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Screening of Natural Plant Phenol Compounds as Better Interacting Agents in Cancer Chemotherapy

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Abstract

Cell death is a normal facet of human physiology, ensuring tissue homeostasis by offsetting cell production with cell demise. Neoplasm arises in part because of defects in physiological cell death mechanisms, contributing to pathological cell expansion.Caspase-3 is frequently activated protein in cell death (Apoptosis/Programmed Cell Death) mechanism which is activated by defective cell proliferation. Our goal is to manipulate apoptotic pathway for selectively destroy cancer cells by using bioactive compounds having natural product origin and thus it may improve clinical outcomes. The current study focused on use of computational tools to find bound conformations of ligand to a larger receptor of caspase-3 with a known structure inducing apoptosis in Cancer prevention. Also we depict the side chain amino acids bond strength involved in ligand binding with caspase-3. The docking result says the quercetin is the best interacting molecule with Caspase-3 protein. It shows one best interaction with Tyr197 (A-chain) by bond energies are negetive. We provide the docking algorithmic perspectives for therapeutic target identification and highlight a number of algorithmic advances which have gotten relatively little attention so far with the hope of strengthening the ties between these two research communities. This information is useful for further studies to discover effective drugs without side effects and low cost to cure cancer suffering patients.

Keywords: Cancer, Apoptosis, Caspase-3, CASTp, Auto Dock4.0.

Contents

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1. Introduction

Cells are the building blocks of life's (8), normal cells multiply when the body needs them and die doesn't need them, is regulated through "Apoptosis"(Programmed cell death mechanism). One defining feature of cancer isappears to occur when the growth of cells in the body is out of control then cells divide too quickly, that grow beyond their usual boundaries, which can then invade adjoining parts of the body spread to other organs via lymphatic,blood called metastasis and developed as malignant tumors.Cancer caused by both external factors (tobacco, infectious organisms, chemicals, and radiation) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism),are directly damage genes or combine with existing genetic faults within cells to cause the disease. Approximately five to ten percent of cancers are entirely hereditary (12).For every cell, there is a time to live and time to die. There are two ways in which cells died by injurious agents and other themost important way is induced to commit suicide(Apoptosis) (15).Apoptosis is a regulated cellular suicide mechanism occurs in three ways, are Extrinsic, Intrinsic and Granzyme A,B pathway (4) see Fig1 and characterized by nuclear condensation, membrane blebbing, cell shrinkage, formation of apoptotic bodies and DNA fragmentation. Caspases are crucial mediators of programmed cell death. Among them, caspase-3 see Fig2 (EC No: 3.4.22.0 Hydrolase) is a frequently activated death protease and Fig1 shows the three pathways causes apoptosis by directly activating caspase-3 protein which catalyze the specific cleavage of many key cellular proteins(11).In active site of caspase-3 the catalytic residues are Gln, Ala, Cys, Arg and Gly, which sequence is conserved in all caspases (1).

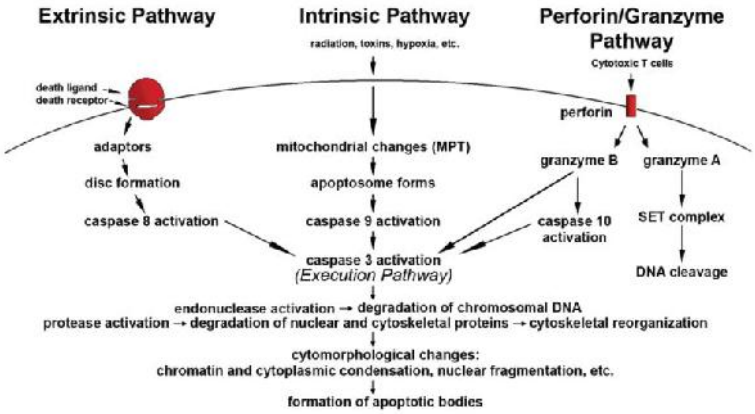


Figure: 1 Diagramatic represantaion of three ways to induce apoptosis via caspae-3 protein

Caspase-3 acts in a tissue selective manner.At least 42 of the 58 known caspase substrates are specifically cleaved by caspase-3, and can process pro-caspases 2, 6, 7 and 9 (2). Caspase-3 is required for proteolysis of alpha-fodrin dimer which is found abundantly in the salivary glands, results of fodrin proteolysis important for protease activation during apoptosis (7). Caspase-3 is essential for gelsolin cleavage, these results causes membrane blebbing (5). Caspase-3 is required for the carboxyl terminal cleavage of ICAD/DFF-45 that is necessary for the generation of the functional endonuclease (13), and is a caspase-3-dependent step of major pathway to DNA fragmentation in apoptosis.Caspase-3 is essential for normal brain development (11) and is important or essential in other apoptotic scenarios in remarkable tissue-cell types. Thus, caspase-3 is essential for certain processes associated with the dismantling of the cell,but it may also function before or at the stage when commitment to loss of cell viability is made.

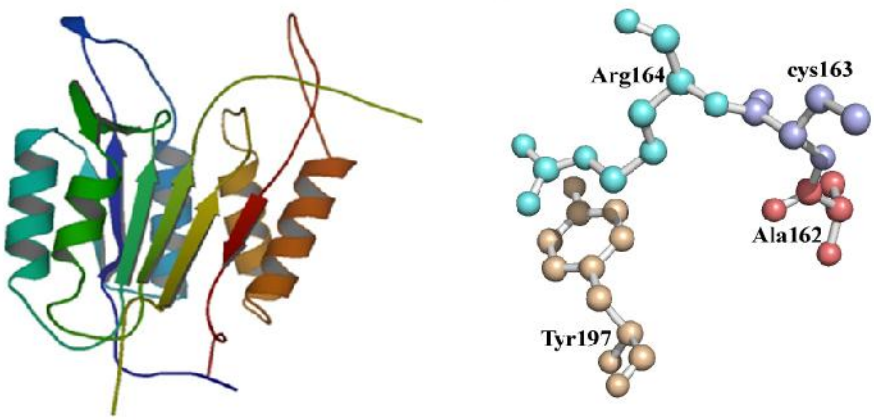


Figure 2: 3D structure of caspase-3 protein and conserved sequence of active siteamino acid residues in all caspses

Several medicinally valuable molecules present in natural plants, includes alkaloids, flavonoids and phenolic compounds which have the antioxidant property, can inhibit the growth of cancer cells as "Chemopreventers", literally proved. Their cancer-preventive effects have been attributed to various mechanisms, including the induction of cell-cycle arrest and apoptosis. Our current study is to predict the best interacting ligand among paradol (Fig3(A)), emodin (Fig3(B)) and quercetin (Fig3(C)) by comparing,using bioinformatics tools like protein-ligand dockingoffline tool kit (ADT) software program Autodock4.0 (Goodsel *et al.*1998), which can induce caspase-3 protein to prevent the cancer cell proliferation by accelerating the apoptosis in cancer patients.

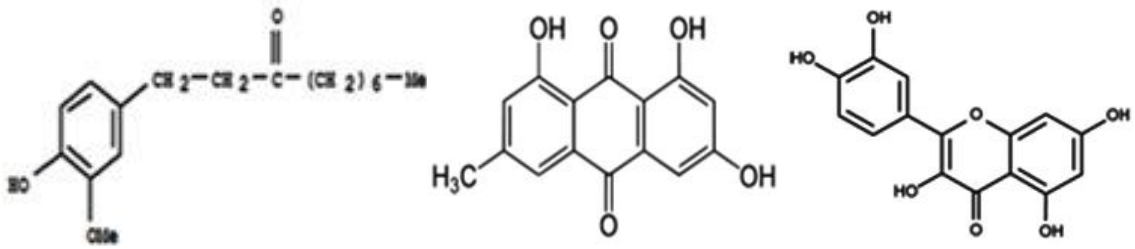


Figure 3: Chemical Structure of natural compounds of **(A)** Paradol **(B)** Emodin **(C)** Quercetine

Tools and System

The docking study was performed on an AMD 64 bit dual processor with Linux operating system and docking files prepared on 32 bit dual processor with Windows OS. Ligand docked with protein was performed on offline software of Autodock 4.0 (9, 14). Figures were developed using Pymol 1.0(*http://pymol.source-forge.net/*).

2. Materials and Methods

Methodology

Retrieving of PDB Files for Ligand and Caspase-3

Required protein caspase-3 of human accession number 1CP3 PDB file was retrieved from online ProteinData Bank (http://www.rcsb.org/pdb),through searching with homologous protein in NCBI (http:// www.ncbi.nlm.nih.gov/) BLASTp (10). Paradol, Emodin, Quercetineligands SDF files were retrieved from online databank of PubChemCompound in NCBI (http://www.ncbi.nlm.nih.gov/pccompound). The PDB files of ligands generated by pasting the ligand sdf file on online Prodrg server (http://davapc1.bioch.dundee.ac.uk/prodrg/) but there is noenergy minimizationof ligand but their charges are full.

Active Site Identification of caspse-3

The active site analysis along with area and volume of caspase-3 active site pocket was performed with Computed Atlas of Surface Topography of Proteins (CASTp) (3) through online server(http://cast.engr.uic.edu). It provides identification and measurements of surface accessible pockets as well as interior inaccessible cavities, for proteins.

Preparation of Docking Files

AutoDock (4.2) employs to predict the docking of a flexible ligand to a binding site of a rigid protein, given the region of the protein containing the binding site to substrate (6). All docking calculations were carried out using the Autodock 4.0/ADT and docking pdb.qt files were prepared by adding all kolluman charges and checked total charges on residues, also added all polar hydrogen's and edited histidine hydrogen's in protein pdb and checked ligand pdb with 50 torsions. The pdb.qt files were uploaded in autogrid and docking runs were set to be 10. During the preparation of files for docking in Autodock4.0 the docking grid parameters are set to 50x50x50 points with a grid spacing of 0.375 A^0. The grid center was placed in the active site pocket center. The grid boxes included the entire binding site of the enzyme and provided enough space for the ligand translational and rotational walk.

Docking

Docking was carried out on Linux OS which can provide environment for molecular simulation of flexible ligand bind towards protein binding site. The binding site on protein was auto gridded and finally docked. Docking was performed up to 10 runs for each ligand and after docking 10 solutions are clustered into groups with the RMSD lower than 0.5 A^0. The clusters were ranked by the lowest-energy representative of each binding mode. The rest of the parameters were set as default values, finally the dock log files were generated. At the end of a docking experiment with multiple runs, a cluster analysis was performed for their binding conformation.

Docking Analysis

The ligand best dynamic interaction with protein was predicted through docking analysis. Docking analysis was based on free energy of binding, lowest docked energy and calculated RMSD values. Besides RMSD clustering, AutoDock also uses binding free energy evaluation to identify the best binding mode. Energy items calculated by AutoDock4.0 include intermolecular energy (constituted by van der Waals energy, hydrogen bonding energy, disolvation energy, and electrostatic energy), internal energy and torsion energy. During all these interactions, the

hydrogen bond between ligand and protein is the most important, because in most cases it can decide binding strength and location of ligand where as the hydrophobic interaction of certain groups can cause the inhibition, specifically to a great extent.

3. Results and Discussion

Active Site Identification of caspse-3

Caspase-3 active site prediction was performed using CASTp program for binding pocket analysis.

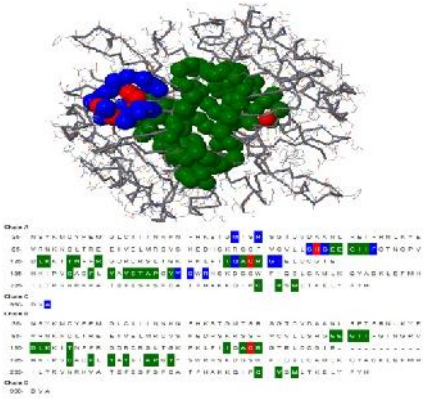


Figure 4: Ligand binding pocket analysis in caspase-3 protein by CASTp server

The predicted ligand binding pocket for 1CP3 is shown in Fig4. The ligand binding pocket containing active site consisted of 26 residues, namely Glu123, Glu124, Gly125, Ile126, Ile127, Leu136, Lys137, Thr140, Asp141, Arg144, Ile160, Ala162, Cys163, Arg164, Asp190, Phe193, Tyr195, Tyr197, Ser198, Thr199, Ala200, Pro201, Tyr203, Cys264, Val266, Met268 . The results of CASTp active site identification was further validated through docking analysis.

Docking Results: The ranking of docking scores to ligands are calculated binding energies for ligands paradol -4.25 kcal/mol, emodin -4.3kcal/mol and quercetin −5.41 kcal/mol.Here the experimental evidence listed in Table. 1indicates the dynamic interactionof paradoland emodin with Arg164 and Tyr197 residues of caspase-3 enzyme,and Quercetin OH group orienting towards hydroxyl group of Tyr197 in A-chain of caspase-3 to form hydrogen bond by removing water molecule as H_2O. The result of Fig5(C) clearly shows that quercetin interact more dynamically with caspase-3 protein than the other ligands. The bonding interaction and that bond energy information are listed in below tables1, 2, & 3.

Table 1: The above table shows the information about the ligands Paradol, Emodin and Quercetin interaction with caspase-3 Protein

S. No.	Ligand Name	Confirmation Number	No. of Hydrogen Bonds	Drug and protein Interaction Information
1.	Paradol	1	2	paradol::DRG1:HAC: 1cp3:B:Tyr197:OH 1cp3:A:Arg164:HH21: paradol::DRG1:OAA
2.	Emodin	44	3	emodin::DRG1:HAD: 1cp3:A:Tyr197:OH 1cp3:A:Arg164:HH21: emodin::DRG1:OAB emodin::DRG1:HAE: 1cp3:B:Tyr197:OH
3.	Quercetin	1	1	quercetin::DRG1:HAG: 1cp3:A:Tyr197:OH

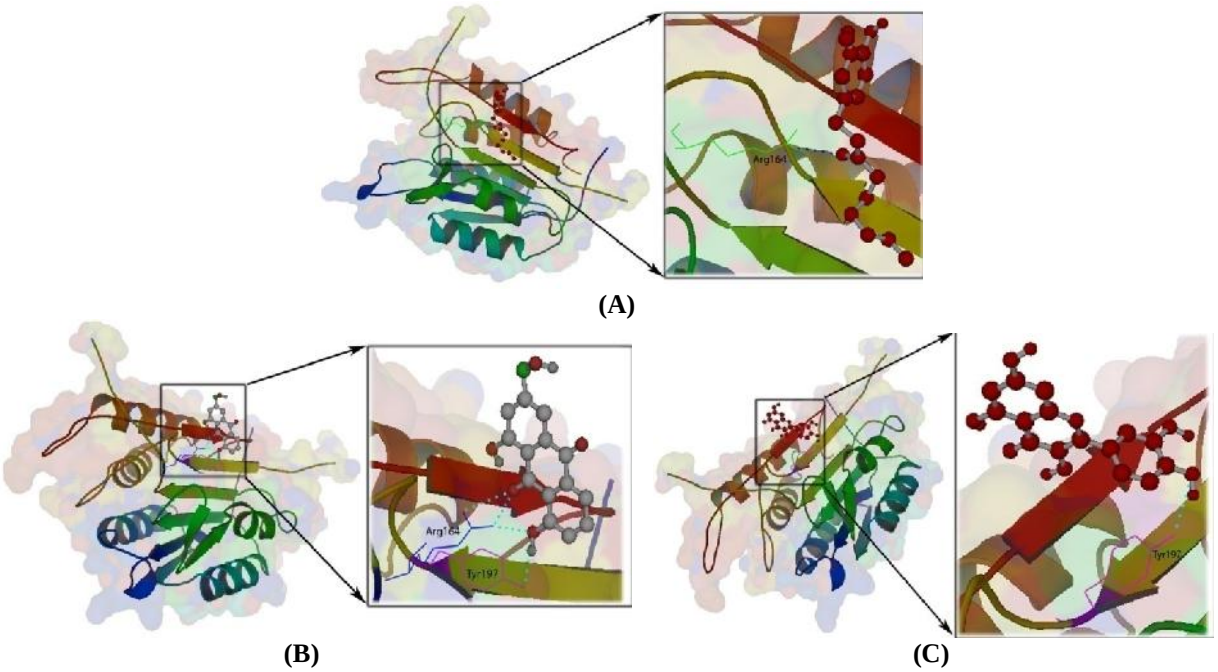
Table 2: Comparison of ligand molecules of Paradol, Emodin and Quercetin with caspase-3 by docked energies in Auto dock analysis

S. No.	Conformation Information	Paradol conformation 1 information (Energy in Kcal/mol)	Emodin conformation 44 information (Energy in Kcal/mol)	Quercetin conformation 1 information (Energy in Kcal/mol)
1.	Binding Energy	-4.25	-4.3	-5.41
2.	Ligand Efficiency	-0.21	-0.22	-0.21
3.	Inhibition Constant	766.97 µM	699.8 µM	108.32 µM

4.	Inter Molecular Energy	-6.57	-4.94	-5.61
5.	Hydrogen Bond Dissolving Energy	-6.45	-4.75	-5.47
6.	Electrostatic Energy	-0.12	-0.19	-0.14
7.	Total Internal Energy	-0.7	-0.47	-1.44
8.	Tensional Energy	3.02	1.1	1.65
9.	dlg Cluster RMS	0.0	0.0	1.4
10.	Reference RMS	25.28	25.33	24.54

Table 3: This table constitutes Hydrogen bond length and energy between ligand and caspase-3 protein

		Paradol	**Emodin**	**Quercetin**
Hydrogen Bond Length	A-chain	Arg164: 2.174	Arg164: 2.036 Tyr197: 2.208	Tyr197: 2.181
	B-chain	Tyr197: 2.15	Tyr197: 2.091	
Hydrogen Bond Energy	A-chain	Arg164: -1.035	Arg164: -5.486 Tyr197: -2.986	Tyr197: -3.487
	B-chain	Tyr197: -2.641	Tyr197: -0.469	



(A) **(B)** **(C)**

Figure 5: The above figures show the Docking interaction of caspase-3 with Paradol (A), Emodin (B) and Quercetine (C). The active site side chain amino acids interactions of caspase-3 with ligand molecules through hydrogen bonds are shown in zoom.

Discussion

In order to screen natural ligands as effective cancer chemotherapeutic drug through AutoDock, after docking the three natural ligands shows best dynamic docking interaction with caspse-3 protein. The total clusters of docking conformations of caspase-3 protein with docked lead molecules showed negative binding energies. The hydrogen bond interaction between ligand and protein information was listed in table. 1.The protein caspase-3 showed the best interaction with predicted amino acids Arg164, Tyr197 of A-chain and Tyr197 of B-chain on attractive site for ligands. The number of hydrogen bonds formed in between protein and corresponding ligands like paradol, emodin and quercetin are 2, 3 and 1 respectively.The docking interaction information of conformation towards corresponding molecule listed in table. 2. Except quercetin, paradol and emodin which has to close energy (higher than -4.2kcal/mol) in conformations, the ligand quercetin has -5.41 kcal/mol binding energy. The inhibition constant of paradol, emodin nearer to 700 µM and quercetin 108.32µM which were compared to less than rest of the two. The total internal energy of quercetin is -1.44kcal/mol, is more than paradol (-0.7 kcal/mol) and emodin (-0.47

kcal/mol).The docking results for hydrogen bond that length and energy information is listed in Table. 3, and the interaction modes of ligands (paradol, emodin, quercetin) with protein (caspase-3) are shown in Fig5(A, B, C) using PyMOL software.Where along with the experimental evidence quercetin have better interaction rate with caspase-3 protein than that of paradol and emodin. Docking analysis reveals that in the arrangements with the lowest docked energy,this ligand can enter into the substrate-binding region of active site andstimulates caspase-3 to further cascade process of apoptosis.

4. Conclusion

The present docking study reveals the mode of binding and binding affinity of three molecules paradol, emodin and quercetin at the active site of caspase-3 protein. A slight difference of lowest docked energy between paradol (-4.25 Kcal/mol) and emodin (-4.3 Kcal/mol) and a wide difference of lowest docked energy to quercetin has been found between these molecules attributed to their different mode of binding and their conformation. Precisely, these molecules interact differentially with amino acids of the active site of caspase-3 protein, which reflects the difference of their binding affinity. Specifically this computational study confirms the existence of hydrogen bonding interaction in ligands, which was predicted experimentally. Overall, the contribution of this docking study indicates, the quercetin is the best interacting ligand among three ligands of paradol, emodin and quercetin. Quercetinan ubiquitous bioactive flavonol, inhibits cells proliferation, induces cell cycle arrest and apoptosis in different cancer cell types. This work requires further research and an interesting future study might involve, todiscovery ofderivatives of quercetine.

5. Acknowledgements

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