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Research Article

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In Silico Analysis of HPV E6 as Drug Target with Natural Antioxidants

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Abstract: Human papillomavirus (HPV) infecting mucosal and cutaneous epithelia and induce cellular proliferation are small, non-enveloped, epitheliotropic, double-stranded DNA viruses. HPV is infectious to humans through sexual contact without showing any physical symptoms. The symptoms of HPV infection comprise warts on the genitals surrounding skin. Infection of HPV accounts to 70% of cervical cancer including cancers of the anus, vulva, vagina, penis and oropharynx. HPV types contain the genomes comprising eight open reading frames (ORFs) transcribed from a single DNA strand; ORF comprises three functional parts; viral replication encoded by the early (E) region comprising E1-E7 proteins; virion assembly encoded by the late (L) region comprising the L1-L2 proteins; and the replication and transcription of viral DNA is encoded by a long control region (LCR) is a non-coding part possessing the cis-elements. The HPV is encoded by three oncoproteins of which E6 protein was identified as an influential oncogene expressed product and is functionally related to the series of events resulting in the malignant conversion of virally infected cells. To understand the route of malignancy in humans by which E6 protein of HPV is resulting, much research is focused on identifying the cellular proteins with which E6 interacts. This study is focused on identifying the best ligands such as carrageenan, curcumin and papain available in natural sources such as fruits and vegetables to target HPV E6 Protein B-chain as drug target against antioxidants in cancerous individuals. This was carried out with the three-dimensional structures of ligands from pubchem and protein from protein data bank and the docking was performed by Autodock Vina. Minimal energy was noticed upon docking with carrageenan and it was the best over the other two selected ligands curcumin and papain.

Keywords: HPV E6, Drug target, carrageenan, curcumin, papain

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1. INTRODUCTION

Human Papillomaviruses (HPVs) infection happens by the cell cycle control taken by the viral particle in the basal layers of stratified epithelia and keratinocytes at various sites of internal organs and the differentiation of infected cells. The best characterized one, the mucosal HPVs of which HPV-16 and HPV-18 are the high-risk types, causing lesions and results in progression to cervical carcinoma. Benign genital warts resulted by low-risk types HPV-6 and HPV-11 and are related to malignancies very rarely.[1] In immune compromised individuals, HPV-5 and HPV-8, a subset of cutaneous HPV types are related to the cancers at sun exposed sites resulting in the progression of human cancers, particularly squamous cell carcinoma (SCC).[2] HPV, because of its limited coding capacity, it utilizes the DNA replication machinery of the host cell to replicate their genomes. In high-risk HPV infections, the replicative phase is confined to more differentiated cells in which the cell cycle had already exited and are non-permissive for DNA synthesis; and in low-risk HPVs, replication begins in cells that are still proliferating.[3] For up regulation, the genes are required for G1/S transition and DNA synthesis is targeted by HPV E7, a high-risk protein, pRb, p107, p130 (pocket protein family) and a number of proteins for cell cycle regulation.[4] The normal response of the cells of the host to this unscheduled induction of proliferation would trigger apoptosis and growth arrest. Regulation of cellular defense mechanisms, terminal differentiation and antiviral defense are targeted by the high-risk E6 protein. Under normal circumstances, viral replication would be carried out by production and release of infectious virions. The life cycle of HPV is intruded rarely and starts off of processes leading to the perpetuation and ultimately to complete transformation of the cell. The E6 proteins of HPV are made up of 150 amino acids containing small polypeptides and possessing two zinc-finger motifs,[5,6] E6 protein function is achieved by its integrity.[7,8] Research on tumours of the cervix and its derived cell lines evinced that E6 was a viral oncoprotein as it retained and expressed after many years of the initial transforming events.[9,10,11] In different assay systems, intrinsic transforming activity of E6 protein was noticed but in rodent cells, weak transforming activity of E6 protein was noticed,[12] efficient cooperation in the transformation of primary rodent cells was activated by ras oncogene under high-risk E6 proteins but not low-risk E6 proteins.[13,14,15] At late passage, E6 immortalized primary human mammary epithelial cells,[16,17] the same was noticed by the low-risk HPV E6 proteins.[18] This study is aimed at identifying the best ligands such as carrageenan, curcumin and papain available in natural available sources such as fruits and vegetables to target HPV E6 protein.

2. MATERIALS AND METHODS

2.1 HPV E6 Protein

2.1.1 *Protein selection and preparation*

HPV E6 Protein (PDB ID=6SIV)[19] at 1.75 Å resolution was retrieved without ligand from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) (RCSB, www.rcsb.org/). Protein preparation is the utmost crucial step of molecular docking protocol. SPDBV was used for the elimination of H_2O molecules, metal ions, cofactors, the addition of hydrogen atoms and charges (http://spdbv.vital-it.ch/).

2.1.2 *Binding Site Prediction*

CASTp was used to characterize the binding sites, measures the area, number, mouth opening circumference of each pocket insolvent and accessible surface of the molecule,[20,21] to identify and measure the obscured volume of proteins, to predict the binding sites on proteins position based on the shape, size and amount of proteins buried, PASS (Putative Active Sites with Spheres) method was used,[22] for predicting the ligand-binding site on a protein which involves the binding of hydrophobic probes to proteins, searching probe clusters on the protein with binding energy of each cluster arranged in order therefore favorable binding is calculated, Q-Site finder was used.[23] To detect pockets on the surface of the proteins, Pocket finder was used. Based on Ligsite algorithm, scanning the probe radius (1.6Å) with a grid resolution 0.9 Å, cubic diagonals and ligands along the proteins and comparison was drawn out by extensive literature search.[23,24]

2.1.3 *Ligand selection and preparation*

Phytochemicals present in naturally available components were taken as ligands. Structures of ligands were retrieved using pubchem (https://pubchem.ncbi.nlm.nih.gov/). The structures were converted to PDB file by Openbabel software hote(www.openbabel.org/). Ligand preparation involves addition of hydrogen atoms, neutralization of the charge groups and elimination of any disperate structure from the ligand. For molecular docking, prepared and optimized structures of ligand and protein were ultimately used. The ligands used for the present study were carrageenan, curcumin and papain (Fig.1).

1. Carrageenan

λ-carrageenan (CGN), extracted from red edible seaweeds belongs to a family of linear sulfated polysaccharide, has diverse biological activities, which include anti-coagulant,[25] antiviral,[26] and anti-tumor effects.[27] In the food industry CGN is used as a stabilizer and an enhancer for gelling and thickening. Because of the CGN strong binding to food proteins, its main application is in dairy and meat products.[28] In USA, under FDA regulations; CGNs are safely used as food additive.[29] Anti-tumor effects of CGN were noticed in mice by stimulating an immune response.[27,30] Treatment with CGN results in the increase of apoptosis in irradiated cancer cell lines and decreases in cell viability.[31]

2. Curcumin

Polyphenolic derivative, curcumin is a rhizome extract of *Curcuma longa* L., whose chemical name is *diferuloylmethane, 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3, 5-dione.* The National Cancer Institute classified it as generally recognized as safe (GRAS) as it is a nontoxic compound. It is an anticancer against prostate cancer, cervical cancer, colorectal carcinoma, leukemia, and human breast cancer cells. Clinical use of curcumin was hampered because of its properties like poor solubility, absorption, bioavailability, and rapid metabolism hampered its clinical use.[32] Anticancer properties of curcumin are exhibited by suppression of cellular transformation,

prevention of cancer cell proliferation, and suppression of carcinogenic effects.

3. *Papain*

Papaya (*Carica papaya* L.) raw fruit and leaves possess papain, a proteolytic enzyme. Because of the presence of papain, papaya consists of high nutritional and medicinal values. Papain can be obtained by eating raw papaya.[33] Papaya leaf extract is consumed for its anti-cancer property.[33,34] Papaya leaf extract in aqueous medium is beneficial for enhancing the longevity of life in patients suffering from cancer of different organs such as blood, liver, pancreas liver and lung.[35] Selective cytotoxic activities on an oral squamous cell carcinoma (SCC25) were noticed with the lyophilized juice extract of papaya leaf in comparison to non-cancerous fion prostate cancer (PCa) cells.[36]

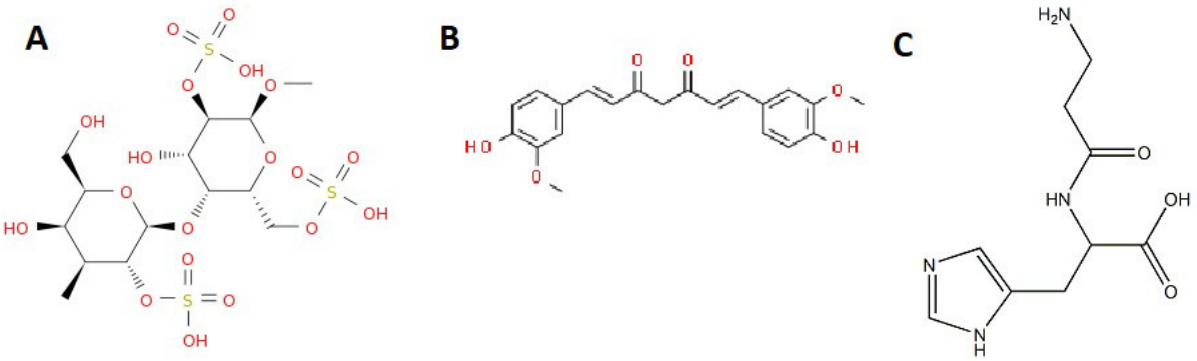

Fig.1: Ligands

A: Carrageenan (Carrageenan consists of alternating 3-linked-β-D-galactopyranose and 4-linked-α-D-galactopyranose units); B: Curcumin (Diferuloylmethane), a natural phenolic compound; C: Papain (Protease present in papaya fruit)

2.1.4 Molecular Docking

The unliganded HPV E6 was subjected to docking against ligands by using thePubchem database. To study protein-ligand interactions for discovering a new drug and its development, docking through computational tools is rapidly used. This process starts with a medicinal important target of known crystallographic structure of an enzyme. Docking enables the prediction of the binding free energy and confirmation of small molecules to the target. Single docking experiments are useful for exploring the function of the target and virtual screening, whereas a large library of compounds may be used to identify new inhibitors for drug development by docking and ranking. Computational docking and virtual screening of small molecules to macromolecular receptors are carried out by AutoDock, a suite of free open-source software. AutoDock Vina (http://vina.scripps.edu) is a turnkey computational docking program based on a simple scoring function and rapid gradient-optimization conformational search. Docking was carried out by AutoDock Vina 1.1.2.[37] Input requires ligand, receptor and docking box, whereas output is a list of poses ranked by ΔG, the predicted binding energy in kcal/mol ('score' = −ΔG).[38]

3. RESULTS AND DISCUSSION

3.1 HPV E6 Protein

Protein crystal structure (6SIV) (Fig.2) was obtained from PDB and "B" chain was pulled out followed by adding polar hydrogens and gustier charges were calculated.
The FASTA format sequence was:

>6SIV:A|PDBID|CHAIN|SEQUENCE
MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPAAA
FQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKY
ENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSAVNYGVTVLPTFKGQ
PSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFW
YAVRTAVINAASGRQTVDAALAAAQTNAAAEDILDELLGEER
>6SIV:B|PDBID|CHAIN|SEQUENCE
GAMFQDPQERPRKLPQLCTELQTTIHDIILECVYCKQQLLRREVYDFARRDLCIVYRDGNPYAVCDKCLKFYSKISEYRHYSYSLYG
TTLEQQYNKPLSDLLIRCINCQKPLSPEEKQRHLDKKQRFHNIRGRWTGRCMSCSRSSRTRRETQL

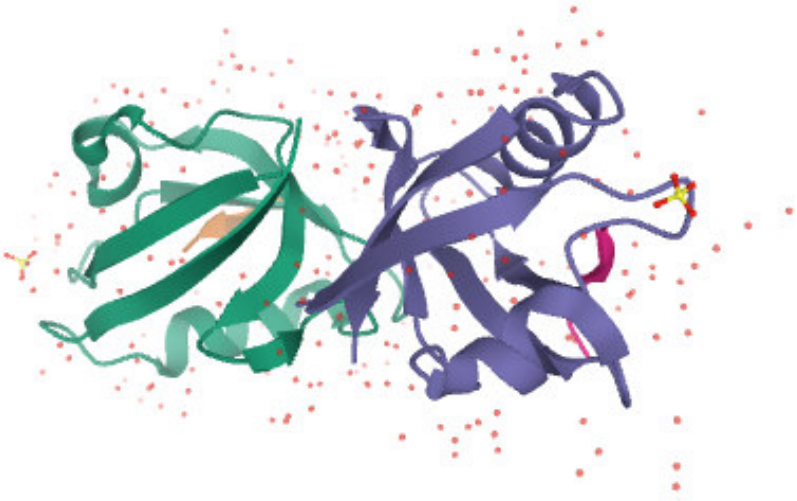

Fig.2: HPV E6 Protein Structure

3.2 Ramachandran Plot

A Ramachandran plot is a way to examine the structural stability of each residue in a protein. The sequence was checked in SWISS-MODEL[48]. The number of residues in most favored region [A, B, L] are 112 making up 90.3%, the number of residues in the additional allowed regions [a, b, l, p] are 10 making up 8.1%, the number of residues in generously allowed regions are 2 making up 1.6% and there were no residues in the diallowed regions. These make up the number of non-glycine and non-proline residues which were 124. The number of end residues (other than glycine and proine) was 4 whereas the number of glycine residues was 4 and prolinne were 6. The total number of residues in the protein in the "B Chain of HPV E6 Protein"was 138. This infers based on the analysis of 118 structures of resolution atleast 2A° and R factor no greater than 20%, a good model would be expected to have over 90% in the most favoured region (Fig.3).

3.3 Docking Results

Fitting ligand molecules into HPV E6 Protein structure, using Autodock Vina resulted in docking files. ADT (AutoDock Tool) was used for reading and analyzing the docking results, for analyzing the docking results. By computing the root mean square deviation (RMSD) between the coordinates of the atoms and creating clustering of the conformations based on the RMSD values, the docked structural similarity was measured. The most favorable docking pose is the one with the lowest binding energy conformation in all clusters. The sum of the total intermolecular energy, total internal energy and torsional free energy minus the energy of the unbound system is the representation of binding energies. Using AutoDock Vina software, protein-ligand interaction studies are carried out by performing energy minimizations to identify the best ligand that interacts with the E6 protein of HPV at minimal energy states. Each ligand interactions with the E6 protein of HPV were carried out for 20 folds to attain the minimal energy state (Table.1). It was noticed that Carrageenan was the best ligand as it showed minimal energy when compared with the other two ligands Papain and Curcumin. They were also effective against E6 protein of HPV (Fig.4)

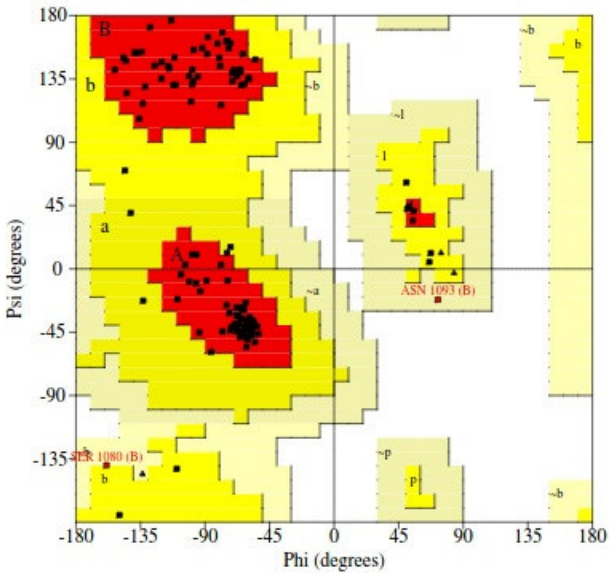

Fig.3: Ramachandran plot of HPV E6 Protein (6SIV: B Chain)

Table 1: Minimal energy states of protein-ligand interactions		
S.No	**Ligand**	**Emergy (k.cal/mol)**
1	Carrageenan	-10.7
2	Curcumin	-3.7
3	Papain	-8

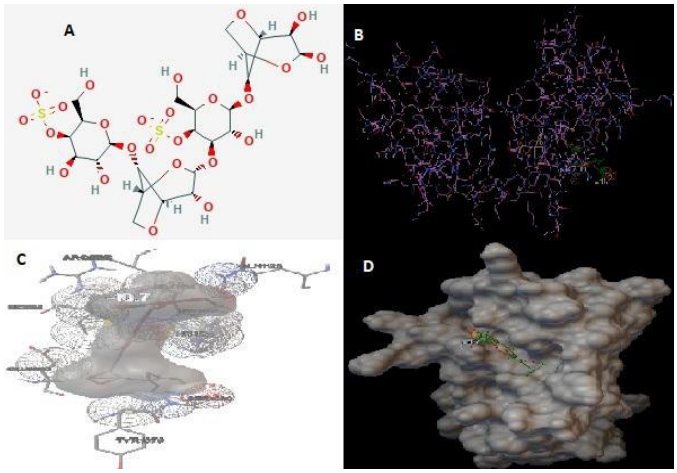

Fig.3: Binding of Carrageenan with HPV E6 Protein

A: Structure of Carrageenan; B: Binding of Carrageenan with HPV E6 Protein B chain; C: Binding affinity of Carrageenan; D: Energy grid

4. DISCUSSION

Cervical cancer resulted by Human papillomavirus (HPV) infection in women is the leading cause of cancer mortality worldwide. [39] Human papillomavirus (HPV) strains of high risk have been the etiologic agent of some anogenital tract cancers.[40] Even Though prophylactic HPV vaccines have been developed and approved for prevention; treatment with drug molecules is a must to overcome the infection and its oncogenic effects.[41] The most studied therapeutic target of HPV is E6 oncoprotein which is a key factor in cell immortalization and tumor progression in HPV-positive cells.[42] HPV circumvents the antiviral response through the possible E6 interaction with IRF3 and abrogates apoptotic activity of p53 by recruiting E6-associated protein. [43,44] There is a necessity to design anticancer drugs against life-threatening infection caused by HPV. Different compounds from natural origin, such as epigallo catechin gallate, indole-3-carbinol, jaceosidin carrageenan, curcumin and withaferin were explored as a hopeful source of anticancer therapy.[45] LxxLL motifs of IRF3 binds within the hydrophobic pocket of E6, percolating Ser-patch phosphorylation, necessary for IRF3 activation and interferon induction was concluded by *in silico* examination of protein-protein and protein-ligand docking, binding energy differences and computational alanine mutagenesis revealing a novel perspective of innate immune suppression in HPV infections and suggests a plausible therapeutic intervention.[46,47] The natural inhibitors against E6 oncoprotein of high-risk HPV-16 in this study represent a new starting point in the development of anti-HPV drugs essential for inactivation of p53 tumor suppressor protein. Docking analysis along with *in silico* validation of natural compounds helps in understanding molecular mechanisms of protein-ligand interactions. It would be better if the same is continued with other antioxidant phytochemicals possessing anticancer activity by molecular docking and to look at reconciling the functional effects observed with HPV E6 protein *in silico*. This requires solvating the HPV E6 protein model prior to any docking studies. Such work is beyond the scope of the current investigation, but our docking with HPV E6 protein permits comparison to other recent studies.

5. CONCLUSION

Molecular docking approach is used to identify and analyze the potential drug molecules. Hence, in this study, it was evidenced that the carrageenan can act as the best drug in the treatment of HPV infection by inhibiting the activity of HPV E6 which may result in cancer. As most of the anticancer drugs exhibit considerable side effects, docking has a merit in search for new drugs. It was concluded that carrageenan was the best ligand as it showed minimal energy over the other two and therefore the E6 protein of HPV exhibiting malignancy can be minimized. The other two ligands Papain and curcumin were also effective against E6 protein of HPV. It was evident from the recent studies that carrageenan kills cancer cells of certain cancer types; however, there is no scientific evidence which is reliable to show that Carrageenan can treat cancer. Carrageenan, curcumin and papain does not only show interactions with identified active residues that are important for catalytic activity of E6 protein of HPV but the free energy of binding ensures a very strong binding with active site of E6 protein of HPV. The current work epitomizes complete interpretations about all known anticancer mechanisms of carrageenan, possible role of naturally occurring carrageenan to fight against cancer and cyanide toxicity is a mistaken belief about exploiting the potential of carrageenan. However, well-planned animal experimentation and clinical trials are to be carried out to prove effectiveness of this substance *in vivo* and to get approval for human use. The main intentions of analyzing these phytochemicals are that these are

antioxidants which are phytochemicals that interact and neutralize free radicals, thus preventing them from causing damage.

6. AUTHORS CONTRIBUTION STATEMENT

This author contribution statement affirms that all individuals listed as authors agree that they have met the criteria for authorship, agree to the conclusions of the study, and that no individual meeting the criteria for authorship has been omitted. Dr. Rajasekhar Pinnamaneni conceptualized and gathered the data with regard to this work. Mr. Dhrub Kumar Yadav carried out the preliminary work such as selection of ligands and protein from the databases in association with the other authors. Mr. Gandham Prasad was instrumental in carrying out the docking studies. Dr. Srinivasulu Kamma analyzed the final data and gave necessary inputs towards the designing of the manuscript.

7. ACKNOWLEDGMENTS

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8. CONFLICT OF INTEREST

Conflict of Interest declared none

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