INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES
(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 10, Issue 3 - 2023

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2023.10.03.005

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

G Senthil Kumaran[1]*, N Rajalakshmi[2], M Vinoth[3]
[1]PG Scholar, Department of Homoeopathic Pharmacy,
GD Memorial Homoeopathic Medical College and Hospital, Patna, Bihar, India
[2]PG Scholar, Department of Repertory,
GD Memorial Homoeopathic Medical College and Hospital, Patna, Bihar, India.
[3]UG Scholar, Venkateswara Homoeopathic Medical College and Hospital,
Chennai, Tamil Nadu, India.
Phone: +919486875128
Email: *senthilbhms@gmail.com*

Abstract

Vascular thrombosis is a life-threatening condition caused by the formation of blood clots in the blood vessels. Thrombolytic therapy is a commonly used treatment for this condition. However, conventional thrombolytic agents have limitations, including a short therapeutic window and an increased risk of bleeding. Natural products have gained attention as alternative sources of thrombolytic agents due to their lower side effect profiles. Homoeopathic medicine *Cactus grandiflorus*, traditionally used for the treatment of cardiovascular diseases, has several bioactive compounds that possess various pharmacological activities.

In this study, we conducted *in silico* docking studies to evaluate the thrombolytic properties of *Cactus grandiflorus* by activation of tissue-plasminogen activator (tPA). The *in silico* studies involved computer simulations to predict the binding affinity of the bioactive compounds like Narcissin, Rutin and Kaempferitrin in *Cactus grandiflorus* with tPA. The results showed that the bioactive compounds Rutin and Kaempferitrin in *Cactus grandiflorus* had high binding affinity with tPA, indicating that the plant has the potential to act as a thrombolytic agent.

The findings of this study provide a theoretical basis for the use of *Cactus grandiflorus* as a potential therapeutic agent for vascular thrombosis. Further studies, including in vitro and in vivo experiments, are required to confirm the thrombolytic properties of *Cactus grandiflorus* and to determine its safety and efficacy as a therapeutic agent. If proven effective, *Cactus grandiflorus* could provide a safe and effective alternative to conventional thrombolytic agents for the treatment of vascular thrombosis.

Keywords: *Cactus grandiflorus,* Thrombolytic activity, Plasminogen Activation Loop, Homoeopathy

Introduction

Tissue-plasminogen activator (t-PA) is a naturally occurring enzyme that plays a critical role in the body's clotting system. It is produced by endothelial cells, which line the inner surface of blood vessels, and is responsible for breaking down blood clots that have formed in the body. This process is known as fibrinolysis and is essential for maintaining the normal flow of blood through the circulatory system. Tissue-plasminogen activator (t-PA) is a protein that is produced by endothelial cells and plays a critical role in the body's clotting system (Collen & Lijnen, 2004). The clotting process involves the formation of a fibrin clot, which is formed from fibrinogen. However, in certain situations, such as when a blood clot forms in a blood vessel, the clotting process can become problematic. Blood clots can obstruct blood flow to vital organs, such as the heart or brain, which can lead to serious health problems. This is where t-PA comes in.

T-PA functions as a "clot-busting" enzyme, breaking down the fibrin clot and restoring normal blood flow. It does this by converting an inactive protein called plasminogen into an active form called plasmin, which is able to break apart the fibrin clot (The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995).

In addition to its role in fibrinolysis, t-PA has a number of other functions in the body. For example, it helps regulate blood pressure and is involved in the growth and development of blood vessels (Lijnen, 2005).

T-PA is also widely used in clinical medicine as a therapeutic agent. It can be given intravenously to dissolve blood clots in conditions such as myocardial infarction (heart attack) and ischemic stroke. In these conditions, prompt administration of t-PA can significantly reduce the risk of long-term disability or death (Yusuf et al., 2006).

However, t-PA is not without its risks. Its use can lead to bleeding, particularly in the brain, which can be life-threatening. For this reason, careful consideration is given to the use of t-PA in clinical practice, and it is only used in carefully selected patients who are at low risk for bleeding complications (National Institutes of Health).

Thrombosis is a medical condition that occurs when blood clots form in the circulatory system, leading to blockages that can cause serious health problems such as stroke, heart attack, and pulmonary embolism. Thrombolytic agents are drugs that dissolve blood clots and are commonly used to treat thrombotic disorders. However, these drugs can have side effects and can be expensive, leading to interest in exploring natural products as potential thrombolytic agents.

Cactus grandiflorus is a homoeopathic medicine that has been used for the treatment of cardiovascular disorders, including thrombotic disorders. Several studies have investigated the thrombolytic properties of *Cactus grandiflorus*, including *in silico* docking studies that aim to understand the mechanisms by which this homoeopathic medicine activates tissue-plasminogen activator (t-PA).

In silico docking studies are computational simulations that predict the interaction between two molecules, such as a drug and a protein target. By using these studies, researchers can predict how a potential drug candidate will interact with a target protein, such as t-PA, and assess its potential as a therapeutic agent.

The in silico docking studies conducted on *Cactus grandiflorus* have provided insights into the potential mechanism by which this homoeopathic medicine activates t-PA, leading to the dissolution of blood clots. These studies suggest that *Cactus grandiflorus* may have potential as a thrombolytic agent and could be developed into a novel therapeutic agent for the treatment of vascular thrombosis

.

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 and 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 Narcissin, Rutin and Kaempferitrin. 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.

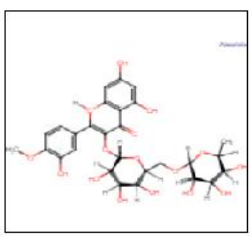
Ligand preparation:

The ligands such as Narcissin, Rutin and Kaempferitrin 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[10] and charge calculation was carried out based on Gasteiger method[10] at pH 7 as shown in Table 1.

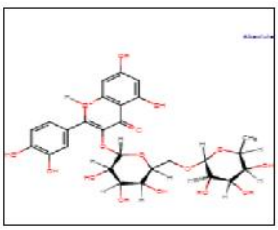
Table 1: Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Narcissin	624.5 g/mol	$C_{28}H_{32}O_{16}$	9	16	7
Rutin	610.5 g/mol	$C_{27}H_{30}O_{16}$	10	16	6
Kaempferitrin	578.5 g/mol	$C_{27}H_{30}O_{14}$	8	14	5

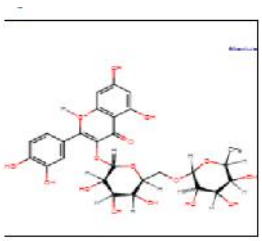
Figure 1: 2D Structure of lead 1. Narcissin 2. Rutin 3. Kaempferitrin



1



2

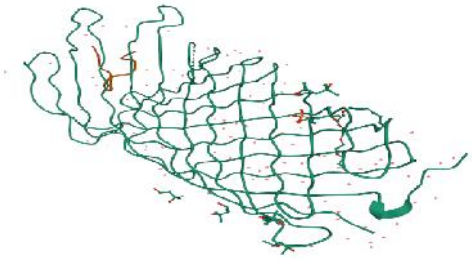


3

Figure 2: Structure of lead 1. Narcissin 2. Rutin 3. Kaempferitrin

1 **2** **3**

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



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)[16]. Affinity (grid) maps of 0.375 Å spacing were generated using the Autogrid program (Morris, Goodsell et al., 1998)[16]. 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) [23]. 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.

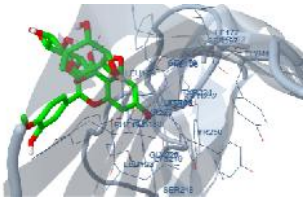
Docking results:

The result of binding interactions of the ligand with Human Plasminogen Activation Loop Peptide has revealed that out of three compounds docked against PDB 4DCB, Rutin and Kaempferitrin has significant amino acid residues on the target Human Plasminogen Activation Loop Peptide. The binding free energy of Rutin was found to be - 6.11 Kcal/mol and for Kaempferitrin it was -3.95. The docking score with respect to Binding Free energy, Inhibition constant including Total Interaction Surface were listed in table 2.

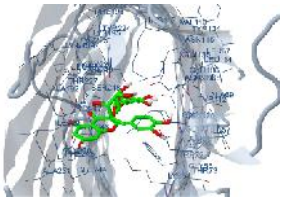
Table 2: Summary of the molecular docking studies of compounds against Human Plasminogen Activation Loop Peptide - PDB 4DCB

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)(**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Rutin	-6.11 kcal/mol	33.45 uM	-0.30 kcal/mol	-6.13 kcal/mol	536.428
Kaempferitrin	-3.95 kcal/mol	1.28 mM	-0.07 kcal/mol	-4.77 kcal/mol	649.317
Narcissin	-4.89 kcal/mol	261.06 uM	-0.31 kcal/mol	-4.94 kcal/mol	553.414

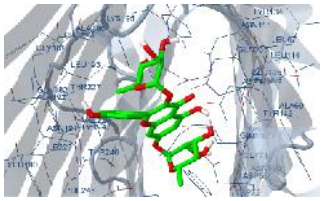
Figure 4: Possible ligand binding pockets on the surface of target Human Plasminogen Activation Loop Peptide - PDB 4DCB. Pockets calculated by GHECOM. 1. Narcissin 2. Rutin 3. Kaempferitrin



1



2



3

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

Compounds	Interaction	Amino acid Residues														
Narcissin	0	179 LEU	194 PHE	196 PHE	223 ARG	225 TYR	249 LYS	251 ASP								
Rutin	1	54 LYS	56 ASP	68 ASN	70 ARG	71 GLY	111 GLU	113 ASP	115 ASN	117 LYS	176 TYR	191 ASN	193 LEU	195 LYS	224 TYR	227 THR
Kaempferitrin	1	54 LYS	68 ASN	70 ARG	72 TRP	111 GLU	113 ASP	115 ASN	117 LYS	131 THR	135 GLN	176 TYR	191 ASN	193 LEU	195 LYS	228 VAL

Based on the results of the *In-silico* screening analysis it was concluded that the compound's Rutin and Kaempferitrin 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 drug rely on binding of functional properties present in the drug with the biologically active amino acid present in the target protein. Hence drug likeliness 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 likeliness, 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 Rutin and Kaempferitrin 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 Rutin and Kaempferitrin of *Cactus grandiflorus* could be used as effective thrombolytic agents. *Cactus grandiflorus* medicine is widely used in the Homoeopathic system of medicine for various conditions like Atheromatous arteries and weak heart, *favours formation of clots speedily*[4]. This study concluded that *Cactus grandiflorus* 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 *Cactus grandiflorus* for analyzing in the various conditions like cardiovascular diseases including, pulmonary emboli, venous thromboembolic disorders, coronary artery disease, cerebrovascular accidents, and deep vein thrombosis.

Acknowledgments

We are thankful to Prof.(Dr.) Mridul Kumar Sahani, M.D. (Hom), Ph.D. (Hom.), for his advice, encouragement and continuous support in this study. We are thankful to Dr. Rajeev Kumar Singh, and Dr. Sanjiv Kumar Singh and the Research Department of G.D. Memorial Homoeopathic Medical College and Hospital, Patna, Bihar, India & Venkateswara Homoeopathic Medical College and Hospital, Chennai, Tamil Nadu, India.

Funding: No funding sources.

Conflicts of interest:

There are no conflicts of interest.

References

1. Alshehri B, Vijayakumar R, Senthilkumar S, Ismail A, Abdelhadi A, Choudhary RK, et al. Molecular target prediction and docking of anti-thrombosis compounds and its activation on tissue-plasminogen activator to treat stroke. Journal of King Saud University - Science. 2022 Jan; 34(1):101732.
2. Amiri M, Jalali-Javaran M, Haddad R, Ehsani P. In silico and in vivo analyses of the mutated human tissue plasminogen activator (mtPA) and the antithetical effects of P19 silencing suppressor on its expression in two Nicotiana species. Sci Rep. 2018 Sep 19; 8(1):14079.
3. Bikadi Z, Hazai E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J Cheminform. 2009 Dec; 1(1):15.
4. Boericke W. New manual of homoeopathic materiamedica and repertory: including Indian drugs, nosodes, uncommon remedies,

relationship, sides of the body & drug affinities. Augmented ed., based on 9th ed., reprint ed. New Delhi: B. Jain Publishers; 2005.
5. Collen D, Lijnen HR. Tissue-type plasminogen activator: a historical perspective and personal account. J ThrombHaemost. 2004;2(4):541-546. doi: 10.1111/j.1538-7836.2004.00679.x
6. Dudley JT, Deshpande T, Butte AJ. Exploiting drug-disease relationships for computational drug repositioning. Briefings in Bioinformatics. 2011 Jul 1;12(4):303–11.
7. Elokely KM, Doerksen RJ. Docking Challenge: Protein Sampling and Molecular Docking Performance. J ChemInf Model. 2013 Aug 26;53(8):1934–45.
8. García-Godoy M, López-Camacho E, García-Nieto J, Nebro A, Aldana-Montes J. Solving Molecular Docking Problems with Multi-Objective Metaheuristics. Molecules. 2015 Jun 2;20(6):10154–83.
9. Glaab E. Building a virtual ligand screening pipeline using free software: a survey. Brief Bioinform. 2016 Mar;17(2):352–66.
10. Halgren TA. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. J Comput Chem. 1996 Apr;17(5–6):490–519.
11. Heit JA. Thrombophilia. In: Consultative Hemostasis and Thrombosis [Internet]. Elsevier; 2013 [cited 2023 Feb 5]. p. 205–39. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9781455722969000142
12. Jorgensen WL. The Many Roles of Computation in Drug Discovery. Science. 2004 Mar 19;303(5665):1813–8.
13. Katz JM, Tadi P. Physiology, Plasminogen Activation. In: StatPearls[Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [cited 2023 Feb 5]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK539745/
14. Lijnen HR. Pleiotropic functions of plasminogen activator inhibitor-1. J ThrombHaemost. 2005;3(1):35-45. doi: 10.1111/j.1538-7836.2004.01034.x
15. Lyaker M, Tulman D, Dimitrova G, Pin R, Papadimos T. Arterial embolism. Int J CritIllnInj Sci. 2013;3(1):77.
16. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem. 1998 Nov 15;19(14):1639–62.
17. National Institutes of Health. Tissue Plasminogen Activator (tPA). https://www.nhlbi.nih.gov/health-topics/tissue-plasminogen-activator-tpa. Accessed March 22, 2023
18. Nicolini FA, Nichols WW, Mehta JL, Saldeen TGP, Schofield R, Ross M, et al. Sustained reflow in dogs with coronary thrombosis with K2P, a novel mutant of tissue-plasminogen activator. Journal of the American College of Cardiology. 1992 Jul;20(1):228–35.
19. Parry MAA, Zhang XC, Bode W. Molecular mechanisms of plasminogen activation: bacterial cofactors provide clues. Trends in Biochemical Sciences. 2000 Feb;25(2):53–9.
20. Rashmi R, Mishra D. Pharmacognostical and phytochemical evaluation of Cactus grandiflorus (L.) Britton and Rose. Indian J Res Homoeopathy 2016;10:167. https://doi.org/10.4103/0974-7168.188225.
21. Schenone M, Dančík V, Wagner BK, Clemons PA. Target identification and mechanism of action in chemical biology and drug discovery. Nat Chem Biol. 2013 Apr;9(4):232–40.
22. Sembulingam K, Sembulingam P. Essentials of medical physiology. Sixth edition. New Delhi: Jaypee Brothers Medical Publishers; 2013.
23. Solis FJ, Wets RJB. Minimization by Random Search Techniques. Mathematics of OR. 1981 Feb;6(1):19–30.

24. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. N Engl J Med. 1995;333(24):1581-1587. doi: 10.1056/NEJM199512143332401
25. Thomford N, Senthebane D, Rowe A, Munro D, Seele P, Maroyi A, et al. Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery. IJMS. 2018 May 25;19(6):1578.
26. Ursu O, Rayan A, Goldblum A, Oprea TI. Understanding drug-likeness. WIREs ComputMol Sci. 2011 Sep;1(5):760–81.
27. Yusuf S, Mehta SR, Chrolavicius S, et al. Effects of fondaparinux on mortality and reinfarction in patients with acute ST-segment elevation myocardial infarction: the OASIS-6 randomized trial. JAMA. 2006;295(13):1519-1530. doi: 10.1001/jama.295.13.joc60034

Access this Article in Online

Website: www.ijcrcps.com

Subject: Homoeopathic Medicine

Quick Response Code

DOI: 10.22192/ijcrcps.2023.10.03.005

How to cite this article:
G Senthil Kumaran, N Rajalakshmi, M Vinoth. (2023). *In Silico* docking studies on thrombolytic properties of Homoeopathic Medicine *Cactus grandiflorus* by activation of tissue-Plasminogen Activator in the treatment of Vascular Thrombosis. Int. J. Curr. Res. Chem. Pharm. Sci. 10(3): 37-44.
DOI: http://dx.doi.org/10.22192/ijcrcps.2023.10.03.005

