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Original Research Article

An Immunoinformatic of Epigallocatechin-3-*O*-gallate as Adjuvant Therapy of Periodontitis: An *in-silico* Study

Mohammed A. Aljunaid[1,2], Rini D Ridwan[3]*, Sidarningsih Sidarningsih[3], Yuliati Yuliati[3], Fianza Rezkita[4], Andari Sarasati[4], Viol Kharisma[5], Alexander P. Nugraha[6], Huda R. Qaid[1,7], Maria J. Anggakusuma[8], Shuhdi G. Alaghbari[2]

[1]*Doctoral program of Dental Medicine, Faculty of Dental Medicine, Universitas Airlangga, Surabaya- Indonesia*
[2]*Lecturer in Department of Dental Medicine, Faculty of Medicine, Taiz University, Taiz-Yemen*
[3]*Oral Biology Department, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia*
[4]*Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia*
[5]*Graduate Student of Biology, Faculty of Mathematics and Natural Science, Universitas Brawijaya, Indonesia*
[6]*Orthodontics Department, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia*
[7]*Faculty of Oral and Dental Medicine, Alsaeed University, Taiz, Yemen.*
[8]*Residence in Periodontic Program, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia*.

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ABSTRACT

Periodontitis results in irreversible bone resorption. Epigallocatechin-3-O-gallate (EGCG) is one of the prominent compounds in green tea and is recognized for its therapeutic efficacy. EGCG supports bone formation and possesses antioxidant and anti-inflammatory properties. EGCG inhibits bone resorption by encouraging osteoclast apoptosis, preventing formation, and supporting the development of mineralized bone nodules. This study investigates the efficacy of EGCG in immunoinformatic as a potential treatment for periodontitis. The 3D chemical structures were obtained from the PubChem database. PyRx v.0.8 software was used to conduct molecular docking simulations. The results showed an inhibitory effect on the protein samples Nuclear Factor Associate T cell-1 (NFATc1), Sclerostin, Tartate Resistant Acid Phosphatase (TRAP), Receptor Activator of kappa beta and ligand (RANK-RANKL), Runt-related transcription factor-2 (RUNX2), Osterix, and Osteocalcin. The docking analysis of target proteins RUNX2, Osterix, and Osteocalcin showed that EGCG exhibited the most negative binding energy, -7.0 kcal/mol, in the RUNX2 domain, potentially enhancing osteonectin activity. The findings indicate that the EGCG inhibits osteoclastic activity by binding and suppressing NFATc1, RANK-RANKL, Sclerostin, and TRAP. Consequently, EGCG substantially enhances osteogenic processes by promoting RUNX2, Osterix, and Osteocalcin *in silico*.

Keywords: Bone regeneration, EGCG, *in silico*, Molecular docking, Medicine, Periodontitis.

Introduction

Bone resorption is a pathologic disease that involves both the dissolution of bone minerals and the degradation of organic bone matrix. [1] Physiologically, there is homeostasis between bone resorption and bone formation. In bone resorption, osteoclasts dysregulate organic and mineral components in the bone matrix.[2] When bone resorption occurs more often than bone formation, it leads to several diseases, including periodontitis. [3]

Periodontitis is a common chronic inflammatory disease in the oral cavity. 20-50% of people in the world suffer from periodontitis. Moreover, there has been an increase in the prevalence of periodontitis to 57.3% between 1990 and 2010.[4] People with periodontitis might have a poor quality of life related to oral health as a result of extreme pain and the loss of teeth.

*Corresponding author. E mail: rini-d-r@fkg.unair.ac.id
Tel: +6281331049714

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The specific cause of periodontitis is well-established, primarily attributed to anaerobic microorganisms, *Porphyromonas gingivalis (P. gingivalis), Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans), Treponema denticola, Tannerella forsythia, and Prevotella intermedia. These bacteria* colonize the subgingival and release lipopolysaccharide, which destroys connective tissue and alveolar bone resorption.[5,6]

Green tea is one of the herbal medicines that contain various compounds, including Epigallocatechin-3-O-gallate (EGCG), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epicatechin (EC), with several medical uses. EGCG is the most prevalent and biologically active catechin in green tea.[7] EGCG can stimulate osteogenesis, which leads to a bone remodeling mechanism that induces osteoclast apoptosis and blocks nuclear factor kappa B (NF-κB) and Interleukin-1 beta (IL-1β), resulting in the blockade of osteoclast formation. Furthermore, EGCG can develop mineralized bone nodules.[8–10] Thus, this study aims to investigate the immunoinformatic of EGCG as an adjuvant periodontitis therapy *in silico*.

Material and Methods

Sample Preparation

In this study, EGCG served as a ligand. EGCG Details such as ID, formula, canonical SMILES, molecular weight, and 3D structures of these compounds were sourced from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The proteins including Sclerostin, Tartate Resistant Acid Phosphatase (TRAP), Runt-related transcription factor-2 (RUNX2), Osteocalcin, Nuclear Factor Associate T cell-1

(NFATC1), Osterix, and Receptor Activator of kappa beta and ligand (RANK-RANKL) were obtained from the Protein Data Bank (PDB) database (https://www.rcsb.org/). The 3D structure for each protein encountered energy minimization using PyRx to achieve a stable molecular structure and then encountered water molecule sterilization and native ligand processing through PyMol software. [11]

Molecular Docking Simulation

PyRx software was used to conduct molecular docking simulations. The simulation involves two models to assess the inhibitor activity and the stimulation activity of EGCG on the targeted protein. When the ligand binds to a protein, predicting the binding energy and the resulting response is possible through docking simulations.[12,13]

Molecular Interaction and Visualization

The docking analysis was used to reveal the bond positions and bond energies between the proteins and EGCG.[13] PyMol software was used to visualize the 3D structures of the molecular complexes and utilized structural representations such as cartoons, surfaces, and sticks.[14]

Results and Discussion

The EGCG details, including ID, weight, formula, and canonical smile, are presented in Table 1. Protein samples, including NFATc1, Sclerostin, TRAP, RANK-RANKL, RUNX2, Osteocalcin, and Osterix, were obtained from the RCSB-PDB database and their details, including ID, visualization method, atom count, resolution, weight, chain, and sequence length, are shown in Table 2**.** The 3D structure of the targeted proteins that encountered sterilization by PyMol is presented in Figure 1**.**

Molecular docking simulations were conducted for NFATC1, Sclerostin, TRAP, and RANK-RANKL proteins. The results showed that EGCG has an inhibitory effect on NFATC1, Sclerostin, TRAP, and RANK-RANKL proteins. The inhibitory effect of EGCG on NFATC1 is attributed to the negative binding energy (-8.1 kcal/mol), indicating a higher affinity of EGCG to NFATC1 than other proteins Table 3**.** In the RANK-RANKL domain, EGCG compounds were found to engage in Van der Waals, hydrogen, unfavorable, and pi anion interactions, influencing the activity of the RANK-RANKL protein Figure 2. Docking analysis results with the target proteins Osterix, RUNX2, and Osteocalcin demonstrated that EGCG exhibited the most negative binding energy (-7.0 kcal/mol) in the RUNX2 domain Figure 3 which suggests the potential to enhance osteonectin activity Table 4**.**

EGCG is a bioactive component in green tea that exhibits an anti-inflammatory effect by promoting the immune cells and stimulating bone tissue regeneration through attenuating osteoclastogenesis and promoting osteoblastogenesis.[9] Preceding research reported the potency of EGCG in suppressing tumour necrosis factor-alpha (TNF-α) and interleukin (IL), including IL-17, IL-6, and IL-1β, while elevating IL-10 expression, which suppresses osteoclast-related molecules, including RANKL, TRAP, and NF_KB while promoting mesenchymal stem cells or osteoblast-related molecules, including RUNX2, Wnt, Osterix, Osteocalcin, and ALP, consequently resulting in bone remineralization and recover bone density.[15,16] Thus, this study investigates the effect of EGCG in periodontal disease through molecular docking simulations to understand the mechanism of EGCG in periodontitis therapy.

Table 1: Results of PubChem ligand sample preparation

Name	PubChem ID	Formula	Weight (g/mol)	Smile Canonical
EGCG	65064	$C_{22}H_{18}O_{11}$	458.4	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C(=C3)O)O)O)OC(=O)C4=CC(=C(C(=C4)O)O)O

Table 2: Results of target protein sample preparation from RCSB-PDB

Name	PDB ID	Visualization Method	Resolution (Å)	Atom Count	Weight (kDa)	Chain	Sequence Length (mer)
RANK-RANKL	3URF	X-ray	2.70	2604	38.38	A	162
Sclerostin	2KD3	NMR	-	767	12.71	A	113
TRAP	1WAR	X-ray	2.22	2564	35.48	A	310
Osteocalcin	1Q8H	X-ray	2.00	378	5.85	A	49
NFATC1	1A66	NMR	-	1891	27.33	A	178
Osterix	6X46	NMR	-	654	14.35	A	121
RUNX2	6VGE	X-ray	4.25	3361	62.53	D	117

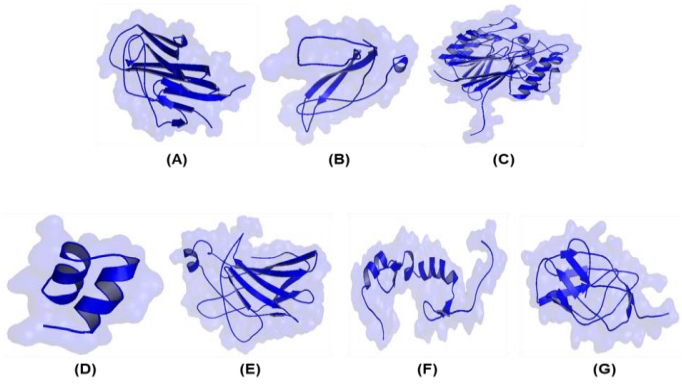

Figure 1: Visualization of the 3D structure of the target protein (A) RANK-RANKL, (B) Sclerostin, (C) TRAP, (D) Osteocalcin, (E) NFATC1, (F) Osterix, (G) RUNX2.

This study's findings align with earlier studies, which indicated that EGCG has significant inhibitory effects on NFATc1 due to its strong binding affinity, resulting in a reduction in osteoclast differentiation.[17] Moreover, EGCG exhibited lower binding affinity to TRAP, Sclerostin, and RANK-RANKL molecules than NFATc1, decreasing osteoclastogenic activities and enhancing canonical Wnt signaling pathway, contributing to bone remodeling.[18] Furthermore, the binding of EGCG affects specific molecular activities, promoting the process of bone remodeling. Molecular docking showed that EGCG has the highest negative binding affinity on RUNX2, promoting osteoblast differentiation and maturation.[19] Moreover, a high binding affinity was also found in osteogenic marker genes, such as Osterix and Osteocalcin.[20]

According to research, the phosphorylation of NFATC1 plays an essential role in activating TRAP, Sclerostin, and RANK-RANKL[21,22] Hence, this study targets phosphorylation domains such as arginine, serine, threonine, and tyrosine for EGCG binding.[23,24]

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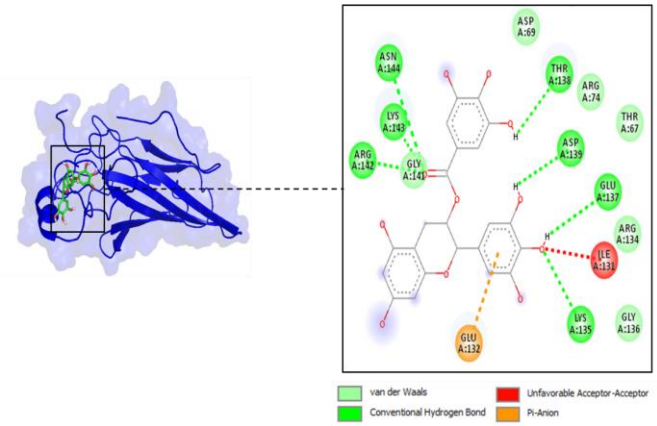

Figure 2: Visualization of the results of the EGCG docking complex with NFATC1. Ligands are displayed as sticks (green) and transparent surfaces and cartoons for target proteins (blue).

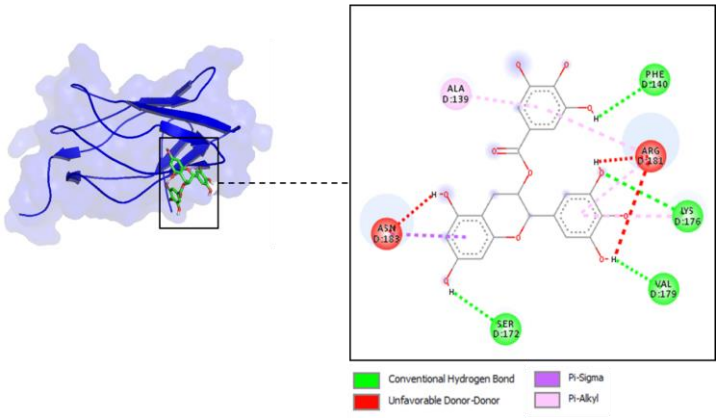

Figure 3: Visualization of the results of the EGCG docking complex with RUNX2. Ligands are displayed as sticks (green) and transparent surfaces and cartoons for target proteins (blue).

Table 3: Results of molecular docking simulations to inhibit the activity

Protein	Ligand	Grid Positions		Binding Affinity (kcal/mol)
		Center	Dimensions	
NFATC1	EGCG	X:15.500 Y:-7.918 Z:1.696	X:53.188 Y:44.923 Z:51.073	-8.1
Sclerostin	EGCG	X:7.231 Y:27.877 Z:-8.988	X:38.970 Y:35.385 Z:58.560	-6.9
TRAP	EGCG	X:68.304 Y:-24.336 Z:17.178	X:25.885 Y:27.644 Z:39.737	-6.2
RANK-RANKL	EGCG	X:8.829 Y:-0.538 Z:17.364	X:37.687 Y:50.684 Z:49.014	-6.4

Table 4: Results of molecular docking simulations to increase activity

Protein	Ligand	Grid Positions		Binding Affinity (kcal/mol)
		Center	Dimensions	
RUNX2	EGCG	X:-50.959 Y:39.758 Z:-15.615	X:50.452 Y:29.127 Z:38.471	-7.0
Osterix	EGCG	X:3.943 Y:3.965 Z:-46.055	X:66.285 Y:36.739 Z:27.656	-6.6
Osteocalcin	EGCG	X:8.069 Y:25.299 Z:22.859	X:24.568 Y:21.085 Z:16.889	-5.4

In addition, Kim *et al.* (2020) and Chen *et al*. (2021) demonstrated that the amino acid residues of proline, serine, and threonine are increased in the RUNX2 domain[25,26], which indicates the potential efficacy of EGCG as an osteogenic agent.

Previous studies demonstrated the effects of EGCG on oral bone regeneration, specifically in extraction sockets and periodontitis, through the inhibition of NFATC1, NFKB, AP-1, MMP-9, ROS, and the promotion of the differentiation of osteogenic mesenchymal stem cells.[9] Furthermore, EGCG's regenerative effect on periodontitis is facilitated by its antibacterial properties, including the inhibition of *P. gingivalis* adhesion and its anti-toxin activity against leukotoxin produced by *A. actinomycetemcomitans*.[27,28] Moreover, EGCG prevents degenerative bone diseases such as osteoporosis due to its osteoprotective activity, leading to a reduction in fracture risk.[29] Therefore, the diverse activity of EGCG demonstrates its effectiveness as a therapeutic agent for oral diseases, particularly periodontitis.

Conclusion

The immunoinformatic analysis showed that EGCG can reduce osteoclastic activity by inhibiting NFATC1, RANK-RANKL, Sclerostin, and TRAP *in silico*. Meanwhile, EGCG can promote osteogenic activity by binding to escalating RUNX2, Osterix, and Osteocalcin *in silico*. Further research is needed to comprehend the potency of EGCG as periodontitis adjunctive therapy.

Conflict of Interest

Authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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