Research Article

Interaction of Hyperoside and β-sitosterol with Alanine Transaminase using Molecular Docking

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ABSTRACT

Alanine transaminase (ALT) is a transaminase enzyme found in serum and in various body tissues. Elevated levels of ALT suggest the existence of disease such as liver damage, diabetes etc. Hyperoside, β-sitosterol was taken as ligand for molecular docking with Type 2 diabetic targets. Alanine transaminase enzyme whose crystallographic structures are available on the PDB database as 3IHJ was used for the docking analysis using the Schrodinger tool. The docking studies of the enzyme Alanine transaminase with two ligand hyperoside and β-sitosterol reveals that these are the good molecules which docks well with targets related to diabetes mellitus. Metformin is used as a standard drug for docking with Alanine transaminase. Hence hyperoside and β-sitosterol can be considered for developing into antidiabetic drug.

Keywords: Alanine transaminase, antidiabetic drug, diabetes, ligand.

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INTRODUCTION

Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced. Classical symptoms are polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). The risk of developing type-2 diabetes increases with age, obesity, cardiovascular disease (hypertension, dyslipidaemia), lack of physical activity, and family history of diabetes. Glycosylated haemoglobin of 6.5% is recommended as the cut point for diagnosing diabetes. A value of less than 6.5% does not exclude diabetes diagnosed using glucose tests.

The docking studies will aid in proper understanding of the binding mode and interaction of diabetic target with ligand (drug) molecules. Hyperoside and β-Sitosterol were chosen as suitable ligand molecules for studying the interaction.

Alanine transaminase or ALT (EC 2.6.1.2) is also called serum glutamic pyruvic transaminase (SGPT) or alanine aminotransferase (ALAT). It catalyzes the transfer of an amino group from alanine to α-ketoglutarate.

Glutamate + pyruvate ⇌ α-ketoglutarate + alanine

Reference range for humans is as follows

Gender	Reference ranges [1].
Female	5–38 IU/L
Male	10–50 IU/L

Significantly elevated levels of ALT suggest the existence of disease such as viral hepatitis, diabetes, congestive heart failure, liver damage etc. ALT is one of the most reliable markers of hepatocellular injury or necrosis. Its level can be elevated in case of hepatic disorders. ALT is thought to be more specific for hepatic injury because it is present mainly in the cytosol of the liver and in low concentrations elsewhere. Its liver enzymes may be involved in several critical processes that affect the risk of developing conditions such as diabetes and cardiovascular disease. Recent Studies suggests that a strong link

exists between certain liver enzymes such as alanine transaminase (ALT) and diabetes [2-4]. These liver enzymes may be involved in several critical processes that affect the risk of developing conditions such as diabetes and cardiovascular disease. Among patients with diabetes, the risk of chronic liver disease is doubled, independent of alcoholic liver disease or viral hepatitis [5]. Structure of ALT is shown (**Fig. 1 & 2**).

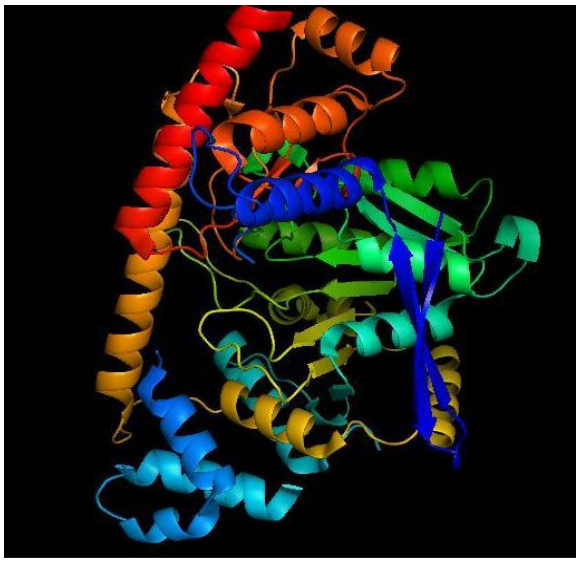

Figure 1: Structure of Alanine transaminase (3IHJ in cartoon representation)

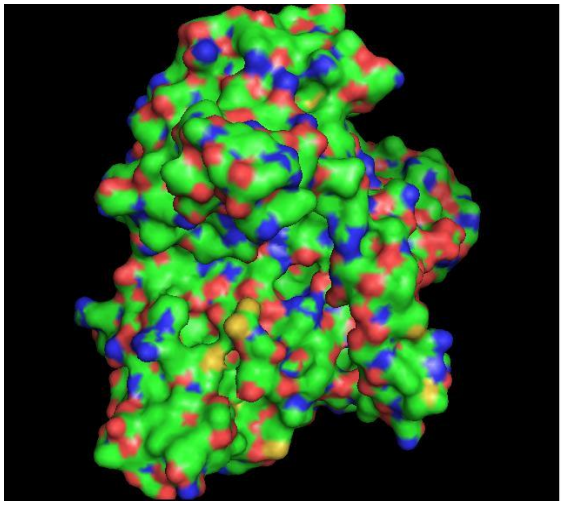

Figure 2: Structure of Alanine transaminase (3IHJ in surface representation)

The deposition of fat in the liver leads to an increase in gluconeogenesis and a decrease in the storage of glucose as glycogen in the liver [6]. Oxidative stress may also play a role in the pathogenesis of diabetes [7]. Studies reported the association between liver enzymes and diabetes and it may differ by sex. Because fatty liver occurs more commonly among men than women and the distributions of concentrations of ALT differ between men and women [8, 9].

Using bio-informatics, Alanine tranaminase enzyme was targeted for docking a ligand at pre-specified regions of its three dimensional structure. Three dimensional structure of Alanine transaminase is shown (**Fig. 3**).

Ligand Molecules

Hyperoside can be isolated from Drosera rotundifolia, from the Lamiaceae Stachys sp. and Prunella vulgari etc. Hyperoside **(**Hyperin) is a flavonol glycoside that has been documented to possess antiviral activity, anti-inflammatory activity, hepatoprotective, and gastricmucosal-protective effects [11].

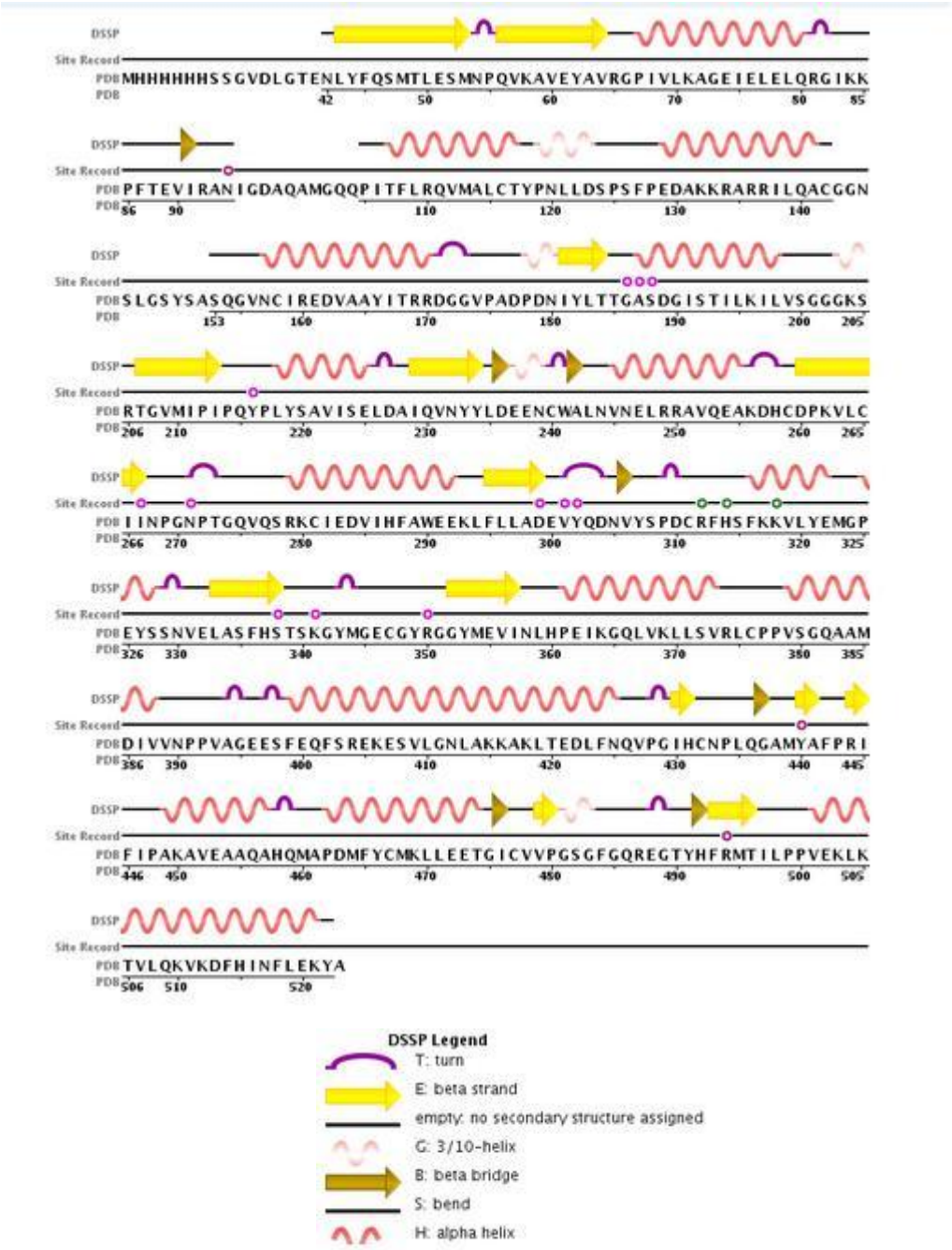

Figure 3: Three dimensional structure of Alanine transaminase enzyme [10].

β-Sitosterol It is widely distributed in the plant kingdom and found in cashew fruit, rice bran, wheat germ, corn oils, soybeans etc. They are hydrophobic and soluble in alcohols. β-sitosterol has been synthesized from stigmasterol, which involves a specific hydrogenation of the side-chain of stigmasterol. Beta-sitosterol is used for heart disease and high cholesterol.

Metformin originally sold as **Glucophage** is an oral antidiabetic drug in the biguanide class. It is used for the treatment of type 2 diabetes. Metformin is sold under several trade names, including Glucophage XR, Riomet, Fortamet, Glumetza, Obimet, Gluformin and Diaformin.

Structure of Hyperoside, Beta sitosterol and Metformin is depicted (**Fig. 4-6**). This paper presents the interaction of ALT–hyperoside and ALT–β-sitosterol complex.

MATERIALS AND METHODS

Protein Data Bank (PDB)

Alanine transaminase structure was downloaded from Protein data bank with

the specific resolution and the PDB id is 3IHJ.

Docking by Glide

The molecular docking tool, Glide (Schrodinger - Maestro v9.3.518) software was used for ligand docking studies in to the Alanine transaminase binding pocket. Glide is one of the most accurate docking tools available for ligand-protein, protein-protein binding studies.

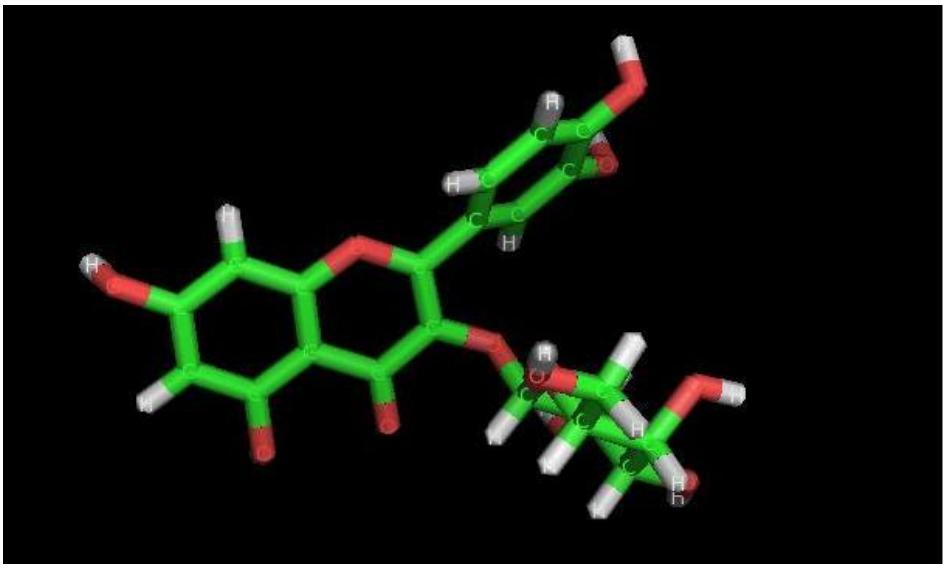

Figure 4: Three dimensional structure of Hyperoside

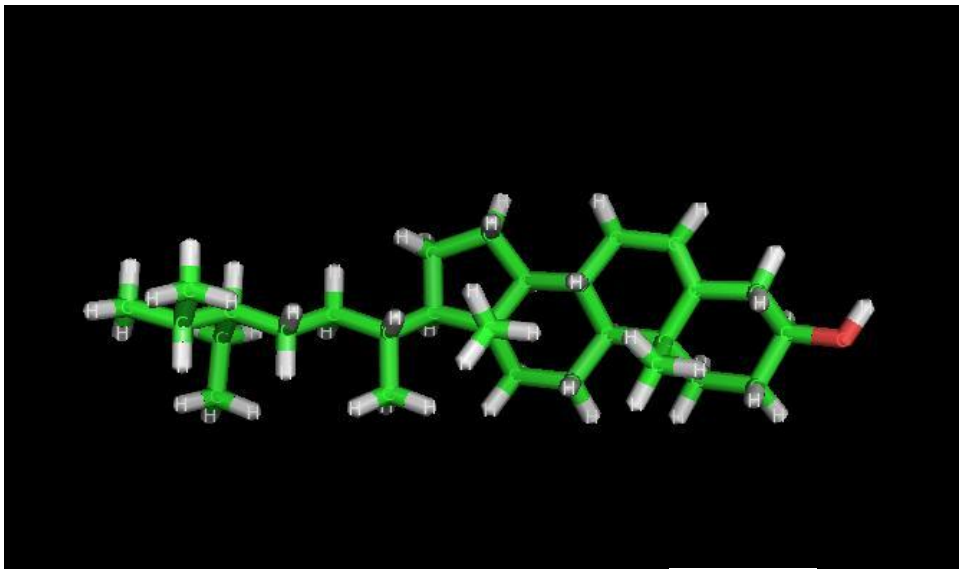

Figure 5: Three dimensional structure of β-Sitosterol

Protein preparation

A typical PDB structure file consists only of heavy atoms, can contain waters, cofactors, and metal ions. The structure generally has no information on bond orders, topologies, or formal atomic charges. Glide calculations use an all-atom force field for accurate energy evaluation.

Ligand preparation

The LigPrep process consists of a series of steps that perform conversions, apply corrections to the structures, eliminate unwanted structures, and optimize the structures. The process like convert the structure format, select the structures, add hydrogen atoms, remove unwanted molecules, neutralize charged groups, generate ionization states, generate low-energy ring conformations to get the output file [12].

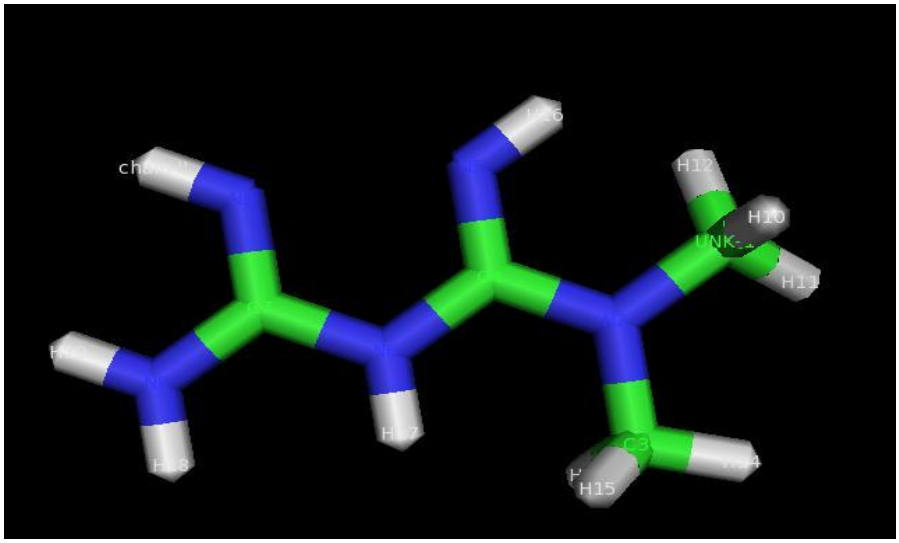

Figure 6: Three dimensional structure of Metformin

Active Site Analysis

Q-site Finder, an online tool which uses hydrophobic probes, was used to predict possible binding sites. Ligand explorer of PDB was also used to study the interactions.

ADMET predictions by QikProp

QikProp is a quick, accurate, easy-to-use absorption, distribution, metabolism, and excretion. QikProp helps in analyzing the pharmacokinetics and pharmacodynamics of the ligand by accessing the drug like properties.

RESULTS AND DISCUSSION

Protein-Ligand interaction plays a significant role in structural based drug designing. In the present work we have docked the ligands (hyperoside and β-Sitosterol) with Alanine transaminase (3IHJ) that are used as the target for Type 2 diabetes. Docking studies on compounds prepared through LigPrep were carried out in the active site of the protein. With the QikProp software, ADME properties of compounds can be predicted.

Table 1: Showing the Properties of the ligands using QikProp software

Properties	Hyperoside	Beta sitosterol	Metformin
Molecular Weight	464.382	414.713	131.18
Dipole Moment (D)	0	0	0
QPpolrz	37.735	47.913	11.168
QPlogHERG	-4.987	-4.445	-3.893
QPPCaco	5.582	3445.496	9.144
QPlogBB	-3.284	-0.328	-0.324
Molecular Volume (A^3)	1224.24	1456.413	497.01
QPlogPoct	31.68	31.68	14.119
QPlogS	-4.032	-7.033	1.496
SASA	653.477	750.024	321.506
max transdermal transport rate	0.002	0	0.02

The ADME prediction of hyperoside and β-Sitosterol shows good result as shown (**Table 1**).

Molecular observation of Alanine Transaminase Protein with Isolated and Control Drug

The docking simulation technique was performed using Glide module (Schrodinger suite) with plant-derived compound hyperoside and β-Sitosterol and it was docked into the protein Alanine transaminase [13].

Table 2: Shows the No of bonds, Bond length and Amino acids involved in the Docking analysis

Protein	Ligand	No. of H bond	Bond Length	Amino Acid	Protein Atm	Ligand Atm	GScore
Alanine transaminase	Hyperoside	8	2.192	ASN 94	O	H	-7.56
			2.198	LYS 341	H	O	
			2.412	LYS 341	H	O	
			1.926	SER 340	O	H	
			2.372	TYR 440	H	O	
			2.143	ASN 271	H	O	
			1.959	TYR 216	O	H	
	βSitosterol	1	2.444	ARG 350	H	O	-5.5
			2.15	GLY 342	O	H	
			2.231	LYS 341	H	N	
	Metformin	6	2.258	SER338	O	H	-5.54
			2.314	ALA 187	H	O	
			1.975	ARG 350	H	N	
			1.941	SER 188	O	H	
			2.141	ARG 350	H	N	

(**Fig. 7** shows the Result for hyperoside vs ALT, β-Sitosterol vs ALT and Metformin vs ALT is shown (**Fig. 7-9**). No. of bonds, Bond length and Amino acids involved in the Docking analysis is given (**Table 2**). The bioactive compounds interacted well with the protein with more number of hydrogen bonds.

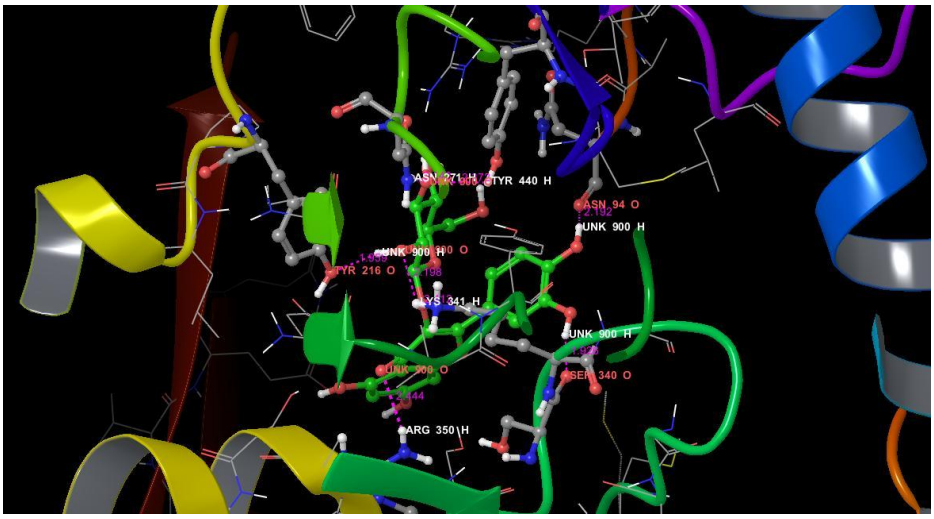

Figure 7: Docking analysis of Alanine transaminase (protein) vs Hyperoside (ligand)

Alanine Transaminase protein interacted with Hyperoside and β-Sitosterol Identified Metformim as control drug to compare the interaction profile. In the docking result, **Hyperoside** H, O, O, H, O, O, H, O atom types bonded with protein residual atom types ASN 94(O), LYS 341(H), LYS 341 (H), SER 340 (O), TYR 440 (H), ASN 271 (H), TYR 216 (O) and ARG 350 (H) with the bond distance of 2.192, 2.198, 2.412, 1.926, 2.372, 2.143, 1.959 and 2.444 correspondingly; **Betasitosterol** H atom types bonded with protein residual atom types GLY 342(O) with the bond distance of 2.15; **Metformin** N, H, O, N, H, and N atom types bonded with LYS 341 (H), SER338 (O), ALA 187(H), ARG 350(H), SER 188(O), ARG 350 (H) with the bond distance of 2.231, 2.258, 2.314, 1.975, 1.941 and 2.141 correspondingly.

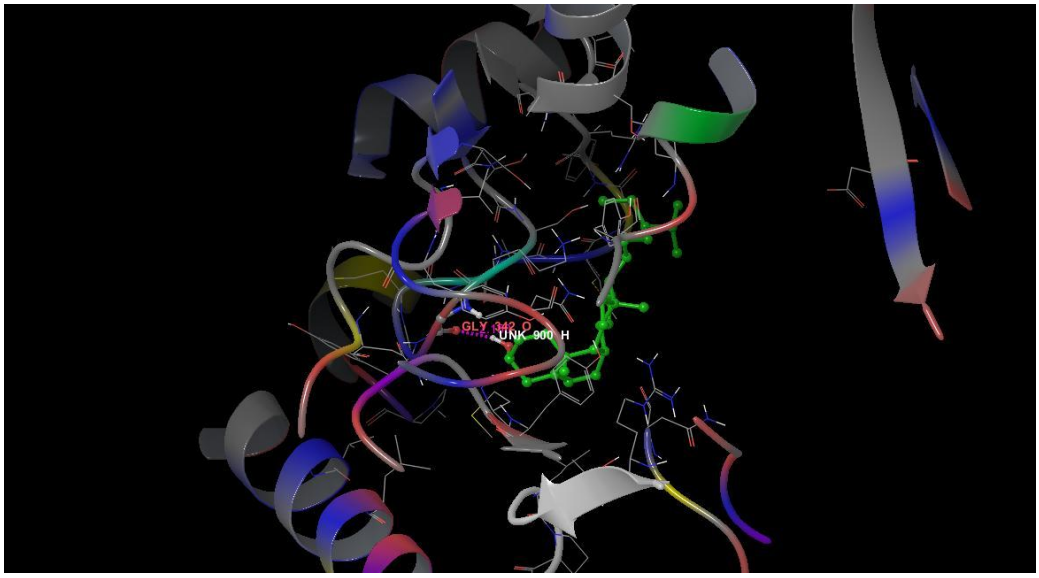

Figure 8: Docking analysis of Alanine transaminase (protein) vs β-Sitosterol (ligand)

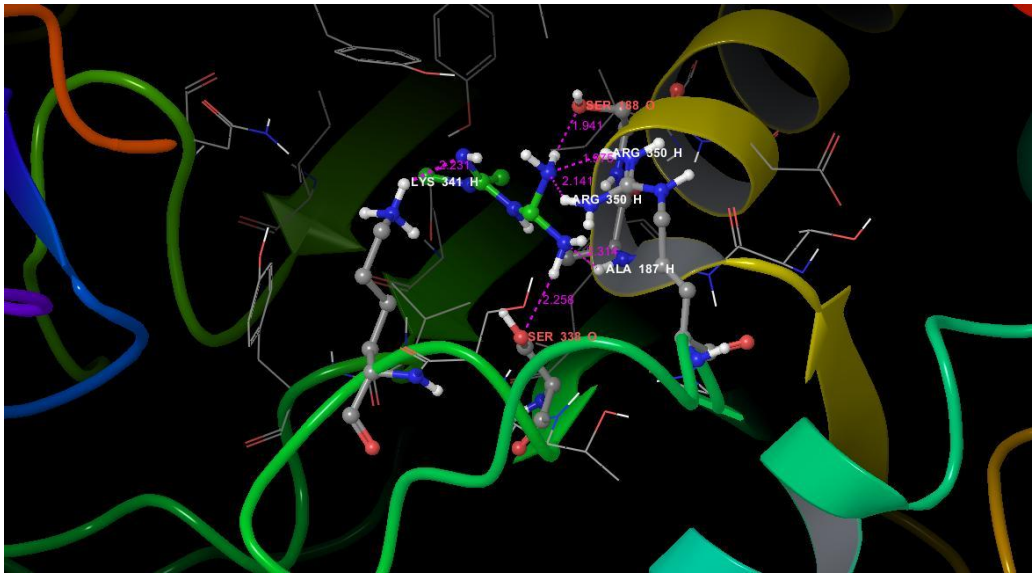

Figure 9: Docking analysis of Haemoglobin (protein) vs Metformin (standard drug)

Descriptor observation from Glide Docking:

In the Glide Docking, Alanine Transaminase subjected to interact with Extra Precision form of Docking in which resulting complex observed Glide Score with Hyperoside and Betasitosterol, Identified Metformim -7.56, -5.5, -5.54 correspondingly with the total addition of Lipophilic EdvW (Chemscore Lipophilic pair term and fraction of the total protein ligand vdW nergy), PhobeEn (Hydrophobic Enclosure reward), PhobeEnHB (Reward for Hydrophobically packed Hydrogen Bond), Hydrogen bond (Chemscore Hydrogen Bond Pair term), Electro (Electro Static rewards), Sitemap (Sitemap ligand/receptor non hydrogen bonding polar/hydrophobic and hydrophobic/hydrophilic complementarity terms), LowMW (Reward for Ligand with Low Molecular Weight), Penalities (Polar Atom burials and desolvation penalities, and Penality for Intra Ligand contact), ExposPenal (Penality for Exposed hydrophobic ligand groups), RotPenal (Rotatable bond Penality values on the whole correspondingly to the compounds).

Final report of the complex

In the total interaction and descriptor observation results, we can infer that

Hyperoside has more Glide Score and hydrogen bonding interactions than other compounds. Control drug metformin also has significant interaction with Alanine transaminase.

CONCLUSION

The docking studies are helpful for understanding the binding mode and interaction of Alanine transaminase with hyperoside and β-Sitosterol. This insilico approach can be further investigated to generate more effective and potential drug through ligand based drug designing approaches. The above results demonstrate that hyperoside and might be potentially used for blood glucose regulation.

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