SUPPLEMENTARY MATERIAL

Molecular Rationale Delineating the Role of Lycopene as a Potent HMG-CoA Reductase Inhibitor: In vitro and In silico study

Sahir Sultan Alvi, Danish Iqbal, Saheem Ahmad and M. Salman Khan*

Clinical Biochemistry & Natural Product Research Lab., Department of Biosciences, Integral University, Lucknow-226026, India

*Corresponding Author
Dr. M. Salman Khan, Ph.D.
Associate Professor
Department of Biosciences
Integral University
Lucknow-226026, India.
mskhan@iul.ac.in
contactskhan@gmail.com

Abstract
The present study initially aims to depict the molecular rationale evolving the role of lycopene in inhibiting the enzymatic activity of β-hydroxy-β-methylglutaryl CoA (HMG-CoA) reductase via in vitro and in silico analysis. Our results illustrated that lycopene exhibited strong HMG-CoA reductase inhibitory activity (IC50 value of 36 ng/ml) quite better than Pravastatin (IC50=42 ng/ml) and strong DPPH free radical scavenging activity (IC50 value = 4.57±0.23 µg/ml) as compared to ascorbic acid (IC50 value = 9.82±0.42 µg/ml). Moreover, the Ki value of lycopene (36 ng/ml) depicted via Dixon plot was well concurred with an IC50 value of 36±1.8 ng/ml. Moreover, molecular informatics study showed that lycopene exhibited binding energy of −5.62 Kcal/mol indicating high affinity for HMG-CoA reductase than HMG-CoA (ΔG:−5.34 kcal/mol). Thus, in silico data clearly demonstrate and support the in vitro results that lycopene competitively inhibit HMG-CoA reductase activity by binding at the hydrophobic portion of HMG-CoA reductase.

Keywords: Lycopene, Hyperlipidemia, HMG-CoA reductase, Antioxidant.
Experimental
Chemical reagents: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was procured from Hi-Media Laboratories, Mumbai, India. Lycopene and HMG-CoA reductase assay kit were procured from Sigma-Aldrich (St. Louis, MO, USA). All the other chemicals used in this study were of analytical grade.

**DPPH radical scavenging activity of lycopene**
The DPPH radical scavenging capacity of the lycopene from MP Biomedicals (India) was determined by the method of (Brand-Williams et al, 1995). Further, IC\textsubscript{50} value represented the concentration of the lycopene that caused 50% inhibition of DPPH radicals and was calculated by interpolation of linear regression analysis.

**In vitro HMG-CoA reductase inhibitory activity of lycopene**
The HMG-CoA reductase assay kit with the catalytic domain of the human enzyme (recombinant GST fusion protein expressed in E. coli) procured from Sigma-Aldrich (St. Louis, MO, USA) was used according to the manufacturer’s instructions, to analyse the HMG-CoA reductase inhibitory activity of lycopene (Iqbal et al, 2014a). The concentration of the purified human enzyme stock solution was 0.52–0.85 mg protein/mL. Reference statin drug pravastatin was used as positive control. To characterize HMG-CoA reductase inhibition under defined assay conditions, reactions containing 4 μL of NADPH (to obtain a final concentration of 400 μM) and 12 μL of HMG-CoA substrate (to obtain a final concentration of 400 μM) in a final volume of 0.2mL of 100mM potassium phosphate buffer, pH 7.4 (containing 120mM KCl, 1mM EDTA, and 5mM DTT), were initiated (time 0) by the addition of 2 μL of the catalytic domain of human recombinant HMG-CoA reductase and incubated in Eppendorf BioSpectrometer (equipped with thermostatically controlled cell holder) at 37 °C in the presence or absence (control) of 1 μL aliquots of lycopene dissolved in DMSO (5%). The rates of NADPH consumed were monitored every 20 sec for up to 15 mins by scanning spectrophotometrically.

**Enzyme kinetics studies**
In order to determine the kinetic properties of HMG-CoA reductase after addition of lycopene, the activity was assayed by using various concentrations of HMG-CoA (100, 200, 300, 400, and 500 μM) in the absence and presence of different concentrations of Lycopene. \(K_m\) and mode of inhibition was determined by double-reciprocal Lineweaver-Burk plot analysis according to Michaelis-Menten kinetics, and \(K_i\) was determined by Dixon plot (Lineweaver and Burk, 1934; Dixon, 1953).

**Molecular docking studies**
HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, a key precursor of cholesterol biosynthesis. The PDB structure of the HMG-CoA in complex with HMG-CoA reductase was retrieved from the Protein Data Bank (PDB ID: 1DQ9) (Brookhaven Protein Data Bank, http://www.rcsb.org). The pdb file was energy minimized. The substrate HMG-CoA (ligand) was also exported in the form of a single sdf file. The separate ligand files of lycopene and Pravastatin were obtained as sdf files from Pubchem database. Molecular docking was performed by using Autodock 4.2 version.

**Statistical Analysis**

For the entire assays, samples were analysed in triplicate and the results were expressed as mean ±S.D. IC₅₀ value was calculated by Origin version 6.0 Professional software.

**References**

Brand-williams, W., Cuvelier, M.E. & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft and Technologie*, 28(1), 25-30.

Dixon, M. (1953). The determination of enzyme inhibitor constants. *Biochem. J.*, 55, 170–171.

Iqbal, D., Khan, M.S., Khan, M.S., Ahmad, S. & Srivastava, A.K. (2014a). An *In Vitro* and Molecular Informatics Study to Evaluate the Antioxidative and β-hydroxy- β-methylglutaryl-CoA Reductase Inhibitory Property of *Ficus virens* Ait. *Phytotherapy Research*, 28(6), 899–908.

Lineweaver, H. & Burk, D. (1934). The determination of enzyme dissociation constants. *J. Amer. Chem. Soc.*, 56, 658–666.
Figure S. S.1: DPPH free radical scavenging activity of lycopene and ascorbic acid. S.2A: In-vitro HMG-CoA reductase inhibitory activity of lycopene and pravastatin. Spectrophotometric time-scans demonstrating the ability of Pravastatin (S.2B) and lycopene (S.2C) to inhibit HMG-CoA reductase activity. S.3A: Lineweaver-Burk double-reciprocal. S.3B: Dixon plot of lycopene against β-hydroxy-β-methylglutaryl-CoA reductase. Each value in the figure is represented as mean ± SD (n = 3). S.4A,B&C: Binding pattern of HMG-CoA, lycopene and Pravastatin within the active site of HMG-CoA Reductase. S.4D: Superimpose image (S.4D) lycopene (Yellow), Pravastatin (Magenta), HMG-CoA (Green) within the active site of HMG-CoA Reductase.