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ORIGINAL ARTICLE

A Therapeutic Approach to Target Mitochondrial Dysfunction using Molecular Docking Studies: Screening of Natural Drugs for Oral Carcinoma

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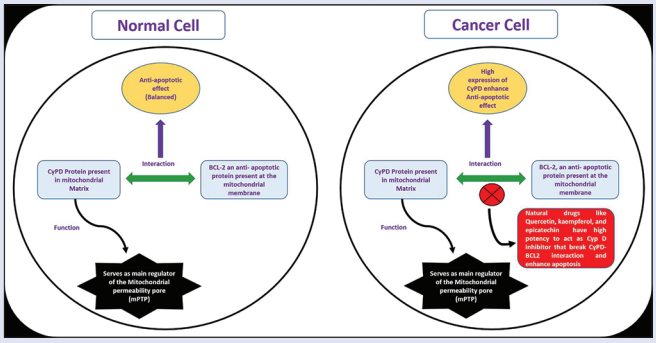
ABSTRACT

Background: Mitochondrial dysfunction is the major cause of various types of cancer, leading to death worldwide. The present study investigated the *in silico* binding potential of natural flavonoids and essential oils with human cyclophilin D (CyPD) protein. CyPD protein is a major molecular marker for apoptosis and has been reported to be elevated in oral carcinoma. **Methods:** PubChem database was used to check the efficacy of different active phytoconstituents (kaempferol, quercetin, eugenol, oxyresveratrol, tanshinone 2a, catechin, epicatechin, cinnamaldehyde, and emodin). These compounds were used as ligands to check their potential as anticancer agents against the inner mitochondrial membrane protein, CyPD. Docking studies were performed with the help of Discovery Studio 2.5 and Autodock. Emodin was used as a reference inhibitor to compare the results. **Results:** The binding energy (B.E.) of the reference inhibitor (known/established drug) emodin was observed −28.9 kcal/mol while novel inhibitors (catechin, cinnamaldehyde, epicatechin, eugenol, kaempferol, oxyresveratrol, quercetin, and tanshinone 2a) exhibited a range from −51.51 to −5.89 kcal/mol. Quercetin, kaempferol, and epicatechin (B.E.: −51.51, −34.79, and −30.62 kcal/mol, respectively) showed strong affinity as compared to reference inhibitor (B.E.: −28.9 kcal/mol). **Conclusion:** Quercetin, kaempferol, and epicatechin can be used as lead inhibitors against targeting CyPD.

Key words: Apoptosis, cyclophilin D pathway, mitochondrial dysfunction, molecular docking, oral cancer

SUMMARY

- The present study investigates the in-silico binding potential of natural flavonoids and essential oils with human cyclophilin D (CyPD) protein
- It was observed that quercetin, kaempferol, and epicatechin showed strong affinity as compared to reference inhibitor emodin.



Abbreviations used: CyPD: Cyclophilin D, BE: Binding Energy, PTPC: Permeability transition pore complex, mPTP: Mitochondrial permeability transition pore.

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INTRODUCTION

Oral cancer is the major cause of cancer morbidity and mortality worldwide. The prevalence of oral cancer in Southeast Asia and in India is approximately 40% of total malignancies.[1] The most affected oral subsite is buccal mucosa and alveolus in the developing countries.[2]

Most of the cancer cells survive and proliferate by manufacturing ATP via glycolysis more willingly than oxidative phosphorylation even in the presence of sufficient oxygen as it was postulated by Warburg.[3,4] Corresponding with this, increase in glycolysis is a suppression of mitochondrial activity in cancer. Under physiological conditions, mitochondria harbor a robust mitochondrial transmembrane potential and a low conductance state of the permeability transition pore complex (PTPC) might contribute to the exchange of small metabolites between the cytosol and mitochondrial matrix, a process that is mainly controlled by mitochondrial solute carriers. PTPC would be composed of voltage-dependent anion channel present in the outer membrane, adenine nucleotide translocase in the inner membrane, and cyclophilin D (CyPD) in the mitochondrial matrix.[5]

Cell apoptosis or survival depends on the balance of pro-apoptotic or anti-apoptotic BCL-2 proteins primarily at the mitochondrial membrane. BCL-2 anti-apoptotic protein is considered as a primary target for anticancer drug development. There are reports for upregulated CyPD in many human cancers that may represent a suppression of apoptosis in cancer cells. It was reported that BCL-2 interacts with CyPD and may be significant for tumor development. A novel function of CyPD is that it

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serves as the main regulator of the mitochondrial permeability transition pore (mPTP). CyPD is proposed to exert an anti-apoptotic effect by binding to BCL-2, a key regulator of apoptosis. A CyPD inhibitor, cyclosporine-A which is a well-known drug, disrupts the CyPD–BCL-2 interaction and enhances the release of cytochrome c from mitochondria, which ultimately leads to apoptosis.[6]

Naturally occurring active compounds have been used for the prevention and treatment of various diseases including cancer. These natural compounds are more beneficial than synthetic compounds due to less toxicity, more availability, and less expensive. Flavonoids and essential oils are a group of natural compounds having antioxidative, anti-inflammatory, and antitumor activity.

Computational and bioinformatics tools became a very useful resource for screening potential therapeutic agent for a particular protein receptor.[7] It has been found that only limited reports of CyPD inhibition with natural active compound are available.[8,9] Therefore, the present study aims to determine the possible interaction and binding-free energy of natural active compounds with CyPD protein using molecular docking approach.

MATERIALS AND METHODS

Preparation of protein and active site identification

The protein selected for the present study is human CyPD (protein data bank [PDB] ID: 4ZSC) whose three-dimensional (3D) structure was obtained from PDB with crystal resolution 1.5 Å. Water molecules in the crystal were removed and the crystal structure was prepared for docking with the help of prepare protein protocol of Discovery Studio 2.5. Active sites were identified with the help of PDB of Japan.[10] The amino acid residues, namely ARG-97, GLN-105, GLY-114, ARG-124, ALA-143, ASN-144, THR-149, GLN-153, PHE-155, and HIS-168, were found to be present in the active sites of the selected protein. CASTp an online tool was also used to measure active site pockets on 3D protein structures, and the area and volume of active sites were found to be 267 Å^2 and 330.1 Å^3 respectively.[11]

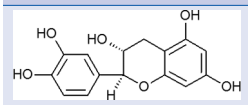
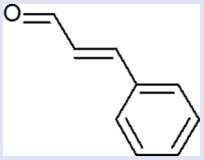
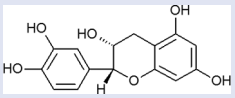
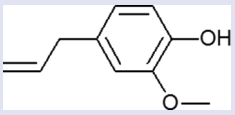
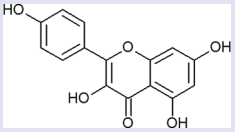
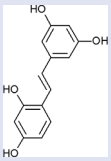
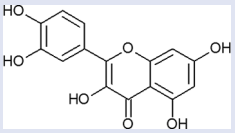
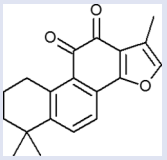
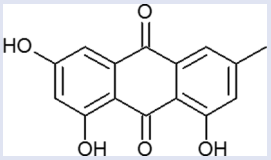
Preparation of ligand

Literature suggested that some of the herbal compounds have anticancer properties; therefore, the present study plans to use these compounds as ligands for the molecular interaction study. Ligands selected for the present study, namely catechin, cinnamaldehyde, epicatechin, eugenol, oxyresveratrol, quercetin, tanshinone 2a, kaempferol, and emodin, were retrieved from PubChem database (www.pubmed.com) as SDF format. Further, compounds were prepared for docking study with the help of "prepare ligand" protocol of Discovery Studio. The 2D structure of the selected ligands is shown in Table 1.

Molecular docking protocol with Discovery Studio

Molecular docking was performed with the help of CDOCKER program of Discovery Studio. It allowed full ligand flexibility to the ligands which help in the generation of ligand conformation. The crystal structure was prepared from the "prepare protein" protocol of Discovery Studio with a default parameter. The selected phytoconstituents used as ligands were prepared in Discovery Studio with the help of "prepare ligand" protocol. Further, docking was performed using the CDOCKER protocol which is a grid-based molecular docking method that uses CHARMm force filed. The default parameters used for the docking study were top hits, 10; random conformations, 10; orientations to refine, 10; force field, CHARMm; and use full potential, false. The obtained results are evaluated on the basis of total docking energy, i.e., CDOCKER energy.

Table 1: Phytoconstituents used as ligand

Structure	PubChem CID number	Name
	CID 73160	Catechin
	CID 637511	Cinnamaldehyde
	CID 72276	Epicatechin
	CID 3314	Eugenol
	CID 5280863	Kaempferol
	CID 5281717	Oxyresveratrol
	CID 5280343	Quercetin
	CID 164676	Tanshinone 2A
	CID 3220	Emodin

Molecular docking protocol Autodock 4.0

Further, docking study was also performed with the help of Autodock to validate our results obtained from Discovery Studio 2.5. The Lamarckian genetic algorithm was used to identify the binding modes and confirmation of ligands in the active site of the protein. Autodock tools were used to prepare the crystal structure of protein.[12] Heteroatoms and water molecules were removed from the crystal structure of the protein, and polar hydrogen and charges were added to the macromolecule during the protein preparation. Ligands torsion was made rotatable to perform flexible docking. Grid maps were set around the active site residues of the receptor using AutoGrid with a grid-point spacing of 0.375 Å and grid box measuring 51 × 51 × 55 Å. Docking studies were performed using a Lamarckian genetic algorithm with a population size of 150 in combination with grid-based energy evaluation method

for the calculation of grid maps. Docking parameters such as mutation rate, crossover rate, and population size were varied to perform docking studies. The best docked conformations were obtained with a mutation rate of 0.02, population size of 150, and crossover rate of 0.8. The results obtained were clustered with a root mean square tolerance of 1.0 Å to obtain final docking results.

RESULTS AND DISCUSSION

Cancer possesses a noteworthy health concern worldwide. Standard treatment strategy for cancer depends on the stage of the disease; more advanced carcinomas often require multimodality therapy with surgery, radiation, and chemotherapy, which can result in very high morbidity. Treatment modalities of cancer have numerous side effects; therefore, development of new treatment modalities such as herbal medicine is crucial for prevention and to reduce mortality.[13]

Docking studies reveal the binding mode of the selected phytocompounds with the human CyPD protein and give an insight to the amino acid residues involved in the binding process. The details of the binding residues involved in the binding process, hydrophobic interaction pattern, their bond energy and their 2D binding forces are shown in Figure 1(a-i) respectively are shown in Table 2. Emodin is docked and used as a standard/reference drug in this study and its binding energy (B.E.) was found to be −28.96 kcal/mol. Recently, Zhang *et al.* suggested that emodin is a potent inhibitor of CyPD through their *in vitro* study on HepG2 cells (liver cancer cell line) and also revealed that three hydrogen bonds exist in between CyPD–emodin complex through molecule docking.[9] The docking results of all the selected phytoconstituents were compared with the emodin. Compounds namely epicatechin, quercetin, and kaempferol were found to show higher B.E. −30.62, −51.51, and −34.79 kcal/mol, respectively, as compared to emodin. The selected phytoconstituents were also docked using Autodock to perform cross-validation of the results. Docking results with Autodock also show that epicatechin, quercetin, and kaempferol show higher B.E. as compared to the other phytoconstituents [Table 2].

Docking study with epicatechin

Docking results obtained from the interaction of human CyPD protein with epicatechin reveal that it shows good binding affinity ΔG −30.62 kcal/mol with the receptor. The docking complex of epicatechin

Table 2: Interaction of human cyclophilin-D proteins with selected phytoconstituents

Ligand	Discovery studio					Autodock	
	Binding energy (kcal/mole)	Hydrogen bond numbers	Hydrogen bonding	Bond length (Å)	Hydrophobic interaction residues	Binding energy (kcal/mole)	Hydrogen bonding residue
Catechin	−28.87	3	HIS-168 ASN-144 GLY-114	2.67 2.48 2.11	PHE-102, LEU-164, PHE-155, MET-103, GLN-105, THR-115, GLN-153GLY-116	−5.95	GLY-114
Cinnamaldehyde	−18.30	2	SER-152 GLN-153	2.24 2.02	THR-149, ASN-150, ALA-143, ASN-144, GLN-105, GLY-114, THR-115, GLY-116, ARG-124	−4.78	GLN-105
Epicatechin	−30.62	4	GLY-114 THR-115 ARG-124 ALA-143	1.96 2.40 2.47 2.25	HIS-96, GLY-116, THR-149, SER-152, GLY-151, ASN-150, MET-142, ASN-144	−5.40	THR-149
Eugenol	−14.28	1	ARG-124	2.58	THR-149, GLY-151, SER-152, ASN-150, GLY-116, GLN-153, HIS-96, GLN-105, GLY-114, THR-115	−4.55	GLY-114, GLN-105
Oxyresveratrol	−25.64	4	THR-115 GLN-153 ALA-143 ASN-144	1.93 3.05 2.17 1.87	GLY-114, ALA-145, ARG-124, GLY-116, SER-152, GLY-151, THR-149, ASN-150, MET-142, HIS-168	−4.88	GLY-114, THR-149
Quercetin	−51.51	2	GLN-153 ASN-144	2.61 3.06	GLN-105, SER-152, GLY-151, GLY-116, THR-115, GLY-114	−6.4	GLN-153
Tanshinone 2a	−5.89	1	GLN-105	2.44	ARG-97, GLN-153, ASN-144, GLY-151, GLY-116, THR-149, ARG-124, THR-115, GLY-114	−5.18	GLN-105
Kaempferol	−34.79	1	GLY-116	2.29	GLY-114, THR-115, ARG-124, GLN-153, GLN-105	−5.83	GLY-114
Emodin	−28.96	2	GLN-105 ALA-143 ARG-124	2.40 2.30 2.41	ARG-97, GLY-114, THR-115, THR-149, GLY-151, ASN-150, SER-152, MET-142, GLN-153	−5.31	ASN-144

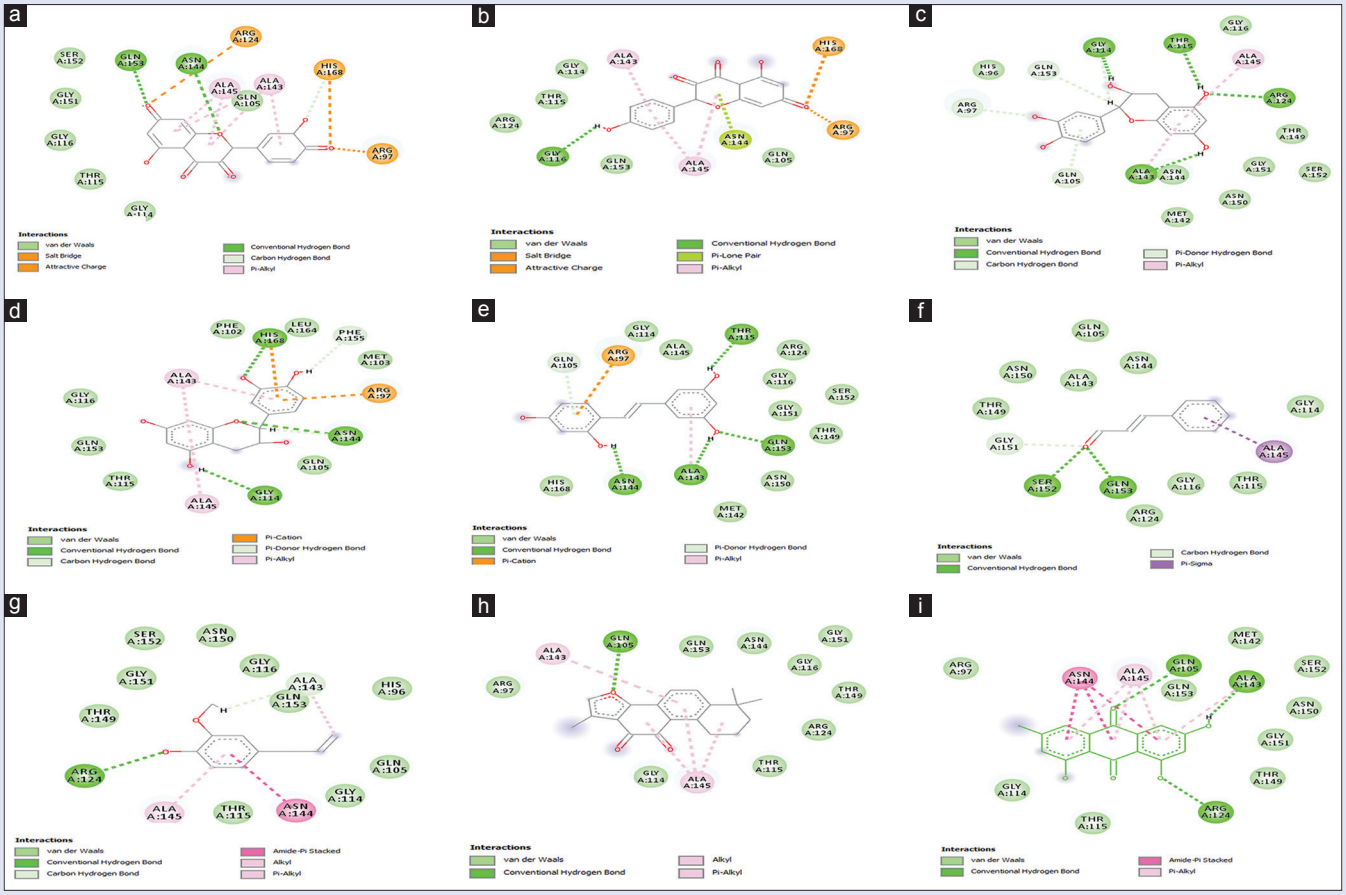


Figure 1: T wo-dimensional interaction map of selected phyoconstituents with human cyclophilin D protein: (a) interaction of catechin with target protein, (b) interaction of cinnamaldehyde with target protein, (c) interaction of epicatechin with target protein, (d) interaction of eugenol with target protein, (e) interaction of kaempferol with target protein, (f) interaction of oxyresveratrol with target protein, (g) interaction of quercetin with target protein, (h) interaction of tanshinone 2a with target protein, (i) interaction of emodin with target protein

and human CyPD and their corresponding interacting residues are shown in Figure 1c. The amino acid residues GLY-114, THR-115, ARG-124, and ALA-143 showed that interaction with the phenolic oxygen of epicatechin and the bond length of this interaction was found to be 1.96, 2.40, 2.47, and 2.25 Å, respectively. From the docking complex, it showed that amino acid residues, namely HIS-96, GLY-116, THR-149, SER-52, GLY-151, ASN-150, MET-142, and ASN-144, were found to be involved with Van der Waals interaction [Figure 1c]. Docking results from Autodock show that epicatechin interacts with the active site residue THR-149, and this binding and interaction pattern support the result of Discovery Studio docking results. B.E. of this interaction was found to be 5.40 kcal/mol which is greater as compared to the emodin ΔG −5.31 kcal/mol.

Docking study with quercetin

Docking result of quercetin with human CyPD protein reveals that it also shows good binding affinity ΔG −51.51 kcal/mol with the receptor. The dock complex and the residues involved in the binding process are shown in Figure 1g. The amino acid residues GLN-153 and ASN-144 showed interaction with the phenolic oxygen of quercetin; bond lengths of this interaction were found to be 2.61 and 3.06 Å, respectively. Docking of quercetin with human CyPD showed that amino acid residues, namely GLN-105, SER-152, GLY-151, GLY-116, THR-115, and GLY-114, were found to be involved with Van der Waals interaction [Figure 1g]. For comparative study, we also performed docking from Autodock, the results obtained shows that quercetin interacts with the active site residue GLN-153, and B.E. of this interaction was found to be 6.4 kcal/mol.

Docking study with kaempferol

The dock complex of kaempferol with human CyPD protein and their interacting residues during the binding process is shown in Figure 1e. The B.E. obtained after the binding process ΔG −34.79 kcal/mol reveals that kaempferol also shows a good binding affinity for the receptor as compared to standard. In the binding of kaempferol with human CyPD amino acid residues, GLY-116 shows interaction with the phenolic oxygen of kaempferol with the bond length of 2.29 Å. In the docking process, Van der Waals interactions are also formed and the amino acid residues involved in this interaction are GLY-114, THR-115, ARG-124, GLN-153, and GLN-105 [Figure 1e]. Docking study of kaempferol with Autodock also shows that it interacts with the GLY-114 residue of the active site and B.E. of this interaction was found to be Δ −5.83 kcal/mol.

Mitochondrial CyPD has appeared as a vital target in cancer chemotherapy because it plays a key role in mPTP, being an integral component of the mPTP. The immunosuppressant CsA is the CyPD inhibitor that has effectively been used for studying the role of CyPD in mitochondria-mediated cell death.[9] CyP-D is proposed to exert an anti-apoptotic effect by binding to BCL-2. The present study revealed that the inhibitors quercetin, kaempferol, and epicatechin have higher

binding potency than emodin; therefore, these drugs may disrupt the CyPD–BCL-2 interaction which may ultimately enhance the apoptotic process.

CONCLUSION

Our finding showed that quercetin, epicatechin, and kaempferol have potential to bind with human CyPD protein, which is a mitochondrial matrix protein and plays a very important role in mitochondrial membrane pore opening. The obtained results reveal that quercetin, epicatechin, and kaempferol have better binding ability than the standard drug emodin against human CypD. Therefore, it is worth mentioning that these phytoconstituents could serve as potential anticancer drugs through mitochondrial dysfunction.

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Conflicts of interest

There are no conflicts of interest.

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