



An *in silico* Evaluation of Anti-diabetic Potential of Vasicine, A Quinazoline Alkaloid of *Justicia adhatoda* Linnaeus

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ABSTRACT

Background: Diabetes mellitus, a chronic metabolic disease with multiple secondary effects, is on the rise. Treatment of type II diabetes mellitus with phytochemicals is gaining importance to avoid secondary complications used in the treatment. *Justicia adhatoda* is said to be an evergreen perennial shrub with a multitude of uses, especially as a stimulant of the respiratory tract.

Methods: Vasicine, a major alkaloid extracted from the leaves of the *adhatoda* was used in the current study to identify its antidiabetic activity through the Computer Aided Drug Design technique. The molecular docking technique was performed between vasicine and five different receptors Protein Tyrosine Phosphate 1B (PTP 1B), Glucose Transporter (GLUT2), Glucagon Like Peptide 1 (GLP-1) and Peroxisome Proliferator Activated Receptors (PPAR γ) which are usually targeted by anti-diabetic drugs. The Libdock score for the interaction between the receptors and vasicine is indicative of the antidiabetic activity of the vasicine in various pathways.

Result: PTP 1B presented the highest LibDock score of 101.46 indicating the antidiabetic property of vasicine.

Key words: *In silico*, PPAR γ , PTP 1B, Type II diabetes, Vasicine.

INTRODUCTION

Diabetes mellitus is one of the major chronic metabolic diseases prevalent in the current times. It is a metabolic disease with glucose intolerance, microangiopathy, neuropathy and insufficient insulin secretion (Hosseini and Abdollahi, 2013).

Justicia adhatoda Linnaeus, commonly known as vasaka is an evergreen perennial shrub found throughout the tropical areas of Southeast Asia including India (Dhankar *et al.*, 2011). In traditional Ayurveda, it has a multitude of uses, leaves of vasaka had a stimulatory effect on the respiratory tract (Gangwar and Ghosh, 2014). For ages, it has been used to treat asthma, chronic bronchitis and other respiratory conditions. In some regions, it is also used to stimulate uterine contractions to speed up childbirth. Leaves of *adhatoda* are considered as potent antimicrobial agents. Apart from these major activities, it is said to have insecticidal, anti-ulcer (Chaturvedi *et al.*, 1983) and wound-healing activities (Zainab *et al.*, 2020). It was observed that the ethanolic extracts of leaves and the roots of the *Justicia adhatoda* plant had antidiabetic activity in diabetic rats (Gulfray *et al.*, 2011). Leaves of *adhatoda* chiefly contain alkaloids such as vasicine and vasicinone. Besides, vasicine and vasicinone, the leaves also contain vasicoline, vasicolinone, vasicinol, *adhatodine*, *adhatonine* and *anisotine* (Yadav and Yadav, 2018).

Due to the lack of phytochemical studies on the interaction of vasicine with potential receptors involved in the treatment of Diabetes mellitus, it is proposed that vasicine can a potential antidiabetic agent with the outcome from the molecular docking studies.

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Conventional drug discovery and development are risky, time-consuming processes that include target identification, lead compound development, preclinical and clinical trials. Recently, Computer-Aided Drug Discovery (CADD) is receiving increasing attention as it can help manage the time and cost issues present in conventional experimental approaches (Tang *et al.*, 2006).

CADD includes the identification of potential drug targets, virtual screening of large chemical libraries for effective drug compounds and their optimization followed by *in silico* assessment of their potential toxicity. The compounds put through these processes are subjected to *in vitro* and *in vivo* experiments. CADD approaches reduce the

number of chemical compounds to be evaluated experimentally by removing inefficient and toxic chemical compounds (Segall and Barber, 2014).

Insulin is a critical hormone that augments multiple functions to control many cellular activities such as glucose homeostasis, protein synthesis, gene transcription and substrate metabolism.

The receptors involved in inducing hypoglycemia in diabetes mellitus such as PTP 1B, GLUT2, GLP-1 and PPAR γ were chosen for docking studies.

MATERIALS AND METHODS

Protein preparation

RCSB (Research Collaboratory for Structural Bioinformatics) Protein Data Bank (PDB) (<https://www.rcsb.org>) was used to obtain the three-dimensional crystal structure of proteins UniProt (<https://www.uniprot.org/uniprotkb>) for the receptors PTP 1B (Protein ID: P20417), GLUT2 (Protein ID: P12336) in PDB format and PPAR γ (Protein ID: 1ZGY), GLP 1 (Protein ID: 5VAI) in PDB format.

Using the protein protocol of Discovery Studio 4.0 (DS 4.0) protein preparation was done by the removal of water molecules and heteroatoms present in the crystal structure. The prediction of the active site in the prepared protein was done using the receptor cavities option in DS 4.0.

Ligand selection

For the interaction with the proteins on the cell membrane, the active phytochemical vasicine from *Justicia adhatoda* was retrieved from the PubChem compound database (<https://pubchem.ncbi.nlm.nih.gov>) (PubChem ID: 667496) in 2-dimensional SDF (Structure Data File) format. Following this, the ligand optimization, energy minimization and conversion of retrieved ligands to 3D PDB format and clean geometry were performed.

Software

The study was performed at Bioinformatics and ARIS cell at Madras Veterinary College, Chennai during 2021-2022. Discovery studio Biovia 2020 (developed and distributed by Dassault systems BIOVIA) was used to visualize and modify receptor and ligand structures and perform docking.

Pharmacodynamic analysis of vasicine

The Pharmacodynamic analysis was carried out in the Prediction of Activity Spectra for Substances (PASS) Online server. The PASS server is opened through the URL: www.pharmaexpert.ru/passonline. The SMILES format is entered into the tool for the prediction. Pa (Probability for active compound) and Pi (Probability for inactive compound) are predicted in the PASS result (Rahim *et al.*, 2020). Activities with Pa > Pi was considered as possibilities for vasicine. The possible activities for vasicine were depicted in the Fig 1.

Pharmacokinetic analysis of vasicine

The ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) prediction was done using the admetSAR

2 online server and significant characteristics of the vasicine was derived from the results (Table 1). For the compound to be considered a good drug it should be efficiently absorbed and distributed for it to be active biologically. The prediction indicates that vasicine can cross the blood brain barrier and the human intestinal absorption is also positive.

The said compound vasicine follows the Lipinski's rule of 5 (Walters, 2012) making it a good drug candidate. The molecular weight of the vasicine is 188.23 Da (≤ 500 Da), A log P 1.30 (≤ 5), H bond acceptor 3 (≤ 10), H bond donor 1 (≤ 5) and rotatable bonds 0 (≤ 10).

RESULTS AND DISCUSSION

Molecular docking was performed using the LibDock protocol of Biovia Discovery studio software 2020 under receptor-ligand interaction. The target protein (enzyme) molecule was the receptor molecule and the identified phytochemical was the ligands. The "LibDock score", binding energy, relative energy and the hydrogen bond distance in the docking interaction were used to identify the quality of molecular docking performed (Table 2). The high positive LibDock score was indicative of good interaction between the ligand and the receptor since the greater the LibDock score indicates the better binding affinity (Cao *et al.*, 2015). Hydrogen bonds were common usually essential for various interactions such as protein-ligand interactions, protein folding and catalysis (Prabhu *et al.*, 2022). By promoting molecular interactions, hydrogen bonds diversify a variety of biological activities. Amino acid residues involved in the receptor-ligand interaction were depicted in Table 3.

After the identification of receptors in the diabetic pathway and phytochemical vasicine, molecular interaction was undertaken using BIOVIA discovery studio 2020.

Protein tyrosine phosphate 1B (PTP 1B)

It plays a major role in the insulin signalling pathway. It interacts with and removes tyrosine phosphates from

All Pa>Pi Pa>0,3 Pa>0,7		
0,682	0,008	Alopecia treatment
0,670	0,025	Ribulose-phosphate 3-epimerase inhibitor
0,653	0,010	4-Hydroxyproline epimerase inhibitor
0,657	0,031	5-Hydroxytryptamine release stimulant
0,638	0,015	(R)-6-hydroxynicotine oxidase inhibitor
0,659	0,069	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0,593	0,005	Antineoplastic (multiple myeloma)
0,665	0,077	Gluconate 2-dehydrogenase (acceptor) inhibitor
0,612	0,032	Glucan endo-1,6-beta-glucosidase inhibitor
0,612	0,042	Pseudolysin inhibitor
0,590	0,023	Pullulanase inhibitor
0,623	0,061	Nicotinic alpha6beta3beta4alpha5 receptor antagonist
0,634	0,074	CYP2J substrate
0,606	0,047	Nicotinic alpha2beta2 receptor antagonist
0,581	0,029	27-Hydroxycholesterol 7alpha-monooxygenase inhibitor
0,594	0,043	Kidney function stimulant
0,604	0,054	Sugar-phosphatase inhibitor
0,561	0,020	Antiasthmatic
0,558	0,019	Polarisation stimulant
0,598	0,063	CYP2J2 substrate
0,552	0,024	(S)-6-hydroxynicotine oxidase inhibitor
0,588	0,068	Glycosylphosphatidylinositol phospholipase D inhibitor
0,498	0,032	Cyclic AMP agonist
0,465	0,009	Antineoplastic alkaloid

Fig 1: Pharmacodynamic analysis of vasicine.

insulin receptors induced by autophosphorylation in response to insulin binding (Wang *et al.*, 2015). It was found to be localized on the endoplasmic reticulum of the cytoplasm of cells. Several synthetic and naturally derived compounds are described as PTP-1B inhibitors. The PTP 1B is an attractive target and is a negative regulator of insulin-receptor and leptin-receptor signalling pathways. The PTP 1B is said to dephosphorylate insulin and leptin receptors, causing their deactivation. Inhibitors of this enzyme may be useful as therapeutics for the treatment of type II diabetes and obesity (Verma *et al.*, 2017).

In the present study, PTP 1B presented the highest LibDock score of 101.46 with zero relative energy during the interaction. This is indicative of the ability of the vasicine to be a potent PTP-1B inhibitor since the LibDock score is said to be an indicator of binding affinity (Cao *et al.*, 2015). Inhibition of PTP-1B could be a very important target to develop new antidiabetic drug candidates (Verma *et al.*, 2017).

In this study, the hydroxyl group of vasicine formed a hydrogen bond with the Ser203 while the hydrogen formed a carbon bond interaction with Gly210 of PTP 1B. It also

formed alkyl and π -alkyl interactions with Pro 206, Arg79, Leu 204 and Ile 211.

Peroxisome proliferator-activated receptors (PPAR γ)

The activity of glucose pathways can be regulated in a co-ordinated pattern at the level of protein-protein interactions or the transcriptional level by nuclear factors and cofactors such as peroxisome proliferator-activated receptors (Bouche *et al.*, 2004). The agonists of PPAR γ on stimulation increase the interactive sensibility of muscle, adipose tissue and liver to the action of insulin (Bermudez *et al.*, 2010).

In the current study, PPAR γ formed a carbon-hydrogen bond with Asp260, a π -alkyl bonds with Lys261 and Ile 341. The libdock score for the interaction and amino acid residues were indicated in Table 2 and 3.

GLUT 2

These are the glucose transporters working through facilitated diffusion. It is expressed mainly in beta cells of the pancreas, hepatocytes and kidney. It plays a major role in the uptake of glucose (Woodbury, 2011). The rate of transportation of glucose by GLUT2 is largely proportional to the ambient glucose concentration, which allows sensing the changes in glucose levels in the postprandial state (Bouche *et al.*, 2004).

In the present study, Vasicine interacted with GLUT 2 receptors that facilitate glucose transportation across the membrane. A conventional hydrogen bond was formed between the Hydroxyl group of vasicine and Asp 51 and a Vander Waals interaction between a hydrogen and Pro 49.

Glucagon like peptide 1

This receptor is expressed in beta cells. It rapidly and potentially stimulates insulin secretion and inhibition of glucagon release by pancreas. It also stimulates insulin gene transcription, islet cell growth and neogenesis (MacDonald *et al.*, 2002). GLP-1 agonist work like natural incretin hormones secreted by the small intestine.

Table 1: ADMET properties prediction of vasicine.

Parameters	Value	Probability
Human intestinal absorption	+	0.9936
Blood brain barrier	+	0.7379
Human oral bioavailability	+	0.6286
Subcellular localization	Mitochondria	0.6652
Carcinogenicity (binary)	-	0.9600
Hepatotoxicity	+	0.6000
Respiratory toxicity	+	0.9333
Reproductive toxicity	+	0.8667
Mitochondrial toxicity	+	0.9625
Nephrotoxicity	-	0.5814
PPAR gamma	+	0.6375
Biodegradation	-	0.8500

Table 2: Libdock score of the receptors with phytochemical vasicine.

Receptors	Active site with high libdock score/Total number of active sites	Libdock score	Relative energy	Absolute energy	H bond distance A°
PTP 1B	2 /6	101.426	0	30.5687	2.0-3.09
GLP 1	7 /45	85.5163	-0.464944	31.0337	2.0-3.2
PPAR γ	2 /5	73.8564	-0.464944	31.0337	2.0-3.9
GLUT 2	5 /5	72.5857	-0.464944	31.0337	2.0-3.09

Table 3: Amino acid residues in the receptor-ligand interaction.

Receptors	Amino acid residues
PTP 1B	Gly A:209, Ser: 203, Arg: 79, Leu :204, Ile :211, Pro :206,
GLP 1	Gln R:234, Ser P:14, Phe R:230, Arg R:299, Trp R: 299, Lys R:288
PPAR γ	Asp A:260, Gly A:345, Ile A: 341, Lys A:261
GLUT 2	Asp A:51, Pro A:49, Thr A: 84, Ala A: 88

GLP-1 stimulation results in insulin production and islet neogenesis. In this study, the LibDock score of the interaction between vasicine and GLP-1 was 85.51. The nitrogen in the vasicine formed a hydrogen bond with Gln234 while π -alkyl and π - π T shaped bonds were formed with Phe230 and Trp297 respectively. π -cation bond was seen between vasicine and Lys288 and Arg299.

CONCLUSION

Justicia adhatoda also known as *Adhatoda vasica* belongs to the family Acanthaceae. It is used mainly as a respiratory stimulant anti-ulcer, wound healing and antimicrobial agent. It was found that leaves and root ethanolic extract was having antidiabetic activity in diabetic rats. Vasicine, a quinazoline alkaloid present in the leaves and roots of *adhatoda* is considered for the present study. *In silico* binding of vasicine with protein receptors involved in insulin production and glucose uptake indicate that it is a potential candidate for inducing insulin secretion. The interaction also provides insight on the potential of vasicine in proliferation of pancreatic islet cells. The other facet of *In silico* evaluation of vasicine depicts suppression of the free glucose and also inhibits non-carbohydrate source conversion to glucose. The study highlights vasicine as a promising drug candidate against insulin-dependent diabetes.

Vasicine can be formulated as a targeted drug delivery system but these studies need validation of *in-vitro* and *in vivo* evaluation.

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Conflict of interest

The authors declare that there is no conflict of interest.

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