming osteoblasts and bone-resorbing osteoclasts. This equilibrium is essential for normal bone development, repair, and adaptation to physiological stress. Among the molecular regulators involved in this process, osteoprotegerin (OPG) plays a pivotal role in preserving bone homeostasis. OPG is a soluble glycoprotein belonging to the tumor necrosis factor receptor superfamily and functions as a decoy receptor for receptor activator of nuclear factor-κB ligand (RANKL). By binding to RANKL, OPG prevents its interaction with RANK on osteoclast precursors, thereby inhibiting osteoclast differentiation, activation, and subsequent bone resorption [1],[2]. The OPG/RANKL/RANK signaling pathway is widely recognized as a central regulatory axis controlling bone metabolism and skeletal remodeling. Dysregulation of this pathway, particularly reduced OPG expression, has been associated with pathological conditions such as osteoporosis, inflammatory bone loss, periodontal destruction, and delayed bone healing, emphasizing its importance as a therapeutic molecular target [3],[4].

In recent years, regenerative medicine has increasingly focused on identifying natural bioactive compounds capable of modulating bone metabolism while minimizing adverse effects commonly associated with synthetic drugs. Plant-derived therapeutics have gained considerable attention because of their antioxidant, anti-inflammatory, and tissue regenerative potential. Among these, Moringa oleifera, commonly known as the drumstick tree, has emerged as a promising medicinal plant due to its rich phytochemical profile, including flavonoids, phenolic acids, isothiocyanates, vitamins, and essential minerals [5]. These bioactive components exhibit diverse pharmacological activities such as free radical scavenging, immunomodulation, antimicrobial effects, and enhancement

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of cellular proliferation and differentiation, suggesting a potential role in bone regeneration and tissue repair [6].

Previous experimental investigations have reported that Moringa oleifera may positively influence bone biology by promoting osteoblast proliferation, increasing alkaline phosphatase activity, enhancing collagen synthesis, and improving mineralization in both in vitro and animal models [7],[8]. Additionally, its antioxidant and anti-inflammatory properties may indirectly support osteogenesis by reducing oxidative stress and inflammatory cytokine-mediated bone resorption [9]. However, despite growing evidence supporting its osteogenic potential, most studies have focused on general cellular or biochemical markers rather than specific molecular mechanisms. In particular, limited research has explored gene-level modulation of osteoclast-regulatory molecules such as OPG following Moringa oleifera treatment. The molecular expression profile of OPG mRNA in osteoblast cells exposed to Moringa oleifera remains insufficiently characterized, representing an important gap in current literature.

Parallel to advances in molecular biology, computational approaches have become integral to modern biomedical research. In silico analysis, including molecular docking and interaction modeling, enables prediction of interactions between bioactive phytochemicals and molecular targets, providing mechanistic insights that complement laboratory findings [9],[10]. These methods allow researchers to better understand ligand–target binding behavior, predict biological activity, and strengthen experimental interpretations in a cost-effective and time-efficient manner. Nevertheless, studies integrating experimental gene expression analysis with in silico evaluation of Moringa oleifera bioactives within the context of OPG-mediated bone signaling remain limited.

Therefore, the rationale for the present study is rooted in the need to identify safe, natural compounds capable of modulating key molecular regulators involved in bone remodeling. Evaluating the effect of Moringa oleifera on OPG mRNA expression in osteoblast cells may provide valuable mechanistic insight into its osteoprotective potential. Furthermore, integrating molecular findings within silico analysis can enhance biological interpretation by identifying possible interactions between phytochemical constituents and bone-related molecular targets. Hence, this study aims to investigate the molecular expression of OPG mRNA in osteoblast cells treated with Moringa oleifera and to support these findings through computational analysis. By addressing current gaps in molecular and computational evidence, this research may contribute to the development of novel phytotherapeutic strategies for bone regeneration and future applications in dental and orthopedic tissue engineering [11],[12].

MATERIALS AND METHODS

This in vitro experimental investigation was done in Dept of Pediatric Dentistry Centre for Trans-disciplinary Research, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai - 600077, Tamil Nadu, India to evaluate the effect of Moringa oleifera leaf extract on osteoprotegerin (OPG) mRNA expression in osteoblast-like cells. The experimental model consisted of three groups: untreated control cells, cells treated with Moringa oleifera extract at 2.5 µg/mL, and cells treated with 25 µg/mL. All experiments were performed using three biological replicates, and each sample was analyzed in triplicate (technical replicates) during qRT-PCR analysis. The experimental workflow included cell culture, treatment exposure, RNA extraction, cDNA synthesis, quantitative real-time PCR, and relative gene expression analysis using the $2^{\Delta\Delta Ct}$ method. Cells were exposed to the treatment for 24 hours, and amplification during qRT-PCR was performed for 40 cycles.

Cell line and culture conditions

The osteoblast-like cell line SaOS2 was procured from the National Centre for Cell Science (NCCS), Pune, India. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and antibiotics–antimycotics. Culture conditions were maintained at 37 °C in a humidified incubator with 5% CO_2 and 95% air. Cells between passages 5–15 were used for all experiments to ensure consistency. For experimental procedures, cells were seeded at a density of 1×10^5 cells per well in 6-well plates and allowed to reach appropriate confluency before treatment. Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Trypsin-EDTA, fetal bovine serum, antibiotics-antimycotics, DMEM, and phosphate-buffered saline (PBS) were purchased from Gibco, Canada. Trizol reagent was obtained from Invitrogen. Chloroform, isopropanol, and ethanol were purchased from SRL, Mumbai. The cDNA synthesis kit was procured from Takara, South Korea, and SYBR Green master mix (4385612) was obtained from Life Technologies.

Preparation of Moringa oleifera extract

Leaves of Moringa oleifera were collected from Vellore District, Tamil Nadu, India. The leaves were washed, shade-dried, chopped, and ground into powder. Approximately 2.5 kg of leaf material was extracted using 95% ethanol through a rotary evaporator-based distillation process, yielding approximately 225 g of concentrated extract. The extract was dissolved in solvent with a final DMSO concentration below 0.05%, diluted with culture medium to the desired concentrations, and sterilized by filtration through a 0.2 µm sterile membrane filter under negative pressure.

Experimental groups and variables

The experimental groups were defined as follows:

Group I: Untreated control (SaOS2 cells)
Group II: Moringa oleifera extract (2.5 µg/mL)
Group III: Moringa oleifera extract (25 µg/mL)

A vehicle control containing culture medium with <0.05% DMSO was included to eliminate solvent-related effects. The independent variable was the concentration of Moringa oleifera extract, while the dependent variable was relative OPG mRNA expression.

RNA isolation, cDNA synthesis, and quantitative real-time PCR

Following treatment, cells were washed with PBS and lysed using 100 µL Trizol reagent. Total RNA was extracted according to the manufacturer's protocol and quantified using a NanoDrop spectrophotometer (Thermo Scientific). RNA purity was assessed using the A260/A280 ratio, and only samples within acceptable purity ranges were used for downstream analysis. Equal quantities of RNA (1 µg per reaction) were reverse-transcribed into cDNA using the cDNA synthesis kit according to manufacturer instructions. Quantitative real-time PCR was performed using an ABI 7500 Real-Time PCR System with SYBR Green master mix. Gene-specific primers for OPG and β-actin (housekeeping gene) used were:

Gene: TNFRSF11B (OPG) [NCBI RefSeq: NM_002546.4]
Forward primer: AGGAGCTGCAGTACGTCAAG
Reverse primer: CCACAGCTGCCTTGTACATG
Gene: ACTB (β-actin) [NCBI RefSeq: NM_001101.5]
Forward primer: CACCATTGGCAATGAGCGGTTC
Reverse primer: AGGTCTTTGCGGATGTCCACGT

PCR cycling conditions consisted of initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. Relative gene expression levels were calculated using the $2^{\Delta\Delta Ct}$ method, normalized to β-actin and expressed as fold change relative to control cells.

Outcome measures

The primary outcome measure of the study was the relative expression of osteoprotegerin (OPG) mRNA, quantified as fold change compared with the untreated control group. Secondary outcome measures included in silico analysis, performed to evaluate the potential molecular interactions between bioactive compounds of Moringa oleifera and bone-related molecular targets, thereby supporting the mechanistic interpretation of the gene expression findings. In addition, morphological observations were carried out to assess treatment-associated changes in cellular structure, attachment, and overall cellular integrity under experimental conditions.

Statistical analysis

Statistical analysis was performed using SPSS version 22, GraphPad Prism software. Data were expressed as mean ± standard deviation (SD). Normality was assessed using the Shapiro–Wilk test. Comparisons among groups were performed using one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. A value of $p < 0.05$ was considered statistically significant.

In-Silico Molecular Docking Analysis

The molecular docking analysis was performed using PyRx (AutoDock Vina) to evaluate the binding interactions of selected Moringa oleifera phytochemicals with mouse RANKL (PDB ID: 1S55), a key regulator of the OPG/RANKL signaling pathway. The crystal structures of these proteins were sourced from the Protein Data Bank (https://www.pdb.org/pdb). Ligand structures were retrieved from PubChem and energy-minimized using the UFF force field. Protein structures were prepared by removing water molecules and heteroatoms, followed by energy minimization. Ligand geometries were optimized using the MMFF94 force field. Docking validation was performed using an RMSD cutoff of < 2 Å. During the docking scrutiny, a grid box with dimensions of 90 Å × 90 Å × 90 Å and a grid spacing of 0.45 Å was used. The docking calculations were carried out using the Lamarckian genetic algorithm (LGA) with 100 genetic algorithm cycles using AutoDock 1.5.4. To visualize the outcomes of the 3D structured complex docking results, BIOVIA Discovery Studio software was utilized.

Ethical Considerations

The study protocol was approved by the Institutional Ethics Committee of Saveetha Dental College and Hospitals, SIMATS, Chennai, India (Approval No.: Number: IHEC/SDC/UG-1917/23/PEDO/187). Ethical approval covered the use of clinical samples and laboratory investigations, and all procedures were conducted in accordance with institutional and international ethical standards.

RESULTS

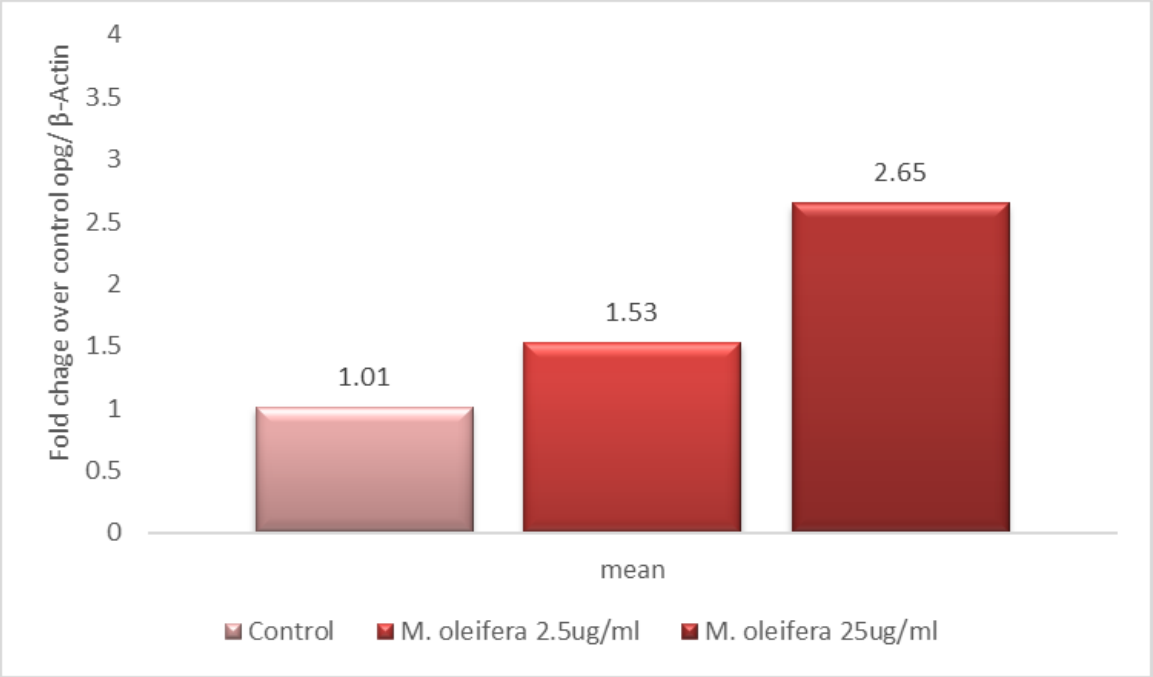

Figure 1: Relative OPG mRNA expression in SaOS2 osteoblast-like cells following treatment with Moringa oleifera extract.

Figure 1 shows a progressive increase in OPG mRNA expression after treatment with Moringa oleifera. The control group exhibited baseline expression (≈1.0 fold), while 2.5 µg/mL produced moderate upregulation (≈1.53 fold) and 25 µg/mL showed marked elevation (≈2.65 fold). This dose-dependent rise suggests that Moringa oleifera enhances osteoprotegerin expression in osteoblast-like cells, supporting its potential role in regulating bone remodeling by modulating the OPG/RANKL signaling pathway.

Group	**Ct OPG (Mean ± SD)**	**Ct β-actin (Mean ± SD)**	**ΔCt (Mean ± SD)**	**p value**
Control	23.50 ± 0.12	18.50 ± 0.05	5.00 ± 0.08	0.552
MO 2.5 µg/mL	22.90 ± 0.10	18.50 ± 0.05	4.40 ± 0.08	<0.001
MO 25 µg/mL	22.10 ± 0.15	18.50 ± 0.05	3.60 ± 0.10	<0.001

Table 1: Comparison of raw Ct values and ΔCt analysis of OPG mRNA expression in SaOS2 osteoblast-like cells treated with Moringa oleifera.

Analysis of qRT-PCR data in table 1 demonstrated a reduction in Ct OPG values with increasing concentrations of Moringa oleifera, indicating higher gene expression compared with control. While Ct β-actin values remained stable across all groups, ΔCt values progressively decreased from 5.00 ± 0.08 in controls to 4.40 ± 0.08 and 3.60 ± 0.10 in the 2.5 µg/mL and 25 µg/mL groups, respectively. This reduction reflects increased OPG transcription following treatment. Significant differences were observed in both treatment groups ($p < 0.001$), suggesting a dose-dependent upregulation of OPG expression in osteoblast-like cells.

Group	**ΔΔCt (Mean ± SD)**	**Fold Change (Mean ± SEM)**	**Significance vs Control**
Control	0.00 ± 0.00	1.00 ± 0.00	ns
MO 2.5 µg/mL	−0.60 ± 0.08	1.52 ± 0.05	$p < 0.05$
MO 25 µg/mL	−1.40 ± 0.10	2.64 ± 0.08	$p \leq 0.001$**

Footnote: Representative graph showing real-time RT-PCR amplification of OPG mRNA expression treated with Moringa oleifera in (SaOS2) Osteoblast-like cells. The 2-ΔΔ Ct method of relative quantification was used to

determine the fold change in expression with β-actin. Values are mean ± SEM of triplicate of 3 independent experiments. '**' denotes statistical significance at the level of p ≤ 0.001 when compared with control.

Table 2: Relative OPG mRNA Expression ($2^{\Delta\Delta Ct}$ Method)

Relative gene expression analysis in table 2 using the $2^{\Delta\Delta Ct}$ method demonstrated a dose-dependent increase in OPG mRNA expression following Moringa oleifera treatment. Compared with the control group, the 2.5 µg/mL group showed moderate upregulation, while the 25 µg/mL group exhibited a marked increase. The progressively reduced ΔΔCt values indicate enhanced transcriptional activation of OPG with increasing extract concentration.

Variable	Group	N	Mean Rank
Ct_OPG	Control	9	13.17
	MO 2.5 µg/mL	9	14.72
	MO 25 µg/mL	9	14.11
p value	(Kruskal–Wallis)	27	0.915
Ct_beta_actin	Control	9	23
	MO 2.5 µg/mL	9	14
	MO 25 µg/mL	9	5
p value	(Kruskal–Wallis)	27	<0.001
fold_change_2ddCt	Control	9	5
	MO 2.5 µg/mL	9	14
	MO 25 µg/mL	9	23
p value	(Kruskal–Wallis)	27	<0.001
delta_Ct	Control	9	23
	MO 2.5 µg/mL	9	14
	MO 25 µg/mL	9	5
p value	(Kruskal–Wallis)	27	<0.001

Table 3: Mean Rank Distribution and Kruskal–Wallis Analysis

Comparison	Mean Difference	95% CI of Difference	Significant	Adjusted p value
group_code vs group_name	−7.167	−13.75 to −0.5836	Yes	0.0276
group_code vs experiment_no	−22.00	−22.48 to −21.52	Yes	<0.0001
group_code vs technical_replicate	−5.704	−6.393 to −5.014	Yes	<0.0001
group_code vs treatment_concentration_ug_ml	−214.3	−224.8 to −203.8	Yes	<0.0001
group_code vs RNA_concentration_ng_ul	−20.83	−21.41 to −20.25	Yes	<0.0001
group_code vs A260_A280_ratio	−16.50	−16.99 to −16.02	Yes	<0.0001
group_code vs Ct_OPG	−2.327	−2.899 to −1.755	Yes	<0.0001
group_code vs Ct_beta_actin	2.673	2.101 to 3.245	Yes	<0.0001

group_code vs delta_delta_Ct	−2.000	−2.674 to −1.326	Yes	<0.0001

Kruskal–Wallis analysis in table 3 showed no significant difference in Ct OPG among the groups, indicating consistent amplification across samples. In contrast, significant differences were observed for Ct β-actin, ΔCt, and fold-change expression. Mean rank distribution demonstrated a clear dose-dependent increase in OPG expression following Moringa oleifera treatment, while ΔCt showed a corresponding decrease, supporting enhanced gene expression in treated groups compared with control.

Table 4: Dunnett's Post hoc analysis with multiple Comparisons

Dunnett's post hoc multiple comparison analysis demonstrated significant differences between group coding and several experimental variables, including treatment concentration, RNA quality parameters, Ct values, and ΔΔCt measurements. These findings indicate consistent group-wise variation across experimental conditions and molecular outcomes, supporting the reliability of the observed treatment-related changes in gene expression. This confirms that group differences were statistically significant and aligned with the dose-dependent response observed in the study.

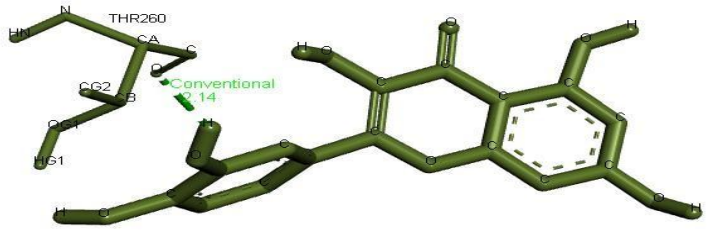

Figure 2: Three-dimensional binding conformation of quercetin within the active region of mouse RANKL (PDB ID: 1S55).

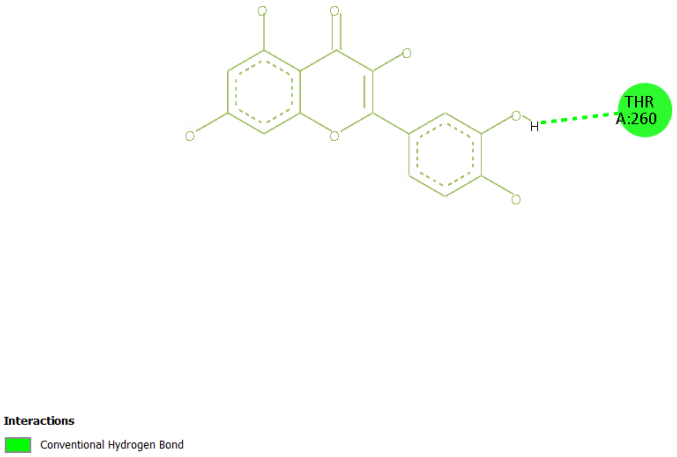

Figure 3: Two-dimensional interaction map of quercetin (PubChem CID: 5280343) docked with mouse RANKL (PDB ID: 1S55).

Molecular docking analysis shown in figure 2 & 3 was carried out using the crystal structure of mouse RANKL (PDB ID: 1S55) with quercetin (PubChem CID: 5280343). The docking results showed stable positioning of quercetin within the protein binding pocket. Interaction analysis identified a conventional hydrogen bond with THR260, suggesting favorable ligand stabilization. The presence of multiple hydroxyl groups in quercetin facilitated polar interactions with surrounding residues, indicating a possible role in modulating RANKL-related signaling pathways involved in bone remodeling.

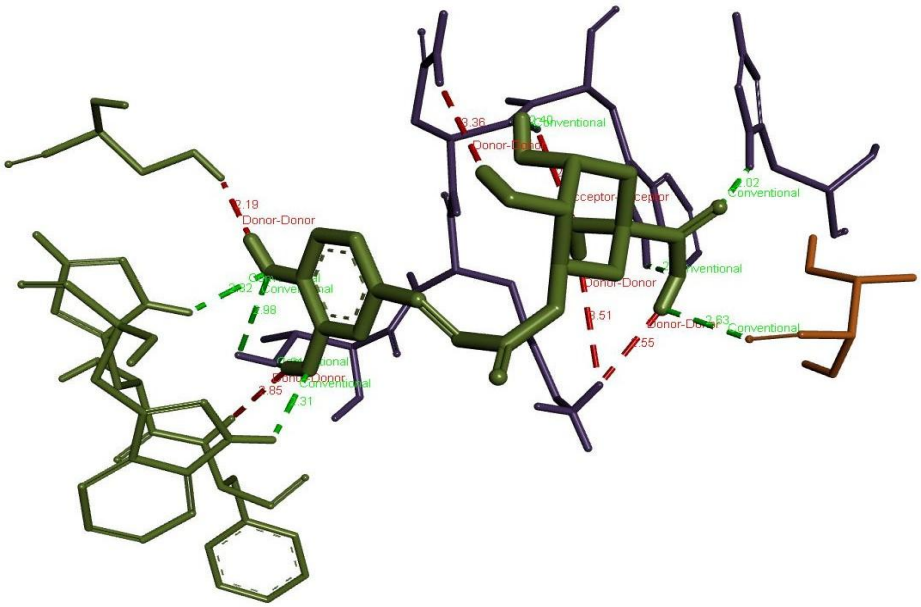

Figure 4: Three-dimensional interaction view of PubChem CID 1794427 docked with mouse RANKL (PDB ID: 1S55).

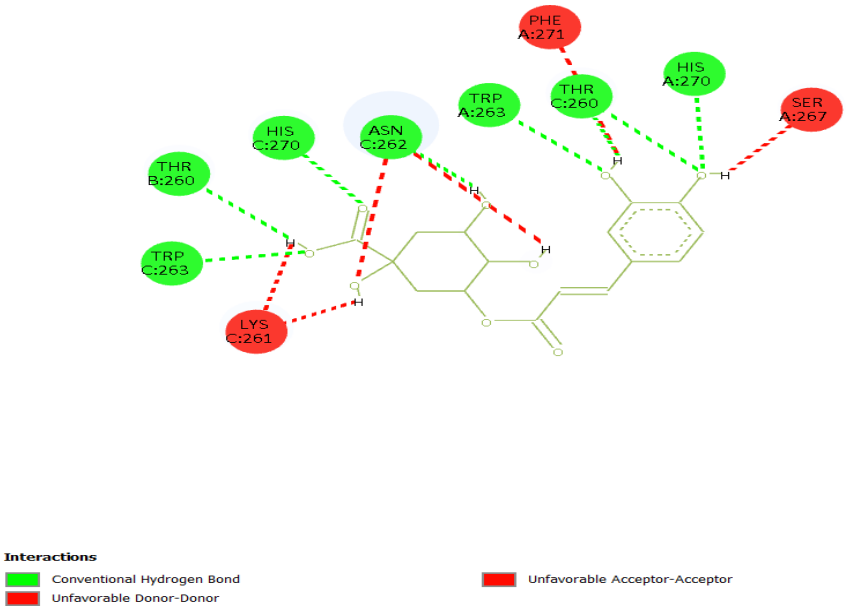

Figure 5: Two-dimensional interaction map of chlorogenic acid (PubChem CID 1794427) docked with mouse RANKL (PDB ID: 1S55).

Molecular docking analysis shown in figure 4 & 5 was performed using mouse RANKL (PDB ID: 1S55) with the ligand corresponding to chlorogenic acid. The docking results showed stable accommodation of the ligand within the binding pocket, supported by multiple conventional hydrogen bonds involving THR260, TRP263, and HIS270. Some unfavorable donor–donor and acceptor–acceptor interactions were also observed with residues such as LYS261, ASN262, PHE271, and SER267, indicating minor steric or electrostatic limitations in ligand orientation. Overall, the ligand demonstrated moderate binding stability, suggesting potential interaction with RANKL-associated osteoclastogenic signaling sites.

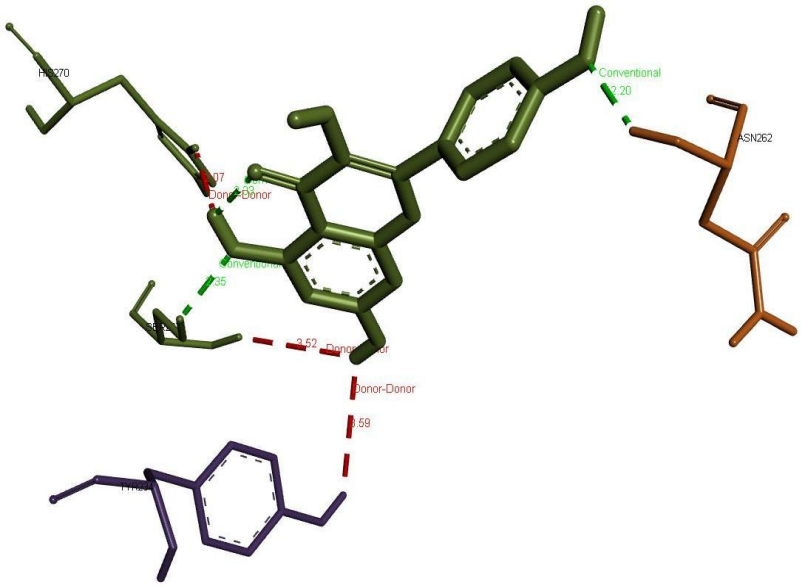

Figure 6: Three-dimensional visualization of the kaempferol–RANKL docked complex showing ligand orientation within the receptor binding pocket.

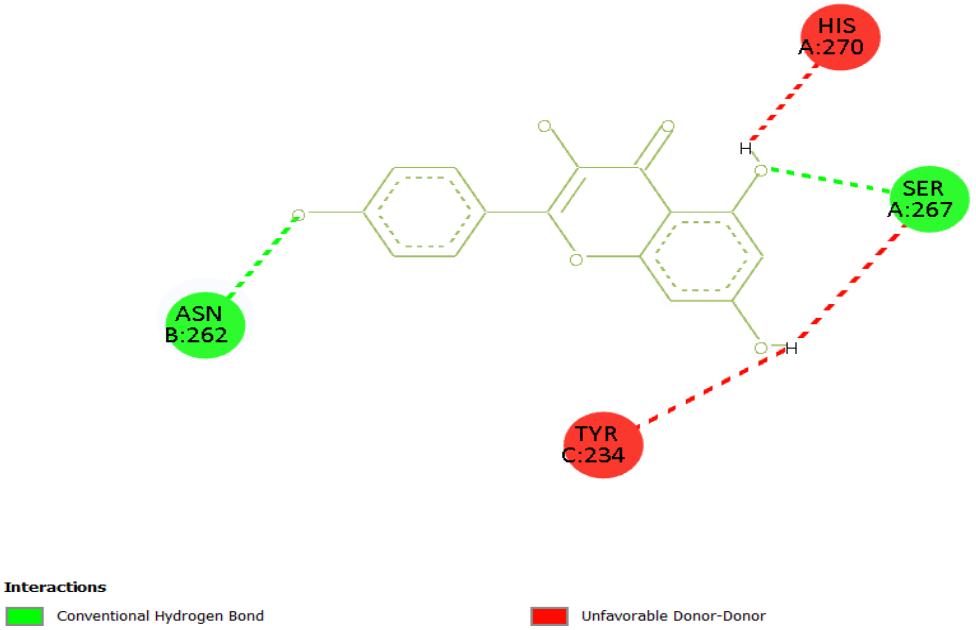

Figure 7: Two-dimensional interaction map of kaempferol (PubChem CID: 5280863) docked with mouse RANKL (PDB ID: 1S55)

Molecular docking analysis of kaempferol (PubChem CID: 5280863) with mouse RANKL (PDB ID: 1S55) showed strong binding affinity, with the best docking score indicating stable interaction within the receptor binding pocket. Interaction analysis revealed conventional hydrogen bonds mainly with ASN262 and SER267, supporting favorable ligand stabilization. A few unfavorable donor–donor interactions were observed with HIS270 and TYR234, suggesting minor steric limitations in ligand orientation. Overall, these findings indicate that kaempferol interacts with functionally important residues involved in RANKL signaling, suggesting a potential role in modulating osteoclastogenic pathways.

Overall, the docking results suggest that these phytochemicals are capable of interacting with key functional residues on the RANKL surface. This supports their possible role in influencing RANKL-mediated osteoclastogenic signaling and is consistent with the experimentally observed increase in OPG expression, indicating a potential bone-protective effect.

DISCUSSION

The present study demonstrated that Moringa oleifera treatment significantly increased OPG mRNA expression in SaOS2 osteoblast-like cells in a dose-dependent manner, with the highest expression observed at 25 µg/mL. Since OPG functions as a decoy receptor for RANKL and suppresses osteoclast differentiation, the observed upregulation suggests a potential anti-resorptive mechanism that may contribute to bone homeostasis. These molecular findings were further supported by in silico docking analysis, which showed stable interactions between selected Moringa oleifera phytochemicals and RANKL, indicating possible modulation of osteoclastogenic signaling.[13],[14] Previous studies have reported similar osteogenic effects of Moringa oleifera. Singh and colleagues [[15]] demonstrated enhanced osteoblast differentiation, increased alkaline phosphatase activity, and improved mineralization following treatment with Moringa oleifera extracts, suggesting activation of bone-forming pathways. The present study extends these findings by demonstrating gene-level regulation through increased OPG mRNA expression, providing further mechanistic insight into the osteoprotective potential of the plant.

Quercetin, one of the key phytochemicals analyzed in this study, showed stable docking within the RANKL binding region and formed hydrogen bond interactions with THR260. This observation supports earlier findings by Wang et al. [16], who reported that quercetin regulates the OPG/RANKL balance and reduces inflammatory bone destruction. Similarly, Chen et al. [17] demonstrated that quercetin inhibits osteoclast activation through modulation of the RANKL/RANK/OPG pathway. Together with our docking findings, these studies suggest that quercetin may contribute to increased OPG expression by indirectly suppressing osteoclastogenic signaling.

Kaempferol demonstrated the strongest binding affinity among the tested ligands in the present study, showing stable interactions with residues such as ASN262 and SER267. These findings are consistent with the work of Kim et al. [18], who reported inhibition of RANKL-mediated osteoclast differentiation by kaempferol through suppression of MAPK signaling pathways. In addition, Choi et al. [19] showed that kaempferol regulates osteoclastogenesis via modulation of autophagy-related signaling. The strong docking affinity observed in our study further supports its role as a bioactive compound capable of influencing bone remodeling.

Comparative studies by Wattel et al. [20] have suggested that both quercetin and kaempferol exert dual effects by enhancing osteoblast activity while inhibiting osteoclast formation. This dual mechanism aligns with the present findings, where increased OPG expression and stable ligand–RANKL interactions collectively indicate a shift toward a bone-protective microenvironment.

Chlorogenic acid and related compounds have also been reported to inhibit RANKL-induced osteoclast differentiation and reduce bone resorption, as demonstrated by Kwak et al. [21]. Furthermore, Zhang et al. [22] reported improved bone microarchitecture and reduced osteoclast activity following chlorogenic acid administration in vivo. These findings support the biological plausibility of our docking results and suggest that multiple phytochemicals within Moringa oleifera may synergistically contribute to modulation of the OPG/RANKL signaling axis.

Similarly, Casado-Díaz et al. [23] demonstrated that flavonoid derivatives such as quercitrin promote osteoblast differentiation while reducing Rankl expression, reinforcing the concept that phytochemicals can simultaneously stimulate bone formation and suppress bone resorption. In addition, Li et al. [24] reported osteogenic effects of chlorogenic acid in dental stem cell models, highlighting the relevance of the present findings to dental and craniofacial tissue engineering applications. Recent integrated in vitro and in silico studies by Sekar et al. [25] further support the value of combining molecular and computational approaches to strengthen mechanistic interpretation, while clinical investigations by Aarthi and Ravikumar [26] emphasize the translational importance of research aimed at improving oral health outcomes.

A major strength of this study is the integrated approach combining in vitro gene expression analysis with in silico molecular docking, providing both experimental and mechanistic insight. However, limitations include evaluation of a single gene marker and reliance on computational predictions without protein-level validation. Future studies should include additional osteogenic and osteoclastogenic markers, protein expression analysis, and functional assays to confirm biological effects. In vivo validation and pathway-level investigations will further strengthen translational relevance for bone regenerative applications.

CONCLUSION

The present study demonstrated that treatment with Moringa oleifera extract enhances OPG mRNA expression in osteoblast-like cells, suggesting a favorable shift toward bone-protective signaling. The in silico docking findings further supported these observations by showing stable interactions between key phytochemicals and RANKL, indicating a possible molecular basis for reduced osteoclastogenic activity. Together, the experimental and computational results provide supportive evidence that Moringa oleifera may influence bone remodeling through modulation of the OPG/RANKL pathway. These findings encourage further investigations involving protein-level validation, functional assays, and in vivo studies to better establish its potential role in bone regeneration and translational dental applications.

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