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Research Article

Angiotensin Converting Enzyme inhibitory potential of Echinosystic acid relevance to Cardiovascular diseases: An in silico study

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ABSTRACT

Plants have been used in treating cardiovascular diseases (CVD) and are recognized for their ability to produce secondary metabolites. Secondary metabolites obtained from different plants have been the starting material for designing different drugs. Echinosystic acid, a pentacyclic triterpene was isolated and identified from the fruits of Gleditsia sinensis Lam. Echinosystic acid has significant cardioprotective effects. Different phytochemicals 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 Echinosystic acid, on the Angiotensin Converting Enzyme (ACE) - the enzyme responsible for various cardiovascular diseases.

The study revealed that Echinosystic acid have high ACE inhibiting potential as compared to other known ACE inhibitors.

Keywords: Phytochemicals, Echinosystic acid, ACE, Cardioprotective, Triterpene

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 affects 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 [4]. 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, 6 &7]. This also relieves other hypertension linked ailments like kidney diseases, diabetes etc. [8].

Meanwhile, herbal based secondary metabolites are constantly being screened for drug discovery with respect to ACE inhibition.

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Echinosystic acid has been reported to be cardioprotective [9]. We, therefore, thought it prudent that Echinosystic acid may inhibit ACE and thus provide cardioprotection. We validate the hypothesis using computational tools.

MATERIALS AND METHODOLOGY

The Ligands

Echinosystic acid was selected for study using available literature, the structure of the ligand was drawn using Chemsketch [10], a chemically intelligent drawing interface freeware was used to construct the structure of the ligands. The three dimensional structure of the compound in PDB formats was generated and converted to SMILES using OpenBabel [11] and then converted to .sdf format again using OpenBabel. Known ACE inhibitors Benazepril, Captopril, Enalapril, Fosinopril, Imidapril, Lisinopril, Quinapril, Ramipril, Trandolapril and Zofenopril [12] were used as reference. 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 rug likeliness of compounds [13]. ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) screening was done using Mobyle@rpbs server [14]. The compounds were loaded in the server in SMILES format using 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 [15].

Protein – Ligand interaction using FlexX

Docking is a term used for computational schemes that attempt to find the best matching between two molecules: a receptor and ligand [16]. The receptor Angiotensin Converting Enzyme (ACE) was docked with the known ACE inhibitors and Echinosystic acid using software FlexX [17]. 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 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.

RESULTS

ADMET screening revealed that Echinosystic acid was non-toxic and obeyed Lipinski's rule (Table:1).

The docked ligand-target complexes were analyzed carefully to identify the interactions. The docking score (Table2 and Table:3) was noted down and docking poses were saved for reference (Figure 1).

Comparative analysis ACE inhibitory potential of Echinosystic acid and known inhibitors: From the docking score of Echinosystic acid and the known inhibitors it was found that

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Echinosystic acid have much more binding affinity compared to the known ACE inhibitors.

DISCUSSION

While considering better ligands, the least score in docking was preferred as it indicates more stability in binding [18]. The interaction of Echinosystic acid was screened based on hydrogen bonding based prediction [19] which shows they binds to the active site residues i.e., ASP377, HIS 383, GLU384, HIS387, GLU162, TYR523, VAL518 etc. which was confirmed by the bonded residues in Flex-X.

After choosing Echinosystic acid as better option on the basis of docking score 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 validate the docking result obtained from Flex X.

Angiotensin Converting Enzyme (ACE) produce Angiotensin II - a very potent chemical that causes hypertension [20]. By decreasing the production of angiotensin II through inhibiting the activity of the enzyme ACE, the function of a failing heart can be improve and thus the chances of hypertension and other CVDs can be reduce. Since, Echinosystic acid binds to the active sites of the enzyme ACE and forms stable bonds therefore; Echinosystic acid may be used as Angiotensin Converting Enzyme inhibitor. Echinosystic acid shows stable bonding pattern in compare to known inhibitors as it shows least score in docking, forms maximum number of hydrogen bonds with the active residues of the enzyme, therefore Echinosystic acid have more ACE inhibitory potentials.

CONCLUSION

Based on present observation of docking score of both Echinosystic acid and known inhibitors, we suggests that Echinosystic acid 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.

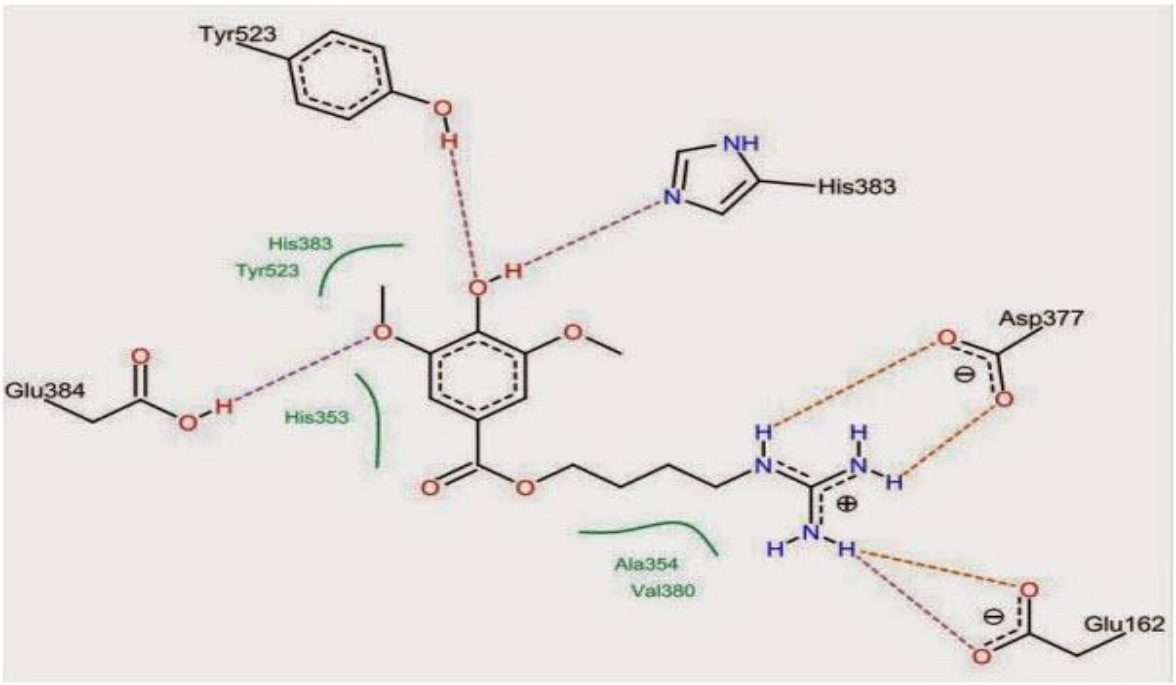

Figure 1: Binding patterns of Echinosystic acid with Angiotensin Converting Enzyme

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Table legends:

Table-1: ADMET Properties of Echinosystic acid

Parameters	MW	Drs	Ars	FB	RB	#R	RL	C	Hetero atoms (H)	C/H	#Chrg	Chrg	LogP	PSA
Parameter standards	200-500	0-6	0-12	0-15	0-50	0-7	0-12	5-12	>2	0.1-1.0	0-3	(-2)-2	(-2)-6	0-150
Echinosystic acid	311.3	5	8	9	8	1	6	14	8	0.21	1	1	0.53	128.63

MW : Molecular weight, Drs : Donors, Ars : Acceptors, FB : flexible bonds, RB : Rigid Bonds, #R : Ring Number, RL : Ring Length, C : carbons, nC : non carbons, C/nC : ratio non carbons/carbons, #Chrg : number of charges, Chrg: Total Charge, LogP : logP (octanol / water), PSA: Polar surface area.

Table-2: Docking of Echinosystic acid with Angiotensin Converting Enzyme

Compound	Total Score (Kcal/mol)	Bond Properties	
		Bonds	Bond Energy (Kcal/mol)
Echinosystic acid	-32.3169	ASP 377 – H43	-8.3
		ASP 377 – H43	-8.3
		GLU 162 – H 40	-4.8
		GLU 162 – H 40	-7.8
		TYR 523 - O 4	-4.5
		HIS 383 - H 33	-2.6

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
		HIS 387 - H 59	-2.2
Captopril	-21.8193	HIS 383 -O 2	-4.7
		GLU 411 - O2	-4.7

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		TYR 523 - O2	-3.5
		HIS 513 - O3	-2.6
		HIS 353 - O3	-4.7
Enalapril	-23.6967	GLU 384 - O4	-3.6
		HIS 383 -O 5	-4.7
		GLU 411 - O5	-4.7
		TYR 523 - O5	-4.0
		GLU 411 - H55	-3.2
		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
		TYR 523 - O5	-2.9
		HIS 387 - O4	-4.7
		GLU 411 - H59	-4.3
		ARG 522 - O3	-3.0
		ALA 356 - H52	-4.1
Perindopril	-15.3663	HIS 383 - O2	-4.7
		GLU 411 - O2	-4.7
		GLU 384 - O3	-4.7
		HIS 513 - O5	-4.5
		ALA 354 - H58	-4.4
Quinapril	-17.6071	HIS 383 - O3	-4.7
		GLU 411 - O3	-3.8
		TYR 523 - O3	-2.8
		GLU 384 - O2	-4.2
		ALA 356 - O5	-2.5
Ramipril	-14.2360	HIS 383 - O5	-4.7
		GLU 411 - O5	-4.7
		TYR 523 - O5	-3.8

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		GLU 384 - O4	-3.5
		HIS 357 - H43	-2.7
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
		ARG 522 - O6	-3.2

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