Integrated *In Silico* and *In Vitro* Evaluation of Beta-Carotene as a Potential Hepatitis B Virus Polymerase Inhibitor

Authors:
Mathew MS Subin[1], Sreekumar S[2], Biju CK[3]

[1, 2, 3] Biotechnology & Bioinformatics Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Kerala, India

Background: Hepatitis B virus (HBV) infection remains a major global health concern despite available vaccines and antiviral therapies. Drug resistance and limited therapeutic efficacy necessitate the discovery of alternative antiviral agents from natural sources. This study evaluated the inhibitory potential of beta-carotene against HBV polymerase using integrated computational and experimental approaches.

Methods: The three-dimensional structure of HBV polymerase was modelled using Modeller 9v15. A total of 141 phytochemicals from *Lycopersicon esculentum* Mill. were subjected to virtual screening and molecular docking using AutoDock 4.2 to evaluate binding affinity toward the target enzyme. The stability of the best protein–ligand complex was assessed through a 50 ns molecular dynamics simulation using GROMACS. Experimental validation was performed in HepG2.2.15 cells. Cytotoxicity was evaluated by MTT assay, and antiviral activity was determined by measuring hepatitis B surface antigen (HBsAg) secretion using ELISA at concentrations ranging from 0.031 to 0.25 µM.

Results: β-carotene exhibited the strongest binding affinity toward HBV polymerase with a docking score of −10.90 kcal/mol. The interaction was predominantly stabilized by hydrophobic interactions, including alkyl, π-alkyl, van der Waals, and π-sigma contacts, without conventional hydrogen bonding. Molecular dynamics simulations confirmed stable complex formation throughout the 50 ns trajectory. RMSD values stabilized between 1.5 and 1.7 nm, while the Radius of Gyration decreased to approximately 3.35 nm after 28 ns, indicating enhanced compactness and conformational stability. However, *in vitro* analysis demonstrated limited antiviral efficacy. β-carotene achieved only approximately 13% inhibition of HBsAg secretion at 0.25 µM, and the IC_{50} value remained above the tested concentration range.

Conclusion: β-carotene demonstrated favourable binding characteristics and stable interaction dynamics with HBV polymerase *in silico*, but only minimal antiviral activity *in vitro*. These findings suggest that strong computational performance may not directly translate into biological efficacy, highlighting the need for structural optimization, improved formulations, or higher-dose evaluations to enhance its anti-HBV potential.

Disclosure of Interest Statement: Financial support for this study was provided by Kerala State Council for Science, Technology and Environment(KSCSTE).