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MOLECULAR DOCKING STUDIES OF ACETIC ACID IN APPLE CIDER VINEGAR FOR ANTI DIABETIC ACTIVITY

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Abstract
Diabetes mellitus is a prevailing problem in most of the countries. It occurs due to the lack of insulin or production of insufficient amount of insulin by pancreatic islets. Insulin is essential for maintaining blood glucose levels in the body. When inadequate amount of insulin is produced it has to be compensated by administration of insulin injections which is a painful procedure. To supplement the insulin needs, small molecules that can be administered through oral route and can mimic insulin action are considered in the present study. The objective of the present study is to perform *In-silico* analysis of acetic acid in apple cider vinegar and to understand it's slow glucose absorption mechanism using Argus Lab docking software.

Key Words
Docking; Disaccharidases; pyMOL; ADME prediction; Argus Lab

Introduction

The earliest known use of vinegar dates to more than 10000 yrs ago. Flavored vinegar has been produced and sold as a commercial product for approximately 5000 yrs. Vinegar ingestion at mealtime reduces postprandial glycemia and increases satiety, metabolic effects that may benefit individuals struggling with diabetes [1-3]. In a research summary, the American Diabetes Association states that vinegar may make food healthier [1]

Diabetes mellitus is most common metabolic disease all over the world and numbers of diabetic patients are still on rise growing at an alarming rate in both developed and developing countries. Diabetes, the third leading cause of death in the world, has many treatment regimens including insulin injections and oral hypoglycemic drugs. Many *in vitro* and *in vivo* data have been accumulated which support the role of apple cider vinegar(ACV) in prevention and management of diabetes, in addition to oral hypoglycemic drugs, the dietary components such as Apple cider vinegar seems to be promising for glycemic control in patients with type 2 diabetes as well as for diabetes related medical conditions. Apple cider vinegar is fermented juice from crushed apples. Like apple juice, it likely contains some pectin; vitamins B1, B2, and B6; biotin; folic acid; niacin; pantothenic acid; and vitamin C. Preliminary clinical research suggests that vinegar can lower postprandial glucose levels in healthy volunteers. Vinegar is thought to affect glucose levels by delaying the gastric emptying rating. Acetic acid in vinegar also seems to suppress disaccharidase activity and increase glucose-6-phosphate levels in skeletal muscle. (www.therapeuticresearch.com). Antiglycemic effects of vinegar have been

known for more than a century and have been demonstrated in animals as well as human studies. Different types of vinegar have protective effect on pancreas and stomach with 15% concentration for 6 weeks. So that using vinegar has a beneficial effect on diabetic patients. Among all types of vinegar, apple vinegar was most effective to decrease glucose total cholesterol, triglycerides, LDL and increases HDL.

Ogawa et al reviewed the effect of acetic acid on Caco-2 cells (a human intestinal cell line) to explore its effect on disaccharidases and glucose transport. Chronic treatment was found not to hinder cell growth or viability, nor did it affect glucose transport (measured using 3-O-methyl glucose), but rather, acetic acid was found to significantly suppressed sucrase in a time and concentration dependent manner. Similarly, the activity of maltase, trehalase, and lactase was found to be significantly decreased. Other acids tested including citric and lactic acid did not suppress sucrase activity [2]. In further exploration, acetic acid was found not to affect the transcription nor translation of sucrose but rather its enzymatic activity [2]. Vinegar is readily available, affordable, and used in a variety of cuisines. Therefore, acetic acid in vinegar may present a viable tool to assist individuals attempting to achieve their glycemic targets [3] and the mechanism of action of vinegar lies in its acidic properties and not the blood level of its salt [4]

Molecular docking is the technique employed for predicting and analysing the interactions between protein receptors and ligands. It provides most detailed possible view of drug receptor interactions and also has created a new rational approach to drug design. Therefore our aim of present study is to find the molecular interaction of acetic acid (apple cider vinegar) with intestinal disaccharidases and explain underlying molecular mechanism of antidiabetic effect of apple cider vinegar by delaying carbohydrate digestion

2. Materials and Methods

2.1. Methods:

An electronic literature search using MEDLINE, PubMed and Google Scholar were conducted. We searched for reviews and randomized controlled trials and prospective cohort studies of vinegar ingestion in populations affected by diabetes. We also searched for *in vitro* and animal experiments that explored vinegar's proposed mechanisms of action. But none of the paper explained underlying molecular mechanism of action of apple cider vinegar antidiabetic effect

2.2. Tools and materials:

In our study we retrieved the data from biological databases like Protein Data Bank (PDB), PubChem. *In silico* studies were carried out using software and online tools like pyMOL, ADME prediction, Argus Lab.

2.3. Preparation of Protein:

The 3D crystal structure of enzymes used in this study was retrieved from RCSB Protein Data Bank (http://www.rcsb.org/pdb). The PDB files were energy minimized using Argus Lab and was used further for docking studies

2.4. Ligands:

Ligands such as drugs (Metformin, Miglitol, Acetic acid) and substrates (lactose, maltose, sucrose, trehalose) were retrieved from website "NCBI PubChem" in SDF format and prepared for docking, geometry optimization of the ligands was carried out in Argus Lab 4.0 (http://www.arguslab.com)

2.5. Preparation of Active Site:

Explicit hydrogen atoms missing in the PDB structure were added using Argus Lab docking software. Furthermore, the atom list of the molecules were prepared, which represents numbers of all the atoms of the active site residues involved.

2.6. Energy Minimization:

Hydrogen added clean files of the proteins were reloaded in the Swiss PDB viewer. The conformations and energy states of the newly added hydrogen were fixed and corrected by minimizing the energy. New energy levels were checked for the RMSD deviation with its actual PDB structures.

2.7. Molecular Docking:

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. The docking was done by using software Argus Lab

2.8. Toxicity Studies:

The toxicity analyses of ligands were carried out by ADME. Based on following ADME properties, drugs were selected further to perform docking studies since they had no side effects.

3. Results and Discussion

Docking analysis scores were obtained from the acetic acid in apple cider vinegar against disacharidases lactase, maltase sucrase and trehalase. The outputs of all ligands were given by energy values in kcal/mol as shown in Table 3. Acetic acid in apple cider vinegar showed good docking scores when compared to standard drugs metformin and miglitol. Docking score of the acetic acid was

compared with the score of the drug metformin and miglitol which are used as a potent drugs for the treatment of diabetes

The physicochemical properties of ligand are shown in Table.1. Ligands toxicity studies was performed to obtain the molecular properties of all ligands as shown in Table 2 using Swiss ADMET software. All ligands were passed and acted as a drug molecule by their adherence to the properties such as Absorption, Distribution, Metabolism and Excretion (ADME) as per the Lipinski Rule Of 5 .The results shows that all the values of analogues fall within the optimal range

Acetic acid was docked with the crystallographic structures of the targets by Argus Lab screening programme as shown in Table 3. The analogues were examined for their binding energies and hydrogen bonding. The conformations with highest binding energies and greater number of hydrogen bonds of the ligands were taken into consideration for ranking the acetic acid.

Potential binding site in Argus Lab screening programme is also used for the identification of most potential active site where the ligand can bind and interact with the target protein in disaccharadases are ARG 59, SER 58, SER 821, ASN 196, TYR 202, GLN 207(Figure 1-5). Docking study also shows that in most of the cases acetic acid showed higher hydrogen bonding with disaccharidases.

6. Discussion

From the Argus lab studies the best pose was obtained with least energy value. The interaction with active site reveals that acetic acid mimics the activity of standard drug metformin in its anti diabetic activity and slowers glucose absorption in the small intestine.

The results of molecular docking (Figure 1-5) showed that acetic acid inhibits disaccharidases lactase, maltase, sucrose, trehalase by binding to its ligand active site and inhibits substrate (disaccharides such as lactose, maltose, sucrose, trehalose) and hence slower their degradation into monosaccharides such as glucose, fructose, galactose, mannose respectively.

7. Conclusion

Flexible docking of ligand to receptor molecules is an emerging approach and is extensively used to reduce cost and time in drug discovery. In this study the approach utilized is successful in finding antidiabetic potential of acetic acid against its receptors. All the compounds show lowest docked energy and hydrogen bonding stabilizes the interactions

The final assessment of drug-likeness and its related parameters helps to confirm the oral activity of compounds. From the Argus Lab dock studies the best pose was obtained with least energy value of -9.62119 kcal/mol for acetic acid binding to trehalose. The interactions with active site residue Ser 821 in sucrose and Arg 59 in lactase indicate temporary inhibition of these disaccharides by acetic acid.

Apple cider vinegar has got an exclusive antidiabetic property and help in preventing diabetic complications. Vinegar is readily available, affordable, and

used in a variety of cuisines. Therefore, its acetic acid may present a viable tool to assist individuals attempting to achieve their glycemic targets.

8. Acknowledgements
We sincerely acknowledge the facility provided by Department of Biochemistry and DBT-Bioinformatics infrastructure facility at Department of Microbiology, Maharani's Science College for Women, Bangalore-560001.

9. References

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Table 1: Physicochemical properties of ligands

Ligand	Molecular formula	MW (g/mol)	Heavy Atoms	Rotatable Bonds	H-Bond Acceptors	H-Bond Donors	MR	TPSA (Å²)
Acetic acid	$C_2H_4O_2$	60.05	4	0	2	1	13.50	37.30
Metformin	$C_4H_{11}N_5$	129.16	9	2	2	3	36.93	91.49
Miglitol	$C_8H_{17}NO_5$	207.22	14	3	6	5	51.08	104.39

Table 2: Drug likeliness of ligands by ADME studies

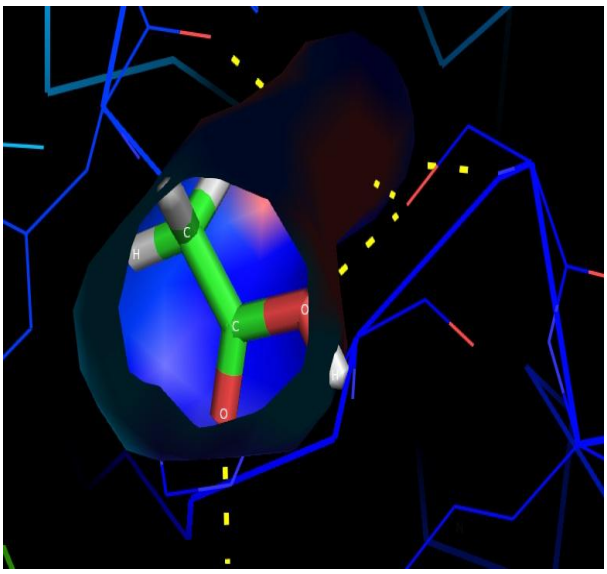
Ligand	Lipinski	Ghose	Veber	Egan	Muegge	Lead-like	Bioavailability Score
Acetic acid	Yes	No	Yes	Yes	No	No	0.56
Metformin	Yes	No	Yes	Yes	No	No	0.55
Miglitol	Yes	No	Yes	Yes	No	No	0.55

Table 3: Binding energy (Kilocalories/mole)

Enzymes	Acetic acid	Metformin	Miglitol	Lactose	Maltose	Sucrose	Trehalose
Lactase	-5.78178 kcal/mol	-4.83844 kcal/mol	-6.52752 kcal/mol	-7.76638 kcal/mol	–	–	–
Maltase	-5.05831 kcal/mol	-5.30214 kcal/mol	-6.2278 kcal/mol	–	-6.91 kcal/mol	–	–
Sucrase	-5.05346 kcal/mol	-5.64527 kcal/mol	-6.44516 kcal/mol	–	–	-8.72268 kcal/mol	–
Trehalase	-5.46408 kcal/mol	-5.42207 kcal/mol	-7.58488 kcal/mol	–	–	–	-9.62119 kcal/mol

Figure Captions

Fig1: Docking of acetic acid with lactase

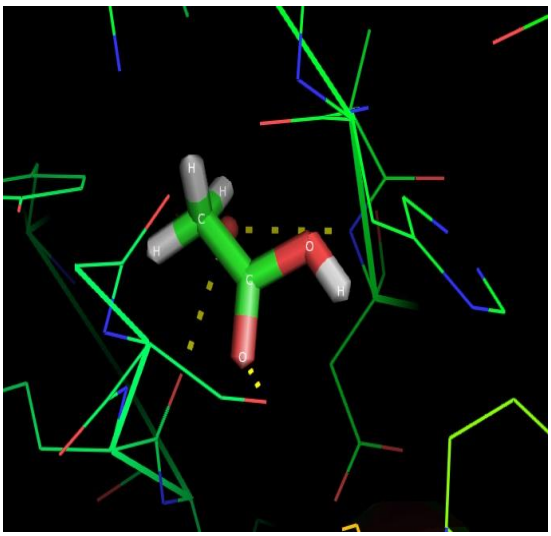


ACETIC ACID WITH LACTASE:

O_2 of acetic acid ⟹ NH_2 of arg 59 of lactase

O_1 of acetic acid ⟹ O(G) of ser 58 of lactase

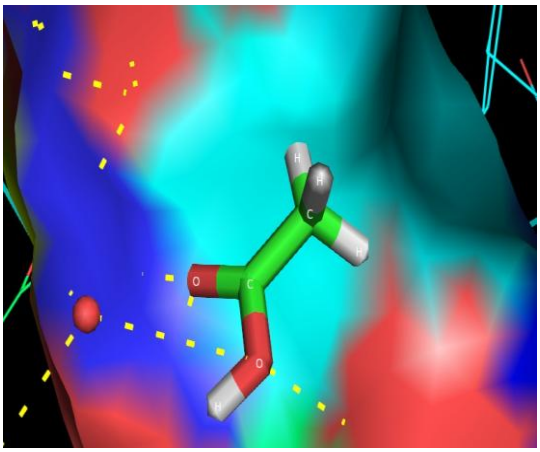
Fig2: Docking of acetic acid with Sucrase



ACETIC ACID WITH SUCRASE:

- O_2 of acetic acid ⟹ O(G) of ser 821 of sucrose
- O_2 of acetic acid ⟹ N of ser 821 of sucrase

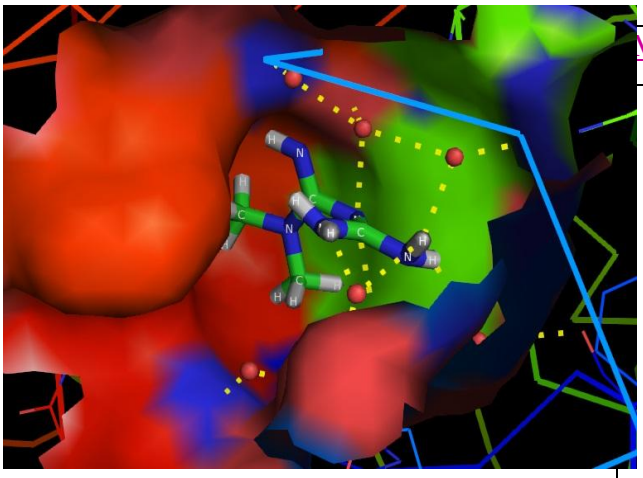
Fig3: Docking of acetic acid with Trehalase



ACETIC ACID WITH TREHALASE:

- O_2 of acetic acid ⟹ N(D2) of asn 196 of trehalase
- O_1 of acetic acid ⟹ OH of tyr 202 of trehalase
- O_2 of acetic acid ⟹ N(E2) of gln 207 of trehalase

Fig 4: Binding of lactase with metformin



METFORMIN WITH LACTASE:

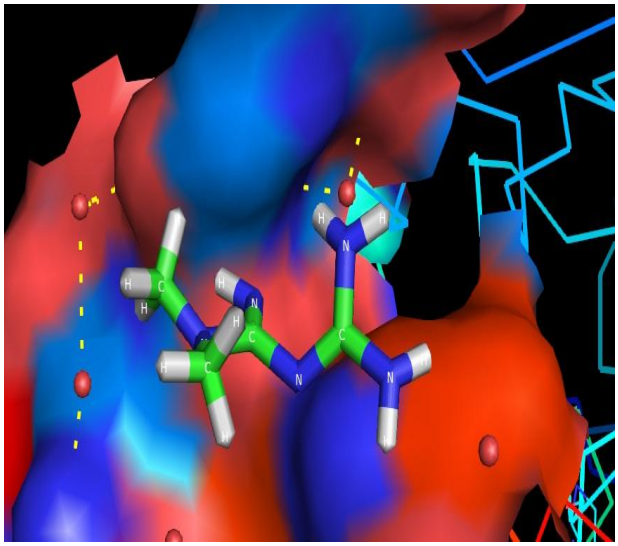
N_2, H_{17}, H_{20} of metformin ⟹ OD1 of asp 101 of lactase

H_{17} of metformin ⟹ water(O) ⟹ NE1 of trp 317of lactase

H_{18} of metformin ⟹ water(O) ⟹ O of ser 58 of lactase

N_2 of metformin ⟹ water (O) ⟹ O of asn 587of lactase

Fig 5: Binding of miglitol with lactase



MIGLITOL WITH LACTASE:

- H_{25}, H_{26} ⟹ NH2 of arg 521 of lactase
- H_{25} ⟹ NH1 of arg 521 of lactase
- H_{30}, O_5 ⟹ NE2 of gln 97 of lactase
- O_3 ⟹ water(O) ⟹ NE2 of gln 95 of lactase