

***In silico* modelling of thymoquinone disruption of PCSK9-LDLR binding for cholesterol regulation**

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Cardiovascular disease remains a leading cause of global mortality, with hypercholesterolemia identified as a major modifiable risk factor. Proprotein convertase subtilisin/kexin type 9 (PCSK9) regulates cholesterol by binding to the EGF-A domain of the low-density lipoprotein receptor (LDLR), promoting its degradation and thereby reducing LDL-cholesterol clearance. Although current PCSK9 inhibitors and statins are effective, their high cost, requirement for injections, and associated side effects limit widespread use. Thymoquinone (TQ), the principal bioactive compound of *Nigella sativa*, exhibits antioxidant, anti-inflammatory, and lipid-lowering properties, making it a potential low-cost therapeutic alternative. However, its molecular interactions with PCSK9, LDLR, and their binding interface remain unclear. In this study, PCSK9 was selected as the primary target, along with LDLR and the PCSK9-LDLR complex. Protein structures were retrieved from the Protein Data Bank and refined using SWISS-MODEL where necessary. Binding site prediction was performed using PrankWeb, ProteinPlus, and COACH-D, and key interface residues were identified through computational prediction and literature validation. A solvated molecular system of PCSK9 was constructed using CHARMM-GUI and subjected to molecular dynamics (MD) simulation using NAMD, indicating receptor stability. Thymoquinone was constructed and

geometry-optimized using density functional theory at the B3LYP/6-31G(d,p) level with GaussView and Gaussian, and subsequently assigned CHARMM parameters using CHARMM-GUI for docking studies. Blind molecular docking was performed using PyRx, and ligand-receptor interactions were analyzed using Discovery Studio Visualizer. Docking analysis revealed binding affinities of -5.1 kcal/mol for the PCSK9-TQ complex, -6.3 kcal/mol for the LDLR-TQ complex, and -6.4 kcal/mol for the PCSK9-LDLR-TQ complex. Targeted docking against the identified interface residues, followed by MD simulation of the most stable complex, is expected to reveal interactions that disrupt PCSK9-LDLR binding, supporting TQ as a promising natural, affordable candidate for cholesterol-lowering therapeutic development.

Keywords:

Thymoquinone; PCSK9; LDLR; molecular docking; molecular dynamics

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