In Silico docking studies on thrombolytic properties of Homoeopathic Medicine *Crataegus oxycantha* by activation of tissue-Plasminogen Activator in the treatment of Vascular Thrombosis

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ABSTRACT: The main aim of this study is to analyse the effective thrombolytic activity of Homoeopathic Medicine *Crataegus oxyacantha* in the treatment of Vascular Thrombosis. A thrombus may form if a vein or an artery or surrounding tissue is damaged. The thrombi can affect the flow of blood through arteries or veins and if the thrombi gets detached from the endothelium of the blood vessel, it lodges in vital organs such as the brain, heart, and lungs, which pose a serious threat (Lyaker et al., 2013)[13]. Plasminogen activation is a key event in the fibrinolytic system that results in blood clot dissolution and promotes cell migration and tissue remodelling. In this study, a total of 4 phytochemical constituents from Crataegus oxycantha were selected for docking studies. Crataegolic acid, Epicatechin, and Vitexin showed significant binding against the target plasminogen. The present work was carried out on computer-aided molecular modelling and the strength of the ligand was validated using binding energy. The bioactive compounds like Epicatechin and Vitexin exhibited good dock scores and these phytochemical constituents of Crataegus oxycantha may exert promising thrombolytic activity. Further in vivo study and RCT are required to validate the thrombolytic activity of Homoeopathic Medicine Crataegus oxycantha.

KEYWORDS: Crataegus oxycantha, Thrombolytic activity, Plasminogen Activation Loop, Homoeopathy

INTRODUCTION

Thrombosis is the formation of a blood clot within the blood vessel, which obstruct the blood flow through the circulatory system[1]. When a thrombus is detached from the endothelium of the blood vessel, it may result in obstruction of blood flow through arteries or veins and will lodge in the vital organs such as the brain, lungs and heart leading to adverse events like cardiovascular diseases including, pulmonary emboli, venous thromboembolic disorders, coronary artery disease, cerebrovascular accidents, and deep vein thrombosis resulting with sudden mortality and morbidity (Nicolini et al., 1992)[15]. Plasminogen is a 92-kDa protein usually present in blood as an inactive precursor of the serine protease plasmin. Plasminogen is converted to plasmin by cleavage of the Arg561-Val562 peptide bond by tissue-type or urokinase-type plasminogen activator (tPA and uPA, respectively)[10]. Dissolving fibrin which is considered to be the main product of Thrombin activity is the function of Fibrinolytic system. The plasmin, serves as the major protease in utilizing fibrin contained within clots as a substrate for proteolysis and producing soluble products and thus maintaining the patency within the vascular system. The precursor to plasminogen, plasmin, serves as zymogen produced by the liver, which circulates throughout the endovascular network and participates in the fibrinolytic pathway serving as both catalytic enzyme and initiating the process of fibrinolysis, and as a substrate, which once cleaved to become plasmin, continues at an accelerated rate in the process of fibrinolysis[10]. The conversion of the zymogen - plasminogen, into plasmin, is highly regulated and then involves several different circulating factors along with feedback mechanisms from the substrate products. Regulation of the activation of plasminogen functions is to control the homeostasis between fibrin deposition and fibrinolysis, particularly in the setting of hemostasis. This regulation is resultant of the molecular structure of plasminogen, its conformational state, the interactions between plasminogen and fibrin, plasminogen activators, inhibitors of activation and the effects of plasmin during fibrinolysis initiating a positive feedback mechanism[10].

Homoeopathic Medicine *Crataegus oxycantha* is widely used in the treatment of Hypertensive disorders and cardiovascular diseases[4]. The pharmacological properties like antioxidant activity, positive inotropic effect, anti-inflammatory effect, anti-cardiac remodelling effect, antiplatelet aggregation effect, vasodilating effect, endothelial protective effect, reduction of smooth muscle cell migration and proliferation, protective effect against ischemia/reperfusion injury, antiarrhythmic effect, lipid-lowering effect and decrease of arterial blood pressure effect were elicited[22]. Drug-target interaction studies by computational simulations using molecular dynamics and in silico approaches are widely used for the screening of various drugs (Jorgensen, 2004)[11]. In silica molecular analysis predicts the relationship between chemical compounds and proteins. Molecular docking incorporates the algorithms like molecular stimulation, molecular dynamic, and fragment-based analysis. Molecular docking is widely utilized to predict the interactions of two different particles and edict the most suited ligand for various pharmacological actions of the drugs. The main objective of this study is the binding of phyto-components with the core amino acid residue 195 LYS which plays a critical role in the recognition of the residues Arg561-Val562 of plasminogen found similar pose in the mutant form. Thereby, phyto-components which bind with the amino acid 195 LYS may expect to medicate the cleavage of zymogen plasminogen at its Arg561-Val562. Further, these leads may be considered potential thrombolytic agents.

MATERIALS & METHODOLOGY:

Several docking tools were used in recent times to find out the structure-based drug design strategies, one among which is auto dock a componential software tool used to analyze the protein Human Plasminogen Activation Loop Peptide and to study the binding energy properties with the following phytochemical component such as Crataegolic acid, Chlorogenic acid, Epicatechin, Vitexin. The Crystalline structure of the target protein Human Plasminogen Activation Loop Peptide - PDB 4DCB was retrieved from the protein data bank and protein clean-up process was done and essential missing hydrogen atoms were added. Different orientation of the lead molecules concerning the target protein was evaluated by the Autodock program and the best dock pose was selected based on the interaction study analysis.

Ligand Preparation:

The ligands such as Crataegolic acid, Chlorogenic acid, Epicatechin and Vitexin built using Chemsketch and optimized

using Docking server online web tool as shown in Figure 1 and 2 for docking studies by using Geometry optimization method MMFF94[9] and charge calculation was carried out based on Gasteiger method[9] at pH 7 as shown in Table 1.

DOCKING METHODOLOGY:

Docking calculations were carried out for retrieved phyto-components against target protein ACE-2. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (Morris, Goodsell et al., 1998)[14]. Affinity (grid) maps of 0.375 Å spacing were generated using the Autogrid program (Morris, Goodsell et al., 1998)[14]. AutoDock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (Solis and Wets, et al, 1981) [19]. The initial position, orientation, and torsions of the ligand molecules were

set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs and were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the study, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

DOCKING RESULTS:

The result of binding interactions of the ligand with Human Plasminogen Activation Loop Peptide has revealed that out of four compounds docked against PDB 4DCB, Crataegolic acid, Epicatechin and Vitexin has significant amino acid residues on the target Human Plasminogen Activation Loop Peptide. The binding free energy of Crataegolic acid was found to be - 6.06 Kcal/mol and for Chlorogenic acid it was -6.49 followed by this Epicatechin with -4.94, and Vitexin with – 5.36. The docking score with respect to Binding Free energy, Inhibition constant including Total Interaction Surface were listed in Table 2.

Table 1: Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Crataegolic acid	472.7 g/mol	C30H48O4	3	4	1
Chlorogenic acid	354.31 g/mol	C16H18O9	6	9	5
Epicatechin	290.271 g/mol	C15H14O6	5	6	1
Vitexin	432.4 g/mol	C21H20O10	7	10	3

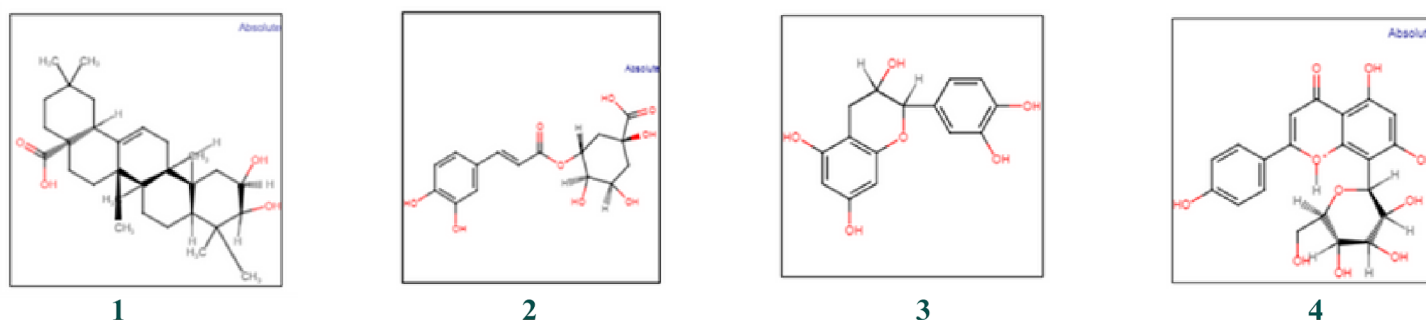*Figure 1: 2D Structure of lead 1. Crataegolic acid 2. Chlorogenic acid 3. Epicatechin 4. Vitexin*

1   2   3   4

Figure 2: 3D Structure of lead 1. Crataegolic acid 2. Chlorogenic acid 3. Epicatechin 4. Vitexin
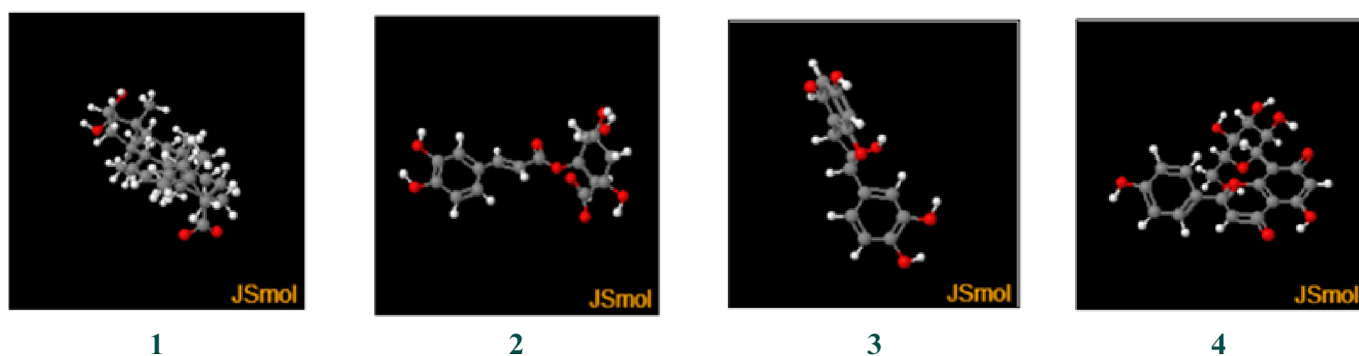
| 1 | 2 | 3 | 4 |

Figure 3: Target protein - 3D- Structure of Human Plasminogen Activation Loop Peptide - PDB 4DCB

Table 2: Summary of the molecular docking studies of compounds against Human Plasminogen Activation Loop Peptide - PDB 4DCB

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki μM (*mM)(**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Crataegolic acid	-6.06 kcal/mol	36.44 uM	-0.04 kcal/mol	-6.86 kcal/mol	622.08
Chlorogenic acid	-6.49 kcal/mol	17.57 uM	-0.42 kcal/mol	-6.29 kcal/mol	544.269
Epicatechin	-4.94 kcal/mol	237.57 uM	-0.44 kcal/mol	-5.08 kcal/mol	486.775
Vitexin	-5.36 kcal/mol	118.01 uM	-0.12 kcal/mol	-4.52 kcal/mol	567.368

Figure 4: Possible ligand binding pockets on the surface of target Human Plasminogen Activation Loop Peptide - PDB 4DCB. Pockets calculated by GHECOM. 1. Crataegolic acid2. Chlorogenic acid 3. Epicatechin 4. Vitexin
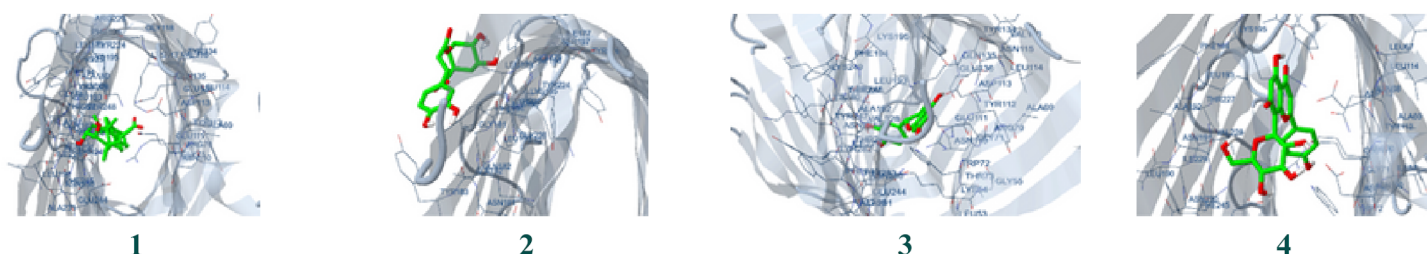
| 1 | 2 | 3 | 4 |

Table 3: Amino acid Residue Interaction of Lead against Human Plasminogen Activation Loop Peptide - PDB 4DCB

Compounds	Interaction	Amino acid Residues														
Crataegolic acid	0	70 ARG	111 GLU	113 ASP	117 LYS	135 GLN	180 ALA	191 ASN	193 LEU	**195 LYS**	224 TYR	228 VAL	230 ASN	244 GLU	246 THR	248 SER
Chlorogenic acid	0	183 TYR	194 PHE	196 PHE	225 TYR											
Epicatechin	1	70 ARG	72 TRP	111 GLU	113 ASP	115 ASN	135 GLN	176 TYR	193 LEU	**195 LYS**	228 VAL	230 ASN	244 GLU	246 THR	248 SER	281 ASN
Vitexin	1	56 ASP	68 ASN	70 ARG	72 TRP	111 GLU	113 ASP	117 LYS	131 THR	176 TYR	191 ASN	193 LEU	**195 LYS**	228 VAL	230 ASN	244 GLU

Based on the results of the In-silico screening analysis it was concluded that Crataegolic acid, Epicatechin and Vitexin bound with active amino acid residue 195 LYS that plays a critical role in the recognition of the residues Arg561-Val562 of target plasminogen.

DISCUSSION:

Docking is a modern scientific approach which involves the prediction of valuable lead towards specific drug target. Docking fundamentally works between the target (enzyme/protein) and lead (drug) interaction. The drug will acts either by antagonistic or agonistic action based on the modern pharmacological principles. These mechanisms of drugs rely on binding of functional properties present in the drug with the biologically active amino acid present in the target protein. Hence drug likeness is the most important property to predict the binding of drug against the receptor.

Identification of active site on to the surface of the target seems to be significant step as this predicts the actual docking score of the molecule. Now a days various online tools are available to predict the drug likeness, ADMET pathway, BBB crossing including structural activity relationship of the potential of the lead for accuracy. The reason for the docking is considerable and important, as it aids in identification of promising lead by involving logical application, active site prediction, and mode of drug action.

The result of binding interactions of the ligand with Human Plasminogen Activation Loop Peptide has revealed that out of four compounds docked against PDB 4DCB, the phytochemical constituents Crataegolic acid, Epicatechin and Vitexin showed significant binding against target plasminogen, which concludes that these compounds may exert promising thrombolytic activity.

CONCLUSION:

The present study revealed that the phytochemical constituents Crataegolic acid, Epicatechin and Vitexin of *Crataegus oxycantha* could be used as effective thrombolytic agents. *Crataegus oxycantha* medicine is widely used in the Homoeopathic system of medicine for various conditions like Haemorrhagic conditions, arteriosclerosis, crustaceous and calcareous deposits in the arteries, and Systemic Hypertension[4]. This study concluded that *Crataegus oxycantha* could be used as effective thrombolytic agents and when this drug is prescribed based on the principles of Homoeopathic system of medicine may exert promising thrombolytic activity and it will be well utilized in the Vascular thrombosis. Further in vivo study and RCT are needed to test the thrombolytic effect of the Homoeopathic Medicine Crataegus Oxycantha for analyzing in the various conditions like cardiovascular diseases including, pulmonary emboli, venous thromboembolic disorders, coronary artery disease, cerebrovascular accidents, and deep vein thrombosis.

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