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RESEARCH ARTICLE

IN SILICO MOLECULAR DOCKING AND ADME/T STUDIES OF SELECTED COMPOUNDS OF *SWERTIA CHIRATA* (GENTIANACEAE) AGAINST TWO RECEPTORS OF TYPE 2 DIABETES.

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Abstract

Background: Diabetes is a metabolic disorder that leads to more complex diseases if kept untreated for a long time. Current oral hypoglycemic drugs are managing diabetes to a satisfactory level although some are associated with intense side effects. Historically natural products are playing the vital role for providing ideal lead molecules for drug development. In silico methods are ahead of conventional drug development methods for screening compounds against a target receptor with higher success rates. Thus this study aims at finding novel compounds from *Swertia chirata* that can be effectively used against diabetes through computational methods.

Material and Methods: The compounds of *S. chirata* were computationally screened by docking with Pancreatic alpha amylase (PDB ID: 1PPI) and Fructose-1,6-bisphosphatase (PDB ID: 2JJK) by Maestro v10.1 of Schrodinger LLC. In addition, ADME/T properties were analyzed through SwissADME. Best docked poses were analyzed and visualized using Discovery studio software.

Results: Molecular docking studies revealed that among the compounds of *S. chirata*, Swertiamarin had the highest docking score of -6.803 against 1PPI and Sweroside had the highest docking score of -5.161 against 2JJK, ensuring strong receptor-ligand binding. Also ADME/T analysis showed that these two molecules possess suitable properties to be considered as drug candidates.

Conclusion: The molecular docking, binding patterns and ADME/T properties of compounds in this study confirms that these phytochemicals can be good lead molecules in the treatment of diabetes.

Introduction:-

Diabetes mellitus is a common endocrine disorder now-a-days which is associated with a number of metabolic sicknesses in which the high blood glucose in plasma is observed. This happens from either deficiency of insulin

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generation, or the body cells don't react legitimately to insulin to lower blood glucose, or the both (Boulton et al., 2005; Woodrow, 2011). Improper insulin level in the body results problems in glucose metabolism which in turn badly affects the body system, particularly blood vessels and nerves. Globally around 422 million individuals (roughly 8.5% of adults) are currently experiencing this disease and the number is on the rise with time (Roglic & World Health Organization, 2016). Diabetes has become an intense medical issue with a substantial financial weight to every nation. Several groups of oral hypoglycemic agents are on the use to manage diabetes which are not devoid of characteristic profiles of side effects (Li et al., 2012; Pickup & Williams, 1991). Thus management of diabetes with fewer side effects is still a big arena where challenges are huge to the medical system. This leads to emerging demand for natural products with anti-diabetic activity as historically they have less side effects. Among the many ethno-pharmacologically used anti-diabetic plants *Swertia chirata* (Family: Gentianaceae) is a good choice as it has been used to control diabetes for a long time (Chandrasekar, Bajpai, & Mukherjee, 1990). Also this plant has hepato-protective (Karan, Vasisht, & Handa, 1999), anti-oxidant (Balasundari, Singh, & Kavimani, 2005), anti-carcinogenic (Saha & Das, 2010) and anti-inflammatory activities (Shivaji, Tapas, Suvra, Prabhash, & Sridhar, 2000). Several phytochemicals have been isolated from *S. chirata* which includes Mangiferin, Swertiamarin, Chiratenol, Episwertenol, Swertanone, Swertenol, Taraxerone, Amarogentin, Amaroswerin and Sweroside.

Pancreatic Alpha-amylase (PDB ID: 1PPI) is the main amylase enzyme found in humans as well as other mammals. This enzyme is involved in the hydrolysis of alpha bonds of large, alpha-linked polysaccharides, such as starch and glycogen, yielding glucose and maltose. It catalyzes the starting step of starch hydrolysis to glucose production and thus is a key enzyme for energy production. For this reason, alpha-amylase has become the target molecule of drug development for type 2 diabetes mellitus (T2DM) treatment. Also its inhibitors and its relationship with the disease have been extensively investigated (Nickavar & Abolhasani, 2013; Yadav, Bhartiya, Verma, & Nandkeoliar, 2013).
Fructose-1,6-bisphosphatase (PDB ID: 2JJK) is a rate controlling enzyme which play major role in gluconeogenesis process (Erion et al., 2005; Hebeisen et al., 2008) and has been the prime target for developing new medicines for T2DM treatment. Endogenous glucose production can be directly influenced by controlling the Fructose 1,6-bisphosphatase enzyme which exerts a unique strategy in treatment of diabetes (Aicher, Boyd, McVean, & Celeste, 2010).

Molecular docking is a fundamental instrument in the virtual screening of new novel compounds that can be potential for treatment of complex disorders. Docking method is a more extensive way of searching novel therapeutically active compounds as it gives molecular level data of ligand-receptor interactions. This is an accurate, fast and cost effective method in the process of drug discovery minimizing the risks of animal trials. Thus the aim of current study is to discover novel compounds against type 2 diabetes through molecular docking interactions and to analyze their ADME/T properties for a safer and effective anti-diabetic medication.

Materials and methods:-

Preparation of receptors

Three dimensional crystal structures of Alpha amylase (PDB ID: 1PPI) and Fructose-1,6-bisphosphatase (PDB ID: 2JJK) involved in T2DM were downloaded in pdb format from the RCSB Protein Data Bank (www.rcsb.org). Structures of the protein targets were prepared and refined using the Protein Preparation Wizard of Schrödinger-Maestro v10.1. Charges and bond orders were assigned, hydrogens were added to the heavy atoms, zero order bonds were created to metals, disulfide bonds were created, selenomethionines were changed to methionines, missing side chains and missing loops were filled using Prime and all waters were eradicated that were beyond 5Å from het groups. Using force field OPLS_2005, minimization was finished converging heavy atoms to 0.30 Å RMSD (root-mean-square-deviation).

Preparation of ligands

A total of 10 compounds were retrieved after thorough literature review. Compounds Mangiferin, Swertiamarin (Bhattacharya, Reddy, Ghosal, Singh, & Sharma, 1976), Chiratenol, Episwertenol, Swertanone, Swertenol, Taraxerone (Chakravarty, Mukhopadhyay, & Das, 1991), Amarogentin, Amaroswerin, Sweroside (Suryawanshi, Mehrotra, Asthana, & Gupta, 2006) were downloaded from Pubchem database in 2D sdf format. Ligprep3.3 wizard in Schrödinger Suite 2015-1 was used to prepare the ligands before performing molecular docking (Madhavi Sastry, Adzhigirey, Day, Annabhimoju, & Sherman, 2013). Three dimensional geometries were created and proper bond orders were assigned for the individual ligands applying OPLS_2005 force field (Shivakumar et al., 2010). Ionization states of the ligands were generated by using Epik3.1 of Schrödinger Suite at pH 7.0±2.0. A maximum of

32 possible stereoisomers per ligand were obtained and 1 low energy ring conformation for each ligand was created using Epik3.1.

Molecular properties and ligand based ADME/T analysis

The molecular properties of compounds play crucial role on the selection of these agents as potential drug candidates. Lipinski's rule of five (RO5) is a useful parameter to evaluate molecular properties of drug compounds for estimation of important pharmacokinetic parameters for drug design and development (Ertl, Rohde, & Selzer, 2000; Lipinski, Lombardo, Dominy, & Feeney, 1997; Veber et al., 2002). Lipinski's rule of five (RO5) filter was applied to screen the ligands as drug candidates, which states that the compound has more permeability and passive absorption if it does not violate more than one of the following conditions: (i) molecular weight (acceptable range: <500); (ii) hydrogen bond donor (acceptable range: ≤5); (iii) hydrogen bond acceptor (acceptable range: ≤10); (iv) high lipophilicity (expressed as log $P_{o/w}$, acceptable range: <5); and (v) molar refractivity should be between 40 and 130. The canonical SMILES for each compound is retrieved from PubChem and analysis was done through swissADME online database (Daina, Michielin, & Zoete, 2017).

Receptor grid generation

As each ligand should bind to a specific active site of the receptor, it is essential to have a computed receptor grid of arranged amino acid residues of proteins for fitting different ligands inside the anticipated active site during docking. Grids were created keeping the default parameters of van der Waals scaling factor 1.00 and charge cutoff 0.25 subjected to OPLS_2005 force field. A cubic box of was generated around the active site of receptor which was defined by the co-crystallized ligand. The box was generated to each direction with a measurement of 14 Å × 14 Å × 14 Å for docking experiments (Kawatkar, Wang, Czerminski, & Joseph-McCarthy, 2009; Repasky et al., 2012).

Glide standard precision (SP) ligand docking

Glide standard precision (SP) ligand docking was performed to analyze the ligand bindings with the receptors. SP docking was performed in Glide of Schrödinger- Maestro v10.1, inside which penalties were connected to non-cis/trans amide bonds. In this docking protocol the ligands are allowed to be flexible while receptor is fixed. Van der Waals scaling component and fractional charge cutoff was chosen to be 0.80 and 0.15, respectively for ligand atoms. Epik state penalties were added to docking scores and post docking minimization was performed. Final scoring was performed on energy minimized poses and showed as Glide score. Ligand poses having the least Glide score was considered as the best docked mode and recorded for each ligand. Finally the best docked poses were further analyzed for 3D binding interactions with amino acid residues using Biovia Accelrys Discovery Studio Visualizer (BIOVIA, 2017) software.

Results:-

This current study was performed to evaluate the binding affinity and binding pose of compounds isolated from the traditionally used medicinal plant *S. chirata*, for finding compounds that are potential for therapeutic activity in diabetes. Molecular docking approach of Schrodinger suite v10.1 was used to find docking score and interactions with the binding site residues. Molecules were screened for their molecular and ADME/T properties prior to docking study to validate them as potential therapeutic targets. Molecular properties of all the ligands screened through Lipinski's RO5 revealed that all compounds were within the acceptence range except Amarogentin, Amaroswerin and Mangiferin (Table 1) and these compounds were discarded from further docking studies.

Table 1:-Molecular Properties of the compounds by SwissADME

Molecule	PID	MW	HBA	HBD	MR	iLog P
Amarogentin	115149	586.54*	13*	6*	142.07*	2.46
Amaroswerin	45359883	602.54*	14*	7*	143.27*	2.61
Chiratenol	14831162	426.72	1	1	134.88*	4.65
Episwertenol	101619548	426.72	1	1	134.88*	4.67
Mangiferin	5281647	422.34	11*	8*	100.7	0.71
Sweroside	161036	358.34	9*	4	80.92	1.77
Swertanone	102285187	424.7	1	0	133.92*	4.48
Swertenol	21726415	426.72	1	1	134.88*	4.67
Swertiamarin	442435	374.34	10	5	82.12	1.74
Taraxerone	92785	424.7	1	0	133.92*	4.55

PID= Pubchem ID; MW= Molecular weight; g/mol (acceptable range: <500); HBA= Hydrogen bond acceptor (acceptable range: ≤10); HBD= Hydrogen bond donor (acceptable range: ≤5); iLogP= High lipophilicity (expressed as LogP, acceptable range: <5); MR= Molar refractivity (acceptable range: 40-130); * denotes violation of acceptance criteria.

Table 2:-Docking scores and receptor ligand complex energy parameters of ligands against 1PPI and 2JJK.

Receptor	Molecule	Docking score	Glide Energy	Glide Ligand Efficiency	Glide Emodel (kcal/mol)	Glide Gscore (kcal/mol)
1PPI	Acarbose	-7.196	-66.325	-0.164	-96.575	-7.564
	Swertiamarin	-6.803	-40.011	-0.262	-56.814	-6.803
	Sweroside	-4.593	-42.353	-0.184	-52.555	-4.593
	Episwertenol	-4.173	-35.214	-0.135	-43.325	-4.173
	Swertanone	-3.947	-36.604	-0.127	-44.428	-3.947
	Chiratenol	-3.814	-30.071	-0.123	-36.091	-3.814
	Swertenol	-3.662	-36.028	-0.118	-42.831	-3.662
	Taraxerone	-3.359	-36.337	-0.108	-42.82	-3.359
2JJK	Glibenclamide	-9.026	-61.751	-0.274	-99.415	-9.047
	Sweroside	-5.161	-35.341	-0.206	-43.964	-5.161
	Swertiamarin	-5.055	-37.69	-0.194	-47.774	-5.056
	Chiratenol	-4.054	-28.259	-0.131	-34.514	-4.054
	Swertanone	-3.502	-32.71	-0.113	-39.717	-3.502
	Swertenol	-3.394	-31.572	-0.109	-37.903	-3.394
	Taraxerone	-3.321	-27.194	-0.107	-32.492	-3.321
	Episwertenol	-3.077	-27.16	-0.099	-31.937	-3.077

Among the compounds Swertiamarin and Sweroside showed highest docking scores against 1PPI and 2JJK respectively (Table 2). Swertiamarin showed best docking score (-6.803) among the ligands against 1PPI which showed both hydrogen bond interaction (GLN63, HIS299, ARG195, ASP197, ASP300, GLU233, HIS305) and hydrophobic interaction (HIS305) with the Pancreatic Alpha-Amylase enzyme (Figure 1). Sweroside showed highest score (-5.161) against 2JJK with hydrogen bond interaction (GLY26, GLY28, LYS112, GLU29, TYR113, ALA24) and hydrophobic interaction (LEU30, ALA24) with Fructose-1,6-bisphosphatase enzyme (Figure 2). Other compounds showed docking scores ranging from -4.593 to -3.359 for Pancreatic Alpha-Amylase and -5.055 to -3.077 for Fructose-1,6-bisphosphatase enzyme.

Table 3:-Binding site and bond interactions analysis of the best docked ligand along with the standard drug against 1PPI.

Hydrogen bonds				Hydrophobic interactions			
Acarbose		Swertiamarin		Acarbose		Swertiamarin	
Residue	Distance (Å)	Residue	Distance (Å)	Residue	Distance (Å)	Residue	Distance (Å)
ILE148	1.78	GLN63	1.97	LEU162	4.23	HIS305	4.00
GLY306	2.61, 2.8	HIS299	2.28				
LYS200	2.32	ARG195	2.08				
GLU240	2.96	ASP197	2.40				
HIS201	2.24	ASP300	2.27, 2.95				
GLU233	1.84, 2.94	GLU233	1.49				
ASP197	2.18, 2.45	HIS305	3.03				
ARG195	2.64						
ASP300	2.04, 2.1						

Table 4:-Binding site and bond interactions analysis of the best docked ligand along with the standard drug against 2JJK.

Hydrogen bonds				Hydrophobic interactions			
Glibenclamide		Sweroside		Glibenclamide		Sweroside	
Residue	Distance (Å)	Residue	Distance (Å)	Residue	Distance (Å)	Residue	Distance (Å)
GLY26	1.97, 2.73	GLY26	3.05	LEU30	4.74	LEU30	4.74, 4.75
THR31	2.30	GLY28	2.81	ALA24	4.48	ALA24	4.61
GLY28	2.07, 2.71	LYS112	2.57	GLU20	4.21		
LEU30	2.46	GLU29	2.79	VAL160	4.53		
GLY21	2.30	TYR113	2.37				
ASP178	2.21	ALA24	1.97, 2.51				
LEU159	2.82						

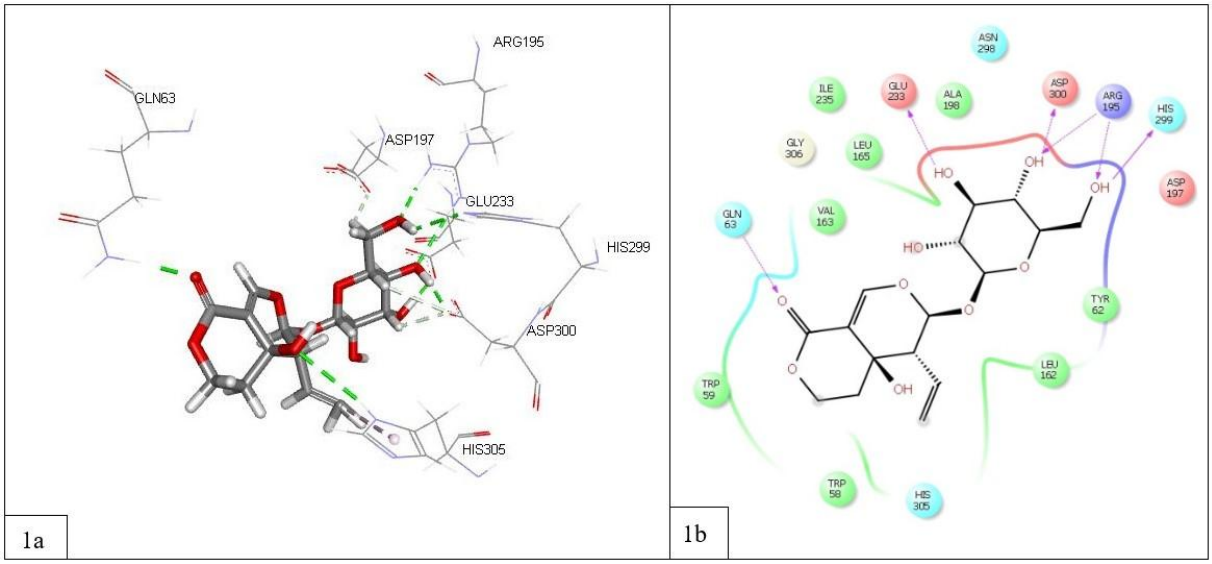

Fig 1:-Interactions of Swertiamarin with the Pancreatic alpha amylase enzyme; a (3D interactions), b (2D interactions)

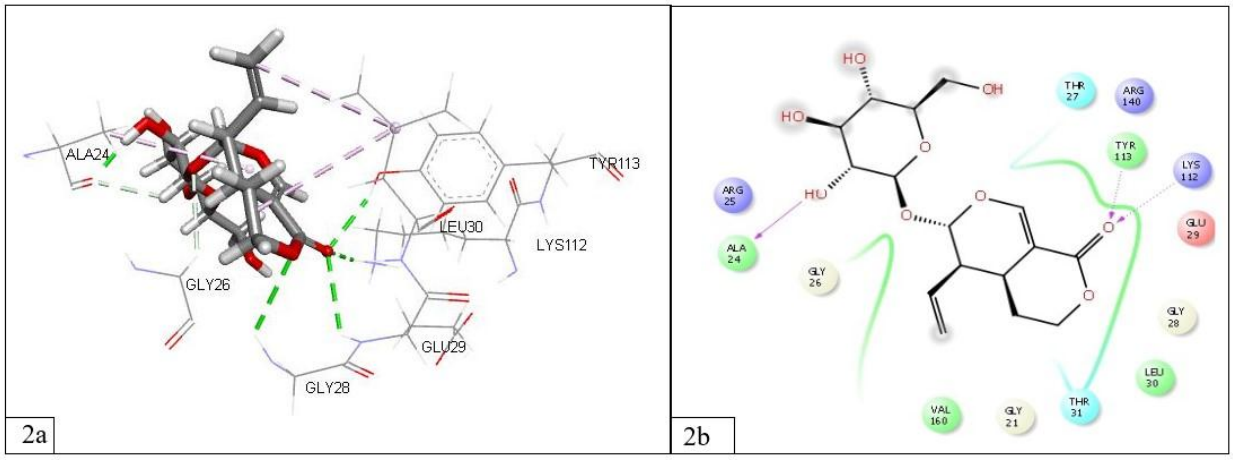

Fig 2:-Interactions of Sweroside with the Fructose-1,6-bisphosphatase enzyme; a (3D interactions), b (2D interactions)

Discussion:-

Virtual screening in drug development has gained popularly as it provides a faster and cost effective method for searching new potential drug molecules (Lionta, Spyrou, Vassilatis, & Cournia, 2014). Molecular docking is an

approach where effective virtual screening can be performed for docking and scoring of compounds against targeted ligands. The technique involves prediction of the binding modes and binding affinities of each compound in the dataset by means of docking to an X-ray crystallographic structure (França, Guimarães, Cortopassi, Oliveira, & Ramalho, 2013). The binding mode of selected compounds against Pancreatic Alpha-Amylase with A carbohydrate Inhibitor (PDB 1PPI) and Fructose-1,6-bisphosphatase complexed with a dual binding amp site inhibitor (PDB 2JJK) were investigated through computational analysis. Glide standard precision (SP) docking had been performed for both the receptors.

Swertiamarin and Sweroside showed most significant docking score compared to other compounds against 1PPI and 2JJK respectively (Table 2). The Glide Emodel scores for the compounds are -56.814 and -43.964 respectively. Lower negative energy indicates a stable system, thus resulting in the likely binding interaction that explains why the ligands are having the best docking scores. In depth evaluation of molecular docking analysis suggested that these compounds bind with the amino acid residues of receptor binding site with favorable distances.

In this docking result Swertiamarin formed eight hydrogen bonds with the amino acid residues of 1PPI which are GLN63 (1.97 Å), HIS299 (2.28 Å), ARG195 (2.08 Å), ASP197 (2.4 Å), ASP300 (2.27 Å & 2.95 Å), GLU233 (1.49 Å) and HIS305 (3.03 Å) and one hydrophobic interaction with HIS305 (4.0 Å) which is a prominent indication that it has a good interactions with Pancreatic Alpha-Amylase (Table 3). In case of interactions with 2JJK, Sweroside formed seven hydrogen bonds with the amino acid residues of 2JJK which are GLY26 (3.05 Å), GLY28 (2.81 Å), LYS112 (2.57 Å), GLU29 (2.79 Å), TYR113 (2.37 Å), ALA24 (1.97 Å & 2.51 Å) and three hydrophobic interaction with LEU30 (4.74 Å & 4.75 Å) and ALA24 (4.61 Å) which is a prominent indication that it has a good interactions with Fructose-1,6-bisphosphatase (Table 4).

It is known that the estimation of ADMET in the discovery phase substantially declines the failures related to pharmacokinetics in the clinical phase (Hay, Thomas, Craighead, Economides, & Rosenthal, 2014). It is notable that, both the two ligands Swertiamarin and Sweroside have suitable properties for oral delivery because of their lower molecular weight which increases the permeation of drugs (Duffy, Devocelle, & Shields, 2015), lower lipophilicity (log $P_{o/w}$) denoting better oral absorption and bioavailability (Daina, Michielin, & Zoete, 2014), and lower hydrogen-bonding interactions relating to higher permeability and absorption (Lipinski et al., 1997). Hence, these two compounds Swertiamarin and Sweroside are very suitable as anti-diabetic drug candidate.

Conclusion:-

Finding newer compounds with less side effects and optimum therapeutic activity is an urgent need in drug development process. The present study expresses molecular docking of important compounds from *S. chirata*, a traditionally used anti-diabetic medicinal plant against two receptors involved in diabetes (Pancreatic Alpha-Amylase and Fructose-1,6-bisphosphatase). The docking scores and binding poses of Swertiamarin and Sweroside evaluates them as suitable lead molecules for anti-diabetic drugs. Further, extensive quantitative structure activity relationship model is required to ensure its efficacy and safety inside animal models. The results suggest that this plant may be an emerging source for the development of new anti-diabetic drugs.

Conflict of interest:-

The authors declare that they have no conflict of interest.

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