MOLECULAR DOCKING & ADMET ANALYSIS OF BIOACTIVE COMPOUNDS OF *TRIGONELLA FOENUM - GRAECUM* ON GLYCOGEN SYNTHASE KINASE-3

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Abstract: Diabetes mellitus is a chronic metabolic disease characterized by persistent hyperglycemia, which may be caused by insulin resistance, decreased insulin production or both. The purpose of this study was to conduct *in silico* analysis for phytoconstituents from *Trigonella foenum – graecum*, as potential treatments for diabetic mellitus and to compare them to the conventional medication, metformin. PyRx and Biovia Discovery Studio software were used to perform *in silico* docking study against the target, GSK 3β retrieved from RCSB database (PDB ID: 1Q4L) and ligand files of phytoconstituents and standard drug (Metformin) were downloaded in sdf. format from PubChem compound database. Furthermore, pharmacokinetic qualities were studied using SWISS ADME and drug likeness properties were tested using DruLiTo. The LC_{50} of fathead minnow, daphnia magna, and oral rat was determined using the Toxicity Estimate Software Tool. The acute toxicity and organ toxicity were evaluated using ProTox-II software. Docking parameters revealed that the trigonelline and 4-hydroxy isoleucine, phytoconstituents of fenugreek had similar binding affinity to that of metformin. Further research on *in vitro* and *in vivo* models of diabetes mellitus could help understand the mechanism of action behind their therapeutic property.

Keywords: Diabetes Mellitus, *Trigonella foenum - graecum*, Metformin, DruLiTo, GSK 3β Molecular Docking.

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INTRODUCTION

India has the second-highest percentage of diabetics worldwide. Currently, more than 74 million Indians, or more than 8.3% of the adult population, have diabetes. According to the National Family Health Survey 4, the prevalence of diabetes among young and middle-aged persons is 6.7%, and the prevalence of pre-diabetes is 5.6%. 42.5 years is the average age of onset. Each year, diabetes claims the lives of upto 1 million Indians [1]. By 2035, 109 million people in India are expected to have diabetes, according to the Indian Heart Association. According to an American Diabetes Association study, by 2030, diabetes cases will rise most

rapidly in India. The high incidence is linked to a blend of genetic predisposition and the increasing middle class in India adopting a high-calorie, low-activity lifestyle [2].

Metformin is a dimethyl biguanide that is used as an antidiabetic medication. It is considered as the first-line treatment for hyperglycaemia and has been used successfully by clinicians all over the world for nearly five decades [3]. Metformin has few side effects; yet, it can produce lactic acidosis, a serious disorder that causes the following signs and symptoms: dizziness, extreme sleepiness, blue/cold skin, muscle discomfort, exhaustion, chills, rapid/difficulty in breathing, flatulence, constipation, dyspepsia, heartburn, nausea, abdominal pain, slow/irregular heartbeat, bloating, and retching are all common side effects of Metformin [4].

Traditionally fenugreek is shown to be antidiabetic in nature and used as an herbal medicinal plant all over India, Africa, and South and Central Asia. Fenugreek seed extract is used to reduce blood glucose levels due to the high content of alkaloid, trigonelline and steroid saponins, 4-hydroxy isoleucine [5]. Glycogen Synthase kinase-3β has been associated with Type 2 diabetes mellitus (T2DM). This makes GSK3β an ideal target for research on diabetes mellitus. Hence this study compared metformin and the phytoconstituents of fenugreek, to their binding nature towards the target GSK3β. Inhibitors of GSK3β act by lowering blood sugar levels by inhibiting the liver's ability to produce glucose and promoting the conversion of glucose to glycogen [6]. ADME properties, toxicity, drug-likeness using in silico methods helped to report properties and differences between allopathic drug and the herbal drug towards anti-diabetic activity.

Materials and Methods

DruLiTo

As ligands for the study, four natural phytoconstituents were chosen, including diosgenin, yamogenin, trigonelline, 4-hydroxy isoleucine, and one common medication now used to treat diabetes mellitus. The 2D structures of the ligands were obtained from the online PubChem database.

The ligands were recorded in Standard Database format (.sdf). All of the selected ligands were screened for drug-like characteristics using the DruLiTo programme [7]. DruLiTo estimates were based on the Lipinski Law, the Veber Rule, the BBB Rule, the CMC-50, and other regulations relating to drug likelihood [8]. The substances underwent testing to determine their drug-likeness properties.

Preparation of Ligand

To get the 2D structures of the phytoconstituents and currently prescribed medication for diabetes mellitus, a PubChem [9] search was carried out. The ligands were downloaded in sdf. format.

Preparation of Protein

The three-dimensional coordinates of GSK 3β (PDB: 1Q4L) were obtained from the Brookhaven Protein Data Bank. The PDB data were created with Biovia Discovery studio by modeling the missing side chains. Water molecules and non-standard residues were therefore

removed, leaving just polar hydrogen. A modest degree of regularisation, adjusting water locations, symmetry and addition of hydrogen were done. Only chain A of the corrected protein target was saved [10].

Molecular docking

Validation in molecular docking protocol is required to verify that ligands attach within the binding pocket in the right conformation, which is performed by confirming the size and center of the coordinates of the grid box surrounding the binding pocket [8]. PyRx was utilized as a simulated screening tool for computational drug research to test the ligand files against the protein [9]. PyRx employs Auto Dock 4 and Auto Dock Vina as docking approaches for the Lamarckian Genetic Algorithm and Analytical Free Energy Scoring feature. PyRx was performed on the projected, energy minimized protein structure using inhibitors of choice. The macromolecular protein structure was created using the PyRx platform and then docked onto the binding site residues within a grid box with X, Y and Z measurements [10].

SWISS ADME

In the input zone, a molecular sketcher based on ChemAxon's Marvin JS (https://chemaxon.com/) allows the user to import (from a file or an external database), sketch, and modify a 2D chemical structure before moving it to a list of molecules. On the same web page, the output panels are all displayed. The values for each molecule are collected in a single panel. When the computation is finished, one molecule at a time is added to it. We studied the pharmacokinetic properties of the compounds in this study [11].

The skin permeability coefficient has been attempted to be estimated (Kp). It is based on Potts and Guy's work [12], who established that Kp is linearly related to molecule size and lipophilicity (R2= 0.67). The less permeable the molecule is to the skin, the lower the log Kp (in cm/s) value. For evaluating active efflux through biological membranes, such as from the gastrointestinal wall to the lumen or from the brain [13], it is crucial to be aware of sub- stances that are substrates or non-substrates of the permeability glycoprotein (P-gp, suggested as the most important member among ATP-binding cassette transporters or ABC-transporters). P-gp serves a crucial purpose in defending the CNS against xenobiotic [14].

It is also necessary to understand how chemicals interact with cytochrome P450 (CYP). This isoenzyme super family plays an important role in drug clearance via metabolic biotransformation. According to the authors, 50 to 90 percent of therapeutic compounds are substrates of five main isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) [15,16]. The inhibition of these isoenzyme undoubtedly contributes significantly to pharmacokinetics related drug interactions [17,18], which can lead to toxic or other undesired adverse effects, , as a result of lower clearance rate and buildup of the drug or its metabolites [19].

TEST (Toxicity Estimation Software Tool)

The TEST software, from EPA ECOTOX allowed us to predict the values for toxicity endpoints. The five ligands were added into the test instrument utilising smiles strings and CAS numbers to swiftly analyse chemical toxicity. The programme computes an estimated LC_{50} threshold based on each model's estimates, as well as a component model consensus average [7]. Here, we adopted the consensus method to predict daphnia magna LC_{50} (48 hr), oral rat

LD_{50}andfathead minnow LC_{50} (96 hr).

ProTox-II

The toxicity of 2D structure of the molecule was predicted byuploading the structure drawn with the help of an embedded chemical editor, by canonical SMILES. Furthermore, ProTox supports the submission of files containing more than one compound in mol. format.

The report is divided into two sections: the first section containing the prediction of acute oral toxicity LD_{50} in mg/kg, classified into a toxicity class ranging from I to VI according to the Globally Harmonised System of classification of chemical labelling (GHS, United Nations, first revised edition 2005) and the second section with indication of possible toxicity targets like organ based toxicity and various toxicological endpoints like carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity have been predicted [20].

RESULT AND DISCUSSION

Worldwide, diabetes mellitus has significant rates of morbidity, prevalence, and mortality [21]. Type 1 and type 2 diabetes are diverse diseases with a wide range in clinical presentation and disease progression. A variety of hereditary and environmental causes may be responsible for the progressive decrease of β-cell mass and/or function, known as hyperglycemia [22]. In organs and tissues such as the retina, heart, kidney, nerves, and blood vessels, persistent hyperglycemia of diabetes mellitus is found to be linked to end organ damage, dysfunction, and failure [23].

Different ailments have been treated with a variety of medicinal plants. A very significant medicinal plant is *Trigonella foenum-graecum*, which has historically been used to treat a number of illnesses, including diabetes [21].

Metformin blocks the mitochondrial respiratory chain at the molecular level in the liver, activating AMPK, improving insulin sensitivity (via effects on fat metabolism), and lowering cAMP, which in turn suppresses the production of gluconeogenic enzymes [24].

The current study reveals a significant contribution of *Trigonella foenum-graecum* phytoconstituents to the treatment of diabetes mellitus. In **table 1**, a comparative evaluation of all the phytoconstituents examined for drug similarity using DruLiTo software is provided .The drug similarity qualities of phytoconstituents were determined using 'The Lipinski law of five'. Trigonelline and 4-hydroxy isoleucine both had drug-like properties. The total number of phytoconstituents violated the rule (i.e., LogP > 5) was 2 i.e., diosgenin and yamogenin, both showed a LogP value of 6.041.

Table 1. A comparison of drug likeness properties utilising DruLiTo

S.	Ligands	Lipinski's rule	Veber Filter	Blood Brain

NO									Barrier likeness
		nHD	nHA	Mol. Wt	LogP	PSA	nRTB	nH	nAcidic
1.	Metformin	3	5	129.1	-0.539	91.49	2	8	0
2.	Diosgenin*	1	3	414.31	6.041	38.69	0	4	0
3.	Yamogenin*	1	3	414.31	6.041	38.69	0	4	0
4.	Trigonelline	0	2	137.05	0.135	43.14	1	2	0
5.	4-Hydroxy isoleucine	3	4	147.09	-2.841	83.55	3	7	1

* fails the Lipinski rule of 5 as LogP value was greater than 5

nHD – number of hydrogen donors

nHA – number of hydrogen acceptors

Mol. Wt – molecular weight

PSA – polar surface area

nRTB – number of rotatable bonds

nH – number of hydrogen bonds

The docking scores of phytoconstituents were evaluated using PyRx and Biovia Discovery Studio given in **table 2**. The docking scores for yamogenin was -8.2, trigonelline had -5.1, 4-hydroxy isoleucine showed -4.4, diosgenin had -10.3 and metformin showed a score of -5, among which diosgenin demonstrated the largest binding affinity. Figure 1-5 shows the binding of phytoconstituents and metformin with GSK 3β.

Table 2. Ligand binding affinity with GSK-3β receptor using Biovia discovery studio

Compound Name	Ligand	Binding Affinity	rmsd/ub	rmsd/lb
Metformin	1q4l_4091_uff_E=136.80	-5	0	0
Diosgenin	1q4l_99474_uff_E=741.60	-10.3	0	0
Yamogenin	1q4l_441900_uff_E=753.48	-8.2	0	0
Trigonelline	1q4l_5570_uff_E=67.47	-5.1	0	0
4-Hydroxy Isoleucine	1q4l_2773624_uff_E=102.68	-4.4	0	0

rmsd/ub – root mean square deviation upper bound

rmsd/lb – root mean square deviation lower bound

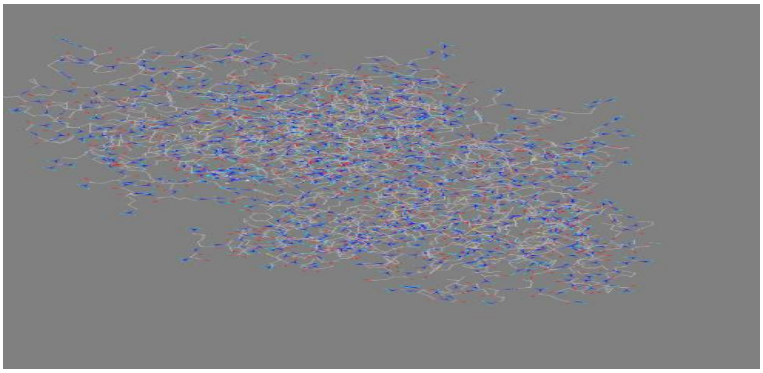

Figure 1. Binding of Metformin with GSK 3β receptor

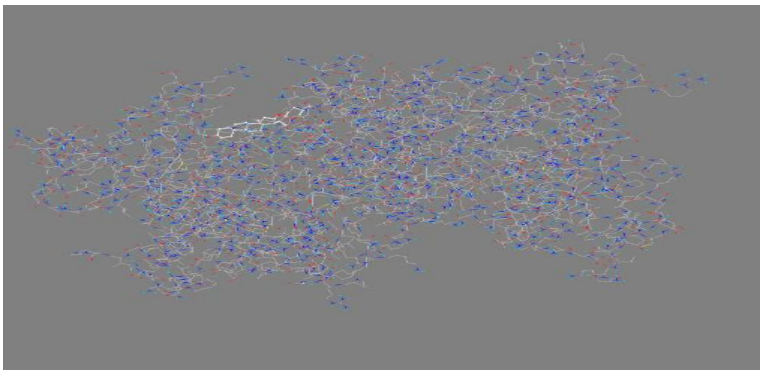

Figure 2. Binding of Diosgenin with GSK 3β receptor

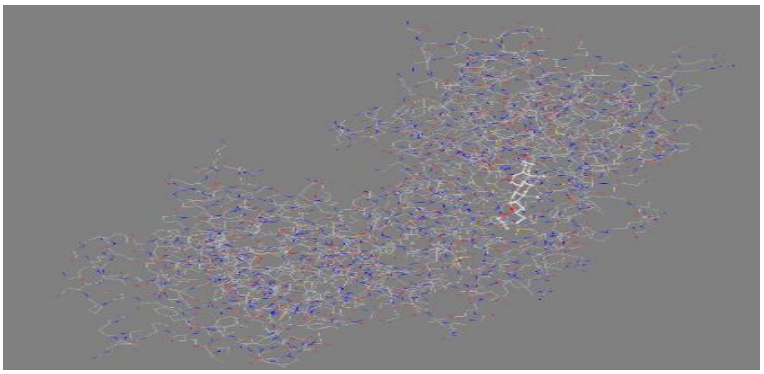

Figure 3. Binding of Yamogenin with GSK 3β receptor

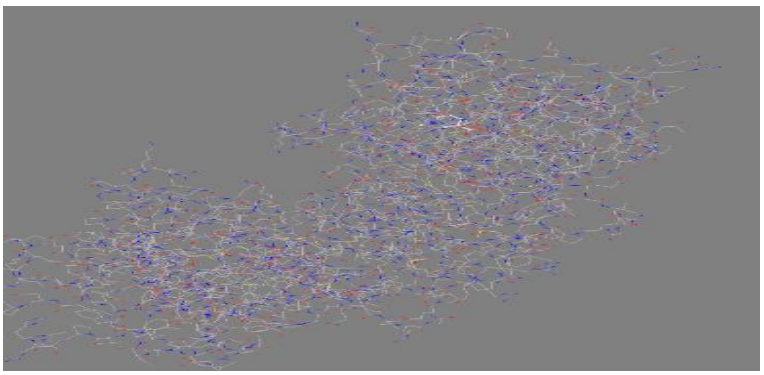

Figure 4: Binding of Trigonelline with GSK 3β receptor

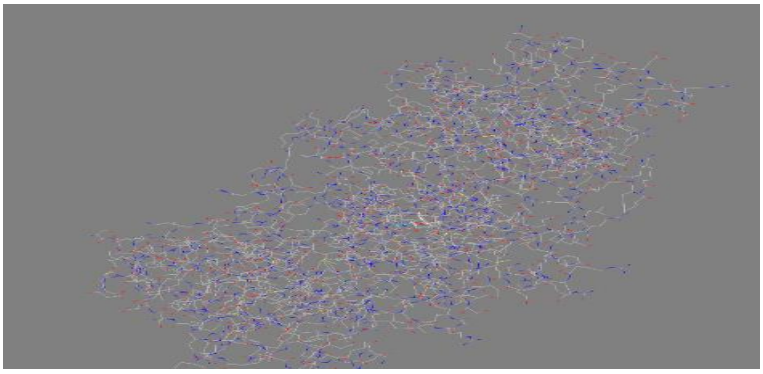

Figure 5. Binding of 4-Hydroxy isoleucine with GSK 3β receptor

The antidiabetic activity of trigonelline was supported by *in vivo* research conducted on genetic mice model of diabetes, where trigonelline was found to improve hyperglycemic condition [25]. *In vitro* antidiabetic studies of 4-hydroxy isoleucine showed remarkable antidiabetic activity [26].

The pharmacokinetic parameters were evaluated using SWISS ADME which is given in **table 3**. The phytoconstituents and metformin has shown high gastrointestinal absorption, diosgenin and yamogenin has shown BBB permeation, there was no P-gp substrate. None of the selected ligands inhibited CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4. The Kp value of all compounds values fall between 4.80 and 6.77 cm/s, while metformin showed a value of 7.99 cm/s, indicating low skin permeability. 4-hydroxy isoleucine had extremely low skin permeability.

Table 3. Pharmacokinetic parameters of compound

Pharmacokinetic parameters	**Metformin**	**Diosgenin**	**Yamogenin**	**Trigonelline**	**4-Hydroxy Isoleucine**
GI absorption	High	High	High	High	High
BBB permeant	No	Yes	Yes	No	No
P-gp substrate	No	No	No	No	No
CYP1A2 inhibitor	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No
Log K_p (skin permeation)	-7.99 cm/s	-4.80 cm/s	-4.80 cm/s	-6.77 cm/s	-9.20 cm/s

The daphnia magna LC_{50} (48 hr),oral rat LD_{50}, fathead minnow LC_{50} (96 hr) values of compounds were computed using test software, as shown in **table 4**, and they revealed tolerable toxicity levels. They can therefore undergo preclinical testing for more accurate findings.

Table 4. Toxicity end points of fathead minnow LC_{50}, daphnia magnum LC_{50} and oral rat LD_{50} using T.E.S.T software.

Ligand Name	Consensus Method End Point		
	Fathead minnow LC_{50} (96 hr) (Log10(mol/L)	**Daphnia magna LC_{50} (48hr) (Log10(mol/L)**	**Oral rat LD_{50} (-Log10(mol/kg)**
Metformin	N/A	2.50	2.92
Diosgenin	5.68	5.35	3.58
Yamogenin	5.68	5.35	3.58
Trigonelline	N/A	N/A	1.68
4-Hydroxy isoleucine	2.19	2.91	1.80

LC – lethal concentration

LD – lethal dose

mol/L – mole per liter

mol/Kg – mole per kilogram

N/A – not applicable

Using ProTox-II software, toxicity predictions have been made for all of the substances. All compounds had acceptable toxicity; however diosgenin and yamogenin revealed immunotoxicity.

Further research could be conducted to evaluate the *in vitro* and *in vivo* activity of the selected medicinal plant as a therapeutic option for type-2 diabetes mellitus, as well as to studyits pharmacokinetic parameters.

CONCLUSION

A total of 5 ligands from *Trigonella foenum – graecum* were docked on protein target, GSK 3β (PDB: 1Q4L), out of which trigonelline showed the best docking scores. This demonstrates that trigonelline could serve as a lead molecule for treating diabetes. Our research might be used as a starting point for more investigation into the antidiabetic potential of *Trigonella foenum-graecum*. The preceding results also support the ethnopharmacological understanding of this plant. As a result, it was concluded that *Trigonella foenum-graecum* has a high potential as an antidiabetic agent.

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Conflicts of Interest: The authors report no conflicts of interest in this work.

Author Contribution:

S.J., A.D. conceived of the presented idea. S.J. encouraged A.D. and M.C. to investigate the effect of phytoconstituents on GSK 3β target protein and supervised the findings of this work. A.D. and M.C. carried out the experiment. Literature search was done by A.D., M.C., K.G., V.A.R, S.M. and M.A. Manuscript was written in by K.G. with support from M.A., S.M. and C.A. Critical review was done by M.A., C.A, M.V & S.S.D. All authors discussed the

results, reviewed and accepted the final manuscript.

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