Original Article

Data-driven dentistry: Computational revelations redefining pulp capping

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Abstract

Introduction: Pulpal and periradicular diseases stem from immune reactions to microbiota, causing inflammation. Limited blood supply hampers dental pulp self-healing. Managing inflammation involves eliminating bacteria and reducing pro-inflammatory mediators especially MMP-9, which has a significant correlation with pulpitis. s. Flavonoids like Hesperidin, Baicalein, Epigallocatechin gallate, Genistein, Icariin, and Quercetin show potential for pulp capping.

Aim: This in-silico study compares various Flavonoids for their anti-inflammatory effects on MMP-9, with Chlorhexidine as a control, a known MMP-9 inhibitor.

Materials and Methods: Protein and Ligand Preparation: The human MMP-9 catalytic domain (PDB ID: 4XCT) structure was retrieved, and necessary modifications were made. Flavonoids from PubChem database were prepared for docking using AutoDock Vina. A grid for docking was created, and molecular dynamics simulations were conducted using Gromacs-2019.4 with GROMOS96 force field. Trajectory analysis was performed, and MM-PBSA calculation determined binding free energies.

Results: Analysis of MMP-9 and ligand interactions revealed Hesperidin's high binding affinity, forming numerous hydrogen bonds with specific amino acids. Molecular dynamics simulations confirmed stability, with RMSD, RMSF, Rg, and SASA indicating consistent complex behaviour over 100 ns. MM-PBSA calculation affirmed favourable energy contributions in MMP-9-Hesperidin interactions.

Conclusion: MMP-9 plays a crucial role in prognosis of pulpitis. Incorporating MMP-9 inhibitors into pulp capping agents may enhance therapeutic efficacy. Hesperidin emerges as a potent MMP-9 inhibitor, warranting further in vivo validation against other agents.

Keywords: Flavonoids; hesperidin; *in silico*; matrix metalloproteinases-9; pulp capping; pulpitis

INTRODUCTION

Treatment of dental caries requires a comprehensive approach involving both disease and lesion management,[1] focusing on bacterial eradication and reducing inflammation.[2] Cariogenic bacteria can penetrate dentinal substrate, activating endogenous Matrix Metalloproteinases (MMPs).[3] MMPs, a family of endopeptidases are expressed at low levels normally but upregulate during inflammation.[4] Success of the therapy relies on dentin bridge quality and pulp response to capping materials.[5] Despite pulp's ability to combat irritants, limited blood supply impedes self-healing.[6] Numerous studies link pulpal inflammation with MMP levels, particularly MMP-9.[7-11]

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The current endodontic therapy prioritizes regenerating a healthy pulp–dentin complex over nonsurgical root canal procedures.[12] Vital pulp therapy is a promising alternative to root canal treatment for teeth with inflamed but vital pulp.[13] Upcoming pharmaceutical research is inclined toward exploring natural agents with Flavonoids, which are plant metabolites that possess anti-inflammatory and anti-oxidative properties gaining attention.[14] Flavonoids such as hesperidin, baicalein, epigallocatechin gallate, genistein, icariin, and quercetin are under scrutiny for their potential as pulp-capping agents.[15-19] Given the correlation of MMP-9 with irreversible pulpitis, inhibiting MMP-9 may positively impact the prognosis of pulpitis.

This study takes an *in silico* approach to compare and assess the anti-inflammatory abilities of these flavonoids in inhibiting MMP-9. Chlorhexidine a cavity disinfectant and a known MMP-9 inhibitor is used as a control.[20]

MATERIALS AND METHODS

Protein and ligand preparation

The *three-dimensional* structure of the human MMP-9 catalytic domain available in the Protein Data Bank (PDB) with the PDB ID: 4XCT was downloaded. This protein was not ready for docking strategies. The addition of hydrogen atoms and assigning of the partial charges were done using MGL tools[21] building the side chains along with the filling of missing loops and energy minimization was done with Swiss PDB viewer.[22] The water molecules present in the crystal structure do not have any coordination with the bound ligand and so they were removed, retaining the zinc and calcium atoms.[23] The prepared protein is represented in Figure 1a. The flavonoids were chosen from the literature and downloaded from PubChem database. These compounds were further prepared for use in docking by energy minimization with AutoDock Vina.[24]

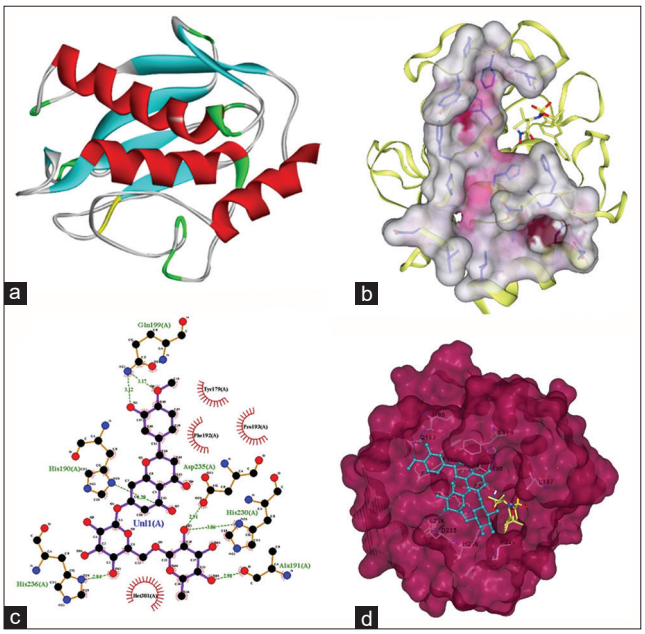

Figure 1: (a) Cartoon representation of MMP-9. (b) Ligand binding cavity of MMP-9. (c) *Two-dimensional* representation of hesperidin binding to MMP-9, Green – Hydrogen bonds, Red – Hydrophobic bonds, Purple – Ligand bonds, Orange – Nonligand bonds. (d) Hesperidin (cyan) binding to the cavity of MMP-9 (maroon)

Receptor grid generation

The X, Y, and Z coordinates of the grid size were 116, 98, and 106 centralized grid 18.715, −9.057, and 13.037, respectively. The grid pacing 1.00 Å with an exhaustiveness of 8. For visualizing, evaluating, and analyzing of molecular dyanamic trajectories PyMOL, QtGrace, and Discovery Studio Visualizer were used.[22]

Molecular dynamics simulation

The protein–ligand complex of MMP-9-hesperidin and MMP-9-chlorhexidine was subjected to molecular dynamics (MD) simulation in Gromacs-2019.4. GROMOS96 force field was used to compute the molecular dynamic simulation. Using the steepest descent algorithm, the system was prepared with vacuum minimization for 1500 steps. Then the complex structures were solvated in a cubic periodic box of 0.5 nm with a simple point-charge water model. The complex systems were subsequently maintained with an appropriate salt concentration of 0.15 M by adding suitable numbers of Na + and Cl counter ions. Each resultant structure from the NpT equilibration phase was subjected to a final production run in the NPT ensemble for 100 ns simulation time. Trajectory analysis was performed using the GROMACS simulation package of protein root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), solvent accessible surface area (SASA), and H-Bond.[24]

Molecular mechanics/Poisson–Boltzmann Surface Area calculation

Interaction-free energy was calculated with Molecular Mechanics/Poisson–Boltzmann Surface Area (MM-PBSA), which is commonly aided in studying the interactions in the biomolecules.[24] The MM-PBSA was calculated

Table 1: Binding energy values of different ligands with MMP-9

Ligand	Target	Binding energy
Hesperidin	MMP-9	−8.1
Epigallocatechin gallate		−7.8
Icariin		−7
Quercetin		−6.9
Genistein		−6.7
Baicalein		−6.6
CHX		−5.9

CHX: Chlorhexidine, MMP-9: Matrix metalloproteinase-9

with molecular dynamic scripts. The g_mmpbsa script of GROMACS was used for calculating the MM-PBSA binding free energies. The equation mentioned below was used in calculating the binding energy:

$$G_{binding} = G_{complex} (G_{receptor} + G_{ligand})$$

where, $G_{binding}$ stands for total energy required for binding the protein–ligand complex, $G_{receptor}$ is free receptor binding energy, and G_{ligand} is the unbounded ligand binding energy.[16]

RESULTS

Analysis of interaction between protein and ligands

In this analysis, docking of the ligands against MMP-9 was done to check the binding affinity. Table 1 summarizes the binding energy of the protein–ligand complexes. A high binding affinity was observed for hesperidin and the least was observed for chlorhexidine. It was also noted that hesperidin has significant conventional amino acid residual interactions. From these data, it is suggested that hesperidin is a potent inhibitor of MMP-9.

Figure 1d represents the complex formation of hesperidin with the substrate binding site of MMP-9. Hesperidin binds to the cavity in the surface representation of the protein are depicted in Figure 1b. Blind docking of MMP-9 with hesperidin was carried out on the entire surface of the protein. The best docking pose was analyzed and chosen.

MMP-9-hesperidin forms most of the interactions (conventional hydrogen bonds [H-Bond]) ASP113 LEU114 TYR179 PRO180 PHE181 LEU187 HIS190 ALA191 PHE192 PRO193 PRO194 ILE198 GLN199 GLU227 GLY229 HIS230 ALA231 LEU232 GLY233 LEU234 ASP235 HIS236 PRO246 amino acid residues of MMP-9. In addition, hydrophobic interactions of the complex with TYR179, PHE 192, and PRO 193 were observed. The two dimensional representation of the bonds between MMP-9 and Hesperidin is represented in Figure 1c.

Molecular dynamics

MD simulation for 100 nanoseconds (ns) was performed to understand the stability of protein–ligand complex. The protein was complexed with selected ligands and the average value of RMSD, RMSF, Rg, and SASA was calculated.

Root mean square deviation

It is an important parameter for the determination of the differences between the two confirmations. The higher the RMSD value, the more the deviation. The RMSD values are calculated against the simulation timescale of 100 ns. These RMSD results represent the relative stability of compound complex. Average RMSD of MMP-9 is 0.21 ± 0.01 nm, MMP-9-hesperidin is 0.26 ± 0.01 nm, and MMP-9-chlorhexidine is 0.32 ± 0.01 nm. Further, all the complexes were stable throughout the simulation. The graph is depicted in Figure 2a.

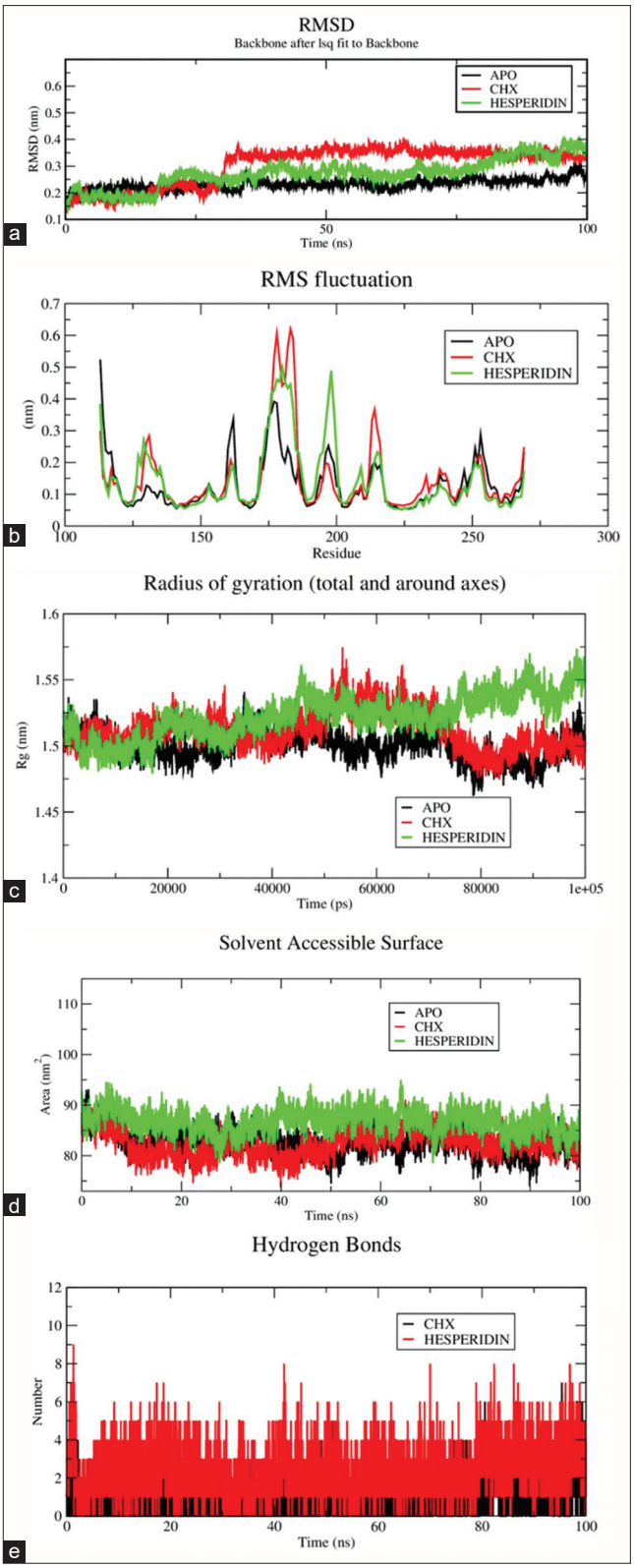

Figure 2: (a-e) Graphical representation of Molecular dynamic simulation analysis of MMP-9 and ligand interaction

Table 2: Molecular mechanics/Poisson–Boltzmann Surface Area calculation of MMP-9 and ligand complexes

Complex	Van der Waal energy (kJ/mol)	Electrostatic energy (kJ/mol)	Polar solvation energy (kJ/mol)	SASA energy (kJ/mol)	Binding energy (kJ/mol)
MMP-9-CHX	−124.333±20.577	−38.981±9.748	83.525±27.358	−16.279±3.242	−96.067±22.172
MMP-9-hesperidin	−243.406±22.592	−32.034±24.313	132.245±38.698	−23.691±1.966	−166.886±19.410

SASA: Solvent accessible surface area, CHX: Chlorhexidine, MMP-9: Matrix metalloproteinase-9

Root mean square fluctuation

RMSF analysis determines which amino acids of the protein make more vibrations, resulting in the destabilization of the protein in the presence and absence of the ligands. The RMSF values are calculated against the simulation timescale of 0–100 ns. The average RMSFs from 0 to 100 ns for MMP-9 is 0.17 ± 0.05 nm, for MMP-9-hesperidin is 0.23 ± 0.05 nm, and for MMP-9-chlorhexidine is 0.22 ± 0.05 nm. The RMSF results are depicted in Figure 2b. The result suggests there were no significant structural changes during the 100 ns simulation.

Radius of gyration

The Rg depicts the overall structure's competence and shape folding at different time points during the simulation. The trajectory can be seen in the Rg plot as illustrated in Figure 2c. Throughout the simulation, complexes exhibited a similar pattern of Rg value. The Rg value from 0 to 100 ns for MMP-9 is 1.51 ± 0.02 nm, for MMP-9-chlorhexidine is 1.56 ± 0.02 nm, and MMP-9-hesperidin is 1.58 ± 0.02 nm.

Solvent accessible surface area

To measure the compactness of the hydrophobic core, the change in SASA was analyzed. The change of SASA of the protein with time is shown in Figure 2d. The SASA value from 0 to 100 ns for MMP-9 is 82.46 ± 2.20 nm^2, for MMP-9-chlorhexidine is 81.46 ± 2.20 nm^2, and for MMP-9-hesperidin is 87.46 ± 2.20 nm^2. No change in structural level protein was observed throughout the simulation.

Hydrogen bond

Protein–ligand complexes are stabilized by the formation of H-Bonds. Here, the H-Bonds formed in the molecular docking analysis were confirmed by the molecular dynamic simulation. The H-Bond results of the complexes are depicted in Figure 2e CHX – 5 H-Bonds, hesperidin – 7 H-Bonds.

Molecular mechanics/Poisson–Boltzmann Surface Area calculation

Table 2 represents the average free binding energy values along with standard deviation. Results of the MM-PBSA analysis showed that all forms of energy aid the interaction between MMP-9-hesperidin.

DISCUSSION

Pulpitis starts with neutrophil infiltration into pulp areas near infected or challenged dentin, leading to microabscess formation and gradual necrosis.[11] Bacteria invade necrotized tissue, accelerating breakdown.[11] Matrix metalloproteinase 9 (MMP-9), a proteolytic enzyme produced by neutrophil granulocytes is present in symptomatic pulpitis but absent in healthy pulps, indicating its role in dental inflammation.[8-10] Inhibiting MMP-9 could open avenues for highly effective pulp-capping agents.

This study compares flavonoids, identified as potential pulp capping agents for their ability to induce odontogenesis through several studies.[15-19] Evaluating their binding energies, hesperidin (−8.1), with the least binding energy, is deemed the most effective inhibitor of MMP-9. As a control, chlorhexidine, a known MMP-9 inhibitor, is included to assess the effectiveness of flavonoids in inhibiting MMP-9. The findings reveal that all the flavonoids outperform chlorhexidine in inhibiting MMP-9.

Computational drug discovery is pivotal in drug research, encompassing target identification, interaction validation, lead discovery, optimization, and preclinical studies.[23] Leveraging large-scale datasets, it offers advantages over time-consuming traditional biological experiments, leading to a trend favoring structure-assisted screening methods.

Through molecular docking analysis, it was found that hesperidin binds to MMP-9 through H-Bonds to amino acid residues and stable hydrophobic packing interactions. From the characterization of the MD parameters such as RMSD, RMSF, Rg, and SASA, it is observed that hesperidin is a potent inhibitor of MMP-9.

The "Average RMSD" and "Average RMSF" values are measures of the overall conformational stability and flexibility of the protein. A lower RMSD value indicates that the protein has a more stable structure, while a higher RMSF value indicates that the protein is more flexible. In this study, two protein–ligand complexes, MMP-9-hesperidin and MMP-9-chlorhexidine were compared against native protein to verify whether binding of the ligand to the protein caused structural deviation and a resulting destabilization in the protein, although insignificant the MMP-9-chlorhexidine complex had less stability compared to MMP-9-hesperidin complex.

The "Rg" value measures the protein's size taking its radius into consideration. Rg plot revealed that throughout the simulation MMP-9 remained tightly packed.

The "Average SASA" value is a measure of the amount of surface area of the protein that is exposed to the solvent (such as water). A larger SASA value indicates that more of the protein is exposed to the solvent. Hesperidin complex had a higher SASA than chlorhexidine.

The free binding energy of the complexes was calculated with MM-PBSA analysis. The energy released during the interaction or bond formation process of ligand and target molecule is presented as binding energy. It is inversely proportional to the ligand and protein binding. The sum of electrostatic, polar solvation, SASA energy, and van der Wall is the final binding energy. The MM-PBSA analysis showed all forms of energy contributed to the interaction between protein and ligand. The MMP-9-hesperidin complex had a lower binding energy and hence a better interaction.

Given that this study is conducted in a simulated environment, it is imperative to verify the findings and ensure their reproducibility in real clinical settings through additional *in vivo* investigations.

CONCLUSION

MMP-9 strongly influences pulpitis prognosis, suggesting MMP-9 inhibitors in pulp-capping agents can enhance therapeutic efficacy. Flavonoids, notably hesperidin, are revealed as a promising MMP-9 inhibitor in this *in silico* study. Further *in vivo* studies, comparing hesperidin with other flavonoids and existing agents, are needed to validate these findings for potential biocompatible pulp-capping agents.

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Conflicts of interest
There are no conflicts of interest.

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