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EVALUATING MULTI-TARGET EFFICIENCY OF PHYTOCOMPOUNDS AGAINST DIABETES MELLITUS - AN *IN SILICO* APPROACH

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Abstract- Diabetes mellitus (DM) is a complex mulitifactorial metabolic disorder resulting from either insulin insufficiency or insulin dysfunction. In the present study, the phytocompounds were selected from traditionally used medicinal plants and nutraceuticals and the selected targets for Diabetes Mellitus were Peroxisome Proliferator Activate Receptor gamma, Fructose 1-6 bisphosphate and Dipeptidyl peptidase IV. Using Glide (Schrodinger module), *in silico* docking of the phytocompounds with these targets was carried out and their efficacy was evaluated against DM. The docking results of the phytocompouds were compared with the corresponding synthetic drugs in order to explore the multi -targeting efficiency of the phytocompounds for treating diabetes. Of the 27 selected compounds from 12 plants, Glucobrassicin present in *Capparis spinosa* and *Brassica oleracea* (Broccoli) and Epigallocatechin gallate (EGCG) present in *Camellia sinensis* showed better interactions and glide score with all the 3 receptors than the corresponding drugs / inhibitor. From the present study it is concluded that the phytocompounds can be used as an appropriate lead molecules against diabetes. Further the nutraceuticals, *Aegle marmelos, Brassica oleracea, Glycine max, Zingiber officinale, Capparis spinosa* and *Trigonella foenum graecum* can be added as food supplement to reduce the risk of diabetes.

Keywords- Diabetes mellitus (DM), Peroxisome Proliferator Activate Receptor gamma (PPARϒ), Fructose 1-6 bisphosphate (FBPase), Dipeptidyl peptidase IV (DPP IV), Glucobrassicin, Epigallocatechin gallate (EGCG)

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia. Insulin and glucagon are the major hormones that help to maintain normal blood glucose level. Defects in insulin secretion, insulin action or both generally lead to impaired carbohydrate and lipid metabolism in the body resulting in elevated fasting and post prandial blood glucose level [1]. When the elevated glucose level remains as such for a certain time period, it results in a condition called hyperglycemia [2]. Some of the long term complications associated with diabetes mellitus are diabetic retinopathy (loss of vision), diabetic nephropathy, peripheral / autonomic neuropathy and cardiovascular diseases [3] and diabetic patients generally have increased risk of atherosclerosis and cerebrovascular diseases [4]. The World Health Organization (WHO) reported that the number of adults with diabetes will rise to 300 million in the year 2025 [5]. By 2025 India, China and United States are expected to have a large number of diabetic patients [6]. One of the world's major killer disease in the next 25 years would be diabetes [7].

Diabetes is of 3 types namely Type I Diabetes (Insulin Dependent Diabetes Mellitus), Type II Diabetes (Non Insulin Dependent Diabetes Mellitus) and Gestational [8]. Type II Diabetes (NIDDM) is also called adult onset Diabetes mellitus, the most common form constituting 90-95% of diabetic population. FBPase is the enzyme that controls the rate limiting step in gluconeogenesis and is specific to gluconeogenesis. Moreover, the inhibition of FBPase does not produce any side effects such as lactemia and hyperlipidemia which is evident from the individuals with genetic deficiency of FBPase [9]. DPP IV, a proline-specific serine dipeptidase which is a cell surface protease that cleaves two amino acids from the N-terminal of GLP-1, produced by glucagon gene that results in GLP-1 inactivation [10]. The activity of GLP-1 is enhanced by inhibition of DPP IV and that leads to the stimulation of insulin production from the beta cells in response to glucose [11] and it reduces the secretion of glucagon [12] thereby regulating hyperglycemia. PPAR exhibits a strong link between lipid/glucose availability and long term metabolic adaptation [13]. PPARγ is present in adipose tissue and to a lesser extent in macrophages, it is almost absent in skeletal muscle [14] and maintains glucose homeostasis thereby serves as the primary receptor to increase the insulin sensitivity [15].

NIDDM patients are usually prescribed with oral antidiabetic agents such as sulfonylureas, metformin, thiazolidinediones, miglitol and acarbose [16]. The treatments with antidiabetic drugs are associated with several side effects such as liver disorders, cardiovascular diseases, weight gain, bloating, flatulence, diarrhea and abdominal discomforts. Moreover none of these glucose-lowering agents adequately control the hyperlipidemia, a condition frequently met with diabetes [17]. As diabetes is a multifactorial disease, multi-targeting is more potential than unidirectional therapeutic approach in the management of diabetes. These limitations as well as the adverse effects of currently available antidiabetic agents either in terms of efficacy or safety have enforced the discovery of new drugs that can manage type II diabetes more efficiently.

About 60% of the world populations rely on traditional medicines for the treatment of various diseases [18] and several medicinal plants have been used in treating diabetes. More than 400 plants have been reported for treating Diabetes, however very few plants had been scientifically evaluated and the fundamental mechanism of these plants in the medicinal systems still remains unclear. Hence

the present study has been designed that relies on computational approach to reveal the mechanism of phytocompounds that are used in traditional system for treating Diabetes mellitus.

Materials and Methods

In this research work the 3-D structure of the target proteins were retrieved from PDB [19] and the active site residues were identified using PdbSum. The synthetic and phytocompounds and inhibitor were retrieved from PubChem database (http://pubchem.ncbi.nlm.nih.gov/). Docking analysis were carried out for the target proteins with the selected synthetic drugs / inhibitor and phytocompounds using Grid based Ligand Docking with Energetics (GLIDE, a Schrondinger module) [20].

Results

The crystal structure of ligand binding domain region of Human Peroxisome Proliferator Activated Receptor gamma (PPARγ) (PDB ID: 2PRG) [21], fructose-1, 6-bisphosphatase (FBPase) in complex with tricyclic inhibitor (PDB ID: 3A29) [22] and Human Dipeptidyl Peptidase IV (DPP IV) complexed with the inhibitor cyanopyrrolidine (PDB ID: 2G5P) [23] were retrieved from the PDB database and the structure was visualized using PyMOL [Fig-1].

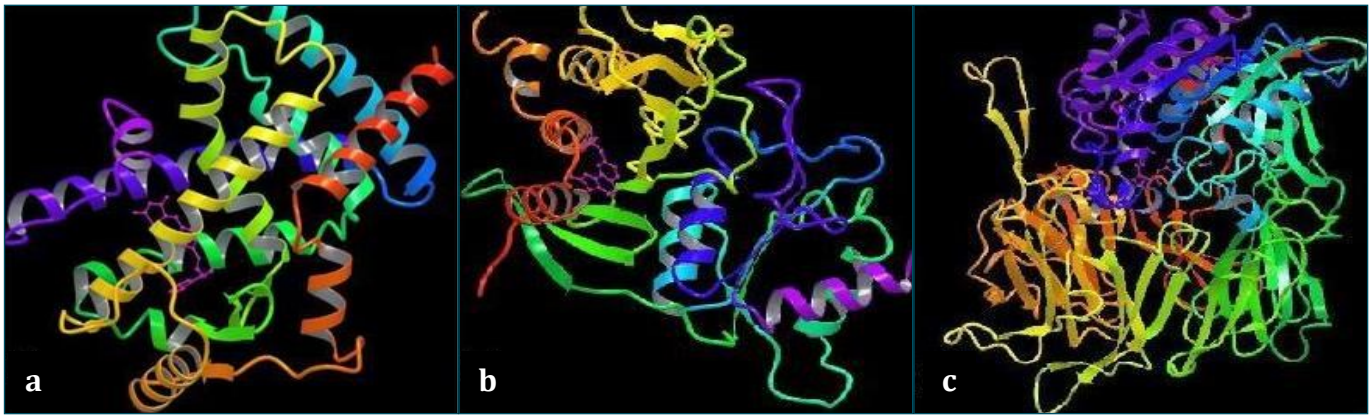

Fig. 1- Crystal structure of a. PPARγ b. FBPase c. DPP IV

Active Site Residues of the Targets

The active site of the target molecules complexed with the corresponding inhibitor are analyzed using PDBSum. The binding site residues of PPARγ are GLN 286, SER 289, HIS 323 and TYR 473 which were found to interact with the antidiabetic drug rosiglitazone. ILE 281, GLY 284, CYS 285, PHE 282, TYR 327, LEU 330, ILE 341, MET 348, MET 364, HIS 449 and LEU 453 exhibited non-bonded interactions with rosiglitazone. The hydrogen bond interactions were observed between tricyclic inhibitor and VAL 17, THR 27, GLY 28, LEU 30, THR 31, LYS 112 and TYR 113 which are the active site residues of human liver fructose-1, 6-bisphosphatase. MET 117, GLU 29, GLY 26, ALA 24, GLY 21 and GLU 20 exhibited non-bonded interactions such as hydrophobic and Vander Waals with the tricyclic inhibitor. In DPP IV, GLU205, GLU 206, TYR 631 and TYR 547 were found to interact with cyanopyrrolidine by means of hydrogen bond formation. ARG 125, HIS 126, SER 209, PHE 357, TYR 631, TYR 662, VAL 656 and TYR 666 exhibited non-bonded interactions such as hydrophobic and Vander Waals with cyanopyrrolidine.

Retrieval of ligand Structures

Twenty seven compounds from traditional medicinal plants and nutraceuticals such as *Andrographis paniculata, Aegle marmelos, Annona squamosa*, *Brassica oleracea, Camellia sinensis, Capparis spinosa, Cinnamomum cassia, Glycine max, Ocimum sanctum, Trigonella foenum graecum, Withania coagulans, Zingiber officinale* [Table-1] have been taken for the present study and the 3D structures of the compounds were retrieved from PubChem database.

Docking Analysis

Molecular docking studies were carried out for all the phytocompounds and specific synthetic drugs/ inhibitor with the receptors PPARγ, FBPase and DPP IV. Based on the glide score and hydrogen bond interactions, 2 compounds have been found to exhibit better glide score with PPARγ when compared with the synthetic drugs, 8 compounds with fructose-1, 6-bisphosphatase and 2 compounds with DPP IV [Table-2]. The glide score of the selected phytocompounds with all the three targets are compared and list [Table -3].

Interaction of the phytocompound Glucobrassicin with the PPARγ, FBPase and DPP IV are shown in [Fig-2], [Fig-3] and [Fig-4].

Discussion

Diabetes mellitus is a major public health problem in the developed as well as developing countries and is ranked seventh among the leading causes of death [24]. NIDDM is a multifactorial disorder associated with a number of common clinical disorders which include impaired glucose tolerance, insulin resistance, hypertension, dyslipidemia and weight gain. With regard to multifactorial diseases, balanced activity on several targets can be more effective and it decreases the side effects compared to the action of single selective ligand (monotherapy) [25].

Finding new therapeutics that hit multiple targets become a new paradigm in drug discovery. Multi-targeting in case of complex diseases such as diabetes can be achieved in 2 ways. First approach is designing a single compound that hit multiple targets which is generally termed as one compound multiple target strategy [26] and the second approach is designing a drug which is a combination of two or more active principles [25]. As NIDDM is a multifactorial disease, multiple target strategy will be more effective to treat this type of disease.

Phytocompounds from medicinal plants exert less side effects and the effect is comparable to synthetic drugs. Hence, it is necessary to identify the phytocompounds which can act on multiple targets. Considerations of ADMET properties (Absorption, Disposition, Me-

tabolism, Excretion and Toxicity) are essential for efficient discovery and the development of new drugs [27]. According to Clardy and Walsh [28] natural compounds are an important exception of ADMET properties or RO5 (Rule Of five). Hence the phytochemical compounds of the nutraceuticals need not to be tested for ADME Tox properties.

Table 1- List of compounds from traditional medicinal plants and nutraceuticals

Compound name	Plant Name
19-hydroxy-3-oxo-ent- labda- 8(17),11,13-trien-16,15-olide	*Andrographis paniculata*
3,19-dihydroxy-ent-labda-8(17),12-dien-16,15-olide	*Andrographis paniculata*
3,19-dihydroxy-15-methoxy-ent-labda-8(17),11,13-trien-16,15-olide	*Andrographis paniculata*
3,15,19-trihydroxy-ent-labda8(17),13-dien-16-oicacid	*Andrographis paniculata*
13,14,15,16-tetranor-ent-labd-8(17)-ene-3,12,19-triol	*Andrographis paniculata*
Beta-Sitosterol	*Glycine max*
Daidzein	*Glycine max*
Eugenol	*Ociumum sanctum*
Gingerols	*Zingiber officinale*
Hydroxychalcone	*Cinnamomum cassia*
Limonene	*Annona squamosa*
Sabinene	*Annona squamosa*
Stachydrine	*Capparis spinosa*
Trigonelline	*Trigonella foenum graecum*
3,18,19-trihydroxy-entlabda-8(17),13-dien-16,15-olide	*Andrographis paniculata*
19-[(b-D-glucopyranosyl)oxy]-19-oxo-ent-labda-8(17),13-dien-16,15-olide	*Andrographis paniculata*
Ent-labda-8(17),13-diene-15,16,19triol	*Andrographis paniculata*
3,19-dihydroxy-14,15,16-trinor-ent-labda-8(17),11-dien-13-oic acid	*Andrographis paniculata*
Aegeline 2	*Aegle marmelos*
Cinnamaldehyde	*Cinnamomum cassia*
EGCG	*Camellia sinensis*
Genistein	*Glycine max*
Glucobrassicin	*Capparis spinosa and Brassica oleracea*
4-Hydroxyleucine	*Trigonella foenum graecum*
α- Pinene	*Annona squamosa*
Sulforaphane	*Brassica oleracea*
Withanolide	*Withania coagulans*

Table 2- Docking results of the phytocompounds and synthetic compounds with the receptors

Sr. No.	Compound name	Glide Score (Kcal/mol)	Interactions (D-H…A)
	Interactions of PPARγ with phytocompounds and synthetic drug		
	Synthetic drug		
1.	Thiazolidinedione	-12.25	GLN286, SER 289, GLU343
	Phytocompounds		
1.	Glucobrassicin	-13.28	GLN286,SER289,HIS323, HIS449,TYR473,TYR473
2.	EGCG	-12.58	CYS285, ARG288
	Interactions of FBPase with phytocompounds and synthetic inhibitor		
	Synthetic inhibitor		
1.	CS917	-7.49	THR27,GLY28,LEU30, TYR113
	Phytocompounds		
1.	3,18,19-trihydroxy-entlabda-8(17),13-dien-16,15-olide	-10.25	THR27,GLY29,LEU30,LYS112, TYR113, VAL160,ASP178
2.	Glucobrassicin	-9.41	GLY28, THR27, LEU30, THR31, LYS112, TYR113, TYR113, ARG140, VAL160
3.	3,15,19-trihydroxy-ent-labda8(17),13-dien-16-oicacid	-9.06	GLY21, GLY26, THR27, GLY28, GLU29, TYR113,
4.	EGCG	-8.99	GLU20, ARG140, ASP178, VAL160
5.	Ent-labda-8(17),13-diene-15,16,19triol	-8.94	LEU30, THR31, TYR113, CYS179
6.	4-Hydroxyleucine	-7.91	THR27, GLY28, LEU30, THR31, TYR113
7.	19-[(b-D-glucopyranosyl)oxy]-19-oxo-ent- labda-8(17),13-dien-16,15-olide	-7.81	TYR113, ASN158, ASP178
8.	3,19-dihydroxy-ent-labda-8(17),12-dien-16,15-olide	-7.50	THR27, LEU30, TYR113, VAL160, ASP178
	Interactions of DPP IV with phytocompounds and synthetic drug		
	Synthetic drug		
1.	Sitagliptin	-10.19	GLU205, SER209, SER630, ASN 710
	Phytocompounds		
1.	Glucobrassicin	-10.85	GLU205, GLU206, TYR547, SER630, TYR662, ASN710
2.	EGCG	-10.72	ARG125, GLU205, GLU206, TYR547, SER630, TYR662, TYR666, TYR669, ASN710

Table 3- The phytocompounds having better glide score with all the three receptors

Sr. No.	Plant Name	Compound Name	PPARγ GLIDE SCORE (kcal/mol)	FBPase GLIDE SCORE (kcal/mol)	DPP IV GLIDE SCORE (kcal/mol)
1	*Capparis spinosa*	Glucobrassicin	-13.28	-9.41	-10.85
2	*Camellia sinensis*	EGCG	-12.58	-8.99	-10.72
3	*Glycine max*	Genistein	-10.76	-7.41	-7.66
4	*Andrographis paniculata*	Ent-labda-8(17),13-diene15,16,19triol	-10.35	-8.94	-7.74
5	*Andrographis paniculata*	13,14,15,16-tetranor- ent-labd-8(17)-ene	-10.06	-5.2	-7.25
6	*Glycine max*	Daidzein	-9.67	-5.32	-4.43
7	*Zingiber officinale*	Gingerols	-7.64	-5.55	-8.06
8	*Aegle marmelos*	Aegeline 2	-7.58	-6.01	-7.72
9	*Trigonella foenum graecum*	4-Hydroxyleucine	-6.56	-7.91	-8.45
10	*Capparis spinosa*	Stachydrine	-5.47	-3.85	-5.82
11	*Trigonella foenum graecum*	Trigonelline	-5.21	-5.2	-5.72

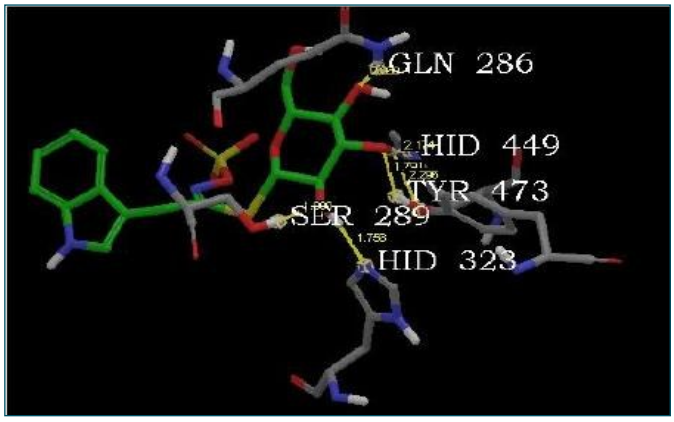

Fig. 2- Interaction of Glucobrassicin with the PPARγ

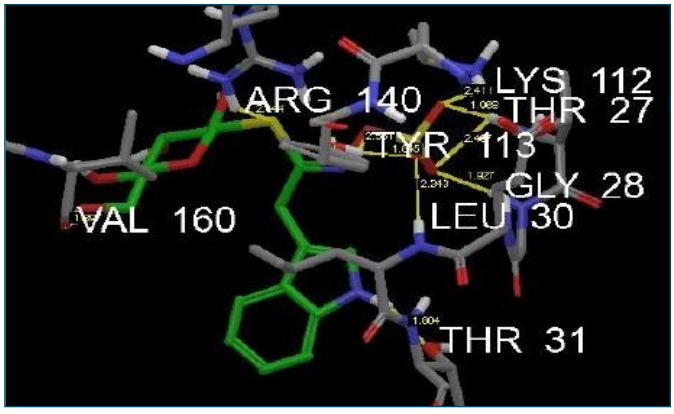

Fig. 3- Interaction of Glucobrassicin with the FBPase

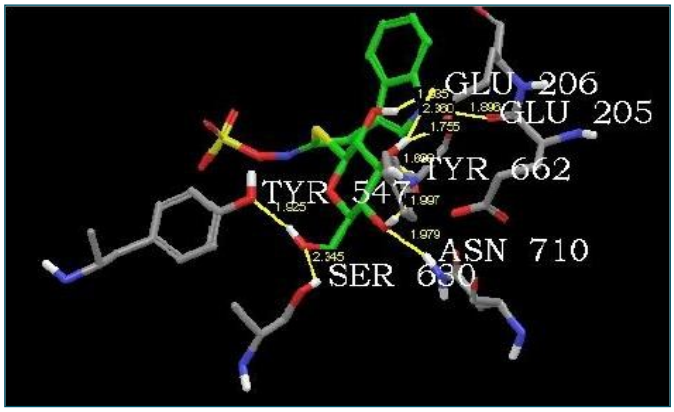

Fig. 4- Interaction of Glucobrassicin with the DPP IV

Glucobrassicin are the main indole glucosinolate present in *Brassica oleracea* [29] which exhibited potential antioxidant and antihyperglycemic activity in STZ induced diabetic wistar rats [30]. The flower buds and young shoots of *Capparis spinosa* also found to contain the bioactive compound glucobrassicin [31]. *Capparis spinosa* has been known as a traditional herbal medicine for its diuretic, antihypertensive, tonic properties [32] and antidiabetic activity [33]. Hence the phytocompound Glucobrassicin was docked with the selected targets. The Glucobrassicin and PPARγ interacted with all the active site residues GLN 286, SER 289, HIS 323, HIS 449 and TYR 473 which had glide score -13.28 kcal/mol and also showed hydrogen bond interactions with fructose-1, 6-bisphosphatase in the active site residues THR 27, GLY 28, LEU 30, THR 31, LYS 112 and TYR 113 with the glide score of -9.41 kcal/mol. The compound Glucobrassicin also exhibited hydrogen bond interactions with the active site residues of DPP IV and showed the glide score of -10.85 kcal/mol. Of the 27 compounds, the phytocompound glucobrassicin interacted with the most of the active site residues of all the 3 targets and showed better glide score when compared to the synthetic drug Thiazolidinedione (-12.25 kcal/mol), inhibitor CS197 (-7.49 kcal/mol) and drug Sitagliptin (-10.19 kcal/mol).

EGCG is the major constituent in *Camellia sinensis* which is found to have many biological properties [34]. Gomes, et al. [35] reported that hot water extract of *C. sinensis* exhibits potential hypoglycemic activity in STZ induced diabetic rats. The phytocompound EGCC present in *C. sinensis* showed better interaction with PPARγ which had glide score of -12.58 kcal/mol when compared with the synthetic drug Thiazolidinedione (-12.25 kcal/mol). The glide score -8.94 kcal/mol was obtained for the interaction of EGCG and fructose-1,6-bisphosphatase with four hydrogen bonds which is relatively better than the synthetic prodrug CS197 (-7.49 kcal/mol). Further, the phytocompound interacted with DPP IV and showed better glide score (-10.72kcal/mol) than the synthetic drug sitagliptin (-10.19 kcal/mol). The phytocompounds Aegeline 2 (-7.58, -6.01, -7.72 kcal/mol), Daidzein (-9.67, -5.32, -4.43 kcal/mol), Genistein (-10.76, -7.41, -7.66 kcal/mol), Gingerols (-7.64, -5.55, -8.06 kcal/mol), Stachydrine (-5.47, -3.85, -5.82 kcal/mol), 4 Hydroyleucine (-6.56, -7.91, -8.45 kcal/mol), Trigonelline (-5.21, 5.20, -5.72 kcal/mol) had glide score comparable to the synthetic drugs with all the three targets PPARγ, FBPase and DPP IV, respectively.

Based on the above *in silico* docking studies, it is concluded that the phytocompounds from *Brassica oleracea, Capparis spinosa* and *Camellia sinensis* may reduce the severity of diabetes mellitus and postpone the onset of disease and can be used as drugs or lead compounds.

Conclusion

From the present study, it is concluded that the phytocompounds Glucobrassicin from *Brassica oleracea* and *Capparis spinosa* and Epigallocatechin gallate (EGCG) from *Camellia sinensis* showed better docking with all the three important targets than the synthetic drugs/inhibitor and also other compounds like Aegeline 2 (*Aegle marmelos*), Daidzein and Genistein (*Glycin max*), Gingerols (*Zingiber officinale*), Stachydrine (*Capparis spinosa*) and 4 Hydroyleucine and Trigonelline (*Trigonella foenum graecum*) had glide score nearer to the drugs/inhibitor and interaction with most of the important residues. These phytocompounds of the nutraceuticals may reduce the severity of diabetes mellitus or postpone the onset of the disease. Hence, it is suggested that diabetic patients can include these plants in their diet as a food supplement for maintaining the blood glucose level.

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Conflict of Interest: There is no conflict of interest.

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