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Original Article

COMPUTATIONAL STUDY ON THE ANGIOTENSIN CONVERTING ENZYME INHIBITORY POTENTIAL OF THE TEA POLYPHENOLS – CATECHINS: RELEVANCE TO CARDIOVASCULAR DISEASES

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ABSTRACT

Objectives: Secondary metabolites obtained from different plants have been the starting material for designing different drugs. Polyphenols are the secondary metabolites that shows myriad varieties of pharmacological activities. Different polyphenols have been found to be cardioprotective and paved the path towards development of cardioprotective formulations. In the present study, we have analyzed the inhibitory potential of tea catechins, a group of polyphenols, on the Angiotensin Converting Enzyme (ACE) - the enzyme responsible for various cardiovascular diseases.

Methods: Binding affinity of catechins against known cardioprotective drug target were calculated by performing the docking experiment using FlexX and IC50 values of the compounds were predicted by QSAR analysis.

Results: ADMET screening revealed that catechins were non-toxic and obeyed Lipinski's rule. The docking studies showed greater affinity of the catechins towards the active site of drug target. QSAR analysis revealed that the catechins have significant IC 50 values.

Conclusion: The study suggests that catechins may be Angiotensin Converting Enzyme targeted potent new drug for treating Cardiovascular diseases.

Keywords: Polyphenols, Catechins, ACE, Cardioprotective, IC50.

INTRODUCTION

Cardiovascular disease (CVD) refers to any disease that affects the cardiovascular system, principally cardiac disease, vascular diseases of the brain and kidney, and peripheral arterial disease. Diseases of the cardiovascular system include those that compromise the pumping ability of the heart, cause failure of the valves, or result in narrowing or hardening of the arteries. Injury or failure of the cardiovascular system, especially the heart, also affect the peripheral tissues that depend on the delivery of nutrients and the removal of wastes through the blood vascular system. CVD is a family of diseases that includes hypertension, atherosclerosis, coronary heart disease, and stroke [1]. Angiotensin Converting Enzyme is the prime target for preventing CVD as the enzyme catalyses conversion of Angiotensin I into Angiotensin II [2]. Angiotensin II is a vasoconstrictor that causes blood vessels to constrict thereby causing hypertension [3]. ACE is expressed in small pulmonary arteries normally. However, during diabetes, obesity, hypertension the expression and activities of the enzyme increases in small pulmonary arteries[4]. This led to the development of ACE inhibitors which show significant cardioprotection through decreasing hypertension [5]. This also relieves other hypertension linked ailments like kidney diseases, diabetes etc. [6, 23]. Meanwhile, herbal based secondary metabolites are constantly being screened for drug discovery with respect to ACE inhibition. Tea polyphenols like catechins, have been reported to confer protection in inflammation, diabetes mellitus, cardiovascular diseases etc. [7]. Polyphenols of tea have been reported to be cardioprotective [8]. Catechins obtained from tea have been found to be cardioprotective. We, therefore, thought it prudent that the polyphenols-catechins may inhibit ACE and thus provide cardioprotection. In the present study, we have analyzed the inhibitory potential of tea polyphenols (catechins) on the Angiotensin Converting Enzyme (ACE) - the enzyme responsible for various cardiovascular diseases and compare the activities with known angiotensin converting Enzyme inhibitors.

MATERIALS AND METHODS

The ligands

Four tea catechins were selected for study using available literature [9], the structure of the ligands was drawn using Chemsketch [10], a chemically intelligent drawing interface freeware was used to construct the structure of the ligands. The three dimensional structures of the compounds in PDB formats were generated and converted to SMILES using Open Babel [11] and then converted to. sdf format again using Open Babel. Known ACE inhibitors Benazepril, Captopril, Enalapril, Fosinopril, Imidapril, Lisinopril, Quinapril, Ramipril, Trandolapril and Zofenopril were used as reference [22]. The structures of these inhibitors were obtained from NCBI PubChem Compound (http: //www. ncbi. nlm. nih. gov/pccompound).

ADME/Tox screening

ADMET screening helps in detecting drug likeliness of compounds [12]. ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) screening was done using Mobyle@rpbs server [13]. The compounds were loaded in the server in SMILES format and the following parameters:

Molecular weight: min 200.0 max 600.0, Hydrogen donors: min 0.0 max 6.0, Hydrogen acceptors: min 0.0 max 12.0, Flexible bonds: min 0.0 max 15.0, Rigid bonds: min 0.0 max 50.0, Ring number: min 0.0 max 7.0, Ring size: min 0.0 max 12.0, No. of Carbons: >2, Hetero atoms: >2, Ratio carbon/hetero: min 0.1 max 1.0, Charge number: min 0.0 max 3.0, Total charge: min -2.0 max 2.0, logP: min -2.0 max 6.0, Polar Surface Area: min 0.0 max 150.0

The receptor

The crystal structure of the drug target Angiotensin Converting Enzyme (PDB ID: 1O8A) was obtained from RCSB Protein Data Bank (http: //www. rcsb. org). The protein has one chain (Chain A) of 589 residues determined by X-ray diffraction method at a resolution of 2.00 Å. It was deposited by: Natesh et al., in the year 2002.

Active site identification

The PDB file was loaded into Q-Site Finder to identify the active site amino acids at default parameter setting [14].

Protein – Ligand interaction using Flex X

Docking is a term used for computational schemes that attempt to find the best matching between two molecules: a receptor and ligand. The receptor Angiotensin Converting Enzyme (ACE) was docked with the known ACE inhibitors and Tea Polyphenols (catechins) using a software FlexX [15,16]. The active site amino acids were defined in the target molecule during the target preparation and residues within a radius of 10 Å were included within the binding site. The SDF file of all the compounds was loaded in FlexX as docking library. The output file gave the energy values in Kcal/mol. For each docked molecule, this value was noted down.

Quantitative Structure Activity Relationship (QSAR) studies

The QSAR analysis was performed by taking the known Angiotensin Converting Enzyme inhibitors *viz.* Benazepril, Captopril, Enalapril, Fosinopril, Imidapril, Lisinopril, Quinapril, Ramipril, Trandolapril and Zofenopril.

The QSAR descriptors *viz.* Polarizability, Molar Refractivity, Molar volume, Molecular weight and LogP were generated for each of the molecule using ACD ChemSketch softwares [17]. The activities have been calculated by taking the inverse logarithm of IC50 values. The descriptors were tabulated in a MS Excel Sheet against their bioactivities (log IC_{50}^{-1}). The descriptors and activities were loaded in Easy QSAR software for multiple linear regression analysis. From the regression, the QSAR equation was generated and the activities of tea polyphenols were predicted [18].

RESULTS

Angiotensin Convertin Enzyme, which is a target for cardiovascular disease was selected based on the literature survey. The structure of Angiotensin Convertin Enzyme was obtained in PDB format. The active site characterization of the enzyme using Q-site finder showed that HIS383, GLU403, HIS387, GLU411, HIS353,ARG522, VAL518, MET223, GLN281, GLY404, ALA356, LYS511, GLU162, ASP377 and ASP415 are the key amino acids forming active site.

For any molecule to become a drug it should not have any toxic or allergenic effects and it should possess all the ADME/Tox properties. The ADME/Tox screening of the compounds has not shown any negative results, which indicates the potentiality of these molecules to become drugs. The results of the ADME/Tox screening is described in table 1**.**

Table 1*:* Molecular weight, Hydrogen donors, Hydrogen acceptors, Flexible bonds, Rigid bonds, Ring number, Ring size, Carbon number, Non carbon number, Ratio carbon/non carbon, Charge number, Total charge, logP and Polar Surface Area of the compounds obeying to Lipinski's rule of drug likeness

Parameters	**MW**	**Drs**	**Ars**	**FB**	**RB**	**#R**	**RL**	**C**	**nC**	**C/nC**	**#Chrg**	**Chrg**	**LogP**	**PSA**
Parameter standards	**200-400**	**0-6**	**0-12**	**0-15**	**0-50**	**0-7**	**0-12**	**>5**	**>2**	**0.1-1.0**	**0-3**	**(-2)-2**	**(-2)-6**	**0-150**
Epicatechin	290.2	5	6	1	17	3	6	15	7	0.47	0	0	1.73	110.38
Epicatechin -3- gallate	442.2	7	10	4	24	4	6	22	11	0.5	0	0	3.61	177.14
Epigallocatechin	306.2	6	7	1	17	3	6	15	7	0.47	0	0	1.54	130.61
Epigallocatechin -3- gallate	458.2	8	11	4	24	4	6	22	11	0.5	0	0	3.42	197.37

Interaction energies between ligand and receptor play the most crucial role in drug designing. In this work, Angiotensin Convertin Enzyme (PDB ID: 1O8A) was selected as drug target and the interactions of the compounds were studied using FlexX.

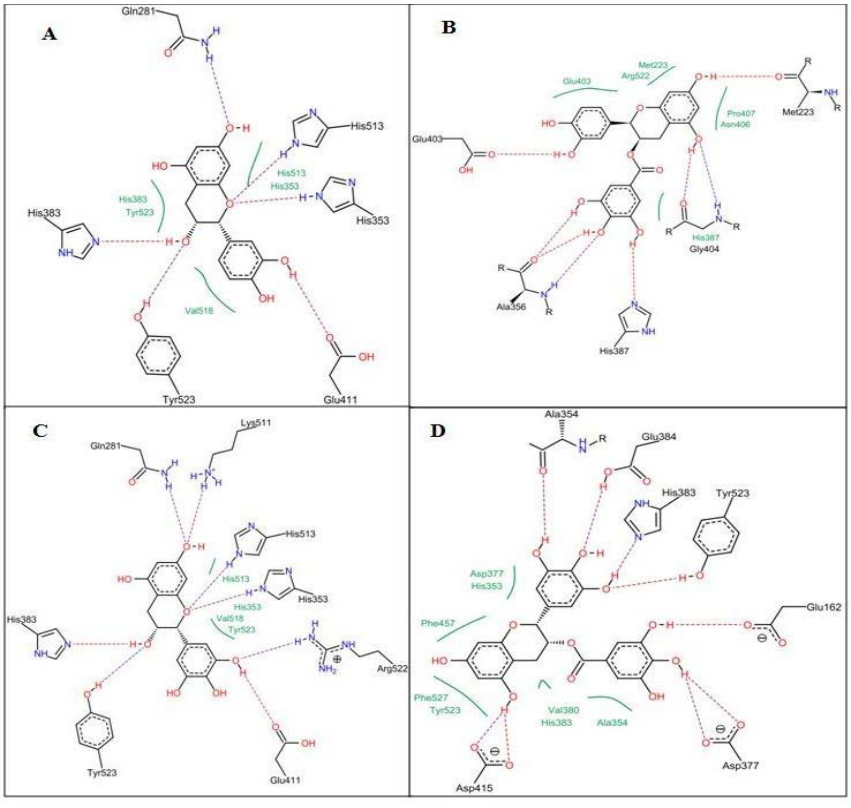
Table 2:Docking results of Catechins with Angiotensin Convertin Enzyme

Compounds	**Total Score (Kcal/mol)**	**Hydrogen Bond Properties**	
		Hydrogen Bonds	**Bond Energy (Kcal/mol)**
Epicatechin (EC)	-23.8671	GLU 411 - H 27	-4.7
		HIS 383 - H 25	-4.7
		GLU 411 - H27	-4.7
		HIS 513 - O 33	-4.1
		HIS 353 - O 33	-4.0
		GLN 281 - O 35	-2.9
Epicatechin -3- gallate (ECG)	-29.1247	GLU 403 - H40	-4.7
		ALA 356 - H30	-4.7
		ALA 356 - H26	-4.7
		HIS 387 - H 37	-4.1
		GLY 404 - H 27	-4.0
		MET 223 - H 23	-4.6
		ALA 356 - O 46	-2.1
Epigallocatechin (EGC)	-21.9804	GLN 281 - O35	-2.3
		HIS 353 -O 36	-3.7
		HIS 383 -H 25	-4.7
		GLU 411 - H 27	-4.5
		LYS 511 - O 35	-2.1
Epigallocatechin-3-gallate (EGCG)	-27.7697	GLU 162 - H 30	-4.3
		ALA 354 - H 40	-2.9
		ASP 377 - H 26	-3.7
		ASP 377 - H 26	-3.9
		HIS 383 - H 38	-3.1
		GLU 384 - O 44	-3.1
		ASP 415 - H 27	-3.3
		ASP 415 - H 27	-4.7
		TYR 523 - O 51	-4.5

Table 3: Docking result of known Angiotensin Converting Enzyme inhibitors

Compounds	**Total Score (Kcal/mol)**	**Hydrogen Bond Properties**	
		Hydrogen Bonds	**Bond Energy (Kcal/mol)**
Benazepril	-13.1522	GLU 411 - H 38	-4.5

		GLU 411 - O 1	-4.3
		HIS 383 - O 1	-4.7
		HIS 513 - O 4	-4.6
Captopril	-21.8193	HIS 383 -O 2	-4.7
		GLU 411 - O2	-4.7
		TYR 523 - O2	-3.5
		HIS 513 - O3	-2.6
Enalapril	-23.6967	GLU 384 - O4	-3.6
		HIS 383 -O 5	-4.7
		TYR 523 - O5	-4.0
		ARG 522 - O3	-4.0
Imidapril	-13.0579	HIS 383 -O 5	-2.5
		GLU 411 - O5	-4.7
		GLU 384 - O3	-4.7
		TYR 523 - O5	-2.4
Lisinopril	-22.7837	HIS 383 -O 5	-4.7
		GLU 411 - O5	-4.7
		GLU 411 - H59	-4.3
		ARG 522 - O3	-3.0
Perindopril	-15.3663	HIS 383 - O2	-4.7
		GLU 411 - O2	-4.7
		GLU 384 - O3	-4.7
		HIS 513 - O5	-4.5
Quinapril	-17.6071	HIS 383 - O3	-4.7
		GLU 411 - O3	-3.8
		TYR 523 - O3	-2.8
		GLU 384 - O2	-4.2
Ramipril	-14.2360	HIS 383 - O5	-4.7
		GLU 411 - O5	-4.7
		TYR 523 - O5	-3.8
		GLU 384 - O4	-3.5
Trandolapril	-2.9183	TYR 523 - O3	-4.6
		GLU 384 - O2	-4.7
Zofenopril	-14.6572	HIS 383 - S1	-2.9
		GLU 411 - S1	-2.7
		TYR 523 - S1	-3.1
		ALA 356 - O4	-4.0



A- Epicatechin, B - Epigallocatechin, C - Epicatechin-3-gallate, D - Epigallocatechin-3-gallate

Fig. 1: Binding patterns of catechins with Angiotensin Converting Enzyme (ACE)

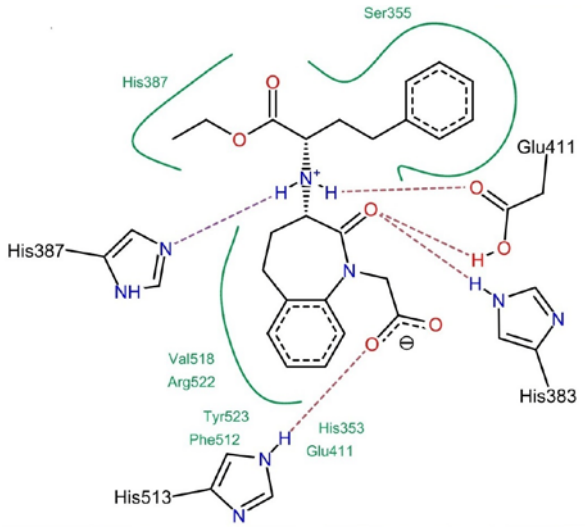


Fig. 2: Binding pattern of known inhibitor (Benazapril)

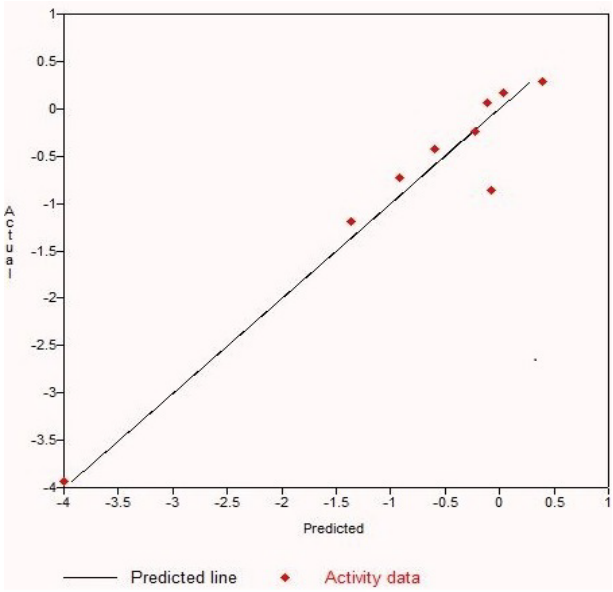


Fig. 3: The multiple regression plots (linear) for ten ACE inhibitors

The QSAR analysis of all the compounds showed significant correlation with R square value of 94.49% (The Rsq value should be definitely high for a good QSAR equation, Higher Rsq means higher fitting of the equation to the given data, Hence better predictions it will provide for new test data). The adjusted Rsq is 85.31 % therefore the difference between Rsq and adjusted Rsq is less (High difference in Rsq and adjusted Rsq indicates weaker overall prediction). The F statistics of the test is 10.29 and the critical F is 3.69 (The F statistics of the test should be greater than Critical F otherwise the generated equation is inefficient) [19].

The equation generated out of QSAR analysis is as follows

Activity = 2.90 -0.168 Molar Volume) + 0.117 (Parachor) + 0.160 (Molar refractivity) + 1.68 (LogP) -0.179 (Molecular Weight).

From the above QSAR equation the IC 50 values of all the four catechins were predicted and the values are 3.890 nM, 0.34 nM, 44.6 nM and 0.21 nM for Epicatechin, Epigallocatechin, Epicatechin-3-gallate and Epigallocatechin-3-gallate respectively.

Comparative analysis ACE inhibitory potential of Catechins and known inhibitors: From the docking score of catechins and the known inhibitors it was found that catechins have much more binding affinity compared to the known ACE inhibitors. The IC 50 values of known ACE inhibitors and the predicted IC 50 of catechins are shown table below.

Table 4: Comparison of IC 50 values of known ACE inhibitors and catechins

Known ACE inhibitors	IC 50 (nM)	Tea Polyphenols (catechins)	Predicted IC 50 (nM)
Benazepril	1.7	Epicatechin	3.890
Captopril	23	Epicatechin -3- gallate	0.34
Enalapril	1.2	Epigallocatechin	44.6
Imidapril	1	Epigallocatechin -3- gallate	0.21
Lisinopril	9900		
Perindopril	1.3		
Quinapril	8.3		
Ramipril	4		
Trandolapril	0.93		
Zofenopril	0.4		

DISCUSSION

While considering better ligands, the least score in docking was preferred as it indicates more stability in binding [15]. The interaction of catechins was screened based on hydrogen bonding based prediction [20] which shows they bind to the active site residues i. e., HIS383, GLU403, HIS387, GLU411, HIS353,ARG522, VAL518, MET223, GLN281, GLY404, ALA356, LYS511, GLU162, ASP377, ASP415 etc. Which was confirmed by the bonded residues in Flex-X.

Activity of the compound in question has been predicted from QSAR model [21] as inverse logarithm of IC50. It showed that the IC50 of catechins was better than the known inhibitors. After choosing catechins as better option on the basis of docking score, IC50 and bonding pattern, cross validation was done by target fishing using Pharm mapper software and found that the target comes in suitable range. This analysis indicates suitability of the chosen ligand for the target in one hand and validates the docking result obtained from Flex X.

Angiotensin Converting Enzyme (ACE) produce Angiotensin II - a very potent chemical that causes hypertension. By decreasing the production of angiotensin II through inhibiting the activity of the enzyme ACE, the function of a failing heart can be improved and thus the chances of hypertension and other CVDs can be reduce. Since, catechins binds to the active sites of the enzyme ACE and forms stable bonds therefore. Catechins may be used as an Angiotensin Converting Enzyme inhibitor. Catechins shows stable bonding pattern in compare to known inhibitors as they shows least score in docking, forms maximum number of hydrogen bonds with the active residues of the enzyme, therefore catechins have more ACE inhibitory potentials.

CONCLUSION

Based on present observation of docking score of both catechins and known inhibitors, IC50 value of known inhibitors and predicted IC50 of the catechins we suggest that catechins may be Angiotensin Converting Enzyme targeted potent new drug for treating

cardiovascular diseases. However, further studies are required to validate the same *in vivo* or *in vitro*.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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