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RESEARCH ARTICLE

MOLECULAR DOCKING STUDIES OF RESVERATROL AGAINST THE HUMAN ORAL CANCER CELL LINE PROTEINS (KB CELLS)

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A R T I C L E I N F O

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A B S T R A C T

Resveratrol compound was identified to have more inhibitory activity against human oral cancer cell line proteins (KB cells) by this docking study. The docking scores were highest in Beta-actin (-4.51 kcal/mol) followed by Nuclear Factor NF-kappa-B p100 subunit (-3.23 kcal/mol), Cytochrome c1 (-3.12 kcal/mol), Caspase 3 (-2.71 kcal/mol) and Caspase 9 (-1.72 kcal/mol). The LogP value and lower hydrogen bond counts, conforming the ability of Resveratrol for binding at the active site of the receptor was determined by *in silico* method. The potential drug candidate can further be validated in wet lab studies for its proper function.

INTRODUCTION

Cancer is an uncontrollable growth of cells that invade and cause damage to surrounding tissue and is a major cause of death throughout worldwide. Oral cancer, which includes cancers of the lips, tongue, cheeks, floor of the mouth, hard and soft palate, sinuses and pharynx (throat), can be life threatening if not diagnosed and treated early. Oral cancer being the 3rd most common type which accounts for over 30% of all cancers in India [1]. Most of the oral cancers are known as squamous cell carcinoma [1, 2], which are malignant and liable to expand rapidly. Oral cancer incidence ranks fifth in the global cancer burden, and a 2- to 3-fold mortality increase has been recorded in eastern and central European countries in the last three decades [2]. In India, oral cancer, ranks first among all cancer cases in males and is the third most common among females in many regions [3-5]. Oral cancer is the sixth most common cancer for both sexes in the general population, and the third most common cancer in developing nations [6].

Resveratrol (3,5,4'-trihydroxystilbene) is a naturally occurring phytoalexin produced by a wide variety of plants such as grapes (*Vitis vinifera*), peanuts (*Arachis hypogaea*), and mulberries. Resveratrol was detected in the leaf epidermis and the skin of grape berries but not in the flesh (7-9).The epidemiologic finding of an inverse relationship between consumption of red wine and incidence of cardiovascular disease has been called the "French paradox" (10, 11). Resveratrol also exhibits antibacterial effects (12), including inhibition of growth of different strains of *Helicobacter pylori* (13-15). Red grapes or red wine are sources of Resveratrol which is well known (16). Resveratrol also shows chemotheraputic properties against different types of cancers [17-21]. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex [22] and docking plays an important role in the rational design of drugs[23] well on the docking studies of Resveratrol against oral cancer cell line proteins is scanty. Hence the present study was taken to fill the lacunae.

Objective of the Study

- To identify the proteins found in the oral KB cell lines.
- To study physicochemical properties of the proteins.
- To carry out docking of five different kinds of proteins viz., caspase 3, caspase 9, NF-kappa B, Beta actin and cytochrome C with Resveratrol and analyse the domain and active sites of binding and evaluate the binding affinity of the Resveratrol compound.

MATERIALS AND METHODS

Preparation of protein structure

The protein information was obtained form Swissprot and the protein structure of oral cell line protein (KB cell) were obtained from RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). The hydrogen atoms were added to the target protein molecule after removing the water molecules for docking. The 3D structure of the proteins were visualised using RASMOL.

Preparation of ligand structure

Ligand is a small molecule, which interacts with protein's binding sites. There are several possible mutual conformations in which binding may occur. These are commonly called binding modes [24]. ChemSketch developed by Advanced Chemistry Development, Inc., (http://www. acdlabs. com) was used to construct the structure of ligands of

Resveratrol. Using draw mode of ChemSketch, the ligands were generated and three dimensional optimizations were done and then saved in.mol file and TORSDOF was used in calculating the change in free energy caused by the loss of torsional degrees of freedom upon binding. After all the above conditions are set and the ligand was saved in "pdbq" format.

Preparation of receptors

The receptor file used by AutoDock must be in " pdbqs" format which is pdb plus "q" charge and "s" solvation parameters: AtVol, the atomic fragmental volume, and AtSolPar, the atomic solvation parameter which are used to calculate the energy contributions of desolvation of the receptors ie macromolecule by ligand binding was also calculated using Open Babel .

Preparation of grid parameter file

The grid parameter file tells AutoGrid the types of maps to compute, the location and extent of those maps and specifies pair-wise potential energy parameters. In general, one map is calculated for each element in the ligand plus an electrostatics map. Self-consistent 12-6 Lennard Jones energy parameters - Rij, equilibrium internuclear separation and epsij, energy well depth are specified for each map based on types of atoms in the macromolecule. If we want to model hydrogen bonding, this is done by specifying 12-10 instead of 12-6 parameters in the "gpf" format. The grid parameter were set using AutoGrid.

Starting Auto Grid

AutoGrid and AutoDock must be run in the directories where the macromolecule, ligand and parameter files are to be found.

Preparation of docking parameter file

The docking parameter file tells AutoDock which map files to use the ligand molecule to move, what its center and number of torsions are where to start the ligand, which docking alogrithm to use and how many runs to do. It usually has the extension, "dpf". Four different docking algorithms are currently available in AutoDock: SA, the original Monte Carlo simulated annealing; GA, a traditional Darwinian genetic algorithm; LS, local search; and GA-LS is also known a Lamarckian genetic algorithm or LGA, because children are allowed to inherit the local search adaptations of their parents.

Starting AutoDock

AutoGrid and AutoDock must be run in the directories where the macromolecule. ligand, gpf and dpf files are to be found.

Analysing the docking results

The key results in a docking log are the docked structures found at the end of each run, the energies of these docked structures and their similarities to each other. The similarity of docked structures are measured by computing the root-mean-square deviation (RMSD) between the coordinates of the atoms. The docking results consist of the PDBQ of the Cartesian coordinates of the atoms in the docked molecule along with the state variables that describe this docked conformation and position and this was done by PyMOL.

RESULTS AND DISCUSSION

In this present study, the interactions between the Caspase 3, Caspase 9, NF -kappa- B p100 subunit, Beta - actin, Cytochrome C1 and oral cancer cell lines (KB cells) were studied to explore their binding mode, docking study was performed using Auto Dock with PyMol Tool.

Oral cancer cell line (KB cells) protein structures were derived from PDB Database and used as a target for docking simulation. The details of resveratrol compound are mentioned in Table.1. The ligands were created and prepared for docking studies using ChemSketch. The structures of the ligands obtained from the ChemSketch indicated in the Fig. 1 and 2.

Table 1 Properties of Resveratrol compound

Molecular Weight	**228.24328g/mol**
Molecular Formula	$C_{14}H_{12}O_3$
X LogP3	3.1
Hydrogen Bond Donor Count	3
Hydrogen Bond Receptor Count	3
Rotatable Bond Count	2
Exact Mass	228.078644g/mol
Monoisotopic Mass	228.078644g/mol
Heavy Atom Count	17
Description of the compound	Phytonutrient, polyphenol compound found in the skin of red grapes. It is an antioxidant and it has many therapeutic uses.

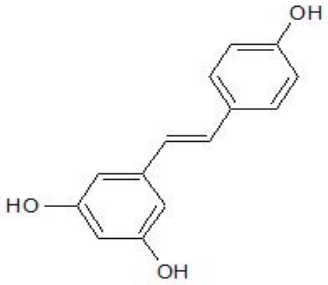

Figure 1 2D Structure of Resveratrol

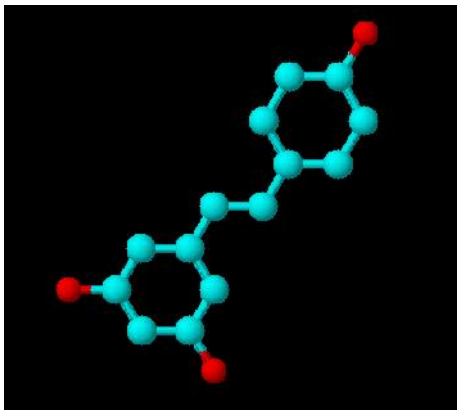

Figure 2 3D Structure of Resveratrol

The detection of ligand - binding sites is the initial step for novel drug discovery. Here the Q-Site Finder predicted the active site of the oral cell line proteins (KB cells) precision as showed in Fig. 3 to 7.

The active sites of human oral cell line protein (KB cells) have the amino acid sequences are as follows:

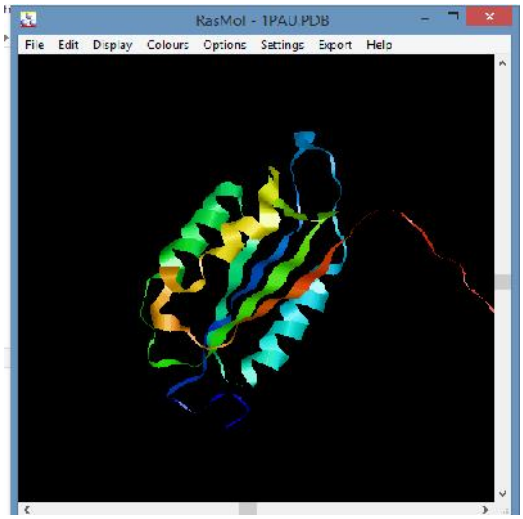

Figure 3 Active site residues of Caspase 3

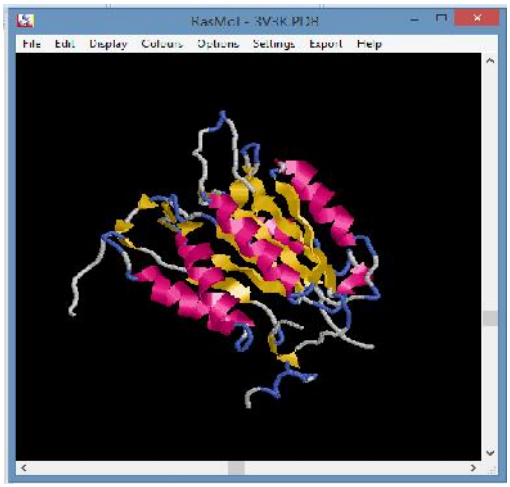

Figure 4 Active site residues of Caspase 9

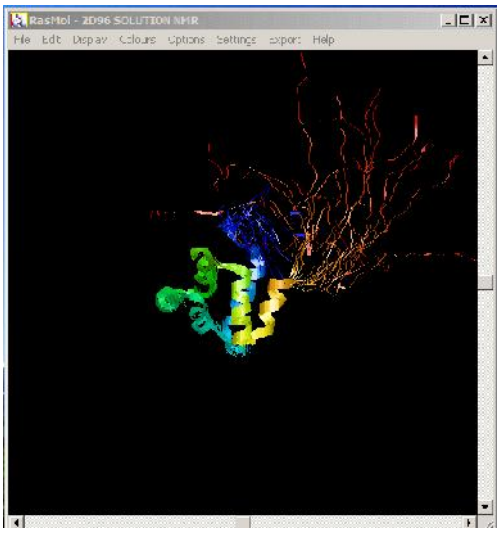

Figure 5 Active site residues of NF-kappa- B p100 subunit

Caspase 3 - Fasta sequence

>sp|P42574|CASP3_HUMAN Caspase-3 OS=Homo sapiens GN=CASP3 PE=1 SV=2

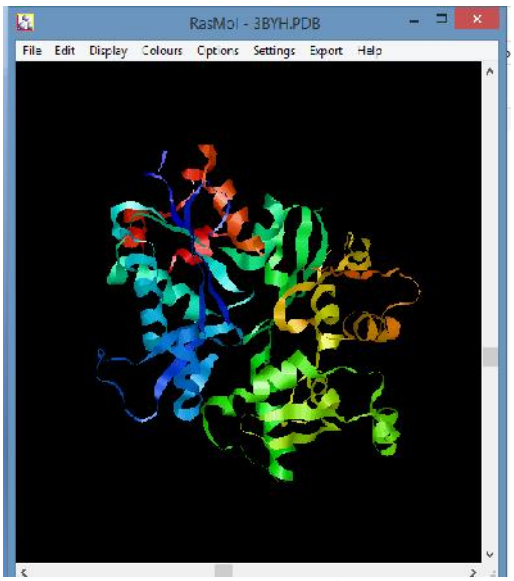

Figure 6 Active site residues of Beta - actin

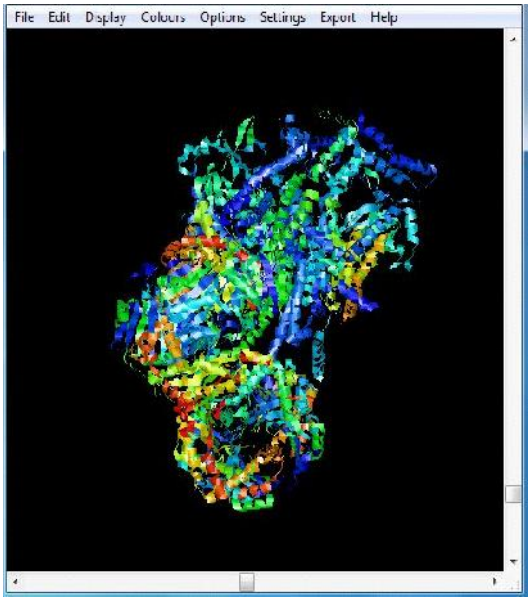

Figure 7 Active site residues of Cytocrome C1

Table 2 Docking Score And Number Of Hydrogen Bonds Formed Between The Proteins And Resveratrol Compound

S.No	Proteins	Resvervatrol	
		Docking score (kcl/mol)	Hydrogen Bond
1	Caspase 3	-2.71	2
2	Caspase 9	- 1.72	1
3	Nuclear factor NF-Kappa-B p100 subunit	-3.23	3
4	Beta - actin	-4.51	1
5	Cytocrome c1	-3.12	2

MENTENSVDSKSIKNLEPKIIHGSESMDSGISLDNSYKM
DYPEMGLCIIINNKNFHKSTG
MTSRSGTDVDAANLRETFRNLKYEVRNKNDLTREEIV
ELMRDVSKEDHSKRSSFVCVLLS
HGEEGIIFGTNGPVDLKKITNFFRGDRCRSLTGKPKLFII
QACRGTELDCGIETDSGVDD
DMACHKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQ
SLCAMLKQYADKLEFMHILTRVN
RKVATEFESFSFDATFHAKKQIPCIVSMLTKELYFYH

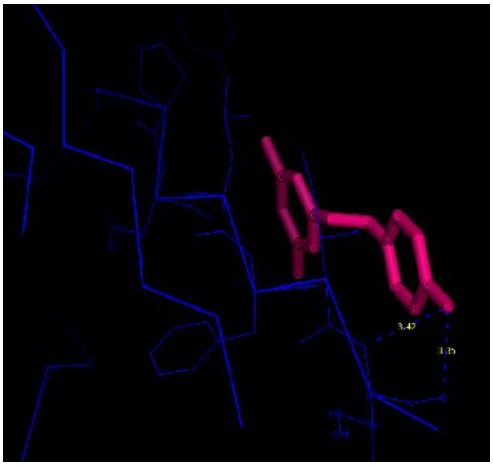

Figure 8 Interaction between Resveratrol (blue) and Caspase 3 (rose)

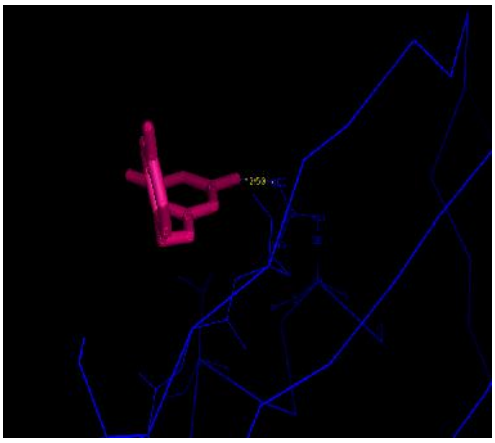

Figure 9 Interaction between Resveratrol (blue) and Caspase 9 (rose)

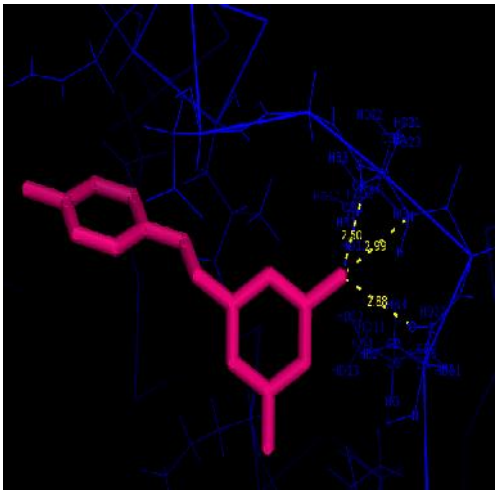

Figure 10 Interaction between Resveratrol (blue) and NF kappa-B p100 subunit (rose)

Caspase 9- Fasta sequence

>sp|P55211|CASP9_HUMAN Caspase-9 OS=Homo sapiens GN=CASP9 PE=1 SV=3

MDEADRRLLRRCRLRLVEELQVDQLWDALLSRELFRP
HMIEDIQRAGSGSRRDQARQLII
DLETRGSQALPLFISCLEDTGQDMLASFLRTNRQAAKL
SKPTLENLTPVVLRPEIRKPEV
LRPETPRPVDIGSGGFGDVGALESLRGNADLAYILSME
PCGHCLIINNVNFCRESGLRTR
TGSNIDCEKLRRRFSSLHFMVEVKGDLTAKKMVLALL
ELAQQDHGALDCCVVVILSHGCQ
ASHLQFPGAVYGTDGCPVSVEKIVNIFNGTSCPSLGGK
PKLFFIQACGGEQKDHGFEVAS
TSPEDESPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSD
IFVSYSTFPGFVSWRDPKSG
SWYVETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIY
KQMPGCFNFLRKKLFFKTS

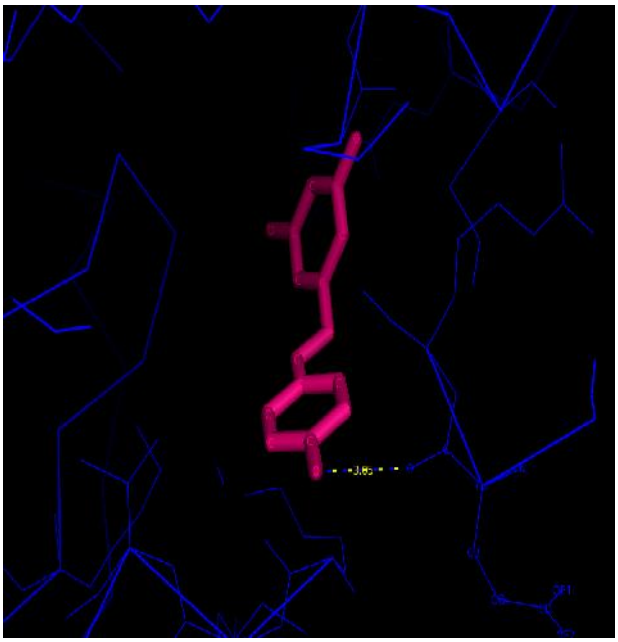

Figure 11 Interaction between Resveratrol (blue) and Beta -actin (rose)

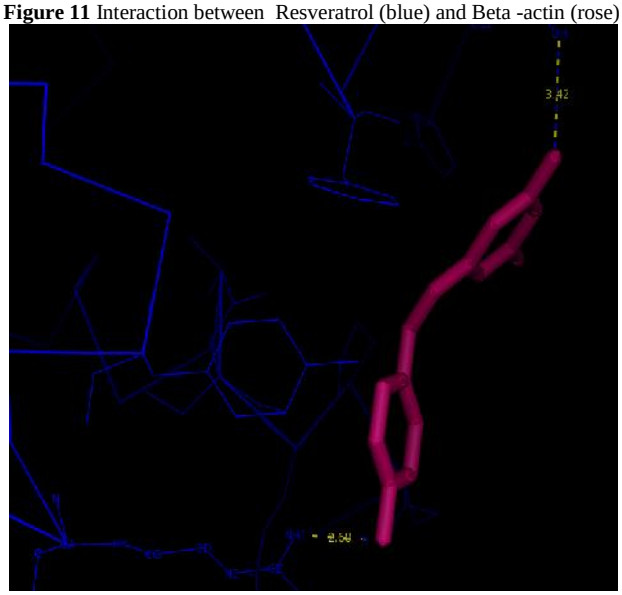

Figure 12 Interaction between Resveratrol (blue) and Cytochrome c1 (rose)

NF-kappa -B p100 subunit - Fasta sequence

>sp|Q00653|NFKB2_HUMAN Nuclear factor NF-kappa-B p100 subunit OS=Homo sapiens
GN=NFKB2 PE=1 SV=4
MESCYNPGLDGIIEYDDFKLNSSIVEPKEPAPETADGPY
LVIVEQPKQRGFRFRYGCEGP
SHGGLPGASSEKGRKTYPTVKICNYEGPAKIEVDLVTH
SDPPRAHAHSLVGKQCSELGIC
AVSVGPKDMTAQFNNLGVLHVTKKNMMGTMIQKLQ
RQRLRSRPQGLTEAEQRELEQEAKE

LKKVMDLSIVRLRFSAFLRASDGSFSLPLKPVISQPIHDS
KSPGASNLKISRMDKTAGSV
RGGDEVYLLCDKVQKDDIEVRFYEDDENGWQAFGDF
SPTDVHKQYAIVFRTPPYHKMKIE
RPVTVFLQLKRKRGGDVSDSKQFTYYPLVEDKEEVQR
KRRKALPTFSQPFGGGSHMGGGS
GGAAGGYGGAGGGGSLGFFPSSLAYSPYQSGAGPMGC
YPGGGGGAQMAATVPSRDSGEEA
AEPSAPSRTPQCEPQAPEMLQRAREYNARLFGLAQRSA
RALLDYGVTADARALLAGQRHL
LTAQDENGDTPLHLAIIHGQTSVIEQIVYVIHHAQDLGV
VNLTNHLHQTPLHLAVITGQT
SVVSFLLRVGADPALLDRHGDSAMHLALRAGAGAPEL
LRALLQSGAPAVPQLLHMPDFEG
LYPVHLAVRARSPECLDLLVDSGAEVEATERQGGRTA
LHLATEMEELGLVTHLVTKLRAN
VNARTFAGNTPLHLAAGLGYPTLTRLLLKAGADIHAE
NEEPLCPLPSPPTSDSDSDSEGP
EKDTRSSFRGHTPLDLTCSTKVKTLLLNAAQNTMEPPL
TPPSPAGPGLSLGDTALQNLEQ
LLDGPEAQGSWAELAERLGLRSLVDTYRQTTSPSGSLL
RSYELAGGDLAGLLEALSDMGL
EEGVRLLRGPETRDKLPSTAEVKEDSAYGSQSVEQEAE
KLGPPPEPPGGLCHGHPQPQVH

Beta -actin - Fasta sequence

>sp|P60709|ACTB_HUMAN Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1

MDDDIAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGR
PRHQGVMVGMGQKDSYVGDEAQS
KRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAP
EEHPVLLTEAPLNPKANREKMT
QIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDG
VTHTVPIYEGYALPHAILRLDL
AGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCY
VALDFEQEMATAASSSSLEKSY
ELPDGQVITIGNERFRCPEALFQPSFLGMESCGIHETTFN
SIMKCDVDIRKDLYANTVLS
GGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSV
WIGGSILASLSTFQQMWISKQ
EYDESGPSIVHRKCF

Cytochrome C1 - Fasta sequence

>sp|P08574|CY1_HUMAN Cytochrome c1, heme protein, mitochondrial OS=Homo sapiens
GN=CYC1 PE=1 SV=3

MAAAAASLRGVVLGPRGAGLPGARARGLLCSARPGQ
LPLRTPQAVALSSKSGLSRGRKVM
LSALGMLAAGGAGLAMALHSAVSASDLELHPPSYPWS
HRGLLSSLDHTSIRRGFQVYKQV
CASCHSMDFVAYRHLVGVCYTEDEAKELAAEVEVQD
GPNEDGEMFMRPGKLFDYFPKPYP
NSEAARAANNGALPPDLSYIVRARHGGEDYVFSLLTG
YCEPPTGVSLREGLYFNPYFPGQ
AIAMAPPIYTDVLEFDDGTPATMSQIAKDVCTFLRWAS
EPEHDHRKRMGLKMLMMMALLV
PLVYTIKRHKWSVLKSRKLAYRPPK

As most of the amino acid residues in the active site are hydrophobic and they are the main contributors to the receptor-ligand interaction.

Details of interaction

The goal of ligand-protein docking is to predict the predominant binding model(s) of a ligand with a protein of known three dimensional structure [25]. To study the binding mode of Resveratrol compound in the binding site of oral cell line protein (KB cells), intermolecular flexible docking simulations were performed and energy values were calculated from the docked conformations of the oral cell line protein-inhibitor complexes. Docking studies yielded crucial information concerning the orientation of the inhibitors in the binding pocket of the target protein. Several potential inhibitors have been identified through the docking simulation. The binding affinity of the oral cell line proteins with the Resveratrol compound were measured by kcal/mol. The docking scores were highest for Beta- actin with -4.51 kcal/mol with the stronger interaction followed by NF-kappa - B p100 subunit (-3.23 kcal/mol.), Cytochrome c1(-3.12 kcal/mol), Caspase 3(-2.71) and the least score was found in Caspase 9 (-1.72 kcal/mol) as showed in the Table 2 and Fig. 8 to 12. Similar type of studies with Quercetin compound was performed by Muthukala *et al*., [26]. The protein-ligand interaction plays an important role in structural based designing [27]. The high docking scores of Resveratrol compound with Beta- actin clearly indicates their binding affinity. Analysis of ligand binding interaction with the oral cell line protein (KB cells) can be useful for new preventive and therapeutic drug for cancer. The results obtained from this study would be useful in both understanding the inhibitory mode as well as in rapidly and accurately predicting the activities of new inhibitors on the basis of docking scores.

CONCLUSION

In this study, the molecular docking was applied to explore the binding mechanism and to correlate its docking score with the activity of Resveratrol compound. The results are helpful for the design and development of novel drug having better inhibitory activity against several types of cancer including oral cancer. From this study we conclude that Resveratrol compound is one of the best anticancer phytochemical agent. These potential drug candidates can further be validated in wet lab studies for its proper function.

Acknowledgement

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References

1. Elango JK, Gangadharan P, Sumithra S, Kuriakose MA. Trends of head and neck cancers in urban and rural India. *Asian Pac J Cancer Prev* 2006;7:108-12.
2. Coleman MP, Esteve J, Damiecki P, Arslan A, Renard H. Trends in cancer incidence and mortality. IARC Sci Publ 1993; 121: 1-806.

3. Indian Council of Medical Research. National Cancer Registry Programme: Biennial Report 1988-1989. ICMR, New Delhi, India, 1992.
4. Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB. Cancer incidence in five continents Vol. VII. *IARC Scientific Publications* 1997; No. 143: IARC, Lyon.
5. Macfarlane GJ, Boyle P, Evstifeeva TV, Robertson C, Scully C. Rising trends of oral cancer mortality among males worldwide: the return of an old public health problem. *Cancer Causes Control* 1994; 5(3): 259-65.
6. La Vecchia C, Tavani A, Franceschi S, Levi F, Corrao G, Negri E.Epidemiology and prevention of oral cancer. *Oral Oncol* 1997; 33:302–12.
7. Langcake P and Pryce RJ: The production of resveratrol byVitis vinifera and other members of the Vitaseae as a responseto infection or injury. *Physiol Plant Pathol* 9: 77-86, 1976.
8. Langcake P and McCarthy W: The relationship of resveratrol production to infection of grapevine leaves by Botrytis cinerea. *Vitis* 18: 244-253, 1979.
9. Creasy LL and Coffee M: Phytoalexin production potential ofgrape berries. *J Am Soc Hort Sci* 113: 230-234, 1988.
10. Kopp P: Resveratrol, a phytoestrogen found in red wine. A possible explanation for the conundrum of the 'French paradox'? *Eur J Endocrinol* 138: 619-620, 1998.
11. Sun AY, Simonyi A and Sun GY: The "French Paradox" and beyond: neuroprotective effects of polyphenols. *Free Radic Biol Med* 32: 314-318, 2002.
12. Chan, MM Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin. *Biochem Pharmacol*, 63: 99-104, 2002.
13. Daroch, F, Hoeneisen, M, Gonzalez, CL, Kawaguchi, F, Salgado, F, Solar, H, and Garcia, A In vitro antibacterial activity of Chilean red wines against Helicobacter pylori. *Microbios*, 104: 79-85, 2001.
14. Mahady, GB, Pendland, SL, and Chadwick, LR Resveratrol and red wine extracts inhibit the growth of CagA+ strains of Helicobacter pylori in vitro. *Am J Gastroenterol*, 98: 1440-1441, 2003.
15. Mahady, GB and Pendland, SL Resveratrol inhibits the growth of Helicobacter pylori in vitro. *Am J Gastroenterol*, 95: 1849, 2000.
16. Celotti, E, Ferrarini, R, Zironi, R, and Conte, LS Resveratrol content of some wines obtained from dried Valpolicella grapes: Recioto and Amarone. *J Chromatogr* A, 730: 47-52, 1996.
17. Gusman, J, Malonne, H, and Atassi, G A reappraisal of the potential chemopreventive and chemotherapeutic properties of resveratrol. Carcinogenesis, 22: 1111-1117, 2001.
18. Bhat, KP and Pezzuto, JM Cancer chemopreventive activity of resveratrol. *Ann N Y Acad Sci*, 957: 210-229, 2002.
19. Fremont, L Biological effects of resveratrol. *Life Sci*, 66: 663-673, 2000.
20. Yang, CS, Landau, JM, Huang, MT, and Newmark, HL Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annu Rev Nutr*, 21: 381-406, 2001.
21. Ulrich S, Wolter F, Stein JM (2005) Molecular mechanisms of the chemopreventive effects of resveratrol and its analogs in carcinogenesis. *Mol Nutr Food Res* 49: 452-461.
22. Lengauer T, Rarey M (1996). "Computational methods for biomolecular docking". Curr. Opin. Struct. Biol. 6 (3): 402–6. doi:10.1016/S0959-440X(96)80061- 3. PMID 8804827.
23. Sharma, N.K., Jha, K.K., Priyanka, 2010. Molecular docking: an overview. *J. Adv. Sci. Res.* 1, 67–72.
24. Kitchen DB, Decornez H, Furr JR, Bajorath J (2004). "Docking and scoring in virtual screening for drug discovery: methods and applications". Nature reviews. Drug discovery 3 (11): 935–49. doi:10.1038/nrd1549. PMID15520816.
25. Mittal RR, McKinnon RA, Sorich MJ. Comparison data sets for benchmarking QSAR methodologies in lead optimization. *J Chem Inf Model* 2009:49:1810-20.
26. Borappa Muthukala, Kanagarajan Sivakumari, Kamalanathan Ashok. In Silico docking of Quercetin compound against the Hela cell line proteins. *Int J Curr Pharma* Res 2015:13-16.
27. Heena V Sanghani, Sunil H Ganatra, Rama Pande. Molecular- Docking studies of potent Anticancer Agent. *J Comput Sci Syst Biol* 2012:5:012-015.

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