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Investigation of Enzymes Binding to "Voglibose- an Antidiabetic Drug" and the Choice of Enzyme to be Used for Biosensing

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Authors' contributions

This work was carried out in collaboration between all three authors. Authors SK, PPG and AP designed the study of work, performed the comparative analysis and wrote the manuscript. Author SK an expert in biosensors planned the work, literature survey, carried out the chemical analysis and compared the results of the chemical analysis with the results of molecular docking. Author PPG being an expert in bioinformatics and cheminformatics carried out the In-silico molecular docking and results were analyzed by all three authors. Authors SK, PPG and AP read and approved the final manuscript.

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ABSTRACT

Aims: This paper mentions of the investigation of the enzymes binding to Voglibose and the choice of the enzyme which is suitable for drug enzyme interaction as used in biosensing. Voglibose is an antidiabetic drug which is reactive and hence used in very low concentrations. The existing methods of analysis of this drug are associated with certain disadvantages. Hence there is a need to establish alternative and simpler method of analysis which could be based on biosensing. The paper focuses on identification of the enzyme binding to the drug Voglibose.
Study Design: In this work there are two enzyme namely alpha amylase and alpha glucosidase which are considered as the potential targets for drug enzyme interaction for the drug Voglibose.

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The study is based on two approaches. The first one is molecular docking process done to verify the inhibition activity of the enzyme by the drug and the next method is chemical analysis to confirm the results obtained in molecular docking.
Place and Duration of Study: This experiment was carried out as a part of the project under Indian Nanotechnology User Program (INUP) at IITB, Mumbai.
Methodology: Molecular docking is a simulation procedure which is used to confirm the inhibition activity of alpha amylase and alpha glucosidase by Voglibose and compare their inhibition activities so as to choose the suitable enzyme for further applications. Chemical analysis is done to re-confirm the same and chose the target enzyme for biosensing applications.
Results: The docking experiments are done to show that Voglibose inhibits both alpha amylase and alpha glucosidase but a more stable complex is formed with alpha amylase and hence alpha amylase is used for the chemical analysis to reconfirm its inhibition by Voglibose. Thus, alpha amylase can be used as the target enzyme for drug enzyme interactions with Voglibose for further applications in biosensing.
Conclusion: The present *In-silico* and *In-vitro* swot analysis indicates that alpha glucosidase and alpha amylase binds to Voglibose. The interaction outcome from molecular docking and chemical analysis suggests that in future one can consider alpha amylase as a choice of target enzyme in applications of biosensing.

Keywords: Voglibose; alpha amylase; alpha glucosidase; molecular docking; chemical analysis.

1. INTRODUCTION

Diabetes Mellitus is an important concern for the health care sector. Postprandial hyperglycemia (which is normally measured two hours after and before eating in a postprandial glucose test) is one of the earliest abnormalities of glucose homeostasis associated with type 2 diabetes and is markedly exaggerated in diabetic patients with fasting hyperglycemia. Postprandial hyperglycemia contributes to the increased risk of both micro- and macrovascular complications in patients with diabetes mellitus. It appears in the literature that managing postprandial plasma glucose is more important in order to prevent the complications of type-2 diabetes [1]. Different types of drug are available for lowering postprandial hyperglycemic levels and amongst the oral drug are the alpha glucosidase inhibitors like Acarbose, Miglitol and Voglibose. These drugs are comparatively inexpensive and can be orally taken for longer durations [2].

The literature mentions that Miglitol and Voglibose have equal efficacy in reducing postprandial hyperglycemia as compared to Acarbose. The clinical benefit of Voglibose are its better safety profile as compared to Miglitol and Acarbose. Voglibose has a better efficacy and hence has a preferential choice in the management of postprandial hyperglycemia for the treatment of type-2 diabetes mellitus [1].

Voglibose [3] is a research product of Takeda Pharma, a Japan based company. It is a highly reactive drug and is recommended in small dosages of 0.2 mg to 0.3 mg per tablet, which is supposed to be lowest concentration in the pharmaceutical industry. The analysis of this drug is of great importance because of its reactivity and low concentration and also as it is an anti- diabetic drug used for the treatment of one of the prevalent ailments in the society.

The literature reveals a number of methods for the analysis of Voglibose. The analysis methods include assay, uniformity of content and dissolution. Assay method of analysis is used for characterizing the quantification of bulk substances in pharmaceutical analysis. Uniformity of Content is done to test the concentration in tablet form and dissolution is the procedure done to know the drug release profiles in the body. All the above mentioned methods are routinely done for all the drugs by analyzing their chromatograms. A number of methods are established for assay and uniformity of content for the above mentioned drug- Voglibose. But currently no methods are available for dissolution studies as in this process the drug gets diluted to greater extent and no chromatograms are obtained.

JP “Japanese Pharmacopeia” describes the post derivatization methods with fluorescent detectors for the estimation of Voglibose which was detected at an excitation wavelength of 350 nm and an emission wavelength of 430 nm [3]. Rao M, et al. [4] explains of UV- Spectroscopic method for estimation of Voglibose at 282 nm in

bulk and tablets. Saikishore et al. [5] developed and validated RP-HPLC (reverse phase - high performance liquid chromatography) method for quantitative analysis of Voglibose in pure and pharmaceutical formulations and was detected at 282 nm. Daswadkar SC, et al. [6] used two different methods (LC_FD and LC_MS) for the analysis and detection of Voglibose. Woo JS, Ryu JK [7] mentions of quantitative determination of Voglibose in tablet using HPLC fluorescence detection with post column derivatization and mass spectroscopic detection and Voglibose was detected at a wavelength of 272 nm.

As mentioned earlier the literature reveals a number of methods available for the analysis of Voglibose for its assay and uniformity of content. But these methods are associated with different challenges. Assay method of analysis has to be performed for every drug and when the concentrations are less than 10mg per tablet, Uniformity of Content (UOC) is also an important parameter to be tested. But the above mentioned methods require expensive instrumentation and skilled personnel to handle the procedures otherwise suffer poor response.

There is another important parameter called the dissolution which is a method of evaluation of absorption rate of drug in the body where the drug is diluted to 900 ml of the solution. Because of its dilution no method of analysis is established for dissolution and even Japanese Pharmacopeia does not mention any method for this study. Thus there is requirement for a new method of analysis to estimate the drug in low concentration using simpler techniques and also to provide a method for dissolution studies [8]. This new method can be developed based on the principle of drug enzyme interaction as used in biosensing [9-11]. In order to develop a new method based on drug enzyme interaction the first step is the identification of a suitable target for the drug. Thus this paper concentrates on the identification and confirmation of the enzyme interacting to the drug- Voglibose.

Three enzymes were considered as targets for the drug enzyme interactions for the drug Voglibose namely CYP450 [12,13,14] series of enzymes, alpha glucosidase (Voglibose is an alpha glucosidase inhibitor AGI) [15] and alpha amylase. Enzymes produced from the cytochrome P450 [12,13,14] genes are involved in the formation (synthesis) and breakdown (metabolism) of various molecules and chemicals within cells. The cytochrome P450 enzymes account for the metabolism of approximately 20% of therapeutic drugs including certain oral antidiabetic drugs (OADs). 2C9 is the CYP450 enzyme for metabolizing antidiabetic drug [12]. Thus this enzyme was chosen as one of the target enzyme. But the CYP enzymes are associated with their disadvantages of instability and are expensive. Thus, these drawbacks limit the use of CYP 450 series of enzymes as potential enzymes to be used for drug enzyme interactions with Voglibose.

The literature strongly mentions that Voglibose is a competitive inhibitor of alpha glucosibase thus it is clear that Voglibose binds to alpha glucosidase [15]. There are references that Acarbose another anti diabetic drug inhibits Alpha Amylase so an attempt was done to test if Voglibose also inhibits Alpha Amylase [16].

The process of binding of alpha amylase and alpha glucosidase could be found by analyzing the inhibition activity of both enzymes by Voglibose. For this two approaches were adapted the first one being molecular docking and the next was chemical analysis. The process of molecular docking is a simulation procedure in which alpha amylase and alpha glucosidase were the target enzymes and their inhibition activity by Voglibose was studied and it was observed that Voglibose inhibits both the enzyme but a more stable complex was formed with alpha amylase. Hence alpha amylase was considered as the target enzyme for chemical analysis [17]. Chemical analysis was done to confirm the inhibition activity of the alpha amylase by Voglibose which was confirmed. After the experiments on molecular docking and chemicals analysis it confirms that alpha amylase can be chosen as a potential target for drug enzyme interaction of the drug Voglibose.

2. MATERIALS AND METHODS

The main aim of the paper was to identify and confirm the enzyme that could be chosen for the drug enzyme interaction for the drug- Voglibose. In this process molecular docking was done to find out the inhibition of alpha amylase and alpha glucosidase by Voglibose as it is mentioned in the literature that Voglibose is a competitive inhibitor [15]. The results of docking showed that Voglibose inhibits both the enzymes although a more stable complex is formed with alpha amylase and hence chemical analysis was done

to confirm the inhibition of alpha amylase by Voglibose. It is well known that alpha amylase digests starch [17] but in presence of Voglibose alpha amylase does not digest the starch as its activity is inhibited by Voglibose. Thus, this shows that alpha amylase interacts with Voglibose.

2.1 *In-silico* Methodology

2.1.1 Receptor protein

The 3D crystallized complex structure of Alpha glucosidase with Acarbose Pdb-id:2QMJ [18] and crystal complex structure of alpha-amylase with Acarviostatin amylase Pdb-id: 3OLD [19] were downloaded from the RCSB Protein Data Bank.

2.1.2 Active site

Identification of an active site or binding site on a protein is a principal analysis in a structure based drug discovery. In present work the default binding site for Acarbose and Acarviostatin was considered for the Voglibose too and is well explained in molecular docking section below.

2.1.3 Ligand preparation

The 3D structure of Voglibose in sketched using Chemsketch 12.0 [20] and optimized the structure using UFF [21] in Argus lab [22] and converted to .Pdb file format.

2.1.4 Molecular docking

Molecular interactions play a key role in all biological reactions. Chemical structure are either mimicking or mitigating the effect of natural ligands binding on to the receptor by exerting the pharmacological reactions. Computational methods are used to understand this mode of binding of ligands to their receptors is known as Molecular Docking [23]. Molecular docking study is carried out in AutodockVina [24] and binding energy is calculated, given by ΔE = Ecomplex – Eligand – Eprotein (ΔE is the ligand binding energy), the energy calculation first considers the receptor, then ligand and finally generated receptor-ligand complex and energy differences is called binding energy [25]. Gasteiger charges are added to the ligand and maximum numbers of calculated rotatable bonds were considered torsions using AutoDock4.0 [26] tool. Kollman charges and the solvation term were then added to the protein structure using the same. Considering the default active site, grid box is adjusted with 72, 42 and 68 Å with a center grid box value for X: -19.206; Y: -5.13 and Z: -5.53 for 2QMJ and grid box value of 40, 40, 40 Å with a center grid box value for X: 11.822; Y: -16.361 and Z: -22.499 for 3OLD. Due to large volume of active site is acquired by Acarbose and Acarviostatin we have increased the spacing value to 1.0 Å from default value of 0.375 Å between grid points to investigate the large volume space. Considering with default parameters the molecular docking process is successfully carried out.

2.2 Chemical Analysis to Verify the Binding of Alpha Amylase with Voglibose

As mentioned earlier chemical analysis was done to confirm the interaction of alpha amylase and Voglibose. The experiments were initially conducted to confirm the activity of alpha amylase (fungal diastase) and then inhibition of alpha amylase in the presence of Voglibose. Experiments were also conducted to show that the inhibition of alpha amylase increases as the concentration of Voglibose increases. This was done to choose the standard concentration of alpha amylase for further experimentation. As the concentration of the drug is about 0.3 mg/tablet, further experiments were concentrated by considering 0.3 mg.ml as the concentration of Voglibose. Thus the inhibition of 0.3 mg Voglibose for varying concentration of alpha amylase has been tabulated and the percentage of inhibition is found to be repeatable for this concentration.

2.2.1 Materials used

Starch used in the experiments was potato starch obtained by HiMedia Ltd. The drug Voglibose was obtained from Tirupati Medicare Pvt Ltd, HP, India as a raw product. Alpha amylase (fungal diastase) was obtained by Anthem Cellutions, Bangalore, India. The buffer used was acetate buffer prepared using acetic acid and sodium acetate with pH of 5.2 as the enzyme (fungal diastase) used in the experiment is active in the acetate buffer with pH range of 5 to 6.

2.2.1.1 Preparation of starch solution

The starch used was potato starch and 50 mg of this was dissolved in 100 ml water with continuous stirring at 100 degree C.

2.2.1.2 Preparation of buffer

The Alpha amylase used is fungal diastase which is active in acetate buffer of pH=5-6. 357 ml of 0.1 M acetic acid was mixed with 643 ml of 0.1 M sodium acetate to get Acetate buffer of pH=5.

2.2.1.3 Preparation of Alpha amylase solution

The Alpha amylase used is fungal diastase with strength of 1:800 100 units equivalent quantity of alpha amylase is weighted and mixed in 200 ml of acetate buffer of pH 5.

10 ml of this solution is diluted to 100 ml using the same buffer solution [17].

2.2.1.4 Iodine indicator

The Indicator used is Iodine Indicator of 2 mM. This was prepared mixing 0.2% Iodine and 2% Potassium Iodide.

2.3 Procedure for Testing the Activity of Alpha Amylase Used in the Experiment (Fungal Diastase)

Starch Iodine Test was used to confirm the activity of the Alpha Amylase [17]. 100 units equivalent quantity of alpha amylase was weighted and mixed in 200 ml of acetate buffer of pH 5. 10 ml of this solution was diluted to 100 ml using the same buffer solution [17]. Different volumes of 3.5 ml, 4.5 ml, 5 ml of alpha amylase solutions were added with 5 ml of starch solution. The test tubes were kept in water bath at 40 degree C for 60 minutes. The absorbance value was noted after the addition of 0.5 ml Iodine Indicator.

2.4 Procedure for Testing Inhibition Activity of Alpha Amylase by Voglibose

A known concentration of Alpha Amylase was prepared. Voglibose of 0.3 mg/ml, 0.4 mg/ml, 0.5 mg/ml were prepared in acetate buffer. Each ml of Voglibose (of the above mentioned concentrations) was mixed with 1 ml of Alpha Amylase and were maintained at 40 degree C for 60 minutes. Later 1 ml of Starch was added and maintained at 40 degree C for 60 minutes. Iodine indicator of 0.5 ml was added and the spectroscopy was obtained to find the absorbance values.

2.5 Procedure for Testing Inhibition Activity of Alpha Amylase by Voglibose with the Concentration of 0.3 mg/ml which is the Concentration Available in Tablets

Known concentrations of Alpha Amylase were prepared and Voglibose of 0.3 mg/ml was prepared in acetate buffer. For every 1 ml of Voglibose, 1 ml of Alpha Amylase (of various concentrations) was added and these maintained at 40 degree C for 60 minutes. Later 1 ml of Starch was added and maintained at 40 degree C for 60 minutes. Iodine indicator of 0.5 ml was added and the spectroscopy was obtained.

The inhibition of Alpha Amylase by 0.3 mg/ml Voglibose was obtained by the formula: A(control)- A(test)/A(control)*100

3. RESULTS AND DISCUSSION

As already mentioned the aim of the paper was identification and confirmation of the choice of enzyme for drug enzyme interaction of Voglibose. Simulation experiments were done by molecular docking and this confirms that both alpha amylase and alpha glucosidase are inhibited by Voglibose and thus both the enzymes can be used as target enzymes for drug enzyme interaction. The binding energy as mentioned in the Table 1 shows that alpha amylase produces a more stable complex with Voglibose and thus the chemical analysis was concentrated on the interaction of alpha amylase and Voglibose.

3.1 Results of Molecular Docking

The 3D optimized structure of Voglibose exhibited a minimum energy of 18.3637 kcal/mol from 210.3295 kcal/mol. Molecular docking studies predicts the interaction between selected ligand molecule and element of an amino acid involved in an active site of a receptor protein. Therefore the optimum binding energy, types of bonding, interaction and as well as pharmacophoric points are equally important in justification and validation of results. The binding energy of Voglibose to Gluco-amylase is -6.1 kcal/mol and Voglibose to Alpha-amylase is -6.4 kcal/mol. Both the generated complexes exhibited a hydrogen bond, vander waal interaction and covalent features. In case of Pdb id 2QMJ Gluco amylase, Voglibose appropriately placed inside the binding site cavity forming a

typical hydrogen, Vander Waal and covalent bond interaction to most of the pharmacophoric amino acid residues. Where ASP 203, ASP 443, ARG 526 are core amino acid for binding the Acarbose within the Gluco amylase by hydrogen bond formation, similar binding activity is exhibited with Voglibose too, such as hydrogen bond: ASP 203, ASP 443, ARG 526. Vander Waal interaction: TYR 299, ILE 364, TRP 441, TRP 539, PHE 575 and Covalent bond with ASP 327, TRP 406, MET 444, ASP 542. Whereas in a crystallized structure Pdb id 3OLD Alpha amylase exhibited a binding affinity with ASP 197, ALA 198, GLU 233, HIS 299, ASP 300, GLY 306 and etc. Here similar binding can be seen with Voglibose too, where ASP 197, ALA 198, GLU 233, HIS 299, ASP 300, GLY 306 formed a hydrogen bond between Voglibose and Alpha amylase, Vander Waal interaction with TRP 58, GLU 60, VAL 98, HIS 101, LEU 162, THR 163, LEU 165and Covalent bond with TYR 62, GLN 63, ARG 195, HIS 299, HIS 305 given in Table (1), Figs. 1 and 2.

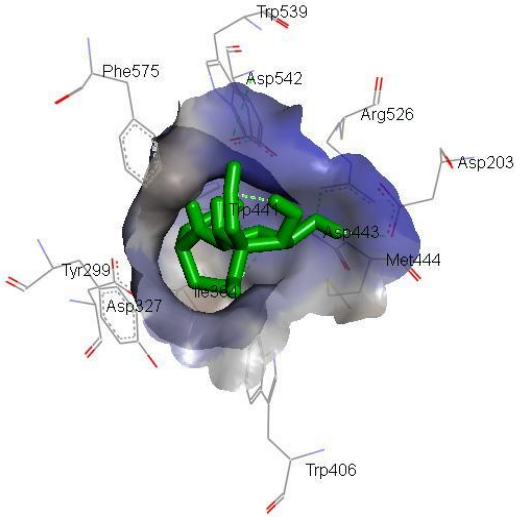

Fig. 1. Interaction of alpha glucosidase with Voglibose

3.2 Results of Chemical Analysis

Molecular docking confirms that the interaction of alpha amylase and Voglibose produce a more stable complex and thus alpha amylase is used as the enzyme for interaction with Voglibose for further analysis. The simulation results were further reconfirmed by chemical analysis.

From Table 2 it can be observed that alpha amylase digests starch and hence it is active in acetate buffer of ph 5. This confirms the activity of alpha amylase. Table 3 shows that as the concentration of Voglibose increases its inhibition activity also increases.

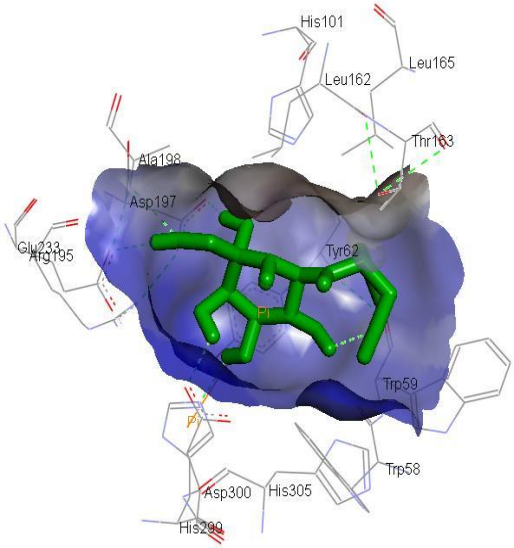

Fig. 2. Interaction of alpha amylase with Voglibose

The Fig. 3 shows that as the concentration of Voglibose increases the absorbance value increase. This is because of retention of the starch in the solution increases as the alpha amylase present in the solution

Table 1. Molecular docking interaction analysis

Complex	Hydrogen bond	Vander waal Interaction	Covalent bond	Binding energy Kcal/mol
Voglibose to 2QMJ	ASP 203, ASP 443, ARG 526	TYR 299, ILE 364, TRP 441, TRP 539, PHE 575.	ASP 327, TRP 406, MET 444, ASP 542	-6.1
Voglibose to 3OLD	ASP 197, ALA 198, GLU 233, HIS 299, ASP 300, GLY 306.	TRP 58, GLU 60, VAL 98, HIS 101, LEU 162, THR 163, LEU 165.	TYR 62, GLN 63, ARG 195, HIS 299, HIS 305.	-6.6

is inhibited by Voglibose and hence alpha amylase cannot digest starch. This shows that the inhibition of alpha amylase increases as the concentration of Voglibose increases.

Table 2. Testing the activity of alpha amylase (fungal diastase) used for the experiment, the absorbance was observed at wavelength of 576 nm

Alpha amylase	Starch	Iodine	Absorbance value
3.5 ml	5 ml	0.5 ml	0.18
4 ml	5 ml	0.5 ml	0.15
4.5 ml	5 ml	0.5 ml	0.148
5 ml	5 ml	0.5 ml	0.11

Table 3. Testing inhibition activity of Alpha amylase by Voglibose, the absorbance was observed at wavelength of 576 nm

Alpha amylases	Voglibose	Starch	Absorbance value
-	-	1 ml	0.565
1 ml	-	1 ml	0.029
1 ml	0.3 mg	1 ml	0.12
1 ml	0.4 mg	1 ml	0.16
1 ml	0.5 mg	1 ml	0.18

3.2.1 Tabulation for testing percentage inhibition of alpha amylase by 0.3 mg/ml of voglibose

The experiments are further concentrated on the inhibition of Voglibose with 0.3 mg/ml as the concentration as this is the drug concentration per tablet and the percentage of inhibition are calculated. Table 4, Table 5 and Table 6 shows the inhibition of 0.3 mg/ml Voglibose for a particular concentration of alpha amylase.

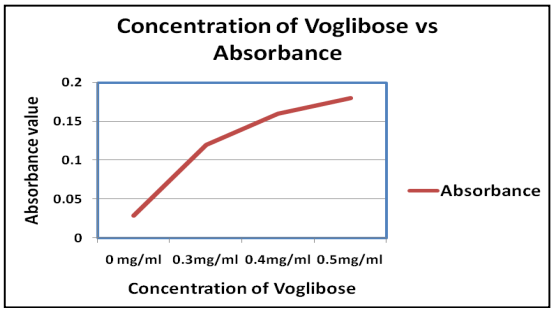

Fig. 3. Graph indicating the increasing in absorbance value with increase in concentration of Voglibose

Experiment 1: Testing the inhibition of 25 ml Alpha amylase in 25 ml buffer by 0.3 mg/ml Voglibose which is shown in Table 4.

Table 4 shows 16.4% inhibition of Alpha amylase by 0.3 mg/ml Voglibose.

Experiment 2: Testing the inhibition of 30 ml Alpha amylase in 20 ml buffer by 0.3 mg/ml Voglibose which is shown in Table 5.

Table 5 shows 15% inhibition of Alpha amylase by 0.3 mg/ml Voglibose.

Experiment 3: Testing the inhibition of 40 ml Alpha amylase in 10 ml buffer by 0.3 mg/ml Voglibose which is shown in Table 6.

Table 6 shows 15.06% inhibition of Alpha amylase by 0.3 mg/ml Voglibose.

Table 4. Inhibition of 25 ml Alpha amylase in 25 ml buffer by 0.3 mg/ml Voglibose

Starch	Alpha amylase	Voglibose	Wavelength	Absorbance value	Percent
1 ml	-	-	578	0.983	100
1 ml	1 ml	-	566	0.229	76.7
1 ml	1 ml	0.3 mg	574	0.39	60.32

Table 5. Testing the inhibition of 30 ml Alpha amylase in 20 ml buffer by 0.3 mg/ml Voglibose

Starch	Alpha amylase	Voglibose	Wavelength	Absorbance value	Percent
1 ml	-	-	578	0.983	100
1 ml	1 ml	-	573	0.34	65.41
1 ml	1 ml	0.3 mg	577	0.487	50.45

Table 6. Testing the inhibition of 40 ml Alpha amylase in 10 ml buffer by 0.3 mg/ml Voglibose

Starch	Alpha amylase	Voglibose	Wavelength	Absorbance value	Percent
1 ml	-	-	578	0.983	100
1 ml	1 ml	-	570	0.409	58.39
1 ml	1 ml	0.3 mg	581	0.557	43.33

4. DISCUSSION

The main aim of the paper was investigation of enzymes binding to Voglibose. There were three enzymes considered for the drug enzyme interaction; amongst which were 2C9 (CYP 450 series of enzymes), alpha amylase and alpha glucosidase. The literature mentions that P450 series of enzymes are expensive and unstable and hence 2C9 was not chosen as the potential target enzyme for drug enzyme interactions with Voglibose. Hence alpha amylase and alpha glucosidase were considered as targets for molecular docking. Molecular docking confirms the drug enzyme interaction of Voglibose with alpha amylase and also Voglibose with alpha glucosidase. The binding energy of the interactions shows that a more stable complex is formed between Voglibose and alpha amylase and thus the confirmation was done by chemical analysis. The chemical analysis was done to confirm the binding of Voglibose and alpha amylase. It has been observed during chemical analysis that the activity of alpha amylase is to digest starch. But in presence of Voglibose (competitive inhibitor), the activity of alpha amylase is inhibited and it cannot digest starch or less amount of starch is digested thus confirming the interaction of Voglibsoe and alpha amylase. It has also been observed that the inhibition activity of Voglibsoe increases as its concentration increases. But the concentration of Voglibose per tablet is about 0.3 mg and hence experiments have been carried out with this concentration, that is to find the inhibition activity of 0.3 mg/ml of Voglibose. It has been found that the percentage of inhibition for this concentration is around 15% and found repeatable. Thus the experiments conducted confirm that alpha amylase is the best choice of enzyme to be used for drug enzyme interaction for the drug Voglibose. Hence for further applications on biosensing alpha amylase can be the potential target enzyme for Voglibose.

5. CONCLUSION

The present *In-silico* and *In-vitro* swot analysis indicates that like Alpha glucosidase even Alpha amylase binds to Voglibose. The interaction outcome suggests that a more stable complex is formed between Alpha amylase and Voglibose and thus in future one can consider Alpha amylase as a choice of target for Voglibose in applications of Biosensing.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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