Current Trends in Biotechnology and Pharmacy
Vol. 15 (2) 213-219 April 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.2.23

A New Insights and Novel Targets for Hyperglycemia from Foxtail Millet (*Setariaitalica* L.) using Molecular Docking Studies

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Abstract

The inhibition of α-glucosidase and α-amylase, enzymes which are implicated in the digestion of carbohydrates, can evidently decrease the post-prandial increase of blood glucose levels. It can be an significant approach in the management of blood glucose level in type 2 diabetic and borderline patients. At present, there is renewed attention in plant-based medicines and functional foods modulating physiological reactions in the prevention and treatment of diabetes and obesity. The plant kingdom is a extensive field to investigate for natural effective oral hyperglycemic agents that have insignificant or no side effects. Consequently, natural α-glucosidase and α-amylase inhibitors from plant sources offer an prominent strategy for the control of hyperglycemia. Nowadays majority of the people divert towards millets and taken the millets as a meal in the place of rice as because they show key results on who are suffering from hyperglycemia. Foxtail millet [*Setariaitalica (L.) P. Beauv.*] is a member of poaceae grass family found in both arid and semi-arid regions which is principally used as a fodder and cereal crop. All the food products prepared from foxtail millet are efficient in reducing the blood glucose levels both in normal as well as diabetic patients. To identify human α-glucosidase and α-amylase inhibitors phenolic compounds (Vanillic acid, Ferulic acid) and flavonoid (Kaempferol) of foxtail millets were screened using the structural based molecular docking approach. The findings of this study revealed that all the tested compounds exhibited inhibitory activity against both the enzymes. Comparatively among the three inhibitors tested kaempferol flavonoid was more effective with -261.79Kcal/mol and -404.66Kcal/mol of binding free energy values against α-amylase and α-glucosidase correspondingly. This insilco study paves the way to further research on foxtail millets (*Setariaitalica* L.) inhibitory compounds associated with hyperglycemia.

Keywords: Foxtail millet, α-Glucosidase, α-Amylase, Vanillic acid, Ferulic acid, Kaempferol, Hyperglycemia.

Introduction

According to WHO modern survey (2012) diabetes mellitus is in the seventh place in causing death worldwide. Nearly 1.5 million people are losing their lives due to diabetes mellitus (http://www.who.int). Diabetes mellitus is a chronic metabolic disease characterized by

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hyperglycemia, resulting from inadequate or inefficient insulin secretion, with some modifications in carbohydrate, protein and lipid metabolism. Studies show that hyperglycemia leads to non-enzymatic glycolisation of various proteins which causes in the promotion of serious complication in diabetes (9,10). Consequently, it is vital to control postprandial blood glucose which is essential for treatment of diabetes and in diminishing severe vascular complications (1,10). One way for reduction postprandial hyperglycemia is to prevent absorption of carbohydrates after food intake. The process of conversion of carbohydrates to blood glucose includes hydrolysis of complex polysaccharides to dextrins by α- *amylases* and further hydrolysis to glucose with the help of α- glucosidase before being absorbed by intestinal epithelium and entering blood circulation. Therefore, inhibition of α-amylase and α-glucosidase help in reduction of post prandial hyperglycemia due to inhibition of enzymatic hydrolysis of complex carbohydrates which delays the absorption of blood glucose. To cure the patients who are suffering with type II diabetes mellitus, acorbose, voglibose and miglitol are injected either alone or in combination with insulin secretogogues (17). But with their usage other side effects like liver disorders, hepatitis, abdominal cramping, flatulence have been reported with several scientific studies there have been reported safer natural α - amylase and α-glucosidase inhibitors from plant material (15).

Millet is an aggregate term alluding to a few little cultivated edible grasses having a place with the family Poaceae, developed on minimal dry grounds in mild, subtropical, and tropical areas of the world (3). Five genera of the subfamily, Panicoideae (Panicum, Paspalum, Setaria, Echinochloa and Pennisetum) and a genus of Chloridoideae subfamily (Eleusine) are together called millets. These millets are valuable in extending the hereditary variety in the food crate and improving food and nutritional security (5).

Foxtail milet [*Setariaitalica (L.) P. Beauv.*], a member of poaceae grass family. In both arid and semi-arid region, it is primarily used as a fodder and cereal crop. It has taken its origin from china and is a vital crop in rain fed areas in India. In India it is cultivated in Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu, Rajasthan, Madhya Pradesh, Uttar Pradesh and north eastern states (16). Foxtail millets serve as staple food in millet delivering regions and are utilized in the making of different conventional foods, for example, porridges, idli, dosa, bread, chakli and papads (20).

Foxtail millet grain contains 351Kcal Energy,11.2 (g) protein, 4(g) fat, 63.3 (g) carbohydrates, 6.7 (g) crude fiber, 31.0(mg) calcium,2.8(mg) iron,3.2(mg) and niacin. (FAO1995) and bran constituted 9.39 per cent crude oil, 12.48 per cent crude protein and 51.69 per cent crude fiber (12).

All the food products made from foxtail millet are observed to be effective in decrease in blood glucose levels in normal as well as diabetic patients [13]. Moreover, wholesome advantages, these grains are store house of various phytochemicals principally phenolic compounds, which help in the administration of ongoing issues like malignant growth, diabetes and cardiovascular illnesses (4).

Three benzoic acid derivatives (Gallic, p-hydroxybenzoic and vanillic acids) and five cinnamic acid derivatives (Caffeic, Chlorogenic, Ferulic, Sinapic and P-coumaric acids) were identified in raw and processed foxtail millet grains. Amongst the phenolic acids, vanillic acid and ferulic acids were the predominant phenolic acids detected in raw barnyard, foxtail (11). Three flavonols (Rutin, Kaempferol and Myrecetin), one flavone (Apigenin) and one flavanone (Naringenin) were detected in raw and processed millets. Kaempferol was the most abundant flavonoid, whereas myrecetin and rutin were minor flavonoids present in foxtail millets (14). Furthermore, the contributions of foxtail millet phenolic and flavonoid inhibitory activities against α- amylase and α-glucosidase were also evaluated (15).

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Structure-based computational methods, including molecular docking, have increasingly been used in the study of bio molecular structure and function, as well as in the design of structure-based rational drugs. In particular, molecular docking contributed to the improvement of several inhibitors and inhibitor candidates that have been highly developed to clinical trials [8], signifying that docking simulation is a useful tool for elevating chemical library with active compounds.

Materials and methods

Molecular docking was conducted using a human α- amylase & α-glucosidase enzymes (Receptors). The human pancreatic α- amylase (PDB Code 1HNY, (2) and α-glucosidase (PDB code, 3L4X, [19] were obtained from same protein data bank (http://www.rcsb.org/). For ligands ferulic acid (ID number-Zinc 58258), vanillicacid (ID number-Zinc 338275), kaempferol (ID number-Zinc 3869768), was downloaded from ZINC Database (http://zinc.docking.org/). MMFF (Merck Molecular Force Field) (7) used for energy minimization. At last energy minimized molecules are used for molecular docking. We used patch dock web server tool (http://bioinfo3d.cs.tau.ac.il/Patch Dock/) for molecular docking (18).

Results and Discussions

The rank of all compounds was determined on the basis of the binding free energy of the lowest energy cluster. The compounds ferulic acid, vanillic acid and kaempfrolshows a binding energy with hydrogen bond within the range.

***Ferulic acid with human* α- amylase and α-glucosidase:** Inhibitor compound ferulic acid, structural based molecular docking with human α- amylase and α -glucosidase shown -154.23 Kcal/mol, -261. 44Kcal/mol of binding free energy correspondingly. Hydrogen bonds were formed between α- amylase oxygen atom of ASN53 residue with one hydrogen atom of ligand ferulic acid with bond length of 1.8Å which is shown on Figure-1 and Table-1. When ferulic acid is docking with α-glucosidase receptor the hydrogen bonds were formed oxygen atom of SER521 with hydrogen atom of ligand and nitrogen atom of MET567 with oxygen atom of ligand with bond lengths1.5Å and 3.1Å respectively which is shown in Figure-2 and Table-1.

***Vanillic acid with human α- amylase and α-glucosidase**:* Inhibitor compound vanillic acid, structural based molecular docking with human α- amylase and α-glucosidase shown -127.42 Kcal/mol, -225.47Kcal/mol of binding free energy respectively. Hydrogen bonds were formed between α- amylase nitrogen atoms of TYR468, SER478 residues with oxygen atoms of ligand vanillic acid with bond length of 3.2 Å and 2.5Å respectively which is shown on Figure-3 and Table-1.When vanillic acid is docking with α-glucosidase receptor the hydrogen bonds were formed between nitrogen atoms of ALA285, nitrogen and oxygen atom of ILE523 with oxygen and hydrogen atom of ligand with bond lengths 3.0Å, 3.3Å and 2.2Å respectively which is shown in Figure-4 and Table-1.

Kaempferol with human* α - amylase and α - glucosidase*: Structural based molecular docking of kaempfrol with human α- amylase and α-glucosidase shown -267.79 Kcal/mol, -404. 66 Kcal/mol of binding free energy, respectively. Hydrogen bonds were formed between receptor α- amylase oxygen atoms of ILE312, ARG346 residues with nitrogen and NH1 atoms of ligand kaempferol with bond length of 3.3Å and 2.8 Å respectively which is shown on Figure-5 and Table-1. When kaempferol is docking with α-glucosidase receptor the hydrogen bonds were formed between OE1 atom of GLN28, NH2 atom of ARG29 with oxygen atoms of ligand with bond lengths 2.4Å and 3.5 Å respectively which is shown in Figure-6 and Table-1.

Conclusions

Findings from our study, signify the potentiality of foxtail millet seed based phenolic (ferulic acid, vanillic acid) and flavonoid (Kaempferol) compoundswithinhibitory potential as anti-diabetic agents. The evidence leads us to propose that α-amylase and α-glucosidase may

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be the targets for the phenolicand flavonoid compounds in hyperglycemia.The result of this study revealed that the tested compounds exhibited inhibitory activity against with both enzymes. Comparatively among the three inhibitors tested kaempferol (flavonoid) was more effective with -261.79 Kcal/mol and -404.66K cal/mol of binding free energy values against α-*amylase* and α-*glucosidase* respectively. This Insilco study paves the way for further studies to evaluate foxtail millet germplasm and its genetic variability for the seed based inhibitory compounds associated with hyperglycemia.

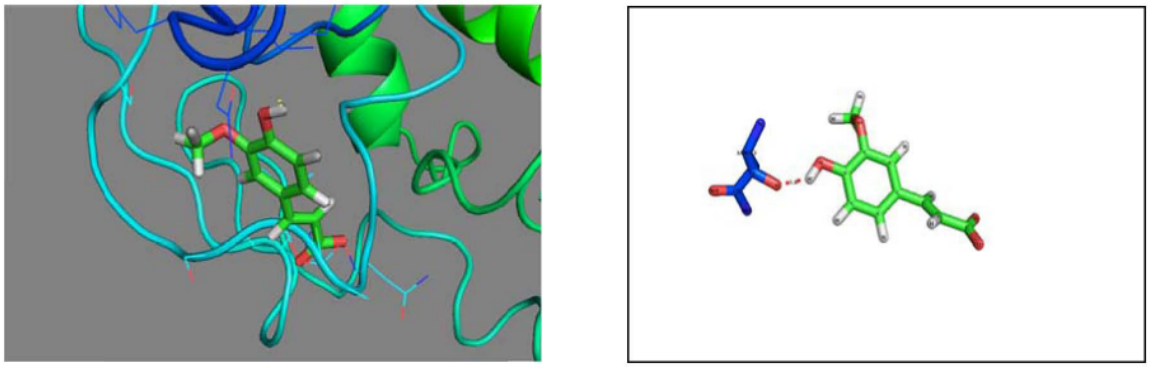

Fig1. (a) The structural overlay of docked compound receptor (ferulic acid)) and ligand human α-amylase **(b)** Hydrogen bond interaction between ASN53-Oxygen residue with hydrogen atom of ligand ferulic acid with bond length of 1.8Å

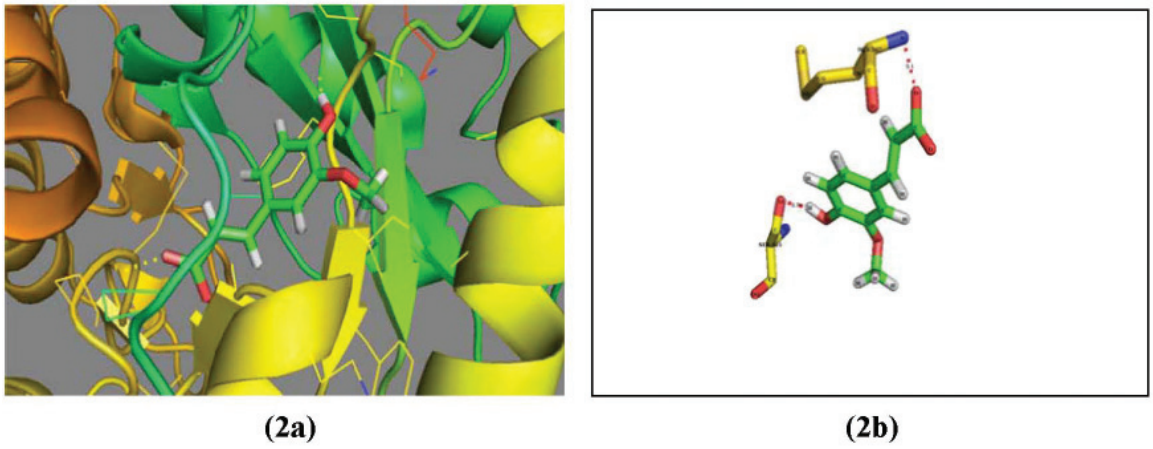

Fig: 2. (a) The structural overlay of docked compound receptor (ferulic acid)) and ligand human α-glucosidase **(b)** Hydrogen bond interaction between SER521-O & MET567-N residues with hydrogen atom& oxygen atom of ligand ferulic acid with bond length of 1.5Å& 3.1Å respectively.

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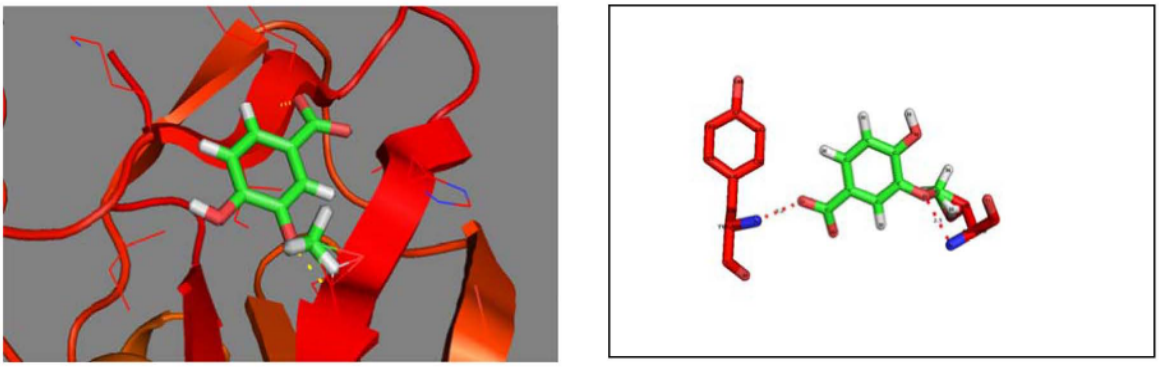
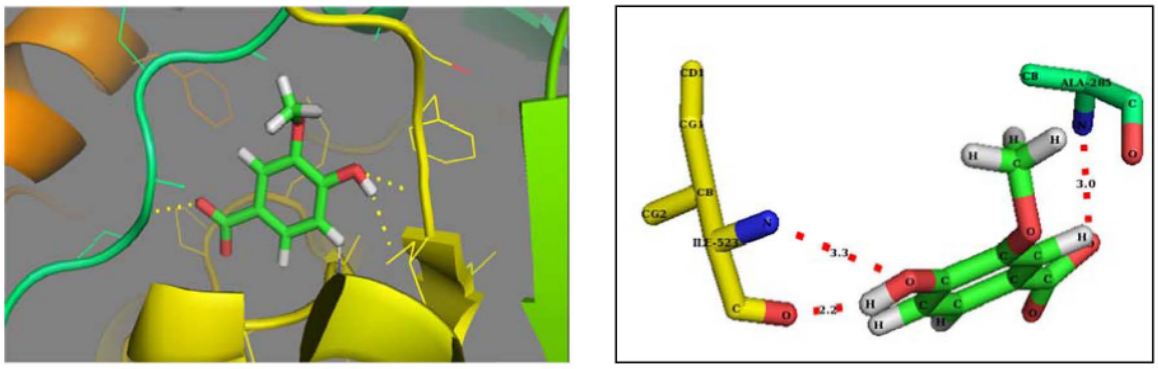


(3a) (3b)

Fig:3 (a) The structural overlay of docked compound receptor (Vanillic acid) and ligand human α-amylase **(b)** Hydrogen bond interaction between TYR468-N & SER478-N residues with oxygen atoms of ligand vanillic acid with bond length of 3.2Å& 2.5Å respectively.



(4a) (4b)

Fig:4 (a) The structural overlay of docked compound receptor (Vanillic acid)) and ligand human α-glucosidase **(b)** Hydrogen bond interaction between ALA285-N, ILE523-N & ILE523-O residues with oxygen & hydrogen atoms of ligand vanillicacid with bond length of 3.0Å, 3.3Å& 2.2Å respectively.

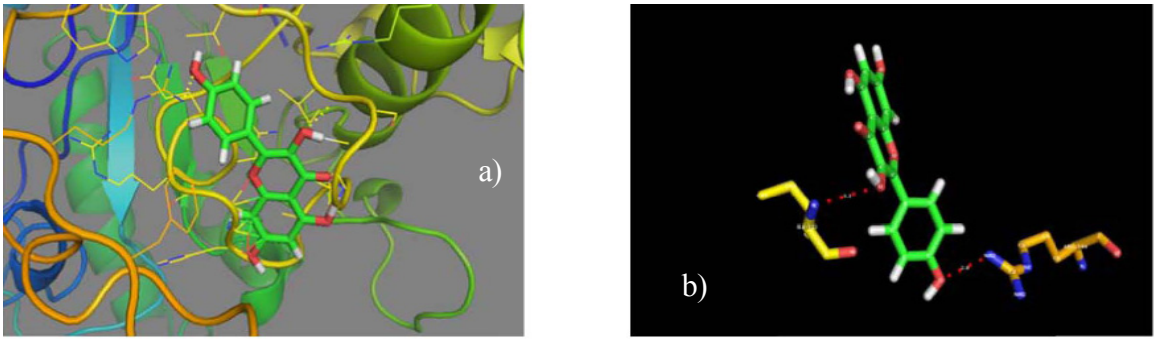


Fig: 5 (a) The structural overlay of docked compound receptor (Kaempferol)) and ligand human α-amylase **(b)** Hydrogen bond interaction between ILE312-O & ARG346-O residues with Nitrogen and NH1 of ligand Kaempferol with bond length of 3.3 Å & 2.8Å respectively

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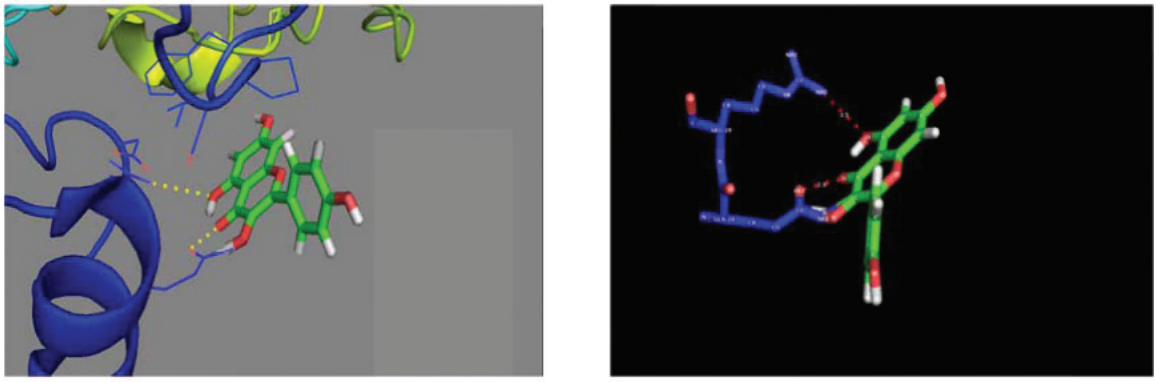

(6a) **(6b)**

Fig:6 (a) The structural overlay of docked compound receptor (Kaempferol)) and ligand human α-glucosidase **(b)** Hydrogen bond interaction between GLN28-OE1 & ARG29-NH2 residues with oxygen atoms of ligand Kaempferol with bond length of 2.4Å& 3.5 Å respectively

Table-1: Compounds binding energies and their enzyme- ligand interaction values

S.No.	Enzyme	Ligand	Binding Energy (kcal/mol)	Receptor Residue Number – Atom	Ligand Atom	Bond Length (Å)
1	α-Amylase	Ferulic Acid	-154.23	ASP253 – H	O	1.8
		Vanillic Acid	-127.42	TYR468 – N	O	3.2
				SER478 – N	O	2.5
		Kaempferol	-261.79	ILE312 – O	N	3.3
				ARG346 – O	NH1	2.8
2	α-Glucosidase	Ferulic Acid	-261.44	SER521 – O	H	1.5
				MET567 – N	O	3.1
		Vanillic Acid	-225.47	ALA285 – N	O	3.0
				ILE523 – N	O	3.3
				ILE523 – O	H	2.2
		Kaempferol	-404.66	GLN28 –OE1	O	2.4
				ARG29 – NH2	O	3.5

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