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UNLOCKING THE POTENTIAL: EXPLORING EUGENOL'S MOLECULAR DOCKING WITH ORAL CANCER TARGETS ARRB1, FLNA, CALM3, AND HTT

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ABSTRACT

Eugenol, a prominent phenolic compound derived from betel leaves (Piper betle), exhibits significant therapeutic and preventive properties in cancer, rendering it a compelling prospect for anticancer interventions. This study aimed to investigate the interactions between eugenol and four oral cancer-associated genes (ARRB1, FLNA, CALM3, and HTT) using molecular docking analysis. The molecular docking simulations were performed using AutoDock software, which predicted the binding interactions between eugenol and target proteins. The binding energies of eugenol with each gene were calculated to determine their affinity towards the ligand. The docking results revealed that eugenol exhibited favorable binding interactions with all four genes, indicating its potential to inhibit their activities. Among the target genes, CALM3 showed the lowest binding energy, indicating the strongest binding affinity with eugenol. Subsequently, the order of increasing binding energy and decreasing binding affinity revealed HTT, ARRB1, and FLNA. Visualization of binding interactions between eugenol and the target proteins provided insights into the molecular interactions and potential mechanisms of action. The identified outcomes underscore the therapeutic potential of eugenol in targeting specific genes implicated in oral cancer development, thereby positioning it as a promising agent for oral cancer treatment.

KEYWORDS: Eugenol, molecular docking, oral cancer, ARRB1, FLNA, CALM3, HTT, Piper betle.

INTRODUCTION

Piper betle, popularly known as betel leaves, have a longstanding history in traditional Indian medicine systems, attributed to their diverse health-promoting attributes. The leaves of Piper betle encompass a range of active phenolic compounds, such as chavibetol, piperol, piperbetol, hydroxychavicol, and eugenol. These compounds have been recognized for their medicinal properties and have traditionally been utilized in herbal remedies.[1]

Eugenol, an active phenolic compound abundantly present in betel leaves, stands out for its significant potential in chemotherapy and chemoprevention. Extensive research has explored its anticancer properties, demonstrating promising outcomes in impeding cancer cell growth. Moreover, eugenol is cost-effective and considered non-toxic, positioning it as a valuable candidate for therapeutic applications.[2] Oral cancer ranks as the sixth most prevalent cancer globally, with the second highest incidence among men.[3,4] Despite advancements in treatments like chemotherapy, radiation, and surgery, the mortality rate associated with oral cancer remains high. These conventional approaches often result in substantial adverse effects on normal cells, manifesting as severe toxicity.[5] Consequently, the utilization of naturally derived molecules for cancer treatment has gained recognition, as they offer less destructive alternatives to conventional methods.[6]

The process of carcinogenesis, a complex and multistep phenomenon, impacts various signalling pathways and induces quantitative alterations in cellular physiology. This intricate process involves genetic changes such as deletions, amplifications, point mutations, and rearrangements of relevant genes.[7] According to conventional understanding, a cell typically requires six or more mutations to undergo malignant transformation. Once cancer develops, the body's regulatory systems lose their control over the affected cell. Genetic alterations

can result in increased functionality of proto-oncogenes, while the recessive component may involve the loss of function in tumour suppressor genes or growth inhibitory genes.

The involvement of four genes, CALM3, ARRB1, HTT, and FLNA, in the oral cancer carcinogenesis pathway has been demonstrated.[8] Among these, the CALM3 gene, encoding the protein calmodulin, plays a crucial role in calcium-dependent signaling, essential for various cellular physiological activities.[9] The widely expressed protein beta arrestin 1 (ARRB1) is indispensable for nuclear transcription in cancer cells.[10] Huntingtin (HTT), a scaffold protein, is associated with Huntington's disease (HD), a neurological condition. In the central nervous system, HTT is involved in cell division, intracellular transport, and transcriptional control.[10] The FLNA gene encodes the Filamin A protein, which serves as a crucial component of the cytoskeleton along with Actin. Together, they contribute to providing structural support to the cell.[11] Moreover, Filamin A performs multiple functions, including cell signaling, phosphorylation, ion channel modulation, proteolysis, and transcriptional regulation. Due to its impact on various systems, mutations in the FLNA gene result in a range of symptoms.[12] Indian traditional medicines have gained significant importance in cancer treatment, particularly in the early stages, offering potential benefits without side effects.

MATERIALS AND METHODS:

Molecular docking involves the process of fitting a molecule into a target structure, exploring various positions, conformations, and orientations. Its objective is to predict the formation of an intermolecular complex between two molecules.[13] Autodock® 1.5.7 is an automated procedure utilized for predicting the interaction between ligands and biomolecular targets. The quality of docking results relies on the initial structure of both the protein and the ligand. To achieve accurate docking outcomes, proper preparation of the protein and ligand structures is crucial, encompassing steps such as protein preparation, ligand preparation, receptor grid generation, and the actual docking process.

Protein preparation

The protein structures of the four genes CALM3, ARRB1, HTT, and FLNA were obtained as docking targets from the primary protein database located at http://www.rcsb.org/pdb/home/hom.do. The respective PDB IDs for these genes are 1ZSH, 3HOP, 3IO6, and 2F3Z. Using the "Load Molecule" option from the menu, the proteins were loaded into the system. The "Read Molecule" function was employed to read the PDB coordinate files. Hydrogen atoms were added to the molecules through the "Edit Menu" to ensure their proper representation. Additionally, the "Edit-Charges" feature was utilized to compute Gasteiger charges for arbitrary molecules, which were tested for integral values. Finally, the prepared macromolecule file was saved in the Autodock® folder for further processing.[14]

Preparation of ligand

The structure of Eugenol was obtained from the zinc15 database in SDF format and subsequently converted into a PDB file using open babel. The ligand was loaded into the system using the "Input" option. The rigid root of the ligand was selected by choosing the appropriate atom through the rigid root selection feature. The rotatable bonds of the ligand were defined using the relevant option for rotatable bonds. Following the preparation of the ligand, the output was saved as a PDBQT file in the Autodock folder for further processing.[15]

Protein-ligand interaction utilizing Autodock:

The docking study was conducted using Autodock tools (ADT) v1.5.7 and Autodock v4.2 programs. The target proteins' search grid was employed to perform the docking process, and the ligand underwent polar hydrogen treatment. After assigning Kollmann charges, parameters for the nuclear solution were added. To introduce internal degrees of freedom and torsion, non-polar hydrogens were combined with carbons and assigned Gasteiger charges. During the docking cycle, the target proteins were kept rigid, while the ligands were allowed to move freely. The blind docking technique was employed, expanding the search to encompass the entire protein. Electrostatic and affinity maps were generated for each atom type using a grid spacing of 1.0 Å. The Lamarckian genetic algorithm was utilized, and the binding energy was employed for data clustering. Cluster analysis based on RMSD values was performed, with the most reliable result determined as the densely populated cluster with the lowest energy content.[16]

RESULTS AND DISCUSSION

In this study, we investigated the impact of eugenol, the active ingredient found in betel leaves, on four genes associated with oral cancer. These genes are interconnected and contribute to a pathway involved in oral cancer development. To assess the effect of eugenol on these genes, molecular docking analysis was performed. AutoDock software was employed to conduct docking simulations between eugenol and the four genes implicated in oral cancer. The docking results were evaluated based on binding energy, and the average binding energy of eugenol with the four genes was recorded in Table 1. The findings revealed that eugenol exhibited favorable binding to the receptor cavities of all four genes, indicating its potential to inhibit their activity. Among the docked genes, the CALM3 gene displayed the lowest binding energy, suggesting a strong affinity between eugenol and the target ligand. Following CALM3, HTT, ARRB1, and FLNA exhibited increasing binding energies and decreasing binding affinities. The interaction between eugenol and the target proteins was visually depicted in Figure 1.

Table 1: Binding energy of ligand with target protein.

PROTEINS	PDB ID	LIGAND BINDING ENERGY
CALM3	1ZSH	-5.43
ARRB1	3HOP	-3.44
HTT	3IO6	-3.8
FLNA	2F3Z	-3.36

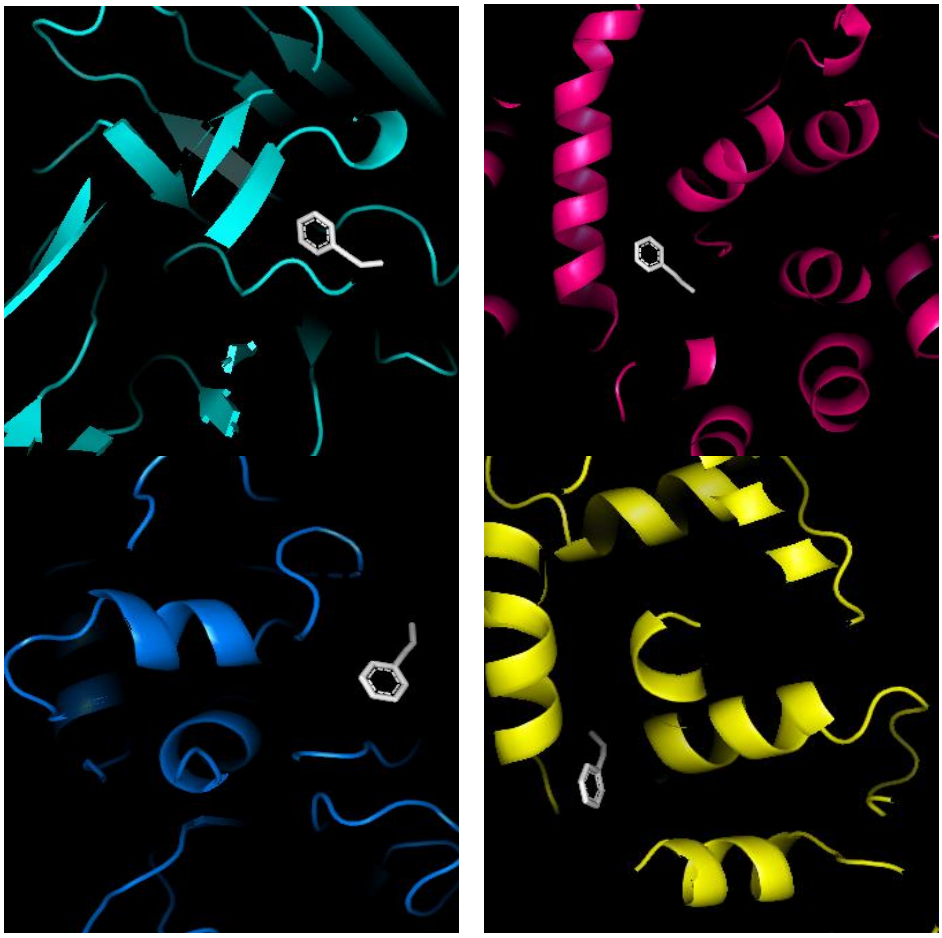

Figure 1: Visualizing Ligand-Protein Interactions.

(a) Eugenol's interaction with 1zsh.
(b) Eugenol's interaction with 3hop.
(c) Eugenol's interaction with 3io6.
(d) Eugenol's interaction with 2f3z.

CONCLUSION

In conclusion, this study explored the molecular docking interactions of eugenol, a phenolic compound found in betel leaves, with four oral cancer-associated genes (ARRB1, FLNA, CALM3, and HTT). The docking results demonstrated that eugenol exhibited favorable binding interactions with all four genes, indicating its potential to inhibit their activities and suggesting its role as a potential therapeutic agent for oral cancer treatment. Among the target genes, CALM3 showed the strongest binding affinity with eugenol, followed by HTT, ARRB1, and FLNA. These findings suggest that eugenol may exert its anticancer effects through modulating the functions of these genes involved in the development of oral cancer. The study results provide valuable insights into the molecular mechanisms underlying the chemotherapeutic and chemopreventive properties of eugenol in oral cancer. However, it is crucial to conduct further experimental investigations to validate these findings and explore the efficacy of eugenol as a therapeutic agent in both preclinical and clinical settings. The use of computational tools like molecular docking enables a better understanding of the interactions between bioactive compounds and target proteins, facilitating the identification of potential drug candidates. This study contributes to the existing knowledge on natural compounds as potential sources of anticancer agents, particularly in the context of oral cancer. Overall, this research highlights the promising role of eugenol in oral cancer treatment and underscores the importance of further investigations to fully elucidate its therapeutic potential and optimize its application in the development of novel therapies for oral cancer patients.

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