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ALPHA-AMYLASE INHIBITORY POTENTIAL OF PRODIGIOSIN: AN *IN SILICO* AND *IN VITRO* STUDY

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Abstract

To find the best alternative, possessing dual activity as a food coloring and anti-diabetic agent, a red bacterial pigment was reported to show many promising potential therapeutic activities viz. anti-protozoan, antibacterial, antifungal, antiviral insecticidal, anti-parasitic, immune modulator, anticancer was studied for α-amylase inhibition potentials which play a key role in raising blood glucose levels. Both *In silico* and *In vitro* studies were performed. Advanced Auto dock software was used for docking studies taking prodigiosin, the red bacterial pigment, and the standard drug acarbose as ligands and pancreatic α-amylase as protein. Similarly, *In vitro* studies were also performed to substantiate the results of *In silico* studies. The test compounds prodigiosin and the standard drug acarbose exhibited binding energies of -7.1 Kcal mol^{-1}, -7.6 Kcal mol^{-1} respectively against the protein 3BC9. In *In vitro* studies, the test compound exhibited strong α-amylase inhibition activity.

Hence, we conclude that prodigiosin has potent human pancreatic alpha-amylase inhibitory activity, which may have an additional impact in the treatment of diabetes and as a food coloring agent.

Key words: Prodigiosin, alpha amylase, food colorant

1. Introduction

Food colors have good demand in food industries (Rana et al., 2021) as they enhance visual appeal, and make the food attractive and grant a unique identity. Some food colorants are also associated with flavor, safety and nutritional value (Sigurdson et al., 2017).Various artificial colors were approved worldwide as food additives for use in food and other industries. However, synthetic colors may have negative impact on human health and cause a hazardous effect on environment (Rana et al.,2021).

Diabetes mellitus (DM) is a chronic metabolic disorder resulting from failure in insulin secretion or a decrease in insulin sensitivity and function affecting carbohydrate and lipid metabolism (Sharma

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et al., 2021). Diabetes is classified into type-1 and type-2 and gestational. Among all the types, type-2 diabetes accounts for 90% of diabetes cases (Hossain et al., 2020) characterized by insulin resistance or failure in the production of insulin ultimately malfunctioning of β-cell cells (Olokoba et al., 2012).Chronic diabetes leads to numerous complications viz. Heart attack, stroke, kidney failure, nerve damage, vision loss, Lipid metabolism disorders and atherosclerosis (Mechchate et al., 2021; Shayegan et al., 2023). The enzymes, alpha amylase and alpha glucosidase break the carbohydrates and increase the blood glucose levels. Alpha-amylase breaks complex carbohydrates into simple sugars whereas; beta-glucosidase plays the final step in releasing glucose (Khanam et al., 2023). The alpha amylase is the key enzyme metabolizing starch. Inhibition of this enzyme in diabetes patients controls blood glucose levels and reduces complications of diabetes. The drugs available today for treating diabetes are found to possess side effects in long term use and have certain limitations (Sharma et al., 2021). Therefore, an ecofriendly and economical alternate is needed that can serve both the purposes i.e. as a food coloring agent with alpha amylase inhibition potentials so that it can also act as medicine to control diabetes.

Hence, in the present study, prodigiosin with many potential pharmaceutical applications is explored for anti-diabetic activity both, *In silico* and *In vitro*.

2. Materials and methods

Prodigiosin and acarbose were gifted by the Department of Microbiology, Vaagdevi, Degree and PG College, Warangal, Telangana State, Hanamkonda. Porcine pancreatic alpha-amylase was procured from Sigma Aldrich Inc. Porcine pancreatic α-amylase was procured from Sigma Aldrich Inc., (St Louis, MO). Dinitrosalicylic acid (DNS) and Tris base were obtained from Himedia Laboratory, Mumbai. All other chemicals were of analytical grade.

2.1.Preparation of Ligand

For docking studies to be performed, prodigiosin was selected as test compound and acarbose, which is α-amylase inhibitor, was selected as standard drug. The structures of both the ligands were drawn using Chem Draw software. The drawn ligand was changed to 3D PDB format using Accelrys Discovery Studio 2.3.

2.2.Preparation of Protein

The pancreatic α-amylase was selected as protein and was downloaded from the protein data bank http://www.rcsb.org/pdb. The bound ligand, hetero atoms, and water molecules were removed and Kollman charges were added, salvation parameters by default were assigned using Auto Dock software.

2.3. Validation of the Software

Before performing *In silico* studies, the validation of docking software was done by redocked the co-crystallized ligand with the same protein thereby reproducing the original interactions of the protein-ligand complexes and comparing the root-mean-square distance of the experimentally determined poses with the docked pose.

2.4. In silico studies: Virtual screening for interaction of Prodigiosin with Pancreatic α-amylase by molecular docking.

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After preparing ligand and proteins, both were converted into pdbqt format using Auto Dock software and were used for docking. To cover the pocket, a grid box was arranged with the main residues of the protein binding site. The grid size of X=40, Y=40 and, Z=40 was maintained. The coordinates used for docking the proteins with the ligands for α-amylase alpha (PDBID: 3BC9) were x= 38.442, y= -16.365, z= 13.631. Advanced molecular docking program Auto Dock software was used to interact the proteins against the ligands. The docking studies were performed (in triplicates) and the average of the best conformations was chosen with the lowest docking energy based on the docking search of ten runs. The interaction of the proteins with the ligand, bond lengths, hydrogen bonds, and root mean square difference (RMSD) was analyzed using PYMOL software.

2.5.α-Amylase inhibition activity

The alpha-amylase inhibitory activity was performed as described by Bhosale et al. (2018) with slight modification. Briefly, 100 µl of prodigiosin and the standard drug acarbose (1 mg/ml) were mixed with 100µl of α-amylase (1mg/ml) and 100µl of 0.1M phosphate buffer (pH 7.0) separately in test tubes. Later, 100µl of starch (0.1%) solution was added and incubated at room temperature for 30min. Further, 1ml of dinitro salicylic acid (DNSA) was added into test tubes separately and incubated in a boiling water bath for 5 minutes to stop the reaction. Later, the test tubes were removed from the water bath, cooled to room temperature, and diluted to 10 ml with distilled water. The absorbance of the product was taken at 540nm. A blank solution was prepared with 100% enzyme by replacing the substrate (prodigiosin) with 100 µl of buffer. A positive control was prepared using the standard drug acarbose (100µg/ml) and the reaction was performed similarly to the reaction of prodigiosin.

The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the equation given below:

$$\% \text{ inhibition} = \frac{\text{O.D of control} - \text{O.D Of test}}{\text{O.D of control}} \times 100$$

3. Results and discussion

In the present study, pancreatic α-amylase inhibition potential of red pigment prodigiosin was performed using acarbose as the standard drug. Initially, *In silico* studies were performed and based on positive results obtained *In vitro* studied were done.

In silico studies

For *In silico* studies, pancreatic α-amylase enzyme was downloaded from protein data bank with PDB ID: 3BC9 (fig.1) and prodigiosin and the standard drug acarbose were used as ligands. The docking of both the protein and ligands was found to be successful and was confirmed based on the formation of the protein ligand complex. The binding energy, hydrogen bonding, bond length, active site residues, RMSD, and orientation of the docked compounds within the active site were visualized. Both the test compound prodigiosin and the standard drug acarbose showed best fit RMSD value of 0.00 indicating statistically significant interaction. The test drug prodigiosin has shown binding energy of -7.6 and the standard drug acarbose has shown binding energy of -7.1 with the protein α-amylase. Hence, it is

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noticeable that prodigiosin is strongly inhibiting α-amylase compared to the standard drug acarbose. The results of the binding energy, hydrogen bonds etc. are presented in table 1 and fig.2.

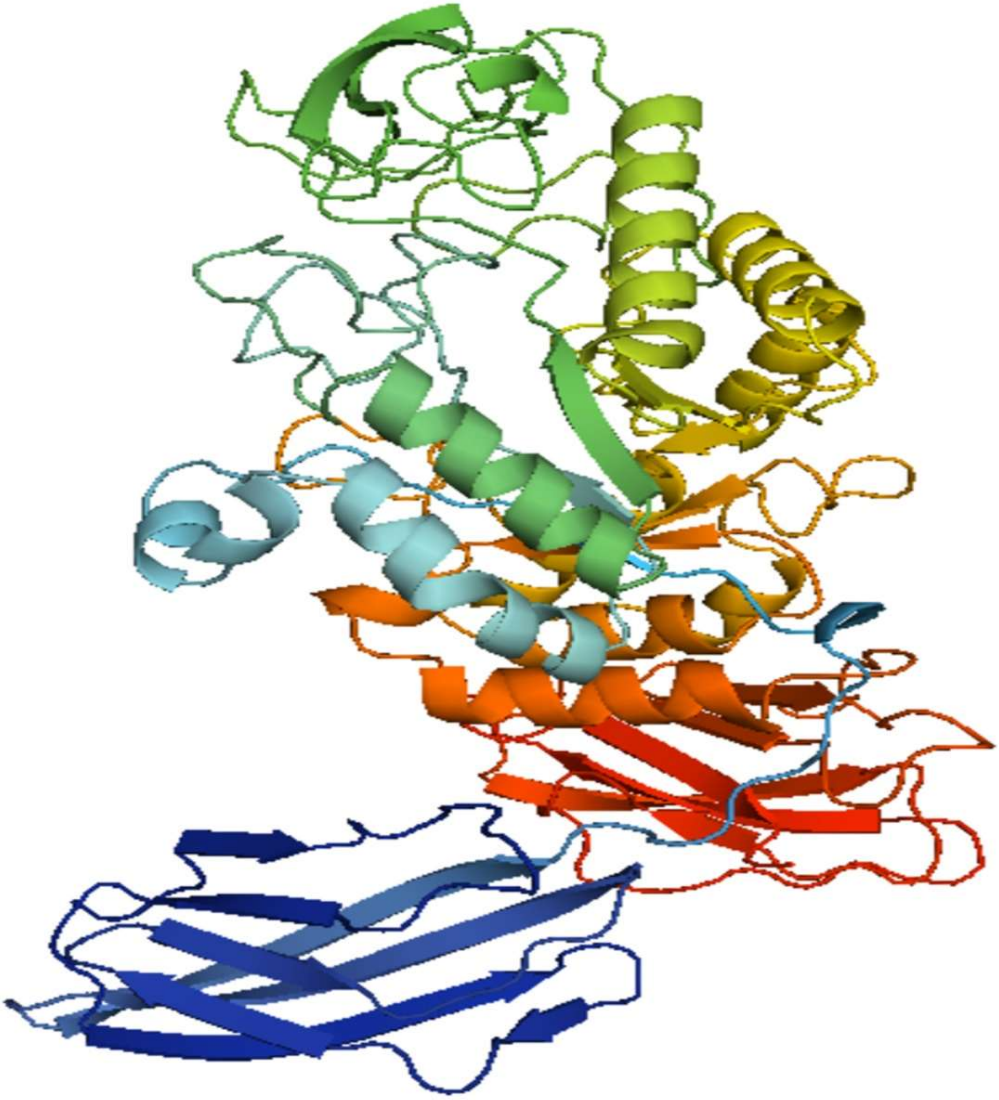

Fig.1. The 3D crystal structure of pancreatic α-amylase enzyme (PDB ID: 3BC9) in cartoon representation

From the table, it is evident that five hydrogen bonds were formed between the ligand prodigiosin and the protein α-amylase. Two hydrogen bonds were formed with the aminoacids Threonine and two bonds with Asperagine and one bond with Histidine. Whereas, the standard drug acarbose formed seven hydrogen bonds with the α-amylase hence, showed a slightly higher binding score compared to the test compound prodigiosin. However, prodigiosin has shown almost equal α-amylase inhibition potentials compared to the standard drug acarbose.

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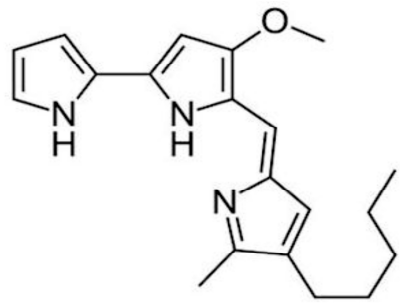
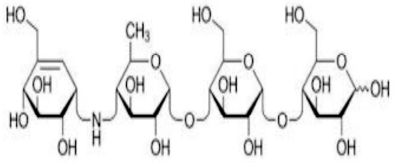
Table.1 showing interacting amino acids, Hydrogen bonds, distance and binding scores of pancreatic α-amylase (PDB ID.3BC9) with test compound Prodigiosin and the standard drug Acarbose.

Name of the Ligand	Affinity kcal/mole	No. of hydrogen bonds	Distance Å	Interacting amino acids
 Prodigiosin	-7.1	05	2.3 2.5 3.0 2.0 2.2	Thr-165 Thr-165 His-123 Asp-222 Asp-222
 Acarbose	-7.6	07	2.4 3.3 2.5 2.0 3.2 2.4 2.5 3.0	Asn-437 Gly-436 Gly-436 Arg-473 Arg-501 Asn-122 Asn-122 Gly-507

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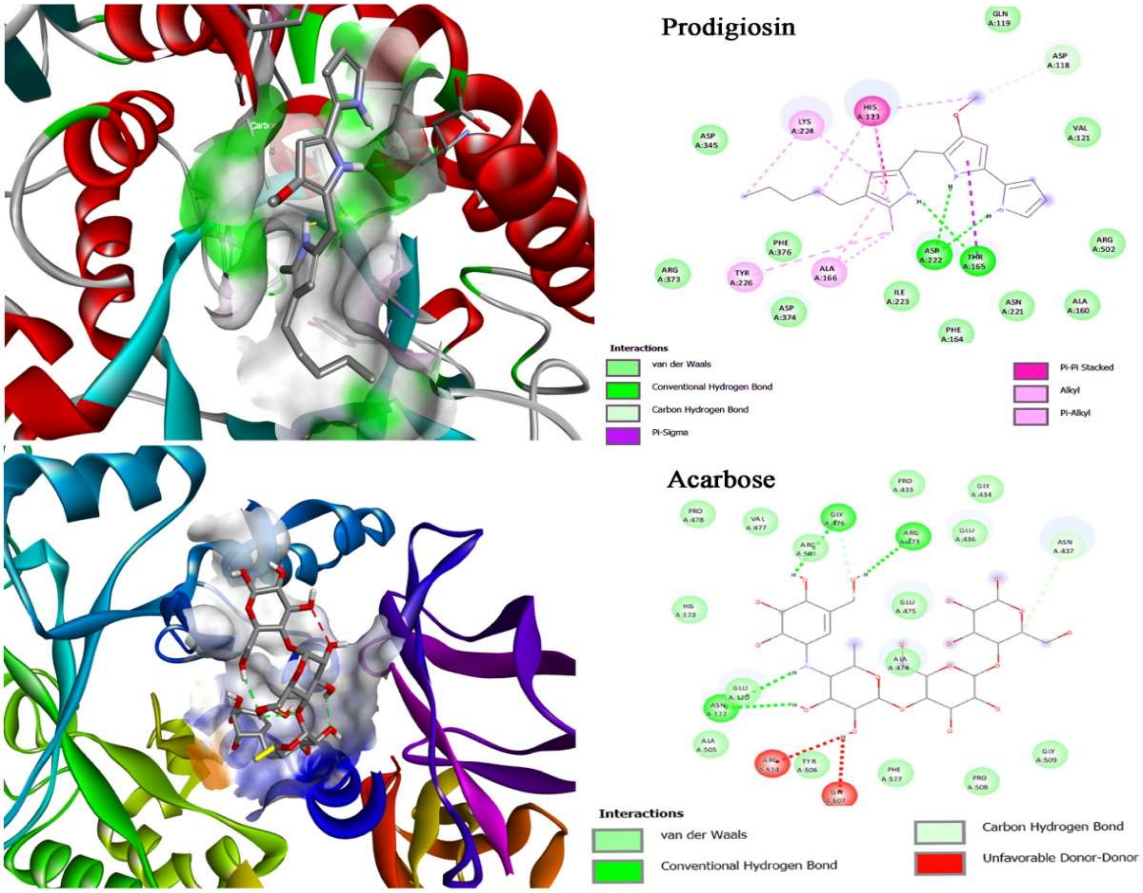

Fig 2. Showing a. Snapshot of docking of Prodigiosin with α-amylase enzyme (PDB ID: 3BC9)
b. Snapshot of docking of Acarbose with α-amylase enzyme (PDB ID: 3BC9)

3.1.In vitro studies

The α-amylase was reported to be the key enzyme which metabolizes the starch. Inhibition of this enzyme controls blood glucose levels and reduce the risk of diabetes. Hence, α-amylase inhibition studies were performed *In silico* and to further to substantiate the results *In vitro* studies were performed using prodigiosin as test drug and acarbose as standard drug and the results are presented in table 2.

From the table it is clear that, both prodigiosin and acarbose when tested at concentrations of 5, 10,15,20,25 mg/ml showed concentration dependent inhibition of α-amylase. The percent inhibition showed by prodigiosin was 5.43, 10.8, 30.4, 51.0, and 66.3 respectively while the standard drug acarbose showed 44.13, 86.64, 91.52 and 100% inhibition respectively and the IC50 values recorded for prodigiosin was 20mg/ml whereas for prodigiosin it is 6mg/ml (Fig 3).

From the studies, it is clear that prodigiosin possess α-amylase inhibition potential hence, use of prodigiosin in food industry serves both purposes viz. as food coloring agent which is safe, ecofriendly and economical and it also helps in treating diabetes patients by inhibiting α-amylase which is key enzyme in metabolising starch.

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Table 2. α-amylase inhibitory activity of Prodigiosin and acarbose

S. No	Concentration of drug mg/ml	% α-amylase inhibitory activity of Prodigiosin	% α-amylase inhibitory activity of Acarbose
1	5	5.43	44.13
2	10	10.8	86.64
3	15	30.4	91.52
4	20	51.0	100
5	25	66.3	100

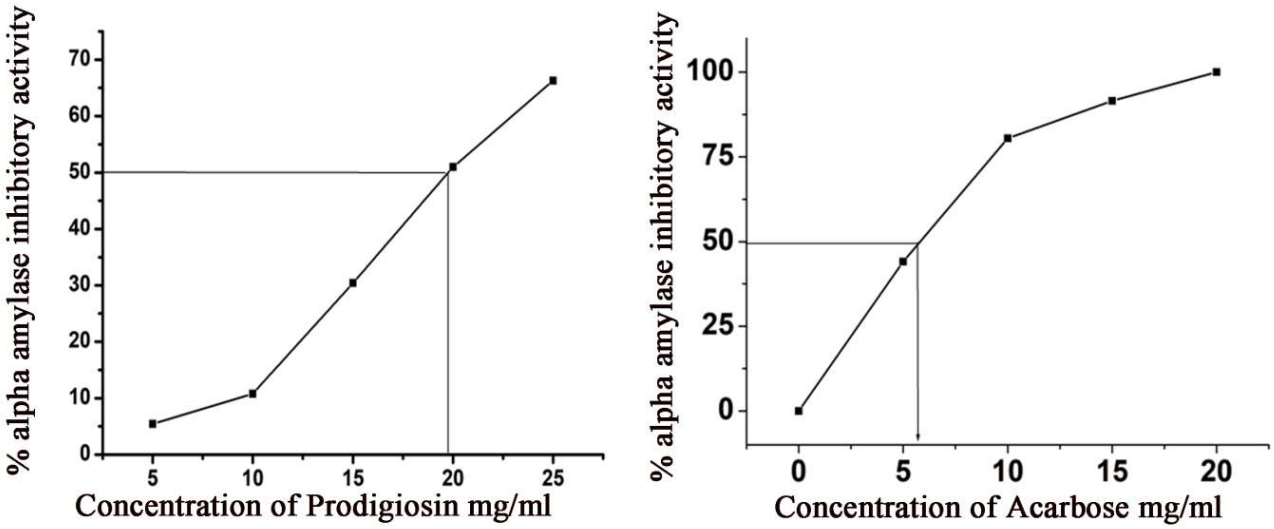

Fig 3. showing IC50 values of prodigiosin and the standard drug acarbose

4.Conclusion

Diabetes which is characterized by high glucose levels poses serious complications viz. kidney damage, vision problems, cardiovascular diseases and other issues. Similarly, different food colorants used today in food industries cause serious side effects and also cause environments issues when discharged in to surroundings. Hence, to solve these issues, the best alternate with duel activity is needed. The bacterial pigment prodigiosin with potential pharmaceutical applications and food coloring

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properties when screened for alpha-amylase inhibition potentials both *In silico* and *In vitro*, showed strong inhibition.

Hence, it can be concluded that prodigiosin can be employed as food coloring agent and as anti-diabetic compound which is ecofriendly and economical.

Conflict of interest

The authors declare that there is no conflict of interest

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