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THE LEAF ALKALOID OF *CATHARANTHUS ROSEUS* LINN. AS ANTIDIABETIC POTENTIAL: *IN SILICO* APPROACH THROUGH QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP MODELLING AND MOLECULAR DOCKING

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ABSTRACT

The medicinal herb, *Catharanthus roseus* Linn. is commonly known as Nayantara in Bengali and the extract of leaf is used for the prevention of type 2 diabetes (T2D) traditionally. The aim of study was to detect acute oral toxicity of rat by quantitative structure activity relationship (QSAR) modelling and identification of lead small molecule by molecular docking for antidiabetic phytocompounds (alkaloids). *In silico* study to detect rat oral acute toxicity of 12 phytocompounds and 2 common synthetic medicines by using ProTox-II webserver and receptor-ligand binding energy and interaction through molecular docking for phytocompounds present in *C. roseus* on tyrosine phosphatase 1B or TP1B (PDB ID: 2BGD) as causative agent for T2D. The molecular docking was performed by using PyRx tool (Version 0.8) to know favorable binding affinity and energy. The molecular interaction was visualized through molecular graphics laboratory (MGL) tool (Version 1.5.6). Present predictive study revealed that Yohimbine (40mg/Kg) obtained high acute toxicity value (LD_{50}) as class II and Glibenclamide (3250mg/Kg) as class V among 14 compounds. The molecular docking showed favorable binding energy in Ibogaine (-8.1Kcal/mol) followed by Yohimbine (-7.9Kcal/mol) when compared to synthetic medicines viz. Glibenclamide (-7.9 Kcal/mol) and Metformine (-5.0Kcal/mol) were obtained on TP1B receptor. In conclusion, the predictions showed Ibogaine could be a suitable lead candidate, which can prevent T2D. The binding was obtained at the active site and this phytoligand can be used for suitable inhibition of TP1B. It is suggested to validate the present prediction with experimental toxicology and pharmacological assay in future.

Keywords: *Catharanthus roseus*; *In silico* study; QSAR modelling; Molecular docking; Tyrosine phosphatase 1B

1. INTRODUCTION

Type 2 diabetes (T2D) is a chronic, metabolic, non-communicable disease. Prolonged untreated T2D can affect eye, heart, kidney, nervous system etc. and possible complication of diabetes is causing neuropathy, cardiovascular disease, retinopathy, nephropathy, diabetic foot etc. [1]. In the world, 425 million people have diabetes and in India over 72,946,400 were diagnosed with diabetes in 2017 [2]. India has been observing and makes feel frightening for increase in incidence of diabetes. WHO [3] documented the prevalence of diabetes in Indian lower middle-income group people were 7.8% and number of T2D deaths ages between 30-69 years for males 75900 nos. and females 51700 nos. and ages >70years males 46800 nos. and females 45600 nos. In West Bengal blood sugar level among adults (age 15-49 years) in women about 7.4% had blood sugar level showed high (>140 mg/dl) and 3.5% had blood sugar level showed very high (>160 mg/dl). In men, 11.4% had blood sugar level showed high (>140 mg/dl) and 5.9 % had blood sugar level showed very high (>160 mg/dl) as per NFHS-4 (2015-2016) data [4].

However, the inhibition of causative proteins viz. glycogen phosphorylase, protein tyrosine phosphatase 1B, etc. are important for the therapeutic efficacy to prevent diabetes [5]. Several antidiabetic synthetic drugs such as Metformin, Glibenclamide, etc. are well known [6-7] but these drugs may have side effects [8-9].

In this context, researchers are emphasizing new drug development by using natural products as per traditional knowledge. The common medicinal herb, *Catharanthus roseus* Linn. is commonly known as Periwinkle [10]. The leaf extract or powder is well known for antidiabetic

agents [11-14] as well as prevent several other diseases [15]. Till date, the exact phytocompound prevents T2D present in leaf aqueous extract or powder is unclear. It is also important to know these phytocompounds are toxic or non-toxic. For this reason, faster screening by using *in silico* prediction can easily be detected the toxic phytocompound(s) and inhibition of particular protein responsible for T2D.

Generally, in recent research, predictive toxicity study can be done through QSAR modelling by using ProTox-II webserver developed by Drwal et al. [16] and further research works done by Banerjee et al. [17], Ghosh et al. [18] and Biswas and Talapatra [19]. On the other hand, molecular docking is used for virtual screening to detect favorable binding energy of ligand through receptor-ligand binding interaction in which lead molecule can easily be identified for new drug design [20]. Few *in silico* studies have been done to know the antidiabetic effect by phytoligands through inhibition of proteins [21-22].

Present *in silico* study was to detect rat oral acute toxicity by QSAR modelling and identify suitable receptor-ligand binding energy and molecular interaction through molecular docking for common bioactive compounds of *C. roseus* on TP1B protein (PDB ID:2BGD).

2. MATERIALS AND METHODS

2.1. Selection of compounds

The selection of leaf phytochemicals (alkaloids) such as Catharanthine, Vindoline, Vindolidine, Vindolicine, Vindolinine, Ibogaine, Yohimbine, Raubasine, Vinblastine, Vincristine, Leurosine and Lochnerine of *Catharanthus roseus* Linn. were done as per literatures [23-24] and antidiabetic synthetic drugs like Metformin and Glibenclamide were selected as per Bösenberg and van Zyl [6] and Pandarekandy et al. [7]. The photograph of medicinal herb (*C. roseus*) is depicted in Fig 1.

Fig. 1: Photograph of *C. roseus* Linn

Table 1: CAS no. and SMILES of studied phytoligands and synthetic ligands

Ligands	CAS No.	Canonical SMILES
Catharanthine	2468-21-5	CCC1=CC2CC3(C1N(C2)CCC4=C3NC5=CC=CC=C45)C(=O)OC
Vindoline	2182-14-1	CCC12C=CCN3C1C4(CC3)C(C(C2OC(=O)C)(C(=O)OC)O)N(C5=C4C=CC(=C5)OC)C
Vindolidine	5231-60-7	CCC12CC(C3C4(C1[NH+](CC4)CC=C2)C5=CC=CC=C5N3C)(C(=O)OC)O
Vindolicine	1362-14-7	CCC12C=CCN3C1C4(CC3)C(C(C2OC(=O)C)(C(=O)OC)O)N(C5=C4C=C(C(=C5)OC)CC6=CC7=C(C=C6OC)N(C8C79CCN1C9C(C=CC1)(C(C8(C(=O)OC)O)OC(=O)C)CC)C)C
Vindolinine	5980-02-9	CC1C23CC(C14C5(C2N(CC5)CC=C3)C6=CC=CC=C6N4)C(=O)OC
Leurosine	23360-92-1	CCC12CN3CCC4=C(C(CC(C3)C1O2)(C5=C(C=C6C(=C5)C78CCN9C7C(C=CC9)(C(C(C8N6C)(C(=O)OC)O)OC(=O)C)CC)OC)C(=O)OC)NC1=CC=CC=C41
Ibogaine	83-74-9	CCC1CC2CC3C1N(C2)CCC4=C3NC5=C4C=C(C=C5)OC
Yohimbine	146-48-5	COC(=O)C1C(CCC2C1CC3C4=C(CCN3C2)C5=CC=CC=C5N4)O
Raubasine	483-04-5	CC1C2CN3CCC4=C(C3CC2C(=CO1)C(=O)OC)NC5=CC=CC=C45
Vinblastine	865-21-4	CCC1(CC2CC(C3=C(CCN(C2)C1)C4=CC=CC=C4N3)(C5=C(C=C6C(=C5)C78CCN9C7C(C=CC9)(C(C(C8N6C)(C(=O)OC)O)OC(=O)C)CC)OC)C(=O)OC)O
Vincristine	57-22-7	CCC1(CC2CC(C3=C(CCN(C2)C1)C4=CC=CC=C4N3)(C5=C(C=C6C(=C5)C78CCN9C7C(C=CC9)(C(C(C8N6C=O)(C(=O)OC)O)OC(=O)C)CC)OC)C(=O)OC)O
Lochnerine	522-47-4	CC=C1CN2C3CC1C(C2CC4=C3NC5=C4C=C(C=C5)OC)CO
Metformin	657-24-9	CN(C)C(=N)N=C(N)N
Glibenclamide	10238-21-8	COC1=C(C=C(C=C1)Cl)C(=O)NCCC2=CC=C(C=C2)S(=O)(=O)NC(=O)NC3CCCCC3

Table 1 tabulates the CAS (chemical abstracts service) No. and SMILES (simplified molecular-input line-entry system) of each compound were taken from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). All the compounds were obtained .pdb file after incorporating SMILES in the CORINA online server (www.mn-am.com/online_demos /corina_demo), which were required in molecular docking study.

2.2. QSAR modelling for acute toxicity prediction

QSAR modeling for predictive toxicity study especially rat oral acute toxicity to know median lethal dose (LD_{50}) as mg/Kg was done by using ProTox-II webserver developed by Drwal et al. [16] and protocol established by Banerjee et al. [17]. The toxicity prediction was carried out for 12 phytoligands and 2 synthetic antidiabetic medicines.

2.3. Selection of protein

The crystal three-dimensional (3-D) structure of protein namely Tyrosine Phosphatase 1B or TP1B (PDB ID: 2BGD) was downloaded from the website of protein data bank (www.rcsb.org). Black et al. [25] experimented and deposited the X-ray diffraction crystallographic structure of this receptor at 2.40Å resolution. The three-dimensional (3-D) ribbon structure of TP1B is depicted in Fig 2 after visualizing in MGL tool developed by The Scripps Research Institute [26]. The attached inhibitor molecule 5-(4-Methoxybiphenyl-3-YL)-1, 2, 5-Thiadiazolidin-3-One 1, 1-Dioxide (T1D) was removed from the target protein to maintain biasness of inhibitory activity during receptor-ligand binding.

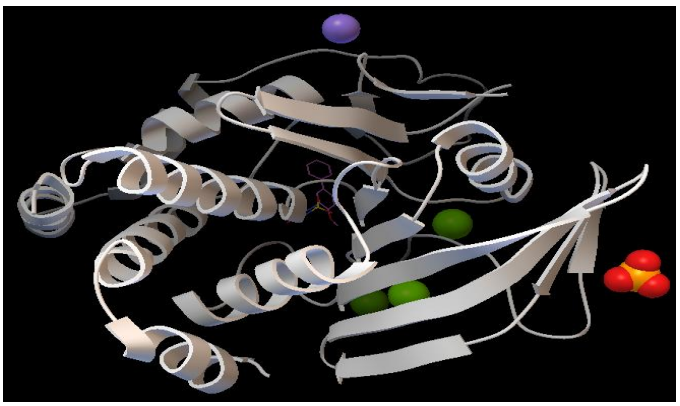

Fig 2: 3-D ribbon structure of target receptor (PDB ID: 2BGD) [chain A = white colour attached with inhibitory molecule (T1D) as line structure at 1298 position; Cl = green ball at 1299-1301 position; PO4 = yellow and red balls at 1302 position; Na = cyan ball at 1303 position]

2.4. Molecular docking and interaction

The molecular docking was done by using PyRx software (Version 0.8) developed by Trott and Olson [27]. The molecular docking was visualized as output .pdbqt file and the result of suitable lead was rendered by using MGL tool [26]. The docking was carried out with 12 alkaloids and 2 synthetic antidiabetic medicines on Tyrosine Phosphatase 1B (PDB ID: 2BGD) receptor was analysed to detect suitable binding energy value. The receptor-ligand interaction of this target protein and phytoconstituents (ligands) were identified to detect the residues involved in each case for the therapeutic efficacy of T2D. The 3-D grid box size values such as X = 67.5882, Y = 51.2883 and Z = 43.5405Å and central position values viz. X = -0.0823, Y = 59.6910 and Z = 15.4450Å respectively for docking site on the studied target protein with a grid spacing of 0.375 Å. Finally, all the 12 phytoligands and 2 synthetic ligands were analysed to detect energy value and binding location along with amino acids interaction by using this tool.

3. RESULTS

3.1. QSAR modelling for toxicity prediction

Table 2 describes the rat oral acute toxicity (LD_{50}) value as mg/Kg, predicted different toxicity classes (I–VI) and prediction accuracy in % for different phyto and synthetic compounds. Among 12 phytocompounds, Yohimbine obtained high acute toxicity value (LD_{50} = 40mg/Kg) as class II, which prescribed fatal after swallowing ($5<LD_{50}\leq 50$) with 100% prediction accuracy. In case of class III compounds (prescribed toxic after swallowing *i.e.* $50<LD_{50}\leq 300$) such as Vindolicine (68mg/Kg), Catharanthine (130mg/Kg), Vindoline (150mg/Kg) and Lochnerine (182mg/Kg) with prediction accuracy 68.07%, 67.38%, 68.07% and 69.26% respectively were obtained. Same LD_{50} value (305mg/Kg) was obtained for Vinblastine, Vincristine and Leurosine with prediction accuracy 100% for former compound and 72.90% for other two compounds as class IV (prescribed may be harmful after swallowing i.e. $2000<LD_{50}\leq 5000$). The LD_{50}value of other class IV compounds viz. Vindolidine and Vindolinine (325mg/Kg), Ibogaine (327mg/Kg), Raubasine (400mg/Kg) and Metformine (680mg/Kg) with prediction accuracy 68.07%, 100% and 54.26% respectively were obtained while only one compound namely Glibenclamide was obtained LD_{50} value 3250mg/Kg as class V (may be harmful after swallowing i.e. $2000<LD_{50}\leq 5000$) with 100% prediction accuracy. According to Drwal et al. [16], these toxicity classes have been mentioned in ProTox-II webserver.

Table 2: Predictive toxicity of studied phytoligands and synthetic ligands

Ligands	Rat oral LD_{50} (mg/Kg)	Prediction class	Prediction accuracy (%)
Catharanthine	130	III	67.38
Vindoline	150	III	68.07
Vindolidine	325	IV	68.07
Vindolicine	68	III	68.07
Vindolinine	325	IV	68.07
Ibogaine	327	IV	100.00
Yohimbine	40	II	100.00
Raubasine	400	IV	100.00
Vinblastine	305	IV	100.00
Vincristine	305	IV	72.90
Leurosine	305	IV	72.90
Lochnerine	182	III	69.26
Metformine	680	IV	54.26
Glibenclamide	3250	V	100.00

3.2. Molecular docking and interaction

In Table 3, the data of favourable binding energy values, two phytoligands such as Ibogaine obtained -8.1 Kcal/mol followed by Yohimbine (-7.9Kcal/mol) when compared to synthetic medicines viz. Glibenclamide (-7.9 Kcal/mol) and Metformine (-5.0Kcal/mol) were obtained on TP1B receptor. Other studied phytoligands showed below energy values compared to above-mentioned two phytoligands.

Table 3: Binding energy value of studied phytoligands and synthetic ligands

Ligands	Binding energy (Kcal/mol) without inhibitor
Ibogaine	-8.1
Yohimbine	-7.9
Raubasine	-7.7
Vincristine	-7.4
Catharanthine	-7.0
Leurosine	-6.8
Vinblastine	-6.7
Vindolidine	-6.5
Vindolicine	-6.5
Vindolinine	-6.5
Lochnerine	-6.4
Vindoline	-5.8
Glibenclamide	-7.9
Metformine	-5.0

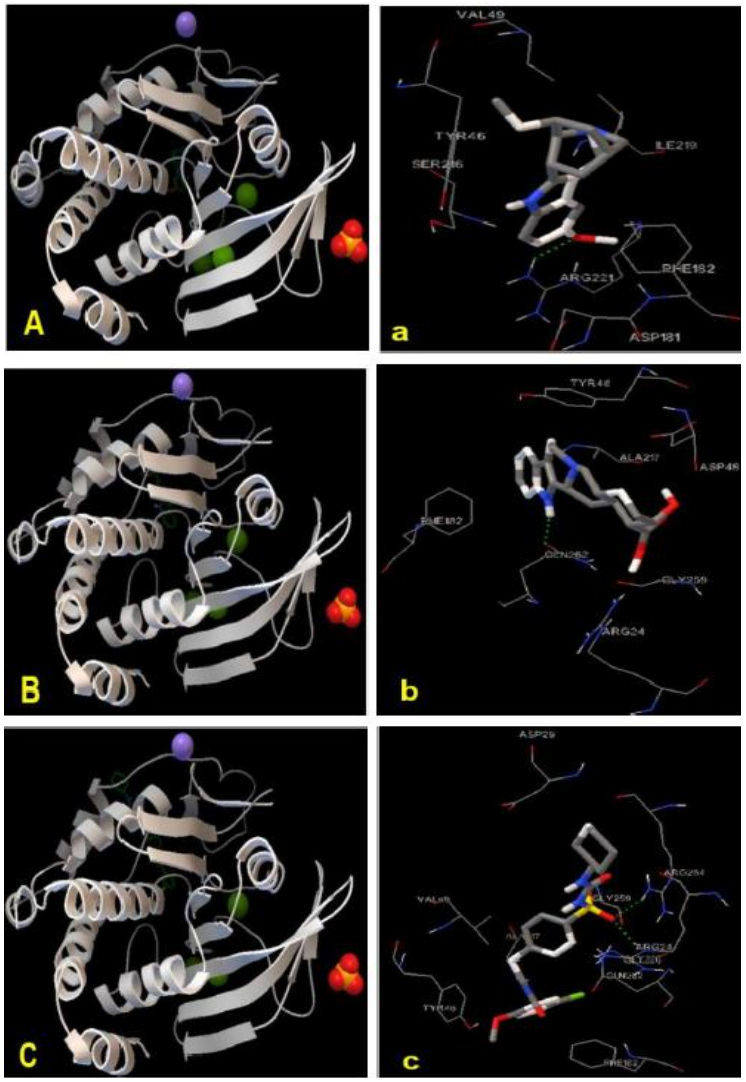

Fig 3: Binding pose and interaction study of favorable energy based phytoligands and synthetic ligand on TP1B (A & a = Ibogaine; B & b = Yohimbine and C & c = Glibenclamide)

In case of receptor-ligand binding pose and interaction study on TP1B the contact residues such as VAL49, TYR46, ILE219; SER216, PHE182 and ASP181 as well as one hydrogen bonding with residue ARG221 were obtained for Ibogaine while the contact residues such as TYR46, ASP48, ALA217, PHE182, GLY259 and ARG24 as well as one hydrogen bonding with residue GLN262 were obtained for Yohimbine. In comparison with Glibenclamide, the contact residues such as ASP29, GLY259, ALA217, VAL49, GLN262, GLY220, PHE182 and TYR46 as well as two hydrogen bonding with residue ARG24 and ARG254 were obtained. The pose and interaction of suitable phytoligands and synthetic ligand is exhibited in Fig 3A-a, B-b and C-c.

4. DISCUSSION

The present predictive toxicity results indicated that all phytoligands (alkaloids) of *C. roseus* and synthetic antidiabetic medicines were observed toxic class of III, IV and V except the phytoligand Yohimbine as lower LD_{50} values (40.0 mg/Kg) as class II obtained by the online webserver (ProTox-II). According to Anderson et al. [28], it was observed that overdoses of Yohimbine led to neurotoxic effects and the concentration in blood found up to 5,000 ng/mL. In earlier study, rat oral LD_{50} was determined 43mg/Kg of Yohimbine [29] and a close similarity was observed in the present toxicity prediction. In several experimental studies, it was reported that leaf extracts or powder of *C. roseus* are suitable for antidiabetic agents [12-15] but it was uncleared about exact phytoligand that is preventing T2D except other *in silico* works on Glucose transporter-4 or GLUT4 and TP1B [30-31].

In the present prediction, the favorable binding energy value was obtained for Ibogaine -8.1 Kcal/mol followed by Yohimbine (-7.9Kcal/mol) when compared to synthetic medicines viz. Glibenclamide (-7.9 Kcal/mol) and Metformine (-5.0Kcal/mol) on TP1B receptors. It was documented that residues such as GLY220, TYR46, VAL49 and ASP48 found inside the active site of protein tyrosine phosphatase1B [30]. In the present molecular docking, the contact residues TYR46 and VAL49 were observed only in phytoligand Ibogaine and synthetic medicine Glibenclamide. In another *in silico* study, the inhibitory activity of TP1B was obtained by the phytoligand namely Diospyrin showed active site of contact residues viz. GLY220, TYR46, VAL49 and ASP48 and in present interaction study revealed two contact residues as TYR46 and VAL49 were obtained in the binding site as supported by previous study by Bawazeer et al. [31].

5. CONCLUSIONS

In conclusion, the result of molecular docking is indicated the antidiabetic potential of Ibogaine found in the leaf of *C. roseus*. Moreover, Ibogaine was obtained LD_{50} value (327mg/Kg) as toxicity of class IV. This small molecule, Ibogaine may be suitable lead compound for T2D therapy because this phytoligand showed lower toxicity value as well as favorable energy and found active site binding of TP1B. Further studies are suggested based on toxicology and pharmacology in *in vivo* and *in vitro* models to validate this prediction.

6. ACKNOWLEDGEMENT

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Conflict of interest

Authors declare no conflict of interest.

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