MEDICINAL PLANTS ARE EFFECTIVE INHIBITORS OF TYPE I AND II DIABETES

Anum Munir[1, 2], Shaukat Iqbal Malik[1*], Sehar Aslam[1], Azhar Mehmood[3], Saeed Amjad[3], K. A. Malik[4], Muhammad Younis[3], Abbas Husain Shah[5], G. Mujtaba Shah[2]

1. *Department of Biosciences, Faculty of health and life sciences, Capital University of Sciences and Technology, Islamabad, Pakistan,*
2. *Department of Bioinformatics, Govt. Post Graduate College Mandian Abbottabad, Pakistan,*
3. *Department of Botany, Hazara University, Mansehra, Pakistan,*
4. *Department of SMME, National University of Science and Technology, Islamabad, Pakistan*
5. *Department of Bioinformatics, Govt. Post Graduate College Mansehra, Pakistan.*

A R T I C L E   I N F O

A B S T R A C T

There are two types of diabetes: Diabetes Mellitus and Diabetes insipidus. Diabetes mellitus occurs because of pancreatic beta-cell destruction and insulin deficiency, Diabetes insipidus occurs because of the deficiency of vasopressin (AVP). This study was conducted to determine the In-silico efficacy and suitability of the chemical constituents of medicinal plants that are used to cure diabetes. The chemical compounds of medicinal plants: Bauhinia Variegata, Prunella Vulgaris, Aloe Vera, and Clematis Montana, used to cure diabetes were checked for toxicity, only 5 chemical constituents lying in non-toxic classes, were further analyzed and docked with mutated Insulin-B and Vasopressin proteins. All the chemical compounds best fit in the pockets of proteins and does not leave the complex, in all the docked complexes the common interacting amino acid residues were ALA, VAL, LEU, HIS, LYS, TRP, and SER. It determines their suitability to be used as medicine against diabetes.

Introduction

Diabetes is a noteworthy worldwide issue because of drastically expanding around the world. Globally, the aggregate number of individuals with diabetes is anticipated to ascend from 285 million in 2010 to 439 million in 2030 relating to an anticipated increase in predominance from 6.4% to 7.7% [1, 2]. Essentially, there are two types of diabetes: Diabetes Mellitus and Diabetes insipidus. Diabetes mellitus refers to a heterogeneous group of disorders. An insulin-dependent diabetes mellitus occurs as a consequence of pancreatic beta-cell destruction and is described by absolute insulin deficiency, an unexpected onset of side effects, proneness to ketosis and reliance on exogenous insulin to manage life. It is the most widely recognized types of diabetes among youngsters and young adults [3]. There is broad and consistent proof that hereditary components play a vital part in modifying an individual's risk for diabetes mellitus [4-8].

A noteworthy research focus for a very long time has been the identification of genes responsible for diabetes risk. Diabetes insipidus is an ailment in which the extensive volume of dilute urine (polyuria) is discharged because of the deficiency of vasopressin (AVP), vasopressin resistance or excessive water consumption. Diabetes insipidus is an uncommon disease with a non-univocal reported pervasiveness of 1: 25,000 [9]. Less than the 10% of diabetes insipidus can be credited to genetic

Corresponding Author: Shaukat Iqbal Malik, Department of Biosciences, Faculty of health and life sciences, Capital University of Sciences and Technology, Islamabad, Pakistan, E-mail: drshaukat @ cust .edu .pk

forms [10]. At present, more than 55 unique mutations resulting in a damaged pro-hormone and a lack of AVP have been distinguished in familial neurohypophyseal central diabetes insipidus [11].

The signs and symptoms of diabetes are dismissed by numerous as a result of the interminable progression of the illness. Individuals don't consider this as a significant problem because of the fact that the numerous different outcomes of hyperglycemia are not showing quickly. Individuals don't know that damage can begin quite a long time before the symptoms appear. The major symptoms of diabetes are, polyuria, polyphagia, and polydipsia happen generally in diabetes mellitus, which has a quick development of extreme hyperglycemia furthermore in diabetes mellitus with abnormally high levels of hyperglycemia. The extreme weight reduction has been normal just in diabetes mellitus or if diabetes insipidus stays undetected for a long time. Unexplained weight reduction, the weakness and restlessness and body pain are likewise regular indications of undetected diabetes [12]. The number of drugs is available in the market as treatment remedy against diabetes, however, the number of side effects are reported. Here the study was conducted to determine the In-silico efficacy and suitability of the chemical constituents of medicinal plants that are used to cure diabetes.

Material and Methods

Protein data and Preparation

Information about proteins involved in diabetes was obtained through literature [13-18] the mutated three dimensional (3D) structures of Insulin-B protein with 5J3H PDB ID, having one mutation and Vasopressin protein with a PDB ID name 1YF4, having 3 mutations downloaded from PDB database [19]. The 3D structures of proteins are shown in Figure 1.
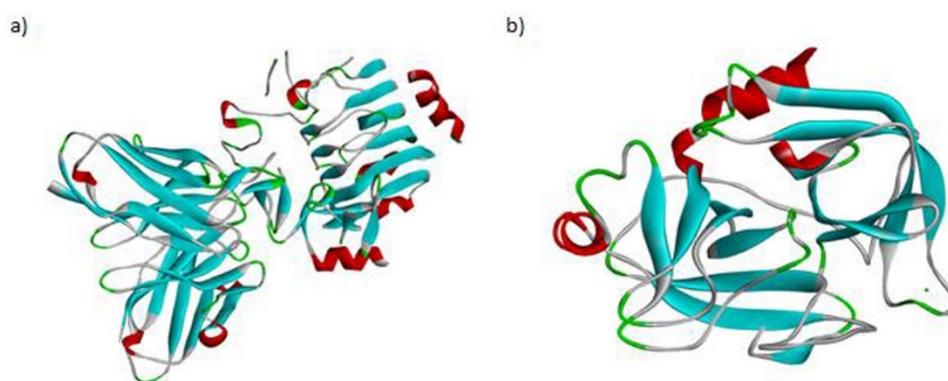
Figure 1: the 3D structures of mutated Insulin-B and vasopressin proteins a) Insulin B b) vasopressin. The alpha helices are represented by red color while the beta strands are represented by the blue color, the gray color represents coil and green color represents turns between strands and coils.

The crystals of water molecules were removed from the protein structures, for Insulin-B, the chain A, and B, while for the Vasopressin, chain A of protein was selected. The hydrogen atoms were added to both of the proteins, the Force Field charges and atom were allocated. Protein structures were prepared using the standard procedure in discovery studio software. Protein structure minimization was done until the average root mean square deviation (RMSD) of non-hydrogen atoms reached in less than 1 Å.

Binding pocket Identification

The effective binding pockets of proteins were identified through Pock Drug; pocket drugability prediction server. The PDB IDs of prepared proteins were input into the server. The effective binding pockets of proteins were selected on the basis of drug score and standard deviation score.

Ethnomedicinal Data Collection

The ethnomedical data about the medicinal plants: Bauhinia Variegata, Prunella Vulgaris, Aloe Vera, and Clematis Montana, used to cure diabetes, was obtained local inhabitants of village sides of Hazara Division, Pakistan by conducting surveys and semi-structured interviews, Approximately 50 informants between that age of 28 - 60 years with a sound traditional knowledge of beneficial wild plants, usually both the native-born or had been living in the place, for more than 30 years, had been interviewed. Questions addressed to the informants about medicinal uses had been specifically centered on local name of the plant, habitats, place of series, the season of collection, components used, classes, the way of drug practice and management, and illnesses cured. Plants were generally collected with the help of Inhabitants and confirmed, to make sure that the proper plants were selected and their uses were confirmed through literature. Informed consent was obtained from all individual participants included in the study, the inhabitants were also told that their provided information about the plants will be published.

Ligand data and Preparation

The effective chemical constituents of these plants were identified and downloaded from the PubChem database [20]. Structural conformations were applied to all the downloaded chemical constituents, the molecular charges were assigned and the chemical constituents were prepared for effective docking, in the discovery studio software.

Toxicity and Genotoxicity Prediction

All the downloaded chemical constituents of selected plants were checked for toxicity level through protox server [21], the genotoxicity hazards of chemical constituents were checked through ACD Labs, both the servers displayed their results in standard classes of toxicity assigned by the general system of classification of chemical compounds, and only those chemical constituents were selected for further study that lied in less toxic classes.

Docking Simulations

The selected chemical constituents were docked into the effective binding pockets of the insulin - B and vasopressin proteins with the help of Patch Dock server [22]. For each docked complex the RMSD value was set at 2 Å. Based on score ranking the docking results with higher score values were downloaded and used for Interaction analysis, pharmacophore and QSAR analysis in discovery studio software.

Identification of the effects that can be produced by the chemical constituents on the human health

All the chemical constituents were selected for further analysis when used can produce several side effects in the body of a living organism. All the selected chemical constituents were checked for their probability of side effects on several organs of living organisms, with the help of an ACD lab server. ACD lab server is an online service used to predict the Insilico physiochemical, ADME and toxicity properties of drug-like compounds

Results and Discussions

After a literature search for the selected medicinal plants, about ten effective chemical constituents of plants were identified and downloaded. The selected chemical constituents are associated with different parts of the plant bodies. The chemical constituents and their plants are shown in Table 1.

Table 1: Effective chemical constituents of medicinal plants used in the treatment of diabetes

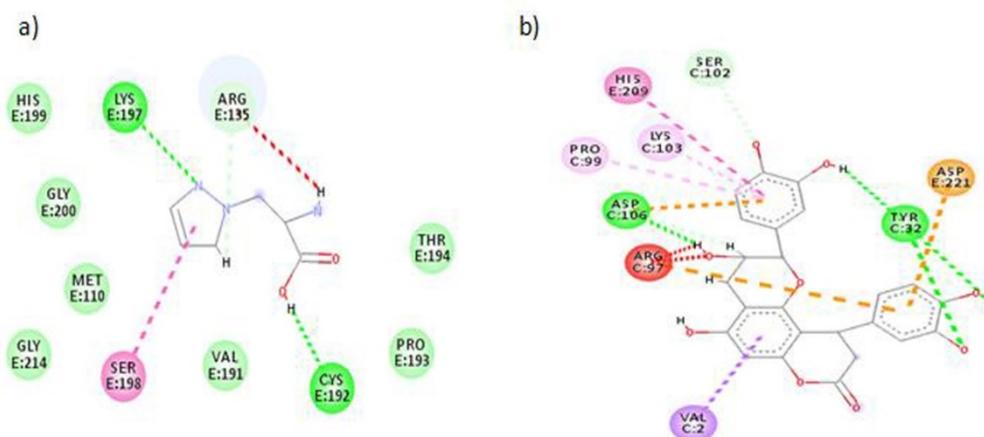| Chemical Constituents	Plants
Roseoside, Beta-pyrazole-1-ylalanine	Bauhinia variegate
Jiangtangsu	Prunella vulgaris
Cinchonain ib, 4-hydroxyleucine	Eriobotrya japonica
Momordicin, prototinosaponins AIII	Aloe vera
isoquinoline alkaloids	Azadirachta indica
Diosgenin, Isobutylamides	Echinops echinatus

The chemical constituents checked for toxicity to determine the LD50 dose, most of the chemicals were lying in non-toxic classes while few reside in the most toxic class. In the screening process of drug molecules, the determination of LD50 is crucial to determine the toxicity level of compounds, the greater the LD50 values safer is the drug compound [23]. Toxic doses are regularly given as LD50 mg/kg body weight.

The LD50 is the deadly dosage, meaning the measurement at which half of the test subjects die upon introduction to a compound. The toxic classes are characterized by the globally harmonized system of classification of labeling of chemicals (GHS): Class 1: deadly if gulped (LD50 $\leq$ 5 mg/kg), Class 2: lethal if gulped (5 < LD50 $\leq$ 50 mg/kg), Class 3: dangerous if gulped (50 < LD50 $\leq$ 300 mg/kg), Class 4: toxic if gulped (300 < LD50 $\leq$ 2000 mg/kg), Class 5: might be hurtful if gulped (2000 < LD50 $\leq$ 5000 mg/kg), Class 6: non-harmful (LD50 > 5000 mg/kg [24, 25]. The toxicity classes of chemical constituents along with prescribed LD50 dosage are shown in Table 2.

Table 2: toxicity classes and prescribed LD50 mg/kg dose values for selected chemical constituents of plants used to cure diabetes

| Chemical Constituents	Toxicity Class	LD50 Dose
4-hydroxyleucine	Class 5	5000 mg/kg
Beta-pyrazole-1-ylalanine	Class 4	593 mg/kg
Cinchonain ib	Class 5	2500 mg/kg
Diosgenin	Class 6	8000 mg/kg
Jiangtangsu	Class 1	3 mg/kg
Isobutylamides	Class 6	6000 mg/kg
isoquinoline alkaloids	Class 3	69 mg/kg
Momordicin	Class 5	5000 mg/kg
prototinosaponins AIII	Class 4	832 mg/kg
Resoside	Class 4	2500 mg/kg

Only 5 chemical constituents: 4-hydroxyleucine, Cinchona in-ib, Diosgenin, Isobutylamides, and Momordicin were lying in class 5 and class 6. Those five chemical constituents were selected for further analysis and docked with Insulin-B and Vasopressin proteins. The docking results of compounds lying in LD50 class 5 and 6 are shown in Fig 2 – 6.
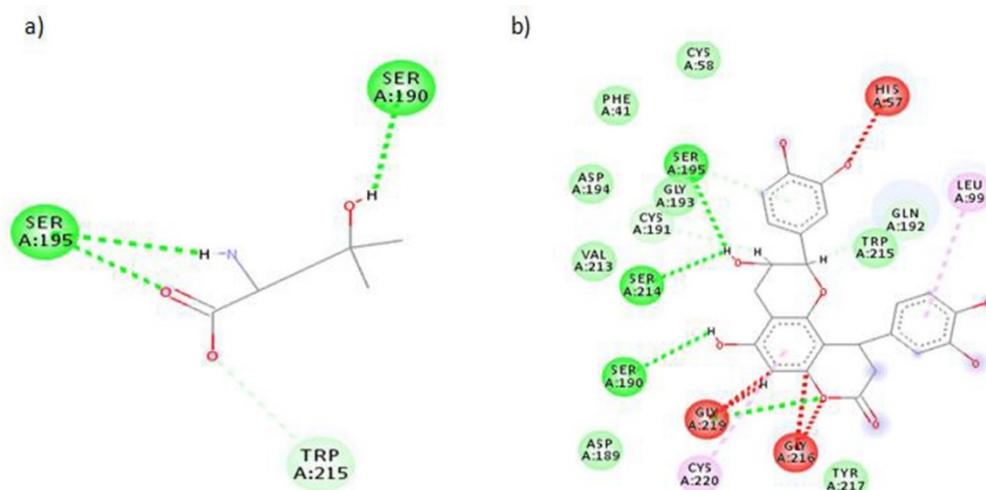
Figure 2: the docking results of chemical constituents along with mutated Insulin-B protein a) 4-hydroxyleucine docking with insulin b, b) Cinchonain ib docking with Insulin-B
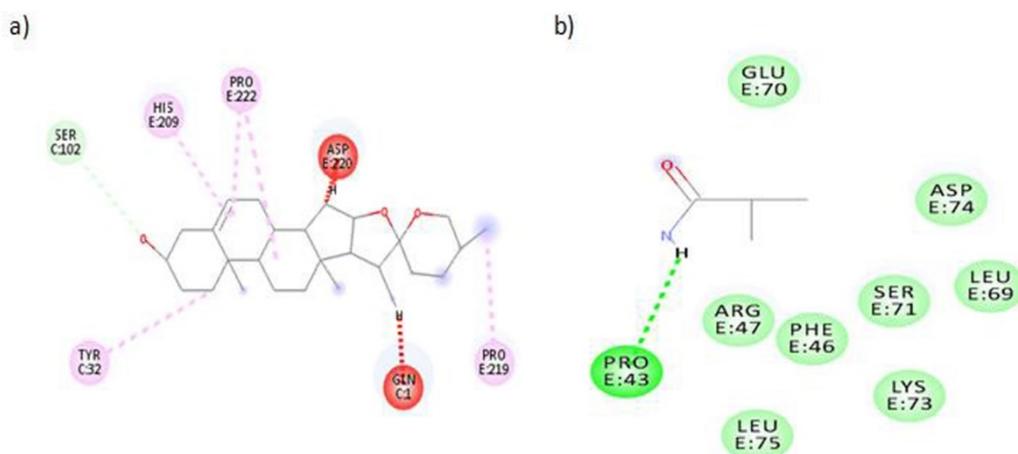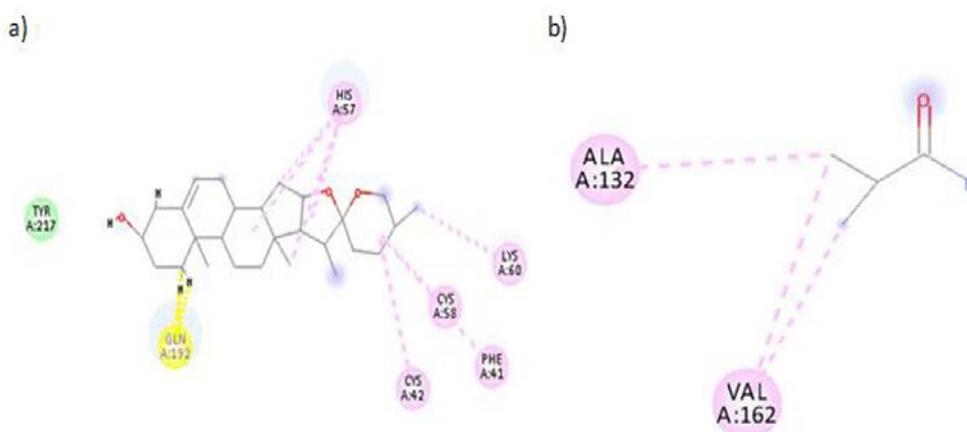
Figure 3: the docking results of chemical constituents along with mutated Insulin-B protein a) Diosgenin docking with Insulin-B, b) Isobutylamides docking with Insulin-B
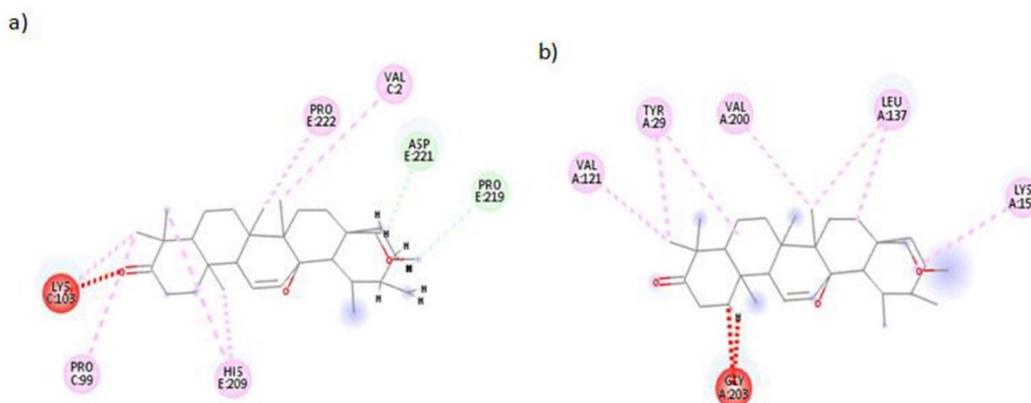
Figure 4: the docking results of chemical constituents along with mutated Insulin-b protein a) Momordicin docking with Insulin-B b) Momordicin docking with vasopressin.

Figure 5: the docking results of chemical constituents along with mutated vasopressin protein a) 4-hydroxyleucine docking vasopressin, b) Cinchonain ib docking with vasopressin

Figure 6: the docking results of chemical constituents along with mutated vasopressin protein a) Diosgenin docking with vasopressin, b) Isobutylamides docking with vasopressin

From figure 2 - 6 several bonding interactions were observed, the purple dash lines represent alkyl bonding, yellow dash lines represented Pi-stacked bonding, conventional hydrogen bondings were represented by a green dash line. Similarly, orange dash lines represent Pi-Sulfur bonding and red dash lines represent sigma bonding. Herbal medicines are utilized for treatment of diabetes in several developing countries where the expense of traditional medical solutions is a burden to the population [26]. In this research work, a computational analysis of herbal plants used to cure diabetes was done.

The selected chemical constituents, docked with proteins were checked for their probability of side effects on several organs. The probability results of side-effects are represented in Table 3.

Table 3: Probability of the side effects might produce by the selected chemical constituents on the several organs of the body

Compounds	Blood	Cardiovascular System	Gastrointestinal System	Kidney	Liver	Lungs
4-Hydroxy Leucine	0.09	0.06	0.13	0.12	0.13	0
Cinhonain ib	0.75	1	1	0.4	0.7	0.6
Diosgenin	0.98	1	1	0.92	0.86	1
Isobutylamine	0.2	0.06	0.29	0.1	0.1	0.03
Momordicin	0.81	1	1	0.9	0.92	0.99

All the selected chemical constituents represent very minimal probabilities of side effects. The more the probability of side effects more is the toxic compounds. Minimal probability ratios confirmed the chemical constituents as non-toxic in nature.

The molecular docking methodology can be utilized to display the connection between a ligand and a protein at the molecular level, which permit us to describe the conduct of ligands in the binding site of target proteins and additionally to

clarify principal biochemical properties [27, 28]. In all the docked complexes alkyl bonding was mostly observed, however, several other bonds like conventional bonds, hydrogen bonds and PI-sulfur bonds and Pi-Stacked bonds were also present. In all the docked complexes the common interacting amino acid residues were ALA, VAL, LEU, HIS, LYS, TRP, and SER. The natural medications with antidiabetic action are yet to be economically figured as advanced pharmaceuticals, despite the fact that they have been acclaimed for their remedial properties in the traditional frameworks of medicine [29, 30].

The medicinal plants have been testified to be beneficial in diabetes universally and have been used as antidiabetic and anti-hyperlipidemic medications. Regardless of the existence of known antidiabetic medication in the pharmacological market, diabetes and the associated problems continued to be a foremost medical problem. Anti-hyperglycemic effects of these plants are endorsed to their capability to reestablish the function of pancreatic tissues by instigating an increase in insulin or prevent the intestinal absorption of glucose or to the enablement of metabolites in insulin-dependent processes. Plant drugs and herbal inventions are often considered to be less toxic and free from side effects than synthetic ones. [31].

All the chemical compounds best fit in the pockets of proteins and does not leave the complex, the strong bonding and common interactions determine their suitability to be used as a remedy for diabetes.

Conclusion

In this research work computational analysis of docking studies on the dataset of chemical constituents found in different plant was done to determine the interactions between the chemical constituents of medicinal plants and mutated proteins are involved in diabetes, only 5 chemical constituents: 4-hydroxyleucine, Cinchona in ib, Diosgenin, Isobutylamides and Momordicin were found to be most effective and safe to be used in the treatment of diabetes.

In all the docked complexes alkyl bonding was observed, in all the docked complexes the common interacting amino acid residues were ALA, VAL, LEU, HIS, LYS, TRP, and SER. On the basis of results, it is suggested that these chemical constituents can be given to diabetic patients for achieving better results. The obtained results are of interest also from the practical point of view.

Acknowledgment

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