Research Article

Molecular Docking Studies of *Xanthium indicum* Bioactive Compounds with Therapeutic Targets of Diabetes Mellitus

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ABSTRACT

Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for diseases. The present study was under taken to investigate the antidiabetic property of *Xanthimum indicum* through the docking simulation of specific target proteins with their bioactive compounds. The bioactive compounds of *Xanthimum indicum* such as palmitic acid, chlorobutanol, stearyl alcohol, α-tocopherol, γ-tocopherol, xanthumin, sitosterol, oleic acid, isohexacosane, β-sitosterol, D-glucoside, 3, 4 dihydroxycinnamic acid, palstoquinone and strumasterol whose SDFs were retrieved from Pubchem database. The molecular targets of diabetes like Glucokinase, Fructose 1, 6- bisphosphatase, Human multidrug resistance protein 1, G- protein activated inward rectifier potassium channel 3, Human mitochondrial ABC transporter, and Cytochrome P_{450} of crystallographic structures were obtained from PDB database for docking analysis by Auto DockVina. The molecular docking studies of these compounds showed that α-tocopherol docks well with various targets related to diabetes mellitus among other bioactive compounds. The present work supports α-tocopherol might act as potential bioactive compound for treatment of the diabetes.

Keywords: α-tocopherol, Docking studies, Diabetes, *Xanthium indicum*.

INTRODUCTION

Diabetes mellitus is a chronic disease emerges in metabolic disorders of multiple aetiologies as a result of inherited or acquired insufficient or ineffective secretion of insulin from the pancreas. It is characterised by chronic hyperglycaemia with disturbances of carbohydrates, fat and protein metabolism.[1] It has been reported that diabetes mellitus is concerned as major health problem throughout the world. According to WHO about 143 million people are suffering from diabetes World wide.[2] This number may increase double by the year 2030. In developing countries including India have shown high pervasiveness and severity of the diabetic disease. India alone has more than 40 millions diabetic individuals which represent nearly 20% of the total diabetes population.[3] Oxidative stress, often to develop the complications of diabetes mellitus including retinopathy, nephropathy and atherosclerotic vascular disease, the leading cause of mortality in diabetes mellitus (DM).[4]

Glucokinase is a hexokinase isoenzyme that facilitates phosphorylation of glucose to glucose-6-phosphate. It is mainly emerged in liver and pancreas and it plays important role in the regulation of carbohydrate metabolism. During diabetic condition irregular activity of glucokinase takes place and it facilitate hypo or hyper glycemia.[5] Fructose1-6-bisphosphatase is focal enzyme in gluconeogenesis via its conversion of fructose 1-6-bis phosphate to Fructose 6- phosphate which permits endogenous glucose production from gluconeogenic amino acids glycerol or lactate.[6] Human multidrug resistance protein-1 involved in lipid metabolism and it is a member of the ATP-binding cassette super family causatively linked to rare and common human genetic diseases including diabetes.[7] Bio transformations of endogenous and exogenous compounds were take place through the mediation of cytochromeP_{450}. In diabetic condition increase in ketones were accompanied with the induction of cytochrome P_{450} expression and catalytic activity.[8,9] Small molecules, ions, hormones, lipids and drugs were across the cell membrane through mitochondrial ABC transporters which play main role in many diseases like juvenile diabetes and cystic fibrosis.[10,11] Antidiabetic compounds inhibit the K^+ flux through K_{ATP} channel pore, membrane depolarization with opening of voltage dependent Ca^{2+} channels and Ca^{2+}dependant release of insulin.[13]

For the treatment of diabetes there are different types of oral hypoglycaemic agents such as bigunides and sulphonylurea are available along with insulin.[14] Although, they may reduce the diabetic complications however simultaneously give the side effects. The management of DM without any side effects is still a challenge to the medical system. However complementary medicine has grown in popularity in recent years. Therefore, the pharmacologists have shown interest to develop the remedies for diabetes by using medicinal plants because of their effectiveness, minimal side effects in clinical experience and relative low costs. Many indigenous Indian medicinal plants have been identified to be useful to successfully manage diabetes and some of them have been tested.[15, 16]

In recent days the bioinformatics offers to find out the therapeutic targets for particular disease by using their software tools and it also used to identify the drugs from the bioactive compounds of medicinal plants by doing

virtual screening. These approaches have played a significant role in computer aided drug design. The field of drug design and discovery from medicinal plants are quicker and efficient.

Xanthium indicum is a weed commonly found in fallow lands. The plant roots were used in folk medicine for treatment of rheumatic pain, inflammation-related diseases[17] and isolated compounds were reported inhibit the prostaglandin E(1) and E(2).[18] Ethanomedicinal uses of plant is popular in Bangladesh to control the blood sugar in diabetic patients. Recent reports have been demonstrated that methanolic extract of this plant has shown anti-bacterial and cytotoxic activities.[19] The present study has made an attempt to investigate the antidiabetic drug properties of bioactive compounds from *Xanthimum indicum* leaves through the docking studies with specific target proteins of the diabetes.

MATERIALS AND METHODS

Protein structure preparation

The crystal structures of diabetic molecular targets like Glucokinase (1V4S), Fructose 1, 6- bisphosphatase(2JJK), Human multidrug resistance protein (2CBZ), G- protein activated inward rectifier potassium channel 3(3QGL), Human mitochondrial ABC transporters (4AYT) and Cytochromes P450(3LC4) whose crystallographic structures are available in the PDB database (http://www.rcsb.org). The proteins were optimized and energy minimized using the protein preparation wizard of the Argus lab 4.0 (http://www.argus lab.com). Protein structure's energies were minimized using steepest descent minimizer of Argus lab Suite.

Ligands preparation

14 natural compounds from *Xanthium indicum* whose structure data files were downloaded from the PubChem database (http://www.pubchem.ncbi.nlm. nih.gov). They are Xanthumin (CID-160533), Isohexacosane (CID-3017639), Chlrobutanol (CID-5977), Stearyl alcohol (CID-8221), β-sitosterol (CID-222284), Palmitic acid (CID-985),Oleic acid(CID-445639),3,4-dihydroxycinnamic acid (CID-689043), β-sitosterol-D-glucoside (CID-5742590), γ-tocopherol (CID-92729), α- tocopherol (CID-14985), Plastoquinone (CID-5375177) and strumasterol (CID-5280794). Glybenclamide (CID-3488) is the known inhibitor for diabetes. It was also used as one of the ligand. Then its energy form were minimized and converted to pdbqt format by Open Babel in PyRx 0.8 as ligand for virtual screening.

Virtual screening of compounds from *Xanthium indicum*

After optimization, the PDB co-ordinate files were taken to be docked against natural compounds from *Xanthium indicum.* AutoDockVina was used through PyRX interface to perform molecular docking and virtual screening, offering partial receptor flexibility providing high performance and accuracy of results. The docking results by using PYMOL tool.[20]

RESULTS AND DISCUSSION

Herbal medicines could simultaneously target multiple physiological processes through interactions between multiple compounds and cellular target proteins. Thus it reverse the biological networks from disease state to health state. Since a group of compounds contained in the herbal medicine could play a therapeutic role, the dosage could be minimized to reduce the toxicity and side effects. In the present study 14 compounds from *xanthimum indicum* leaves were screened against with specific target proteins of the diabetes such as 1V4S, 2JJK, 2CBZ, 3QGL, 4AYT and 3LC4. From the virtual screening, we found out that 4 compounds were shown high inhibition activity towards the target molecules among 14 compounds when compared to the known inhibitor Glibenclamide. The docking results were observed that among 4 compounds CID-14985, CID-92729, CID-3017639 and CID-222284, α-tocopherol has best docked binding energy. The average binding energies varies from -10.2kcal/mol to -7.7kcal/mol.

The best possible binding modes of the six targeted protein's active sites are displayed in Figures 1, 2, 3, 4, 5, and 6 by using PyMol tool v 1.1 (PyMOL Molecular Graphics System, Version 1.1) and corresponding energy values are listed in Table 1 Fig.1 shows the result of docking analysis of human glukokinase (1V4S) with α-tocopherol; it showed the binding site of the protein and ligand with Arg 63, Pro 66,Tyr 61, Ile159, Tyr 214,215, Leu 451, Val 455, Lys 459 and Val 62, 452 amino acid residues.

Glucokinase play a key role in the regulation of glucose homeostatis and it expressed only in liver and pancreatic beta cells. Regulation of hepatic glucose disposal facilitates the glucokinase by maintaining a gradient for glucose transport into the hepatocytes. In the beta cells, glucokinase involves in regulation of insulin release due to the availability of glucose levels in the cell.[21] In diabetic condition insufficient or deficiency of insulin disturb the carbohydrates metabolism which ultimately results in decrease the activities of enzymes which involve in the metabolism including glucokinase resulting in the impaired glucose utilization and augmented hepatic glucose production.[22] Chandramohan *et al.* (2008) reported that diabetic rats treated with 3-HMX active principle from *Xanthimum indicum* increased glucokinase activity. In the same way α-tocopherol increases glucokinase activity, thereby increasing the utilization of glucose leading to decreased blood sugar level.

Fructose 1-6 bisphosphatase is a rate limiting enzyme of the gluconeogenesis pathway. This enzyme catalyses the hydrolysis of fructose 1-6 bisphosphate to fructose 6-phosphate and inorganic phosphate.[23,24] Normally insulin inhibits the hepatic glucose production by suppressing G6Pase and fructose 1, 6-bisphosphatase activity.[25,26] From our results we observed that α-tocopherol interact

with 2JJK Arg 49,Ser 45, Ala 51, Pro 188, Ser 473, Ala 826, Arg 377, Ala 507, Pro 1143, Ser 692, Arg 1014 and Ser1011 amino residues of the protein. This may be the specific target to inhibit the 2JJK activity (Figure 4).

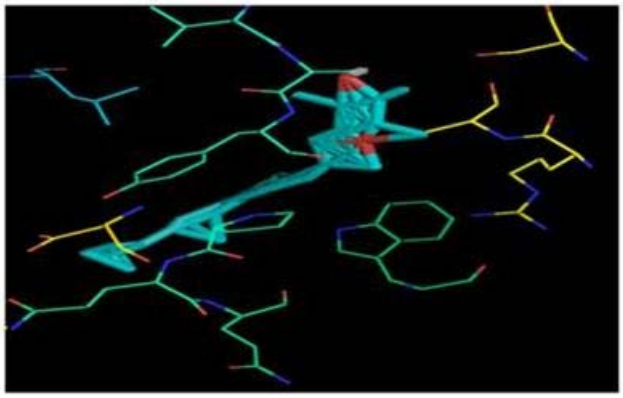

Figure 1: α- tocopherol (CID-14985) interact with binding pocket of Glucokinase (1V4S) and the interacting amino acids are Arg 63, Pro 66,Tyr 61, Ile159, Tyr 214,215, Leu 451, Val 455, Lys 459 and Val 62,452.

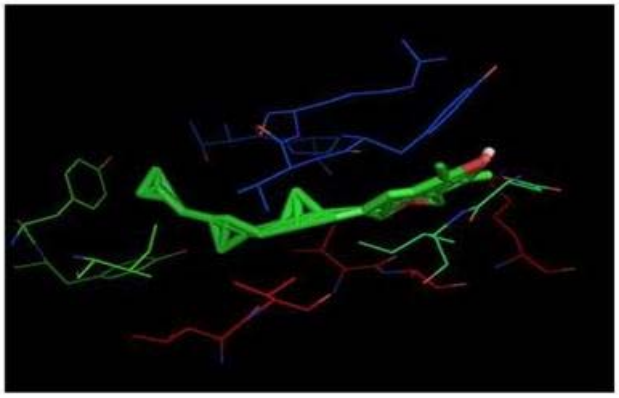

Figure 2: α- tocopherol (CID-14985) interact with binding pocket of Cytochromes P450(2CBZ) and the interacting amino acids are Leu 692, Val 708, Ala 709, Tyr 710, Pro 712, Asp 792, Gln 713,714,Trp 716, Arg 780, Ala781 and Ser 784.

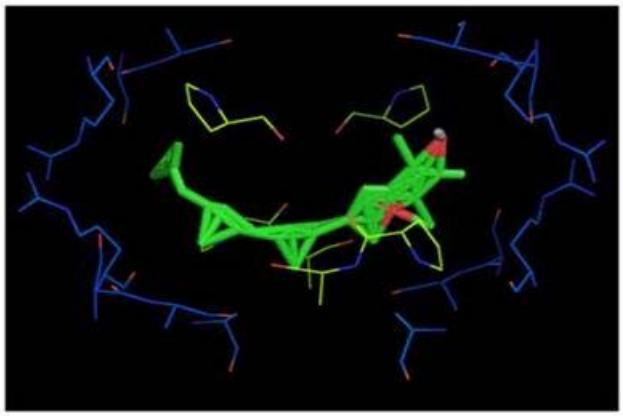

Figure 3: α- tocopherol (CID-14985) interact with binding pocket of Human multi drug resistance protein (3LC4) and the interacting amino acids are Tyr 769, Leu 930, Pro 932, Ile 820, Thr 821, Phe 819, Asp 853, Ser 854, His 544, Thr 521, Asn 859, Pro 865 and Thr 857.

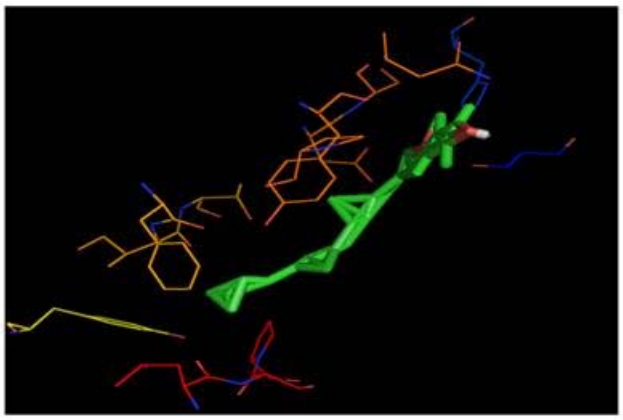

Figure 4: α- tocopherol (CID-14985) interact with binding pocket of Fructose 1, 6- bisphosphatase (2JJK) and the interacting amino acids are Arg 49,Ser 45, Ala 51, Pro 188, Ser 473, Ala 826, Arg 377, Ala 507, Pro 1143, Ser 692, Arg 1014 and Ser1011.

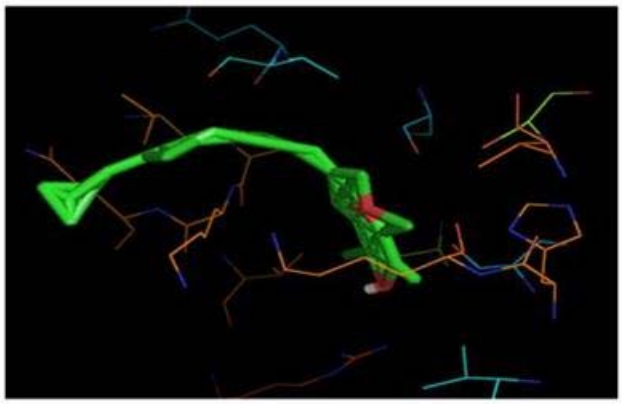

Figure 5: α- tocopherol (CID-14985) interact with binding pocket of G- protein activated inward rectifier potassium channel 3 (3QGL) and the interacting amino acids are Gln 227, Val 228, Thr 280, Gln 283, His 281, Lys 282, Val 285, Ser 406, Val 387, Lys 384, His 583, Val 330 and Arg 289.

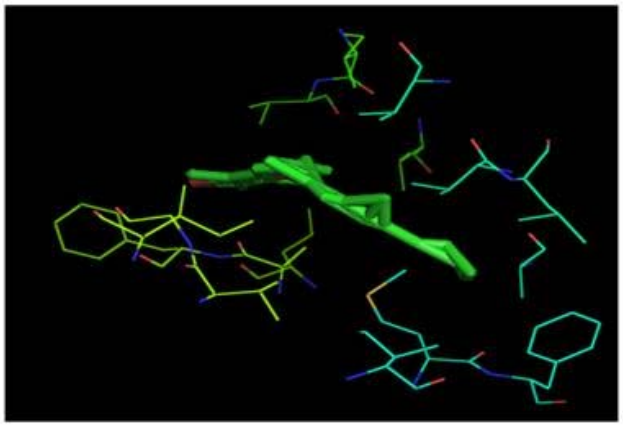

Figure 6: α- tocopherol (CID-14985) interact with binding pocket of Human mitochondrial ABC transporter (4AYT) and the interacting amino acids are Ile 445, Gly 442, Val 441, Ile 304, Phe 439, Ala 438, Ile 435, Val 413, Phe 308, Ala 315, Val 322,318, Ile 409, Met 307 and Leu 319.

Multi drug resistance protein-1 (MRP-1) is a multi specific organic anion transporter with oxidized glutathione in

endothelial cells and inhibition of MRP-1 prevent the oxidative damage of endothelial cells induced by reactive oxygen species. Therefore, MRP-1 may represent a therapeutic target in treatment of diabetes induced vascular dysfunction.[27] In the present study, Figure 3 illustrates the docking analysis of the human multi drug resistance protein 1; it showed binding interaction of α-tocopherol with target protein Tyr 769, Leu 930, Pro 932, Ile 820, Thr 821, Phe 819, Asp 853, Ser 854, His 544, Thr 521, Asn 859, Pro 865 and Thr 857 amino acid residues. These interactions may inhibit the expression of MRP-1 and reduce the diabetic induced vascular dysfunction.

Table 1: Binding energies of *Xanthium indicum* bioactive compounds towards the diabetic molecular targets in Kcal/mol. Binding affinities of plant compounds were analyzed and ranking has given according to lower energies.

X.i.bioactive compounds	1V4S	3LC4	2JJK	2CBZ	3QGL	4AYT
α-tocopherol	**-10.2**	**-8.2**	**-9.9**	**-9.6**	**-8.4**	**-9.4**
γ-tocopherol	-9.5	-8.2	-8.5	-9.5	-8.2	-8.8
Isohexacosane	-9.6	-7.7	-9.5	-8.8	-7.9	-7.4
β-sitosterol D-glucoside	-8.1	-7.6	-7.8	-8.9	-7.8	-8
Glybenclamide	-7.7	-8	-8.5	-8.7	-8.2	-8.6
Strumasterol	-7.1	-6.7	-7.5	-8.3	-8.1	-8.1
β-sitosterol	-6.4	-6.7	-6.8	-7.7	-7.7	-8
Plastoquinone	-7.1	-4	-5.5	-6.7	-6.6	-7.4
Xanthumin	-6.7	-6.1	-7.1	-6.6	-6.6	-6.3
3,4dihydroxy cinnamic acid	-6.5	-5.8	-6.4	-6.5	-6.2	-5.6
Palmitic acid	-6.3	-3.9	-5.6	-5	-4.6	-4.4
Stearyl alcohol	-6.2	-3.9	-3.9	-4.4	-4.7	-4.5
Oleic acid	-5.2	-4	-5.9	-6.8	-4.7	-4.9
Chlrobutanol	-4.1	-3.8	-4.4	-4.3	-4.1	-4.2

Rank 1: α-tocopherol; Rank 2: γ- tocopherol; Rank 3: Isohexacosane; Rank 4: β-sitosterol-D-glucoside.

Production of ketone bodies by diabetes would result in increased expression and catalytic activity of CYP2E1[8]. Numerous studies in animal models have confirmed the induction of CYP2E1 in diabetes, but this was reversed by insulin treatment. In our study, α-tocopherol interaction of human cytochrome P_{450} 2E1 protein with Leu 692, Val 708, Ala 709, Tyr 710, Pro 712, Asp 792, Gln 713,714, Trp 716, Arg 780, Ala781 and Ser 784 amino acid residues indicates good protein ligand interaction. It could may reduce the cytochrome P_{450} activity.

Mitochondrial ABC transporters were reducing the oxidative stress by preventing free radical formation in the mitochondria.[28,29] Figure 6 depicts the docking analysis of Human mitochondrial ABC transporter (4AYT) with α-tocopherol. It shows the interaction of α-tocopherol with Ile 445, Gly 442, Val 441, Ile 304, Phe 439, Ala 438, Ile 435, Val 413, Phe 308, Ala 315, Val 322,318, Ile 409, Met 307 and Leu 319 amino acid residues of 4AYT protein. These interactions were enhanced the protein expression, so it could act as good ligand for 4AYT which suggest that the incidence of diabetes complications can minimized due to decrease in the oxidative stress.

G- Protein activated inward rectifier potassium channel regulates the extra cellular concentration of potassium. Antidiabetic compounds inhibit the K^+ flux through K_{ATP} channel pore, membrane depolarization with opening of voltage dependent Ca^{2+} channels and release the insulin.[12] In the present study, Fig -5 illustrates the α-tocopherol interaction with Gln 227, Val 228, Thr 280, Gln 283, His 281, Lys 282, Val 285, Ser 406, Val 387, Lys 384, His 583, Val 330 and Arg 289 amino acids of 3QGL. It could act as good inhibitor for 3QGL and it may control the K^+ ions flow and release the ca^{2+} dependent insulin.

CONCLUSION

The present work was taken up to determine the potential efficacy of antidiabetic activity of bioactive compounds from *Xanthimum indicum* of leaf compounds through insilico studies with specific target proteins of the diabetes. Among 14 bioactive compounds, α-tocopherol has been observed as a good molecule which docks well with various targets related to diabetes mellitus. Thus, α-tocopherol can be considered for developing a potent antidiabetic drug and it can be used for further studies with pharmacological approach.

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