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Mechanistic Insight Hypolipidemic Potential of Ascorbic Acid: *In-Silico* Molecular Docking

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ABSTRACT: Ascorbic acid, also known as vitamin C, is a crucial part of a balanced diet. The history of vitamin C is intertwined with that of the human ailment scurvy, which was perhaps the first to be identified as a deficiency condition in humans. Its signs include diarrhoea, overall weakness, severe bleeding of the tissues and gums, and tiredness. Ascorbic acid is a water-soluble chemical molecule essential to numerous biological functions. The exact mechanism of action for the lipid lowering action of ascorbic acid was still not revealed. With intent to propose the most probable mechanism of action of ascorbic acid the docking based computational analysis has been performed against the lipid lowering drug targets like ATP citrate lyase enzyme, lanosterol 14α-demethylase enzyme, squalene synthase enzyme, and Niemann Pick C1 like Protein. The docking analysis, chemical interactions, followed by the physicochemical based pharmacokinetic profiling has revealed that the ascorbic acid is executing its lipid lowering action via inhibiting the squalene synthase enzyme.

Keywords: Ascorbic acid, ATP citrate lyase enzyme, lanosterol 14α-demethylase enzyme, squalene synthase enzyme, and Niemann Pick C1 like Protein & Molecular docking.

RESEARCH PAPER

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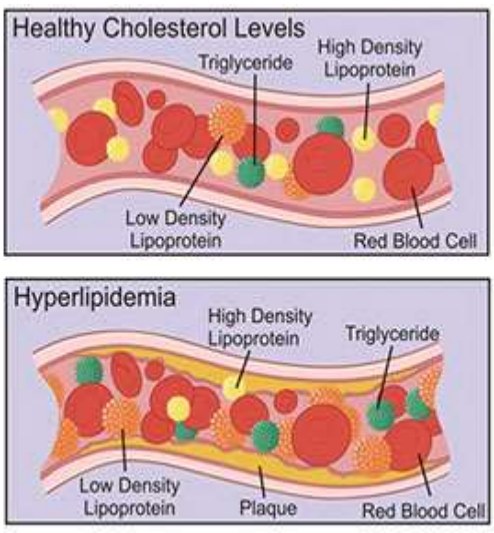
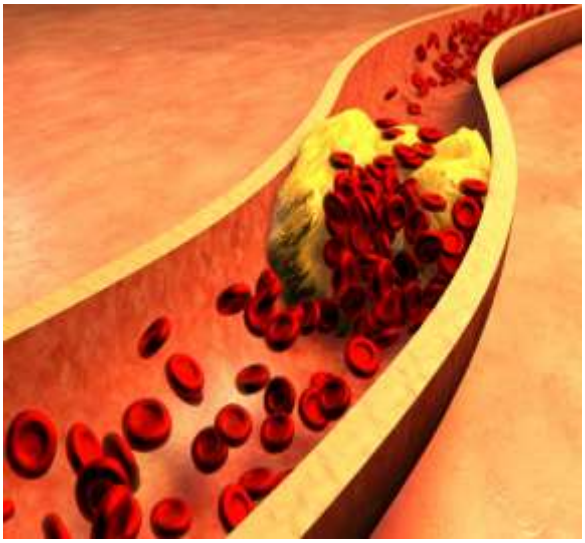
INTRODUCTION

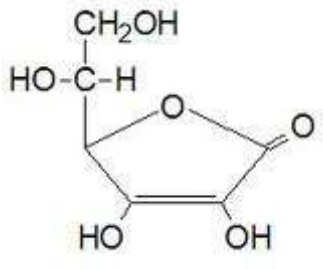
Traditional herbal medicine practitioners have reported how numerous indigenous plants are effective in treating a variety of diseases1. Traditional herbal medicine and synthetic medications are both made from natural ingredients. In some regions of the world, they continue to be the primary healthcare system2. In India, the usage of plants as natural treatments is based on local empirical knowledge of their medical characteristics. Many people in India and around the world agree that consuming plant items can result in positive therapeutic results. For thousands of years, plants have served as the foundation for many traditional medicines around the world, and they continue to offer humans new treatments [1]. Hyperlipidemia is recognized as one of the major risk factors for cardiovascular disease (CVD). Cardiovascular disease accounts for one-third of all deaths worldwide and is projected to be the leading cause of death and disability worldwide by 2020.Hyperlipidemia is an increase in one or more plasma lipids, such as triglycerides, cholesterol, cholesterol esters, phospholipids, or plasma lipoproteins, such as very-low-density lipoprotein or low-density lipoprotein, and high-density lipoprotein is a decline in the level of [2]. Hyperlipidemia [Lipid = Fat Emia = Excess]. Hyperlipidemia is excessive amounts of fatty substances in the blood (aka Hyperlipemia). It is a medical condition characterized by an elevation of any or all lipid profile and/or lipoproteins in the blood. It is also called hypercholesterolemia/hyperlipoproteinemia. Although elevated low density lipoprotein cholesterol (LDL) is thought to be the best indicator of atherosclerosis risk, dyslipidemia (abnormal amount of lipids in the blood) can also describe elevated total cholesterol (TC) or triglycerides (TG), or low levels of high density lipoprotein cholesterol (HDL) [3].





Hyperlipemia

Complications due to hyperlipidemia are Atherosclerosis, Coronary Artery Disease, Stroke, and Heart Attack (which can result in death) [4]. Due to its anti-inflammatory and antioxidant qualities, vitamin C, also known by its chemical names ascorbic acid and ascorbate, is a six-carbon lactone that is an important food ingredient. It serves a significant role as an antioxidant that is a part of the body's defensive mechanism against reactive oxygen species and free radicals, avoiding tissue damage. It also aids in the formation of connective tissues, bones, teeth, and blood vessels. It is frequently utilized to treat a number of illnesses, including scurvy, the common cold, anemia, hemorrhagic disorders, wound healing, and infertility. Ascorbic acid, also known as vitamin C, is a crucial micronutrient that the body needs in order to function normally metabolically. Therefore, a lack of this vitamin leads to scurvy symptoms and eventual death [5]. With as little as 10 mg of vitamin C each day, which is easily attained by eating fresh fruits and vegetables, this potentially fatal condition can be avoided. Although 60 mg of vitamin C per day might delay the onset of scurvy for roughly a month in a diet deficient in vitamin C, the current recommended dietary allowance (RDA) for this vitamin is established at 60 mg per day to offer an adequate margin of safety. A number of enzymes involved in the manufacture of collagen, carnitine, and neurotransmitters require vitamin C as a cofactor [6].



Ascorbic acid

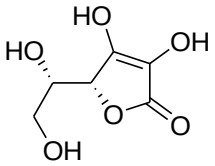
A water-soluble chemical molecule called ascorbic acid is crucial for many biological processes. Ascorbic acid lowers cholesterol; however the precise mechanism of action is still unknown. The docking-based computational research against lipid-lowering pharmacological targets such as ATP citrate lyase enzyme, lanosterol 14-demethylase enzyme, squalene synthase enzyme, and Niemann Pick C1 similar Protein was done with the intention of proposing the most likely mechanism of action of ascorbic acid.

Experimental work

Molecular docking studies

Ligand Preparation:

2D Structure of ligands ascorbic acid was drawn by using ChemDraw [7].The two-dimensional structures of ligand was converted into 3-D structures with optimized 3D geometry by using Chem3D software. The optimized structure was saved in PDB format for AutoDock compatibility [8-10].



Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing between grids points can be adjusted with another thumbwheel, the value in the study taken is given in table 1.[10-14].

Table 1: The grid-coordinates of the grid-box used in the current study

Proteins	x-D	y-D	z-D	Spacing (Å)	x center	y center	z center
7lj9	40	50	56	0.497	93.43	96.924	72.7
6uez	40	40	40	0.392	-29.059	-33.0	15.889
1efz	40	40	40	0.336	-12.596	43.348	32.219
5u74	40	40	40	0.336	48.867	35.064	-21.902

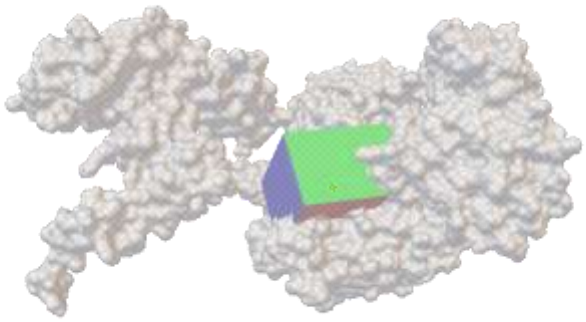


Figure 1: Grid box covering all active sites in ATP citrate lyase enzyme (7lj9)

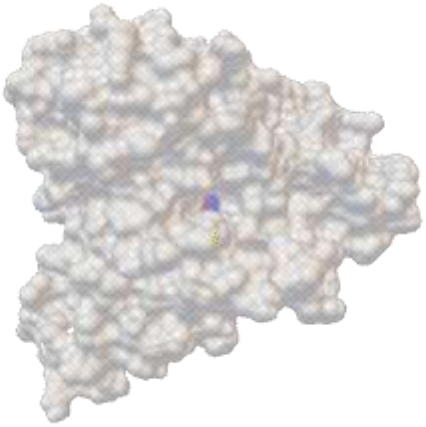


Figure 2: Grid box covering all active sites in lanosterol 14α-demethylaseenzyme (6uez)

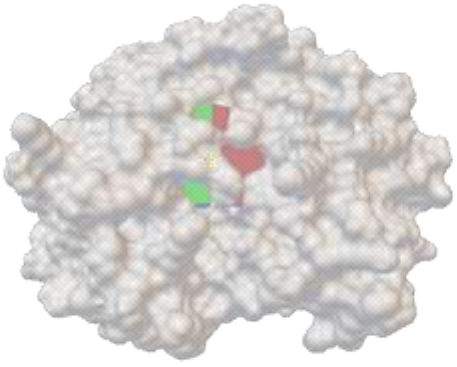


Figure 3: Grid box covering all active sites in squalene synthase enzyme (1efz).

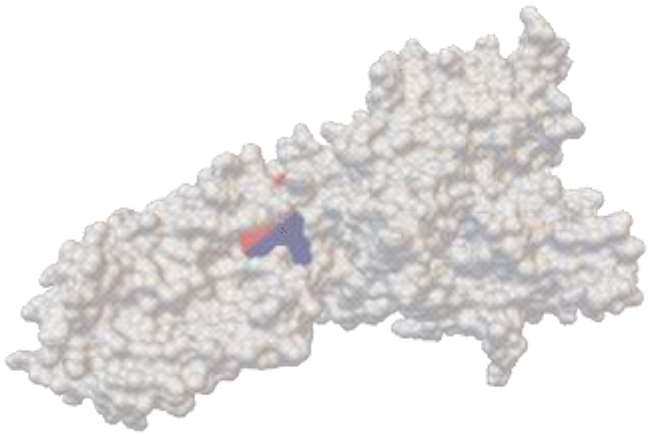


Figure 4: Grid box covering all active sites in Niemann Pick C1 like Protein (5u74)

Preparation of the docking file

All the calculations were carried out by using Autodock4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [15-18].

Macromolecular structure

ATP Citrate Lyase

The crystal structure of the ATP citrate lyase enzyme consisting of macromolecular receptor associated with bound endogenous ligand acetyl CoA is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (7lj9.pdb) registered in the Protein data bank was used [19-24].

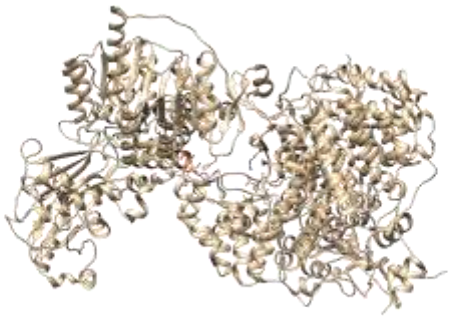


Figure 5: Crystal structure of ATP citrate lyase enzyme with bound ligand acetyl CoA (PDB ID-7lj9)

Lanosterol 14α-demethylase

The crystal structure of the lanosterol 14α-demethylase enzyme consisting of macromolecular receptor associated with bound substrate ligand lanosterol is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (6uez.pdb) registered in the Protein data bank was used [25-28].

Figure 6: Crystal structure of lanosterol 14α-demethylase enzyme with bound substrate ligand lanosterol (PDB ID-6uez)

Squalene Synthase

The crystal structure of the squalene synthase enzyme consisting of macromolecular receptor associated with bound inhibitor ligand is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (1ezf.pdb) registered in the Protein data bank was used [29].

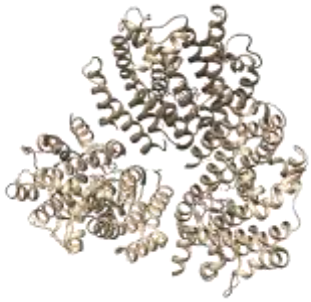


Figure 7: Crystal structure of squalene synthase enzyme with bound inhibitor ligand (PDB ID-1ezf)

Niemann Pick C1 like Protein

The crystal structure of the Niemann Pick C1 like Protein enzyme consisting of macromolecular receptor associated with bound substrate ligand MES is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (5u74.pdb) registered in the Protein data bank was used [30-31].

Figure 8: Crystal structure of Niemann Pick C1 like Protein with bound substrateligand MES (PDB ID-5u74)

Molecular Docking Simulation Studies

Docking of ligand ascorbic acid was performed against ATP citrate lyase enzyme, lanosterol 14α-demethylase enzyme, squalene synthase enzyme, and Niemann Pick C1 like Protein was performed by Autodock to establish its probable mechanism of action for their lipid lowering effect. All the bonds of ligand ascorbic acid were kept flexible, while no residues in receptor were made flexible [31].

Toxicity & ADME-T Studies

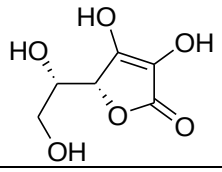
The pharmacokinetics of ligand molecule was studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME-T properties [32].

RESULTS AND DISCUSSION

To date, molecular docking studies have been used recently in predicting the potential inhibitor that has been compared with marketed Anti-hyperlipidemic drugs as which by seeking these natural compounds as new Anti-hyperlipidemic agents with little or no adverse effects for the long term effect. Molecular docking is an important computational method for predicting possible drug-protein interactions with the selected ligand that represent bioactive compound containing in the medicinal plants. Bioinformatics tools, like molecular docking experiments, which involve study and analysis of ligand-receptor interactions, play important role in identifying the molecular targets (receptors) for different ligand. We have periodically reviewed some such novel molecular targets for anti-hyperlipidemic drug research. It was thought worthwhile, to assess the interaction of compound LM-1554, with few such molecular targets through, its *in-silico* docking experiments and gain some insight on its probable mechanism of action. Six such molecular targets related to hyperlipidaemia were selected for this study. These were Niemann Pick C1 like1 protein (NPC1L1), ATP citrate lyase (ACL), C-reactive protein (CRP), lanosterol 14α-demethylase (LDM), squalene synthase (SqS) and farnesiod X-receptor (FXR). The X-ray crystal structures of these targets complexed with their respective co-crystallized native ligands were available from the RSCB-Protein Data Bank (PDB).In the present study ascorbic acid isolated from *S.acuta* was taken for evaluation of antilipidaemic potential by molecular docking technique. Docking study of ascorbic acid with Niemann Pick C1 like1 protein (NPC1L1), ATP citrate lyase (ACL), lanosterol 14α-demethylase (LDM) and squalene synthase was carried out by Auto dock to establish its probable mechanism of action. The grid box cover all active enzyme was tabulated in table1 & fig.1-4, whereas crystal structure of all enzyme shown in fig.5-8.The outcome of docking of ascorbic acid against Niemann Pick C1 like1 protein (NPC1L1), ATP citrate lyase (ACL), lanosterol 14α-demethylase (LDM) and squalene synthase showed the binding energy -3.68,-3.76, -3.637 &- 4.69 respectively (table 2).Molecular interaction of ascorbic acid with selected receptors showed in fig 9-20. Molecular simulation revealed that the ascorbic acid bind strongly with squalene synthase receptor having covalent bond with Ser53, Thr50, Tyr73, Ser53, along with vander

waal's interaction with Arg77, Phe54, Arg52, Met295, Ala55, Pro292 present in receptor. The pharmacokinetic profiling of the ascorbic acid ligand has revealed that it is having good pharmacokinetic profile associated without the presence of major toxic effects like mutagenic, reproductive effects, irritant effect, and tumorogenic properties. The pharmacokinetic and toxicity profiling results of ascorbic acid was shown in figure 21.

Table 2: Results of docking of ATP citrate lyase enzyme, lanosterol 14α-demethylase enzyme, squalene synthase enzyme, and Niemann Pick C1 like Protein

S. No	CompoundName	Structure	Binding Energy			
			ATP citrate lyase (7lj9)	lanosterol 14α-demethylase (6uez)	squalene synthase (1ezf)	Niemann Pick C1 like Protein(5u74)
1	Ascorbic acid		-3.76	-3.63	-4.69	-3.68
	Interacting Residues		Arg576, Asp226, Leu575, Tyr227, Lys230, Ala223	Met381, Met378, Ile379, Met487, Ile377, Ile488, Thr135, Phe234	Ser53, Phe54, Arg52, Thr50, Tyr73, Arg77, Ser51, Met295, Ala55, Pro292	Ser1218, Asp620, Glu688, Phe1221, Tyr1225, Ser617

Interactions

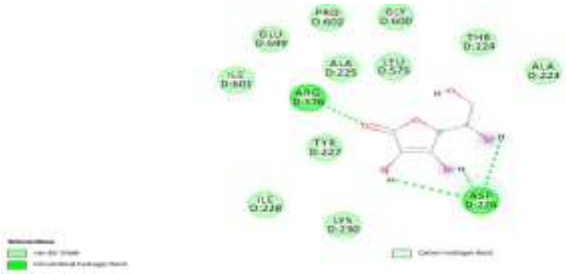


Figure 9: Two-dimensional binding interaction of ascorbic acid with ATP citrate lyase enzyme

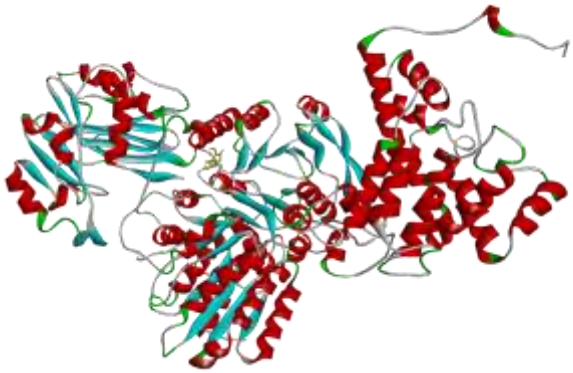


Figure 10: Three-dimensional binding interaction of ascorbic acid with ATP citrate lyase enzyme

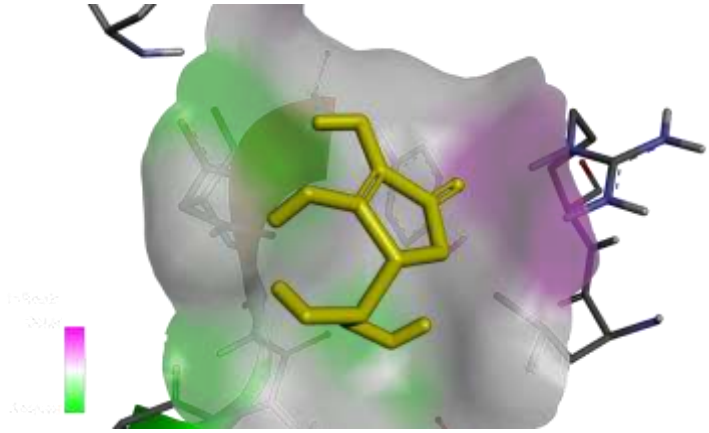


Figure 11: Binding conformation of ligand ascorbic acid with ATP citrate lyase enzyme

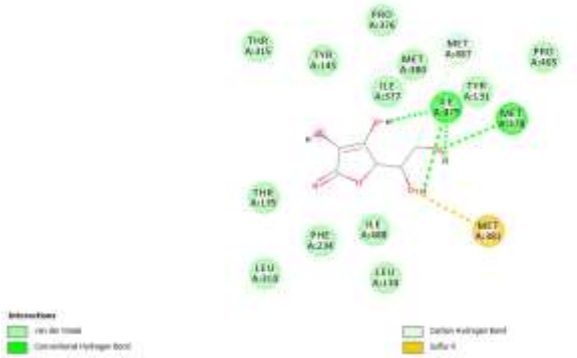


Figure 12: Two-dimensional binding interaction of ascorbic acid with lanosterol 14α-demethylase enzyme

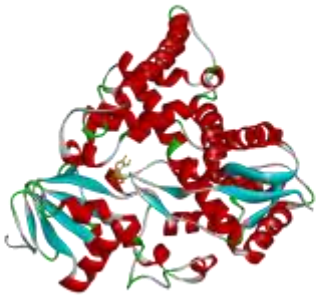


Figure 13: Three-dimensional binding interaction of ascorbic acid with lanosterol 14α-demethylase enzyme

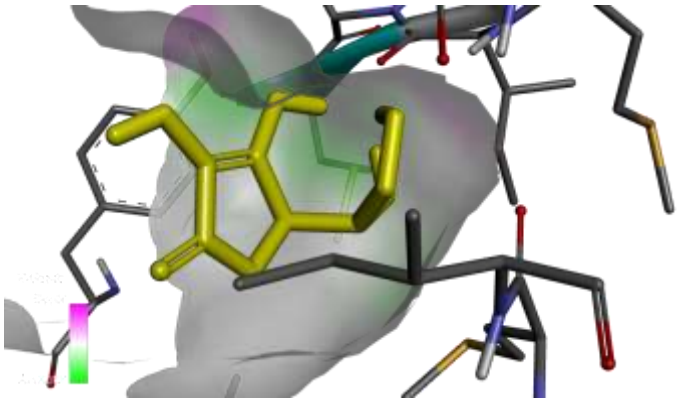


Figure 14: Binding conformation of ligand ascorbic acid with lanosterol 14α-demethylase enzyme

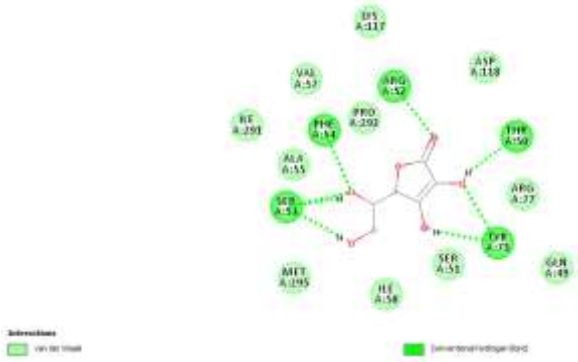


Figure 15: Two-dimensional binding interaction of ascorbic acid with squalene synthase enzyme

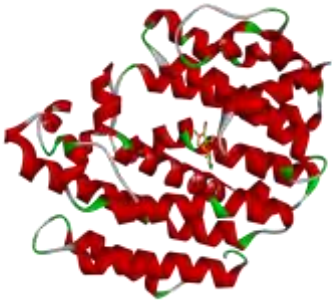


Figure 16: Three-dimensional binding interaction of ascorbic acid with squalene synthase enzyme

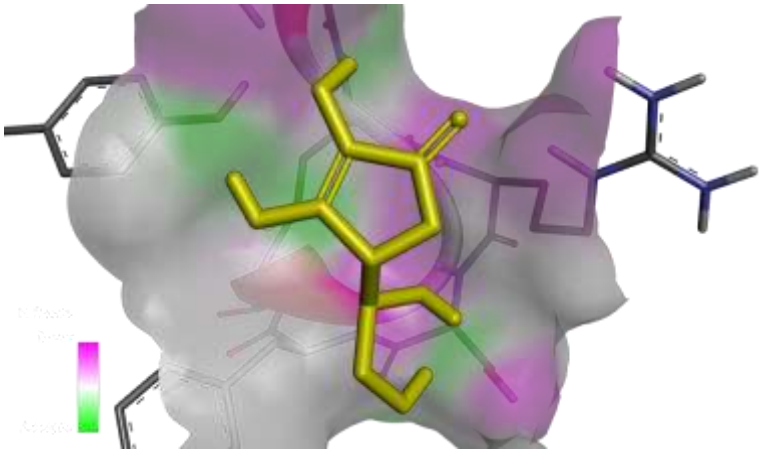


Figure 17: Binding conformation of ligand ascorbic acid with squalene synthase enzyme

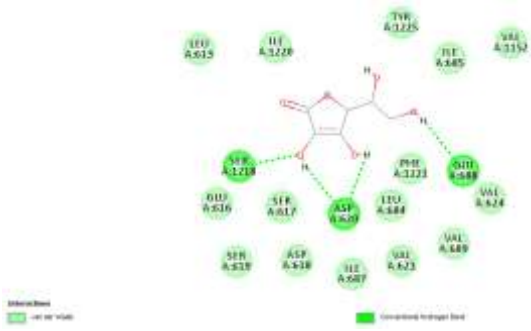


Figure 18: Two-dimensional binding interaction of ascorbic acid with Niemann Pick C1 like Protein

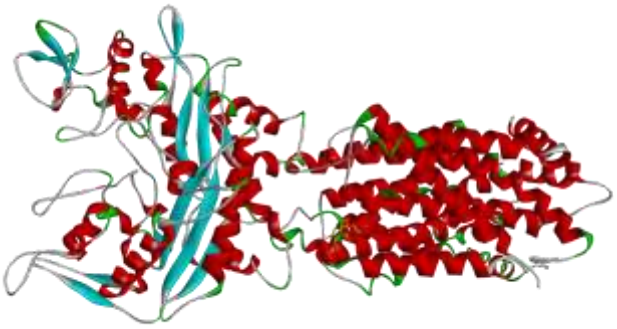


Figure 19: Three-dimensional binding interaction of ascorbic acid with Niemann Pick C1 like Protein

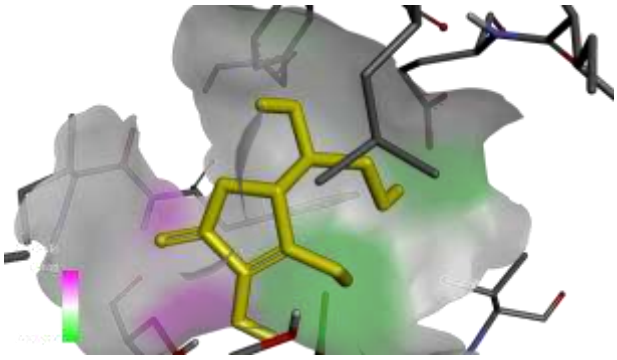


Figure 20: Binding conformation of ligand ascorbic acid with Niemann Pick C1 like Protein

Toxicity & ADME-T Studies

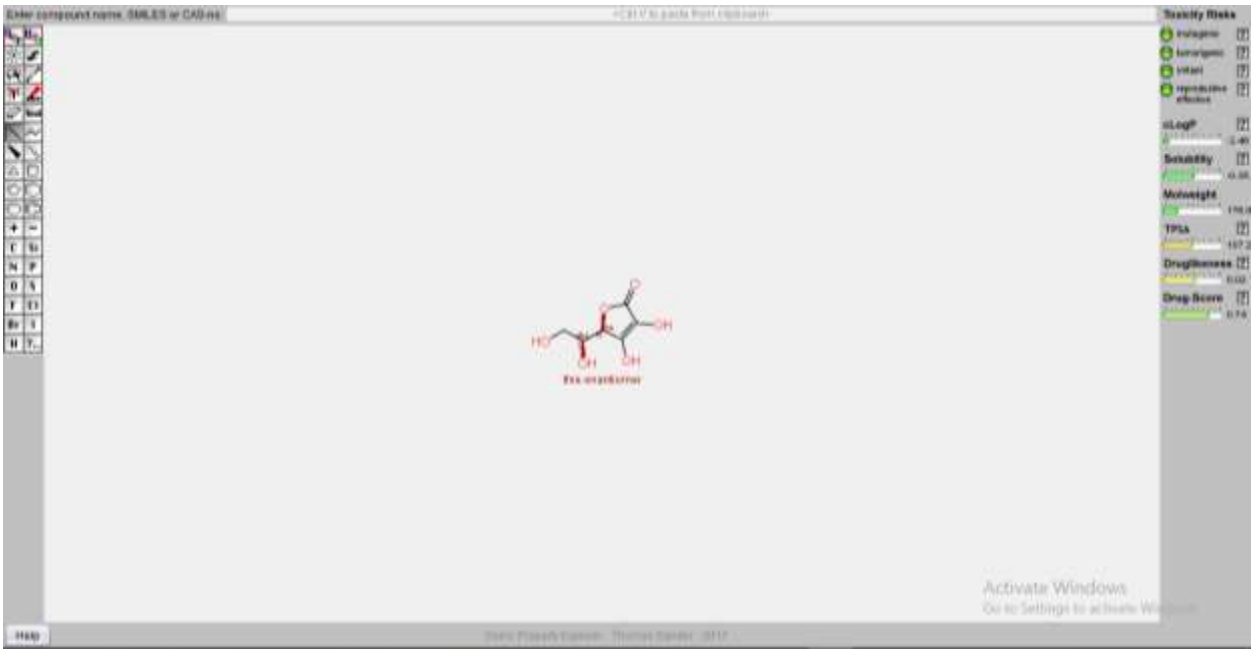


Figure 21: Pharmacokinetic and toxicity profiling of ascorbic acid.

CONCLUSION

The plants containing ascorbic acid are traditionally utilized for the cure of hyperlipidemia and related disorders from the immortal time. The exact mechanism of action for the lipid lowering action of ascorbic acid was still not revealed. With intent to propose the most probable mechanism of action of ascorbic acid the docking based computational analysis has been performed against the lipid lowering drug targets like ATP citrate lyase enzyme, lanosterol 14α-demethylase enzyme, squalene synthase enzyme, and Niemann Pick C1 like Protein. The docking analysis, chemical interactions, followed by the physicochemical based pharmacokinetic profiling has revealed that the

ascorbic acid is executing its lipid lowering action *via* inhibiting the squalene synthase enzyme.

REFERENCE

1. Himesh Soni etal. Characterization of Azadirachtin from ethanolic extract of leaves of *Azadirachta indica*.. Journal of Pharmacy Research, 2012,5(1),199-201.
2. Mishra, P. R., Panda, P. K., Apanna, K.C.,Panigrahi, S. Evaluation of acutehypolipidemic activity of different plantextracts in Triton WR-1339 inducedhyperlipidemia in albino rats.Pharmacologyonline., 3: 925-934 (2011).4.
3. Onwe PE ., Folawiyo MA., Anyigor -Ogah CS., Umahi G., Okorocha AE and Afoke AO Hyperlipidemia: Etiology and Possible Control. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 14(10) ;93-100.
4. Dugdale, D.C. (2010). High blood cholesterol and triglycerides. Retrieved February 16, 2011, from http://www.nlm.nih.gov/medlineplus/ency/article/000403.htm.
5. Soni Himesh etal. Quantification of Ascorbic acid in leaves of Annona squamosa.. International Journal of Pharmacy and Pharmaceutical Sciences. Vol 4, Issue 3, 2012;144-147.
6. Soni Himesh etal. Quantification of ascorbic acid in Salad components.2012,IJCPR .4(1),43-47.
7. K.R. Cousins, ChemDraw Ultra 9.0. CambridgeSoft, 100 CambridgePark Drive, Cambridge, MA 02140. www. cambridgesoft. com. See Web site for pricing options, ACS Publications, 2005.
8. Jain, R., & Mujwar, S. (2020). Repurposing metocurine as main protease inhibitor to develop novel antiviral therapy for COVID-19. *Structural chemistry*, *31*(6), 2487-2499.
9. Mujwar, S. (2021). Computational repurposing of tamibarotene against triple mutant variant of SARS-CoV-2. *Computers in Biology and Medicine*, *136*, 104748.
10. Mujwar, S., Deshmukh, R., Harwansh, R. K., Gupta, J. K., &Gour, A. (2019). Drug repurposing approach for developing novel therapy against mupirocin-resistant Staphylococcus aureus. *ASSAY and Drug Development Technologies*, *17*(7), 298-309.
11. Kaur, A., Mujwar, S., & Adlakha, N. (2016). In-silico analysis of riboswitch of Nocardia farcinica for design of its inhibitors and pharmacophores. *International Journal of Computational Biology and Drug Design*, *9*(3), 261-276.
12. Sharma, K. K., Singh, B., Mujwar, S., &Bisen, P. S. (2020). Molecular docking based analysis to elucidate the DNA topoisomerase IIβ as the potential target for the ganoderic acid; a natural therapeutic agent in cancer therapy. *Current computer-aided drug design*, *16*(2), 176-189.
13. Minaz, N., Razdan, R., Mujwar, S., Hammock, B.D. and Goswami, S.K., Impact of diabetes on male sexual function in streptozotocin-induced diabetic rats: Protective role of soluble epoxide hydrolase inhibitor, Biomedicine & Pharmacotherapy,.115, 1-6.
14. Mujwar, S., &Harwansh, R. (2022) In-silico bioprospecting of taraxerol as a main protease inhibitor of SARS-CoV-2 to develop therapy against COVID-19. Structural Chemistry, 33 (2), 1-10.
15. Mujwar, S., & Kumar, V. (2020). Computational Drug Repurposing Approach to Identify Potential Fatty Acid-Binding Protein-4 Inhibitors to Develop Novel Antiobesity Therapy. Assay and Drug Development Technologies.
16. S. Mujwar, K. Shah, J.K. Gupta, A. Gour, Docking based screening of curcumin derivatives: a novel approach in the inhibition of tubercular DHFR, International Journal of Computational Biology and Drug Design, 14 (2021) 297-314.
17. Mujwar, S., & Tripathi, A. (2022). Repurposing Benzbromarone as antifolate to develop novel antifungal therapy for Candida albicans. Journal of Molecular Modeling, 28(7), 1-9.
18. Mujwar, S. and Pardasani, K.R., Prediction of riboswitch as a potential drug target for infectious diseases: an insilico case study of anthrax, Journal of Medical Imaging and Health Informatics, Vol. 5(1), pp. 7-16, Feb 2015.
19. Mujwar, S. and Pardasani, K.R., Prediction of riboswitch as a potential drug target and design of its optimal inhibitors for Mycobacterium tuberculosis. International Journal of Computational Biology and Drug Design Vol.8(4). 2015, 326-347.
20. Mujwar, S. (2021). Computational bioprospecting of andrographolide derivatives as potent cyclooxygenase-2 inhibitors. Biomedical and Biotechnology Research Journal (BBRJ), 5(4), 446.
21. P. Pradhan, N.K. Soni, L. Chaudhary, S. Mujwar, K.R. Pardasani, In-silico prediction of riboswitches and design of their potent inhibitors for H1N1, H2N2 and H3N2 strains of influenza virus, Biosciences Biotechnology Research Asia, 12 (2015) 2173-2186.
22. K. Shah, S. Mujwar, J.K. Gupta, S.K. Shrivastava, P. Mishra, Molecular Docking and In Silico Cogitation Validate Mefenamic Acid Prodrugs as Human Cyclooxygenase-2 Inhibitor, Assay Drug Dev Technol, 17 (2019) 285-291.
23. K. Shah, S. Mujwar, G. Krishna, J.K. Gupta, Computational Design and Biological Depiction of Novel Naproxen Derivative, ASSAY Drug Development Technologies, 18 (2020) 308-317.
24. N. Soni, K.R. Pardasani, S. Mujwar, Insilico analysis of dietary agents as anticancer inhibitors of insulin like growth factor 1 receptor (IGF1R), J Pharm Pharm Sci, 7 (2015) 191-196.

25. E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF Chimera--a visualization system for exploratory research and analysis, J Comput Chem, 25 (2004) 1605-1612.
26. U.P. Agrawal N, Mujwar S, Mishra P,, Analgesic, anti-inflammatory activity and docking study of 2-(substituted phenyl)-3-(naphthalen1-yl)thiazolidin-4-ones, Journal of Indian Chemical Society, 97 (2020) 39-46.
27. T. Sander, J. Freyss, M. von Korff, J.R. Reich, C. Rufener, OSIRIS, an entirely in-house developed drug discovery informatics system, J Chem Inf Model, 49 (2009) 232-246.
28. Kciuk, M., Mujwar, S., Szymanowska, A., Marciniak, B., Bukowski, K., Mojzych, M., &Kontek, R. (2022). Preparation of Novel Pyrazolo [4, 3-e] tetrazolo [1, 5-b][1, 2, 4] triazine Sulfonamides and Their Experimental and Computational Biological Studies. International Journal of Molecular Sciences, 23(11), 5892.
29. Mujwar, S., Sun, L., &Fidan, O. (2022). In silico evaluation of food-derived carotenoids against SARS-CoV-2 drug targets: Crocin is a promising dietary supplement candidate for COVID-19. Journal of Food Biochemistry, e14219..
30. Fidan, O., Mujwar, S., &Kciuk, M. (2022). Discovery of adapalene and dihydrotachysterol as antiviral agents for the Omicron variant of SARS-CoV-2 through computational drug repurposing. Molecular Diversity, 1-13.
31. Shah, K., & Mujwar, S. (2022). Delineation of a Novel Non-Steroidal Anti-Inflammatory Drugs Derivative Using Molecular Docking and Pharmacological Assessment. Indian Journal of Pharmaceutical Sciences, 84(3), 642-653.
32. Kciuk, M., Gielecińska, A., Mujwar, S., Mojzych, M., Marciniak, B., Drozda, R., &Kontek, R. (2022). Targeting carbonic anhydrase IX and XII isoforms with small molecule inhibitors and monoclonal antibodies. Journal of Enzyme Inhibition and Medicinal Chemistry, 37(1), 1278-1298.