

Investigation of Enzymes Binding to “Voglibose- an antidiabetic drug” and the choice of enzyme to be used for biosensing.

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. 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

20 hyperglycemic levels and amongst the oral drug are the alpha glucosidase inhibitors like Acarbose,
21 Miglitol and Voglibose. These drugs are comparatively inexpensive and can be orally taken for longer
22 durations [2].

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

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

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

40 JP “Japanese Pharmacopeia” describes the post derivatization methods with fluorescent detectors
41 for the estimation of Voglibose which was detected at an excitation wavelength of 350 nm and
42 an emission wavelength of 430 nm [3]. Rao M *et al.* explains of UV- Spectroscopic method for
43 estimation of Voglibose at 282nm in bulk and tablets [4]. Sai kishore *et al.* developed and
44 validated RP-HPLC (reverse phase - high performance liquid chromatography) method for
45 quantitative analysis of Voglibose in pure and pharmaceutical formulations and was detected at
46 282nm [5]. Daswadkar S C *et al.* used two different methods (LC_FD and LC_MS) for the
47 analysis and detection of Voglibose [6]. Woo J S, Ryu J K. mentions of quantitative
48 determination of Voglibose in tablet using HPLC fluorescence detection with post column
49 derivatization and mass spectroscopic detection and Voglibose was detected at a wavelength of
50 272nm [7].

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

60 There is another important parameter called the dissolution which is a method of evaluation of absorption
61 rate of drug in the body where the drug is diluted to 900ml of the solution. Because of its dilution no
62 method of analysis is established for dissolution and even Japanese Pharmacopeia does not mention any
63 method for this study. Thus there is requirement for a new method of analysis to estimate the drug in low
64 concentration using simpler techniques and also to provide a method for dissolution studies [8]. This new
65 method can be developed based on the principle of drug enzyme interaction as used in Biosensing [9]-
66 [11]. In order to develop a new method based on drug enzyme interaction the first step is the identification
67 of a suitable target for the drug. Thus this paper concentrates on the identification and confirmation of the
68 enzyme interacting to the drug- Voglibose.

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

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

84 The process of binding of alpha amylase and alpha glucosidase could be found by analyzing the inhibition
85 activity of both enzymes by Voglibose. For this two approaches were adapted the first one being
86 molecular docking and the next was chemical analysis. The process of molecular docking is a simulation
87 procedure in which alpha amylase and alpha glucosidase were the target enzymes and their inhibition
88 activity by Voglibose was studied and it was observed that Voglibose inhibits both the enzyme but a more
89 stable complex was formed with alpha amylase. Hence alpha amylase was considered as the target

90 enzyme for chemical analysis [17]. Chemical analysis was done to confirm the inhibition activity of the
91 alpha amylase by Voglibose which was confirmed. After the experiments on molecular docking and
92 chemicals analysis it confirms that alpha amylase can be chosen as a potential target for drug enzyme
93 interaction of the drug Voglibose.

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95 **2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY**

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97 The main aim of the paper was to identify and confirm the enzyme that could be chosen for the drug
98 enzyme interaction for the drug- Voglibose. In this process molecular docking was done to find out the
99 inhibition of alpha amylase and alpha glucosidase by Voglibose as it is mentioned in the literature that
100 Voglibose is a competitive inhibitor [15]. The results of docking showed that Voglibose inhibits both the
101 enzymes although a more stable complex is formed with alpha amylase and hence chemical analysis was
102 done to confirm the inhibition of alpha amylase by Voglibose. It is well known that alpha amylase digests
103 starch [17] but in presence of Voglibose alpha amylase does not digest the starch as its activity is
104 inhibited by Voglibose. Thus, this shows that alpha amylase interacts with Voglibose.

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106 **2.1 In-Silico Methodology.**

107 **2.1.1. Receptor Protein:**

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

111 **2.1.2. Active site:**

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

115 **2.1.3. Ligand preparation:**

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

118 **2.1.4 Molecular docking:** Molecular interactions play a key role in all biological reactions. Chemical
119 structure are either mimicking or mitigating the effect of natural ligands binding on to the receptor by
120 exerting the pharmacological reactions. Computational methods are used to understand this mode of
121 binding of ligands to their receptors is known as Molecular Docking [23]. Molecular docking study is
122 carried out in AutodockVina [24] and binding energy is calculated, given by $\Delta E = E_{\text{complex}} - E_{\text{ligand}} - E_{\text{protein}}$ (ΔE is the ligand binding energy), the energy calculation first considers the receptor, then ligand
123 and finally generated receptor-ligand complex and energy differences is called binding energy [25].

124 Gasteiger charges are added to the ligand and maximum numbers of calculated rotatable bonds were
125 considered torsions using AutoDock4.0 [26] tool. Kollman charges and the solvation term were then
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127 added to the protein structure using the same. Considering the default active site, grid box is adjusted
128 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
129 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.
130 Due to large volume of active site is acquired by Acarbose and Acarviostatins we have increased the
131 spacing value to 1.0 Å from default value of 0.375 Å between grid points to investigate the large volume
132 space. Considering with default parameters the molecular docking process is successfully carried out.

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134 **2.2 Chemical Analysis to verify the Binding of Alpha Amylase with Voglibose.**

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

144 **2.2.1 Materials used**

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

150 *2.2.1.1 Preparation of Starch Solution:*

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

153 *2.2.1.2 Preparation of buffer*

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

156 *2.2.1.3 Preparation of Alpha amylase solution*

157 The Alpha amylase used is fungal diastase with strength of 1:800

158 100 units equivalent quantity of alpha amylase is weighted and mixed in 200ml of acetate buffer of pH 5.
159 10ml of this solution is diluted to 100ml using the same buffer solution [17]

160 *2.2.1.4 Iodine Indicator*

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

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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 200ml of acetate buffer of pH 5. 10ml of this solution was diluted to 100ml using the same buffer solution [17]. Different volumes of 3.5ml, 4.5ml, 5ml of alpha amylase solutions were added with 5ml 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 0.5ml Iodine Indicator.

2.4 Procedure for testing inhibition activity of Alpha Amylase by Voglibose.

A known concentration of Alpha Amylase was prepared. Voglibose of 0.3mg/ml, 0.4mg/ml, 0.5mg/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 1ml of Starch was added and maintained at 40 degree C for 60 minutes. Iodine indicator of 0.5ml 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 0.3mg/ml which is the concentration available in tablets.

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

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

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.

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202 3.1 Results of Molecular docking

203 The 3D optimized structure of Voglibose exhibited a minimum energy of 18.3637 kcal/mol from 210.3295
204 kcal/mol. Molecular docking studies predicts the interaction between selected ligand molecule and
205 element of an amino acid involved in an active site of a receptor protein. Therefore the optimum binding
206 energy, types of bonding, interaction and as well as pharmacophoric points are equally important in
207 justification and validation of results. The binding energy of Voglibose to Gluco-amylase is -6.1kcal/mol
208 and Voglibose to Alpha-amylase is -6.4kcal/mol. Both the generated complexes exhibited a hydrogen
209 bond, vander waal interaction and covalent features. In case of Pdb id 2QMJ Gluco amylase, Voglibose
210 appropriately placed inside the binding site cavity forming a typical hydrogen, Vander Waal and covalent
211 bond interaction to most of the pharmacophoric amino acid residues. Where ASP 203, ASP 443, ARG
212 526 are core amino acid for binding the Acarbose within the Gluco amylase by hydrogen bond formation,
213 similar binding activity is exhibited with Voglibose too, such as hydrogen bond: ASP 203, ASP 443, ARG
214 526. Vander Waal interaction: TYR 299, ILE 364, TRP 441, TRP 539, PHE 575 and Covalent bond with
215 ASP 327, TRP 406, MET 444, ASP 542. Whereas in a crystallized structure Pdb id 3OLD Alpha amylase
216 exhibited a binding affinity with ASP 197, ALA 198, GLU 233, HIS 299, ASP 300, GLY 306 and etc. Here
217 similar binding can be seen with Voglibose too, where ASP 197, ALA 198, GLU 233, HIS 299, ASP 300,
218 GLY 306 formed a hydrogen bond between Voglibose and Alpha amylase, Vander Waal interaction with
219 TRP 58, GLU 60, VAL 98, HIS 101, LEU 162, THR 163, LEU 165 and Covalent bond with TYR 62, GLN
220 63, ARG 195, HIS 299, HIS 305 given in Table (1), Figure (1) and (2).

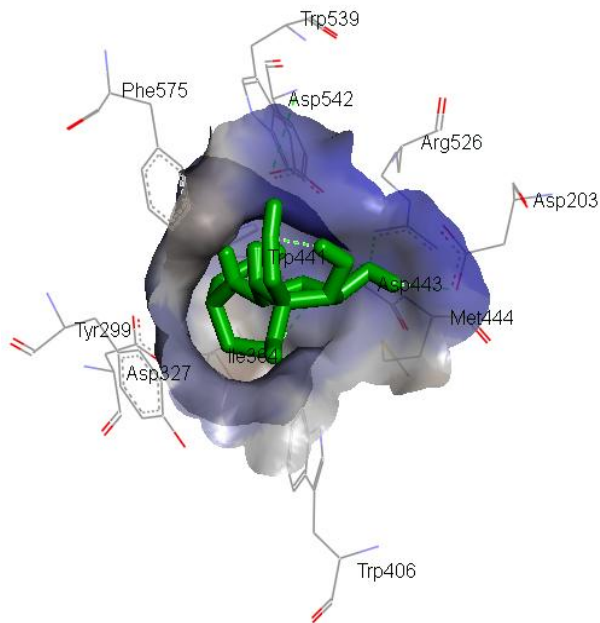
221 **Table 1: Molecular Docking Interaction Analysis**

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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

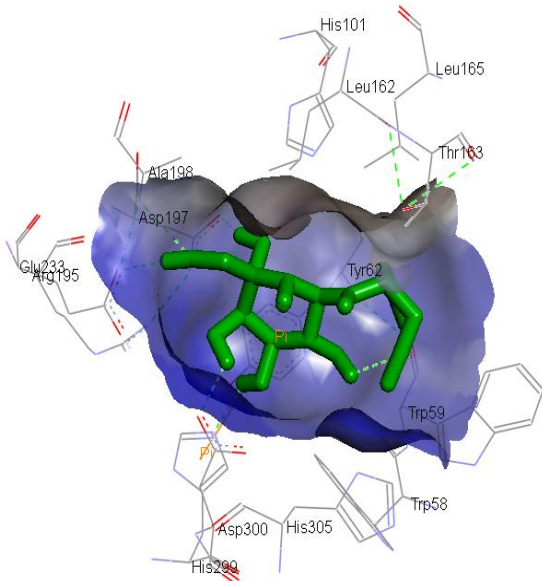
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226 **Fig 1: Interaction of Alpha Glucosidase with Voglibose**



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228 **Fig 2: Interaction of Alpha Amylase with Voglibose**

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230 **3.2 Results of Chemical Analysis**

231 Molecular docking confirms that the interaction of alpha amylase and Voglibose produce a more stable
 232 complex and thus alpha amylase is used as the enzyme for interaction with Voglibose for further analysis.

233 The simulation results were further reconfirmed by chemical analysis.

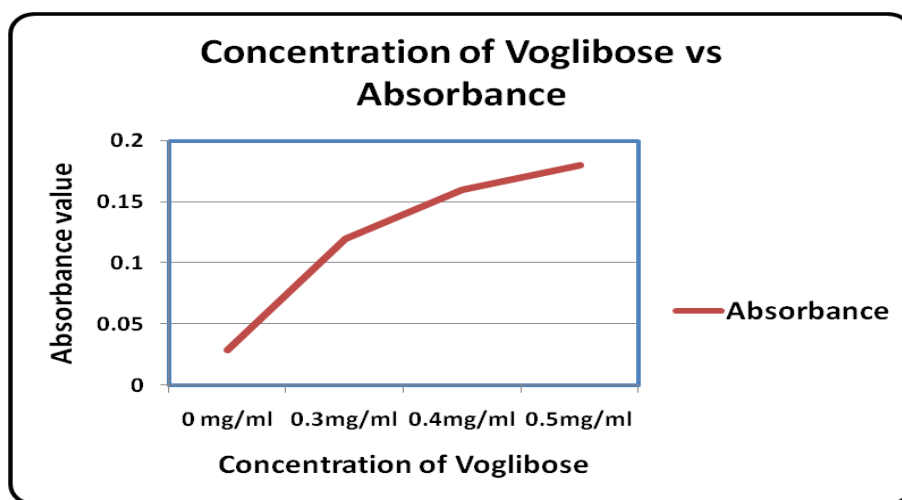
234 From Table 2 it can be observed that alpha amylase digests starch and hence it is active in acetate buffer
 235 of pH 5. This confirms the activity of alpha amylase. Table 3 shows that as the concentration of Voglibose
 236 increases its inhibition activity also increases.

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 238 **Table 2: Testing the activity of Alpha Amylase (Fungal Diastase) used for the experiment, the**
 239 **absorbance was observed at wavelength of 576nm**

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

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 241
 242 **Table 3: Testing Inhibition activity of Alpha Amylase by Voglibose, the absorbance was observed**
 243 **at wavelength of 576nm.**

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



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 246 **Fig 3: Graph indicating the increasing in absorbance value with increase in concentration of**
 247 **Voglibose.**

248 The Fig 3 shows that as the concentration of Voglibose increases the absorbance value increase. This is
 249 because of retention of the starch in the solution increases as the alpha amylase present in the solution is
 250 inhibited by Voglibose and hence alpha amylase cannot digest starch. This shows that the inhibition of
 251 alpha amylase increases as the concentration of Voglibose increases.

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 253 **3.2.1 Tabulation for testing percentage inhibition of Alpha Amylase by 0.3mg/ml of Voglibose.**

254 The experiments are further concentrated on the inhibition of Voglibose with 0.3mg/ml as the
 255 concentration as this is the drug concentration per tablet and the percentage of inhibition are calculated.

256 Table 4, Table 5 and Table 6 shows the inhibition of 0.3mg/ml Voglibose for a particular concentration of
 257 alpha amylase.

258
 259 **Experiment 1:** Testing the inhibition of 25ml Alpha Amylase in 25ml buffer by 0.3mg/ml Voglibose which
 260 is shown in Table (4).

261
 262 **Table 4: Inhibition of 25ml Alpha Amylase in 25ml buffer by 0.3mg/ml Voglibose**

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

263
 264 This shows 16.4% inhibition of Alpha Amylase by 0.3 mg/ml Voglibose.

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 266 **Experiment 2:** Testing the inhibition of 30ml Alpha Amylase in 20ml buffer by 0.3mg/ml Voglibose which
 267 is shown in Table (5).

268
 269 **Table 5: Testing the inhibition of 30ml Alpha Amylase in 20ml buffer by 0.3mg/ml Voglibose**

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

270
 271 This shows 15% inhibition of Alpha Amylase by 0.3mg/ml Voglibose

272 **Experiment 3:** Testing the inhibition of 40ml Alpha Amylase in 10ml buffer by 0.3mg/ml Voglibose which
 273 is shown in Table (6)

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275 **Table 6: Testing the inhibition of 40ml Alpha Amylase in 10ml buffer by 0.3mg/ml Voglibose**

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

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277 This shows 15.06% inhibition of Alpha Amylase by 0.3mg/ml Voglibose.

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279 **Discussion:**

280 This paper shows that alpha amylase can be used as the target enzyme for drug enzyme interactions
281 with Voglibose. As mentioned earlier Voglibose is an alpha glucosidase inhibitor. But this paper confirms
282 that Voglibose binds to alpha amylase as it produce a more stable complex on comparing the binding
283 energy between the two enzymes and Voglibose. This has been confirmed by molecular docking process
284 and the same thing has been reconfirmed by performing the chemical analysis. It can be seen from table
285 3 and Fig 3 that the inhibition of alpha amylase increases as the concentration of Voglibose increases. In
286 the experiments conducted by considering 0.3mg/ml of Voglibose with various concentrations of alpha
287 amylase which is as tabulated in Table 4-6, it can be seen that the percentage of inhibition is repeatable
288 for all the experiments. Thus, alpha amylase can be used for interacting with Voglibose which can have
289 further applications in the field of biosensing.

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291 **4. CONCLUSION**

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

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