Docking Studies of Green Tea Flavonoids as Insulin Mimetics

Jayasree Ganugapati
Associate Professor
Department Of Biotechnology
Sreenidhi Institute of Science and Technology
Ghatkesar. A.P, INDIA

Sirisha Mukkavalli
Department Of Biotechnology
Sreenidhi Institute of Science and Technology
Ghatkesar.A.P., INDIA

A.Sahithi
Department Of Biotechnology
Sreenidhi Institute of Science and Technology
Ghatkesar.A.P., INDIA

ABSTRACT
Diabetes Mellitus is a metabolism disorder where glucose, principal source of energy cannot enter the cells due to deficiency of insulin. In the present study, insilico approach was used to asses the use of natural green tea flavonoids as potential agents that could act as insulin receptor activators and reduce the harmful effects of diabetes. Docking studies of green tea flavanoids were carried out using Auto Dock 4.0 and Argus lab 4.0.1. Analysis of the results of both the docking softwares suggested that epicatechin can act as a potent insulin receptor activator.

Keywords
Green tea flavonoids, Diabetes mellitus, Interdisciplinary Research, Applications of Computer Science, Techniques for medical diagnosis, Docking, AUTO DOCK.

1. INTRODUCTION
Diabetes Mellitus is a metabolism disorder where glucose , principal source of energy for our bodies cannot enter our cells .This is because the body does not produce insulin or the insulin produced may be insufficient. Insulin is prerequisite for the activation of insulin receptor which facilitates transport of glucose from bloodstream to cells, hence they get deprived of glucose and the blood-glucose levels increases.

Insulin receptor is a transmembrane receptor that belongs to the large class of tyrosine kinase receptors and is activated by insulin. The decrease in insulin receptor signaling leads to the onset of diabetes mellitus type 2. Hence one possible solution that can be provided to help cure type-2 diabetes could be the identification of molecules that mimics insulin.

2. GREEN TEA FLAVONOIDS AS INSULIN MIMETICS
Tea (*Camellia sinensis*) is a popular beverage world wide. Recent studies indicate that tea has a wide range of preventive effects on diabetes and obesity for animal and human health (5,10). A number of studies have suggested that green tea polyphenols mimic insulin action. Rat epididymal adipocyte assays indicate that green tea extract has an insulin-potentiating activity on the utilization of glucose (1,2). Both polyphenolic and polysaccharide components in tea are considered to be hypoglycemic and experiments on rats results indicate that polyphenolics were responsible for the insulin-like activity.(2) Several studies also indicate that tea extract and its major polyphenol EGCG have insulin-potentiating activity in in vitro and animal models. Although insulin has become one of the most important therapeutic agents known to medicine, there is a continuing effort to find insulin substitutes, secretagogues, or sensitizers from synthetic or plant sources for the treatment of diabetes.(7)

In the present study various flavonoids (9) **(Table 1)** present in green tea were analysed for their action as insulin receptor activators.

Table 1 The list of the flavonoids present in dried green tea leaves obtained from USDA Database

S.NO	FLAVAN-3-OLS	FLAVONES	FLAVONOLS
1	Epicatechin	Apigenin	Kaempferol
2	Epicatechin-3-gallate	Luteolin	Myricetin
3	Epigallocatechin		Quercetin
4	Catechin		
5	Theaflavins		
6	Theaflavin-3,3'-digallate		
7	Theaflavin-3'-gallte		
8	Theaflacin-3-gallate		
9	Thearubigins		

3. DOCKING STUDIES

To study the nature of interactions, binding mode and selectivity of insulin receptor protein with individual flavonoids, docking was carried out with, Autodock 4.0 and Arguslab 4.0.1.

3.1 Protein

The structure of Insulin receptor protein complexed with peptide substrate (PDB Code: 1IR3) was obtained from PDB

3.2 Ligands

The CID files of the Ligands were obtained from NCBI PUBCHEM. The minimization of the prepared ligands was carried out with the GROMOS96 implementation of Swiss-PDB Viewer (3, 4).

Mol Inspiration, an online tool, (11) was used to identify suitable biological targets .Based on Lipinski's Rule of Five probable ligands were selected.

3.3 Active Site Analysis

Q-site Finder, an online tool which uses hydrophobic probes, was used to predict possible binding sites. Energetically favorable probes sites were clustered and then ranked according to the sum of interaction energies. Ligand explorer of PDB was also used to study the interactions.

4. DOCKING SOFTWARES

4.1 Autodock 4.0

Autodock 4.0 predicts the interaction of small molecules with macromolecular targets. Autodock performs the docking of the ligand to a set of grids (pre-calculated by Autogrid) describing the target protein. The energy grid was built within a cubic box of dimensions 40X16X50 A° with a spacing of 1.0 A°. The docking was performed based on Lamarckian Genetic Algorithm. (6)

4.2 Arguslab 4.0.1

Arguslab 4.0.1 is Molecular modeling and Drug Docking software. It is very flexible and can reproduce crystallographic binding orientations. Arguslab, provides a user friendly graphical interface and uses Shape Dock algorithm, to carry out docking studies. (8)

5. RESULTS

5.1 Mol Inspiration

The Mol Inspiration data of the compounds was then analyzed using Lipinski's Rule of Five. Those compounds that had many violations (i.e.thearubigins, theaflavins and its derivative), were eliminated from the present study**.(Table 2)**

5.2 *Q-Site Finder AND Ligand Explorer*

Q-site finder predicted 10 different sites. The most probable binding site was analysed and the residues that were identified in the binding site were LEU1002,GLY1003,GLN1004,SER1006,PHE1007,LYS1030,HIS 1057,HIS 1058,TYR 1087, ARG 1101 ,ASP 1143 ,THR 1145, THR 1105,GLU 1077,MET 1079,ASP 1083,ASN 1137,ASP 1150 **(Figure 1)**

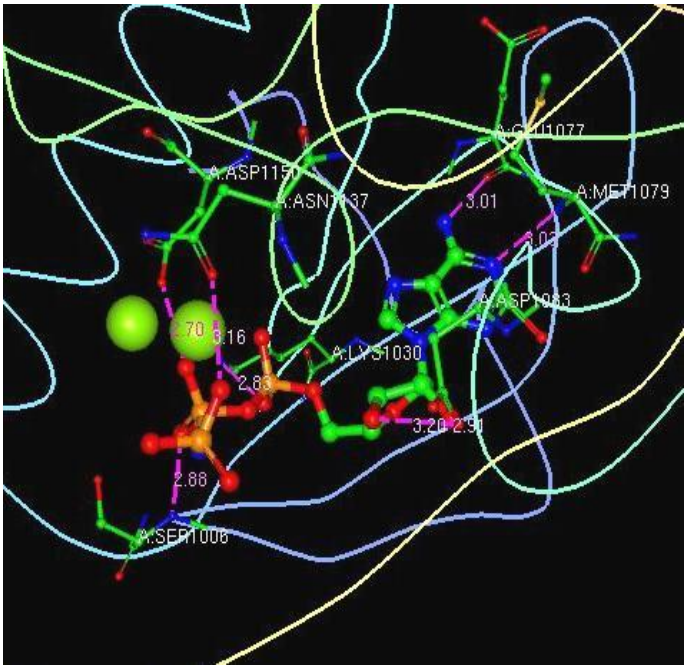

Figure 1 Active site analysis using Ligand Explorer

5.3 DOCKING RESULTS

The green tea flavonoids were then docked using the docking softwares. The energy values from the docking softwares are indicated in **Table 3.** The binding energies that were obtained for the interaction between flavonoids andinsulin receptor by using Autodock ranged from-2.78 to -8.03 kcal/mol and Arguslab yielded energies ranging from -7.87 to -9.45 kcal/mol. From the results epicatechin can be considered as a potent insulin receptor activator.

The interaction of epicatechin with insulin receptor is indicated in **Figure 2.**

Table 2 Molinspiration Results

SNo	Compound	milogP	TPSA	natoms	MW	nON	nOHNH	nviolations	nrotb
1	(-)Epicatechin-3-gallate	2.537	177.135	32	442.376	10	7	1	4
2	(-) Epigallocatechin-3-gallate	2.245	197.363	33	458.375	11	8	3	4
5	Apigenin	2.463	90.895	20	270.24	5	3	0	1
6	Kampferol	1.683	131.351	22	302.238	7	5	0	1
7	Quercetin	1.683	131.351	22	302.238	7	5	0	1
8	Caffeine	0.063	61.836	14	194.194	6	0	0	0
9	Luteolin	1.974	111.123	21	286.239	6	4	0	1
10	Myrcetin	1.392	151.579	23	318.237	8	6	2	1
11	(-)-Epicatechin	1.369	110.374	21	290.271	6	5	0	1
12	(-)-Epigallocatechin	1.077	130.602	22	306.27	7	6	1	1
13	(+)-Catechin	1.369	110.374	21	290.271	6	5	0	1
14	Theaflavin-3-gallate	3.278	284.352	53	730.631	16	11	4	5

Table 3 Docking Results

Name of Compound	**ARGUSLAB Energy(kcal/mol)**	**AUTODOCK ENERGY(Kcal/mol)**
Epicatechin	-9.45	-8.03
Epicatechin-3-gallate	-9.04	-4.13
Epigallocatechin	-8.72	-4.27
Epigallocatechin – 3-gallate	-8.28	-2.78
Catechin	-8.90	-4.27
Apigenin	-8.87	-5.23
Luteolin	-9.39	-5.43

Kampferol	-8.86	-5.77
Myrcetin	-7.87	-4.96
Quercetin	-8.53	-5.16

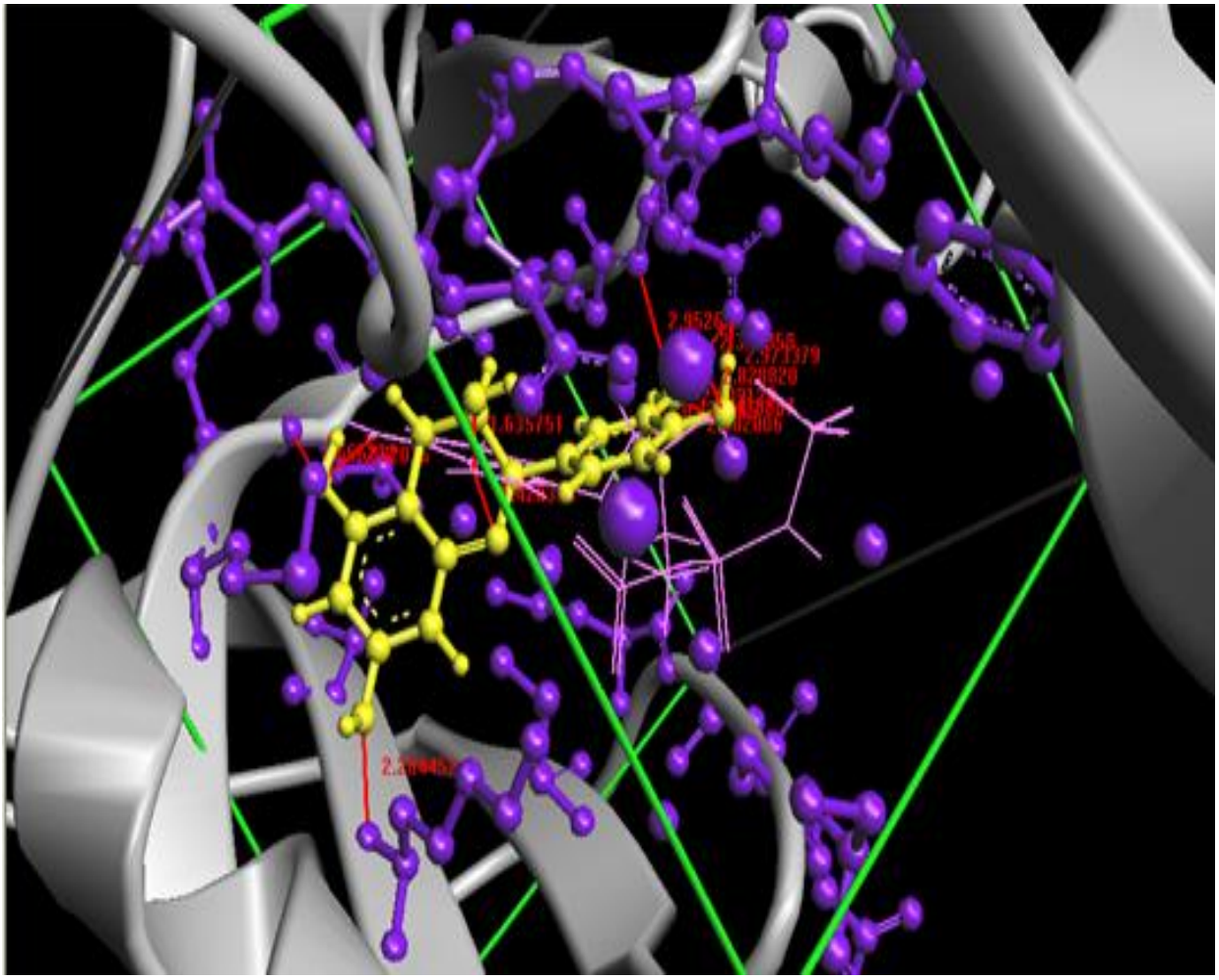

Figure 2: Epicatechin bound to the active site :Yellow – Epicatechin **Purple -** Active site **Red solid lines –**hydrogen bonds

6. DISCUSSION

The aim of the study was to identify a potent insulin receptor activator or an insulin mimetic.

1. We performed docking studies of insulin receptor 1IR3 with all flavonoids including the flavon-3-ols, flavonones and flavonols from green tea.
2. Two polyphenols, epicatechin and epicatechin-3-gallate and a flavone luteolin were identified as best flavonoids and docked well into the active site of 1IR3.

3. From the binding energies obtained we propose that these 3 flavonoids can be considered as insulin mimetics since they effectively interact with the active site region of insulin receptor by hydrogen bonding interactions.

4. Docking results of docking softwares indicated epicatechin as a potent insulin receptor activator

7. CONCLUSIONS

1. The best binding green tea flavonoids were identified by best binding energies obtained in docking studies of flavonoids with insulin receptor protein.

2. The docking studies are also helpful for understanding the binding mode and interaction of insulin receptor with green tea flavonoids.

3.The present study also provides an insight into the mechanism of action of oral insulin mimetics.

4. This insilico approach can be further investigated to generate more effective and potential insulin receptor activators through ligand based drug designing approaches.

5. Hence it can be concluded that green tea flavonoids can act as insulin receptor activators.From this analysis it was found that epicatechin can bind more effectively with insulin receptor

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