Original Article

Molecular Docking Study Of Thymoquinone With Target Proteins Involved In Cholesterol Biosynthesis

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Abstract

Hypercholesterolemia is a hazardous disorder that increases the risk of coronary heart disease, heart attack, and stroke in persons with normal cholesterol levels. Nigella sativa (N. sativa) (Family Ranunculaceae) is a medicinal herb that is widely used worldwide. Numerous researchers have conducted extensive research on N. sativa, elucidating a broad range of its pharmacological actions, which may include antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, renal protective, gastroprotective, and antioxidant properties. Thymoquinone (TQ) is a significant bioactive component found in the Nigella sativa (NS) black seeds. We examined the efficacy of TQ in the treatment of hypercholesterolemia in the current study. The TQ was docked with 20 proteins involved in cholesterol production. The docking data indicated that TQ had the greatest docking score with the majority of the target proteins and also exhibited a favourable interaction pattern with the target proteins. Thus, upon experimental validation, TQ may be an effective treatment for hypercholesterolemia.

Key words: Hypercholesterolemia, Nigella sativa , Thymoquinone, Molecular docking

Introduction

Hypercholesterolemia is a condition of abnormally increased levels of total cholesterol up to ≥ 240 mg/Dl .Hypercholesterolemia has been reported as an influential factor for cardiovascular disease. Treatment for hypercholesterolemia is generally performed by consuming statin drug targeting HMG CoA reductase in the cholesterol biosynthesis pathway [Gavin et al., 2000]. The use of statin group causes muscle ache (myopathy). Therefore, a drug called lapaquistat is developed to inhibit squalene synthase that also resides in the cholesterol biosynthesis pathway so that other non-sterol products are not inhibited [Hebert et al., 1997]. A clinical test report indicated that lapaquistat application in a dose of 100 mg had a toxic potential towards the liver. Due to the side effects of synthetic drugs, people currently tend to choose herbal medicine to reduce LDL cholesterol levels in the blood.

Among various medicinal plants, Nigella sativa (N. sativa) (Family Ranunculaceae) is emerging as a miracle herb with a rich historical and religious background since many researches revealed its wide spectrum of pharmacological potential. N. sativa is commonly known as black seed. Thymoquinone (TQ) is a chief bioactive constituent of black seed oil (Nigella sativa). TQ holds promising pharmacological properties against several diseases. It exhibits outstanding antioxidant, anti-inflammatory, anticancer, and other important biological activities.

Insilico models have potential use in the discovery and optimization of novel molecules, with affinity to the target, clarification of absorption, distribution, metabolism, excretion and toxicity properties as well as physiochemical characterization [Venkatachalam et al., 2003]. Docking is an important in the study of protein ligand interaction properties such as binding energy, geometry complementarity, electron distribution, hydrogen bond donor acceptor, hydrophobicity and polarizability thus molecular docking contribute a major role in the drug discovery in the identification of innovative small molecular scaffold, exhibiting the important properties with selectivity for the target together with reasonable ADME profile, lead and or drug likeness [Krvoat et al., 2005]. Hence, in the present study molecular docking studies was carried out between the TQ and target proteins from cholesterol biosynthesis to identify the hyper Hypercholesterolemia activity of TQ.

S.no	**Protein name**	**PDB ID**	**Binding Energy kcal/mol**	**Hydrogen bond forming amino acids residues**
1	Acat2	1wl5	-4.8	-
2	Cyp51	6uez	-6.2	
3	Dhcr24	AF-Q15392-F1	-7.2	
4	Dhcr7	AF-Q9UBM7-F1-model_v1	-6.6	
5	Fdft1	1ezf	-4.9	
6	Fdps	2qis		
7	Ggps1	6r4v	-4.2	HIS-80
8	Hmgcr	1dq8	-5.2	
9	Hmgcs1	2p8u	-5.6	
10	Hsd17b7	AF-P56937-F1-model_v1	-6.1	ILE-14
11	Lbr	2dig	-5.6	
12	Lipa	6v7n	-6	
13	Lss	1w6j	-7.4	THR-704(H-bond) TYR-98(Pi-Alkyl) PHE-444(Pi-Pi) TRP-581(Pi-Sigma) VAL-453(Pi-Alkyl)
14	Mvd	3d4j	-5.4	SER-275
15	Mvk	3d4j	-5.5	SER-275 ALA-279
16	Nsdhl	6jkg	-4.5	ASN-242
17	Pmvk	3ch4	-6.2	ARG-81 ARG-111
18	Sc5d	AF-O75845-F1-model_v1-SC5D	-6.3	TYR-180
19	Soat1	6l47	-7.4	PHE-145(Pi-Pi) PHE-382(Pi-Pi) PHE-378(Pi-Alkyl)

				TYR-142((Pi-Alkyl)
20	Sqle	6c6r	-6.3	
21	Tm7sf2	AF-O76062-F1-model_v1-TM7SF2	-5.5	ARG-305

Table 1: Molecular docking results of proteins in cholesterol biosynthesis pathway with thmoquinone

Materials and Methods

Protein Preparation

We downloaded the crystal structures of twenty proteins involved in cholesterol biosynthesis from the RCSB protein data bank (http://www.rcsb.org). The PDB ids for each protein were listed in Table 1. Separation of protein structure inhibitors was accomplished by releasing the.PDB file's atomic coordinates. All water molecules have been deleted, and ADT software has been used to prepare the required files for AutoDock Vina by assigning hydrogen polarities to protein structures, calculating Gasteiger charges on protein structures, and converting protein structures from the.PDB file format to the.PDBQT file format. [Trott O and Olson et al., 2010]

Ligand Preparation

Thymoqunione's chemical structure was downloaded in sdf format from the pubchem database. Using an online smiling translator, the.SDF format was converted to the.PDB format. ADT was then used to study the structures of ligands in terms of their combinations with non-polar hydrogens, Gasteiger alterations, and rotatable bond structures. The PDB files were then converted to ligand format. PDBQT format with ADT support, compatible with AutoDock4 (AD4) and AutoDock Vina. [Morris and Hueyet al., 2009].

Docking Methodology

The AutoDock Vina application was used to execute the docking of the molecules. For each receptor, the ligand was docked using grid coordinates (grid centre) and grid boxes of varying sizes. When interacting with macromolecules under rigid circumstances, the ligand was in a flexible state. By opening notepad and running AutoDock Vina, the configuration file was accessed. ADT was tasked with the responsibility of preparing the input. PDBQT file for proteins and to configure the grid box's size and centre. The structures of the proteins contain Kollman charges and polar hydrogen atoms. Docking was accomplished using the grid box's default specifications. PDBQT was used to save the prepared file. Ligand binding affinities were predicted using the AutoDock Vina scoring tool as negative Gibbs free energy (G) scores (kcal/mol). PyMOL was used to depict post-docking analysis, which revealed the sizes and positions of binding sites and hydrogen-bond interactions. Compounds were docked to target proteins' active sites. Following that, the binding poses of each ligand were observed and their interactions with the protein were defined, and the best and most energetically favourable conformations of each ligand were chosen.

Results and discussion

Hypercholesterolemia, or high cholesterol, occurs when there is too much cholesterol in the body. Cholesterol is a soft, waxy, fat-like substance that is a natural component of all the cells of the body. Molecular docking is used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. The

present study is deals with the molecular docking of TQ which is the active component of the Nigella sativa is against 20 target protein involved in cholesterol biosynthesis using AutoDock software**.**

Nigella sativa is a well-known medicinal herb that has been used to cure a variety of ailments for thousands of years. Nigella sativa and its major bioactive thymoquinone (TQ) have been shown to exert their pharmacological effects by enhancing critical biological functions such as anti-inflammatory, immunomodulatory, antitumor, analgesic, antihypertensive, antidiabetic, antimicrobial, antioxidant, and tissue protective properties in a variety of disorders [Zhang et al., 2015]. We wanted to investigate the Hypercholesterolemia, benefits of TQ utilising insilico molecular docking experiments based on innovative findings from earlier studies that demonstrate the critical function of TQ in disease management. Using the Autodock vina software, TQ was docked with proteins involved in cholesterol production.

In this study, we used open-source software to perform a computational protein–ligand docking analysis and displayed the interactions of the potential ligand thymoquinone with proteins implicated in the cholesterol biosynthesis pathway. Protein–ligand binding occurs spontaneously only when the free energy difference between the complexed and unbound free states is negative, and the difference between the G levels of the complexed and unbound free states is proportional to the protein–ligand interaction's stability. As a result, when G is low in the system, both protein folding and protein–ligand binding occur [Sergeev et al., 2014]. Thus, negative G scores imply that the resultant complexes with receptor molecules are stable, which is a necessary property of effective medicines [Muthu et al., 2016].

The proteins involved in cholesterol biosynthesis-thymoqunione interactions exhibited the highest negative G values in the current study, although significant negative changes in G levels indicated a strong binding affinity. Thus, we hypothesise that the majority of the proteins evaluated in this pathway have the ability to form robust and stable complexes with thymoqunione. While G values provide information about ligand docking in the active pocket of a protein, other types of molecular interactions with essential amino acid residues, such as hydrogen bonds, hydrophobic interactions, and electrostatic interactions, provide information about ligand docking in favourable conformations [Hariono et al., 2016]. Hydrogen bonding also contributes to protein stability, but less than hydrophobic interactions do, even in the tiniest globular proteins. Our findings indicate that distinct amino acid residues mediate hydrogen bond interactions. LSS and SOAT1 in particular had a high binding affinity with a binding energy of -7.4 kcal/mol. All of these proteins' hydrogen bond interactions were depicted in Figure 1 and the amino acid residues implicated in hydrogen bonding were reported in detail in table 1. Lanosterol synthase is an oxidosqualene cyclase (OSC) enzyme that catalyses the conversion of 2,3-oxidosqualene to a protosterol cation and then to lanosterol. [5] Lanosterol is a critical four-ringed intermediate in the production of cholesterol. Lanosterol synthase (LSS) transforms (S)-2,3-epoxysqualene to lanosterol in the cholesterol synthesis pathway, starting with acetyl-CoA.

SOAT1 and SOAT2 have distinct functions: SOAT1 is expressed at varying levels in a variety of organs and preferentially catalyses the esterification of free cholesterol with unsaturated fatty acids, which are then deposited as intracellular lipid droplets (LD). Thus, LDs act as a buffer for free cholesterol, which would impede cellular function if it interfered with membrane fluidity [Beloribi-Djefaflia et al., 2016]. SOAT2 is mostly expressed in the liver and gut and is involved in lipoprotein secretion [Temel et al., 2007].

Figure Caption 1 : Molecular interaction of TQ with a)LSS

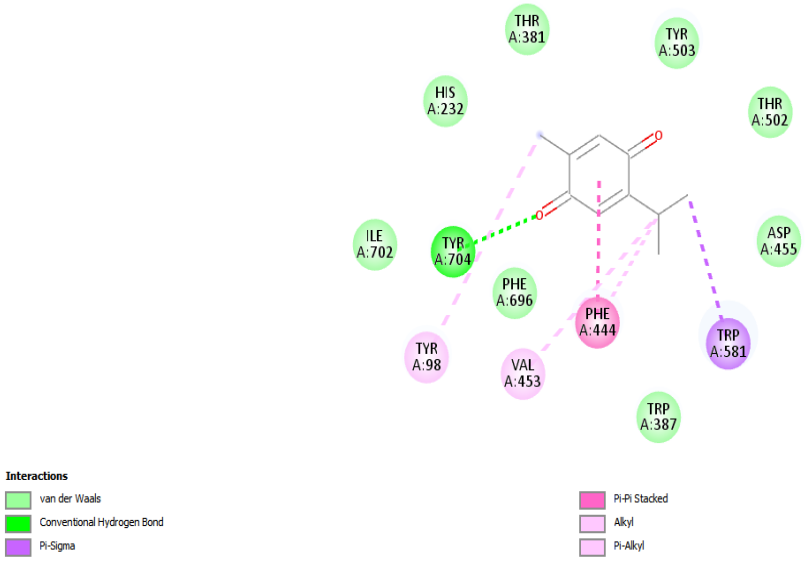
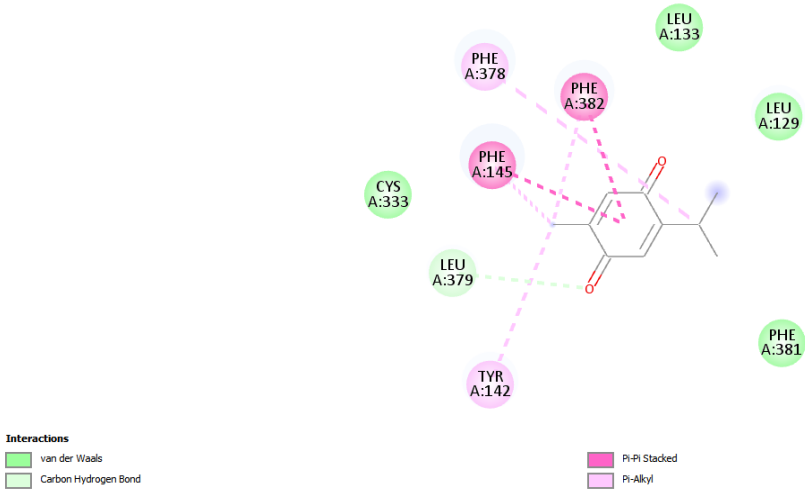


Figure Caption 1: Molecular interaction of TQ with b) SOAT1



Conclusion

Cardiovascular disease or coronary heart disease has been linked to hypercholesterolemia. The current study examined the interaction of natural chemicals TQ with target proteins involved in cholesterol production. Three-dimensional molecular interactions between TQ and target proteins were modelled using autodock software in order to gain a better understanding of TQ's structural-based functionality and the molecular mechanism of interactions at the enzyme's active site. TQ is the best suited medication based on molecular docking interactions. This type of study, conducted with the aid of computer-aided drug discovery tools, enables the detection of target-ligand interactions at the molecular level, providing a deeper insight into the drug molecule's subsequent optimization with improved pharmacokinetic profiling.

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