ejpmr, 2016,3(2), 275-278

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

INTERACTION STUDIES OF CATECHIN WITH HPV E6 AND E7 BY MOLECULAR DOCKING

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Article Received on 10/12/2015 **Article Revised on 01/01/2016** **Article Accepted on 20/01/2016**

ABSTRACT

Cervical cancer remains one of the major gynaecological disorder worldwide and these cases tend to progress at an alarming rate every year. Saraca *indica* belonging to the family Caesalpiniaceae is a universal panacea to the Ayurveda medicine. The plant bark tend to possess anti-cancer, anti-menorrhagia, anti-oxytocic, anti-microbial activity and has extended uses in all traditional medicinal formulations. Catechin is a polyphenol antioxidant found to be present in the bark of the plant. This insilico study was performed to identify the interaction and binding pattern of catechin compound with antigenic E6 and E7 of Human Papilloma viral proteins.Catechin, structure obtained from the database was docked with E6 and E7 using the Glide module of Schrödinger. Glide score of -7.7 was obtained for E6 with catechin compared to E7 oncoprotein Glide score -5.5, suggesting a higher interaction with E6.This study concludes that catechin functionally interacts with E6 and E7 proteins and further studies may help to understand the binding pattern and in developing an efficient drug from the compound.

KEYWORDS: Catechin, E6, E7, *Saraca indica.*

INTRODUCTION

Cervical cancer remains the third most common cancer among women in the world and the seventh overall. Though the incidence and mortality of cervical cancer has been significantly reduced from 1950 in the developing countries it has been approximated that 530,000 cases are newly diagnosed and 275,000 women die each year from cervical cancer, about 88% of which occur in developing countries.[1] There are more than 100 genotypes of HPV, which are associated with the development of malignant lesions and classified as "high-risk" for their ability to promote cancer. DNA from high-risk HPV has been found in over 95% of cervical cancer cases. Approximately, 50% of all cervical cancers contain HPV-16.[2] The HPV genome codes for six early proteins including E6 and E7 that are able to interfere with the p53 and pRb cell replication regulatory proteins, respectively, and the cascade controlled by them. The interaction of the viral oncoproteins E6 and E7 with cell cycle regulatory proteins, as well as the role of the viral proteins E1 and E2 are responsible for the initiation of the viral DNA replication.[3] The complex relation between the virus and its host cell is a potential target for the development of therapeutics mainly focusing to interfere with virus replication or with cell proliferation.[4]

S. indica (Ashoka) is an evergreen tree belonging to the Caesalpiniaceae subfamily of the legume family.[5] It grows throughout India up to an altitude of 750 m.[6] The bark of this plant yields alkanes, esters and primary alcohols. It gave n-octacosanol, tannin, catechin, (+)-catechol, (-) epicatechin, (-)-epicatechol, leucocyanidin, leucopelargonidin, procyanidin derivatives, methyl and ethyl cholesterol derivatives.[7] It also contains flavonoids and sterols.[8,9] Catechin is a polyphenolic antioxidant plant metabolite. The term catechin is also commonly used to refer to the related family of flavonoids and the subgroup flavan-3-ols (or simply flavanols). The crude bark extract of this plant possess significant anti-cancerous effect against HeLa cervical cell lines.[10] Catechin is a hepatoprotective agent, known for its antioxidizing capabilities and membrane-stabilizing properties.[11] Catechin compounds have been shown to exhibit cytostatic properties in many tumor models.[12] This insilico study was designed to study the interaction of the flavonoid catechin with the oncogenic proteins E6 and E7and to assess the stability.

MATERIALS AND METHODS

Docking studies were performed for the catechin compound with target HPV, E6 and E7 proteins by using the molecular docking tool, Glide (Schrodinger - Maestro v 9.3.518) software. Molecular docking involves the following steps- LigPrep, Protein preparation wizard, Glide Grid generation and Docking.

Protein and ligand retrieval

The three dimensional structures for Human Papilloma virus E6 oncoprotein (1VZN) and Human Papilloma virus E7 oncoprotein (2B9D) were retrieved from PDB (Protein Data Bank) and the structure of Catechin (CID-9064) compound was retrieved from the PubChem database.

Ligand and protein preparation

LigPrep is a robust collection of tools designed to prepare high quality, all-atom 3D structures for large numbers of drug-like molecules, starting with 2D or 3D structures in SDF or Maestro format. By using the LigPrep module (Ligprep, version 2.3, 2009), obtained ligand was geometrically optimized. The ionisation and tautomerisation were performed using Epik module for pH range of 7 ± 2, OPLS(Optimized Potentials for Liquid Simulations) force field was used for charges, bond angle and torsion parameters. The chirality of the input molecule was retained.

Protein preparation

The Protein preparation wizard of Schrodinger suite has been utilized to prepare the proteins. It converts a target protein in its PDB form into suitable for docking with ligand and modelling calculations. A typical PDB structure consists only of heavy atoms and may include a co- crystallized ligand, water molecules, metal ions and cofactors. The multimeric complexes are simplified after which it is determined whether the protein-ligand complex is a dimer/multimer. The geometrical errors are fixed in the protein and the missing residues near the active site are repaired. The protein structures are subsequently checked for metal ions and cofactors. The charges, correct atom types, bond orders and formal charges for any cofactors are adjusted as required. The orientation of disoriented groups (such as amide groups of Asn and Gln) is fixed.

E6, E7 protein preparation

The structures of E6, E7 oncoprotein were pre-processed prior to docking and molecular simulation. The E6 protein possessed a single chain and no hetero atom whereas E7 protein was found to be dimer. One chain was deleted, hetero atoms were removed and the missing side chains were added.

Protein-Ligand Interaction using Glide module

Grid Generation for E6, E7 oncoprotein- Grid Generation Panel

The E6, E7 oncoprotein grid was set up and generated from the Receptor Grid Generation panel. In the Grid panel, it allows to define the receptor structure by excluding any co-crystallized ligand that may be present to determine the position and size of the active site as it will be represented by receptor grids, set up Glide constraints, set up flexible hydroxyl groups, scale the Van der Waals radii for non-polar parts of the receptor.

Ligand Docking

Glide uses a hierarchical series of filters to search for possible locations of the ligand in the active region of the E6, E7 oncoprotein. The shape and properties of the protein are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. Conformational flexibility is handled in Glide by an extensive conformational search, augmented by a heuristic screen that rapidly eliminates unsuitable conformations, such as conformations that have long-range internal hydrogen bonds. Based on this consistency, ligand and protein were subjected to dock together utilizing Extra Precision value observation to find out the optimized parameter for the complex.

Molecular simulation of Protein-ligand complex using Schrödinger Suite (Macro Model)

MacroModel is a force field based Molecular Modelling program with applicability to a wide range of chemical systems. The methodology and parameterization for molecular simulation of E6, E7 oncoprotein and E6, E7 oncoprotein-catechin complex are carried out. Initially the structure was minimized to a low gradient using PRCG method with 500 maximum interactions and at 0.05 convergence threshold and the potential energy calculation was performed using OPLS-2005 force field in water solvent. The charges were taken from OPLS-2005 and extended cut-off was selected for Van der Waals and electrostatic calculation.

No Constraints was maintained for atoms, bonds, angle or dihedral. The options for molecular dynamics and None SHAKE were selected; total trajectories were set to 10 samples. The equilibration time was done for 1 Pico second and total simulation period was set up to 100 Picoseconds at 400 K temperature. This state of observation was created with TIP4P (water) environment to check the stability. The post observation targeted Root Mean Square Value of each trajectories in super imposed manner and the relational difference of each complex will be notified to analyse the protein structure stability. RMSD is calculated using the following formula, where M is summation of each complex, m denotes individual trajectories and r is atom types coordinates.

$$RMSD = \sqrt{\frac{1}{M}\sum_{i} m_i \left| \left| r_{i,1} - r_{i,2} \right| \right|}$$

RESULTS AND DISCUSSION

On performing the docking studies the interaction profile has been evaluated. The interaction profile of E6 oncoprotein with catechin showed that the ligand interacted at four sites of the protein from GLU 82 (OE1), GLU48 (OE1, OE2), GLN 97 (OE1) and PRO 116 (OE1) via H (21A, 20A, 19A, 9A and 18A) atom types forming hydrogen bonds with the bond distance of 2.43 Å, 2.37 Å, 2.35 Å, 1.79 Å, 2.00Å and 2.12Å respectively (Fig 1). Whereas, the interaction profile of E7 oncoprotein with catechin drug showed that the ligand interacted at three sites of the protein from ARG 60(O), LEU 58(O), PRO45(O, OE1) via H(21A, 20, 9A, 19A) atom types forming hydrogen bonds with the bond distance of 3.46, 2.92, 1.97 and 2.40 Å respectively(Fig 2).The higher interaction of E6 oncoprotein with catechin can be noticed from the Glide score -7.7 as compared to E7 oncoprotein Glide score -5.5.

Molecular dynamics were performed to look into the stability of E6, E7 oncoprotein and that of E6 oncoprotein-ligand complex in water solvent. After 1.0 Pico second equilibration a trajectory for 100 Picoseconds was generated for ten samples. RMSD drawn from the super imposed conformational structure for protein and protein-ligand complex showed that E6 oncoprotein is unstable in water whereas E7 oncoprotein is quite stable within 2.5 Å. The E6, E7 oncoprotein - catechin complex were found to be stable in water medium (Fig 3).The limitation of G Score defines the higher negative value which has more than -4, gives more interactive profile.

The preliminary docking studies establish that the catechin binds effectively to the target proteins and the glide score value of -7.7 seemed to be significant. E6 and E7 play an important role in viral replication by interacting with other cell regulatory proteins. The pro-apoptotic protein p53 is central to cellular defense mechanism by up-regulating expression of apoptotic proteins in response to cellular stress. In case of HPV-16 infections, E6 protein complexes with the cellular factor E6-AP (E6-Associated Protein) and forms an ubiquitin ligase that specifically binds to and targets p53 for ubiquitin mediated degradation, thus evading the p53 mediated defense mechanism. The E6 proteins also alter the transcriptional pattern of a variety of cellular and viral promoters, which seems to be in large part mediated by its interaction with E6-AP. E6 thus represents an excellent target for development of antiviral agents.[13] In this study, interaction profile clearly shows that the plant compound, catechin is effectively interacting with the proteins and the glide score value of E6 and catechin is significant. Catechin is also known to have antitumor properties. Further studies in the interaction pattern and additional properties may use to develop a potential lead drug/inhibitor from this compound against cervical cancer.

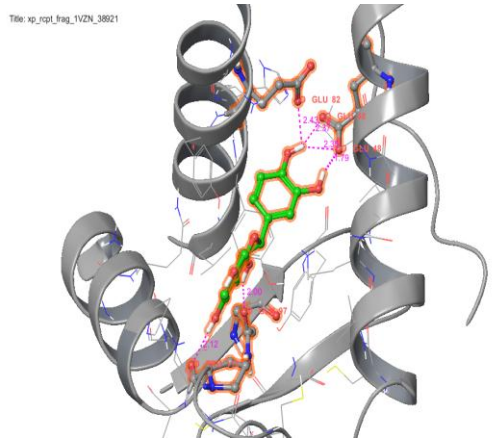

Fig 1: Catechin binding with E6 protein.

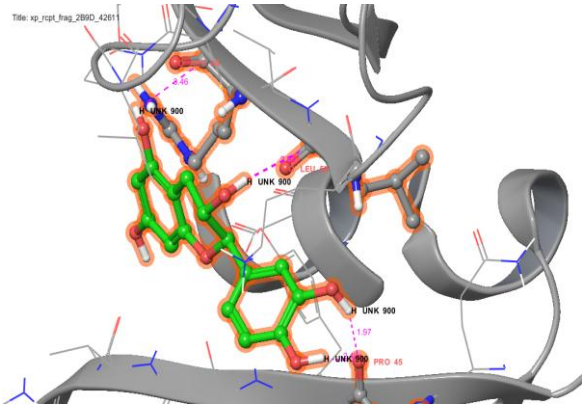

Fig 2: Catechin binding with E7 protein.

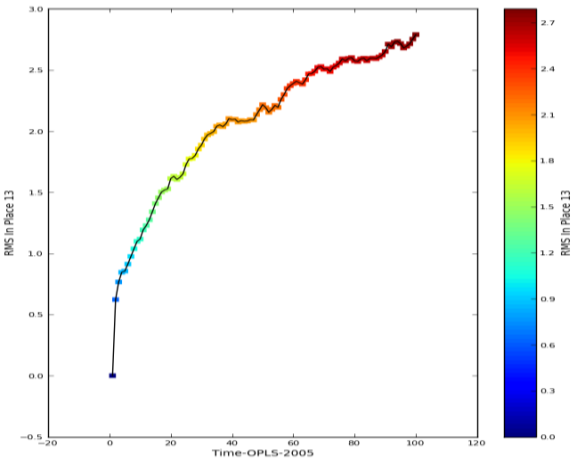

Fig 3: RMSD Graph for E6-Catechin Complex for picosecond observation.

CONCLUSIONS

The insilico interaction studies are helpful for understanding the binding mode and interaction of catechin with E6 and E7 proteins expressed by Human Papilloma Viruses. This computational approach can be further investigated to generate more effective and potential drug through ligand based drug designing methods. The above results demonstrate that catechin

might be potentially used as lead drug for developing a cure against cervical cancer.

ACKNOWLEDGEMENTS

We would like to acknowledge Prof. Shanmughavel, Centre for Bioinformatics, Department of Bioinformatics, Bharathiar University, Coimbatore in helping us in accessing the Schrodinger tool and Mr. Murugesh Easwaran, Department of Bioinformatics, Bharathiar University, Coimbatore for helping us throughout the study.

CONFLICT OF INTERESTS: Declared none.

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