Research article

In silico analysis of ferulic acid against therapeutic target proteins PPAR-γ, SIRT1, FOXO1 and LXR-α for the treatment of hyperlipidemia

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ABSTRACT

Introduction and Aim: Hyperlipidemia is a worth-mentioning risk factor for a variety of rapidly spreading diseases such as cardio-vascular diseases, myocardial infarction, impaired glucose tolerance and metabolic syndrome**.** Peroxisome proliferator-activated receptor - gamma (PPAR-γ), sirtuin 1 (SIRT1), forkhead box factor 1(FOXO1), and liver X receptor-alpha (LXR-α) are the important determinants of hyperlipidemia by regulating a plethora of transcriptional factors in metabolically active tissues such as adipose tissue, liver, and skeletal muscle. The present study aimed to evaluate the binding affinity of 4-hydroxy-3-methoxy cinnamic acid (ferulic acid) with therapeutic target proteins of hyperlipidemia using an in silico approach.

Materials and Methods: The *in silico* docking studies were performed between ferulic acid (PubChem CID: 445858) and PPAR-γ, SIRT1, FOXO1, LXR-α with PDB ID of 3ADX – A chain, 4ZZI- A chain, 4LG0– A chain, and 3IPQ-A chain respectively by using Autodock 4.2 docking tool.

Results: The results revealed that ferulic acid exhibited maximum binding affinities with FOXO1 (-8.63) followed by SIRT1(-6.18), PPAR-γ (-5.79), and LXR-α (-5.79) kcal/mol respectively. Ferulic acid interacted with FOXO1 with amino acids ASN 204, TYR 165 with a distance of 2.01 Å and 1.86 Å. Furthermore, the molecular interaction of ferulic acid with SIRT1 was at residues SER 441(2.20 Å), GLN 345(2.79 Å), and LXR-α was at amino acids ASP 444 (1.85 Å) and SER 418 (1.98 Å). Also, to activate the action of PPAR-γ ferulic acid interacts with it at residues VAL 450(2.04) and GLN 454 (2.80).

Conclusion: These *in vitro* findings suggest that ferulic acid could be used as a lead structure for designing and developing more powerful hypolipidemic medicines.

Keywords: Ferulic acid; PPAR-γ; SIRT1; FOXO1; LXR-α.

INTRODUCTION

Human beings are constantly confronting against life-threatening mortalities such as type II diabetes mellitus, metabolic syndrome, cardiovascular diseases and cancer. Above all, hyperlipidemia is the major contributing factor for the development of coronary heart diseases as well as myocardial infarctions. Elevated plasma triglycerides (TG), low-density lipoproteins (LDL), free fatty acids (FFA) along with small amounts of HDL (high-density lipoproteins) cholesterol are the major causes for the cardiovascular related diseases (1). To combat these morbidities, a highly effective and safer pharmaceutical treatment is essential. Drug development is a complex and tedious process and the search for novel effective compounds is easy due to the availability of numerous docking tools and computer assisted approaches has narrowed the gap to a certain extent (2).

Barley (*Hordeum vulgare*) is a magnificent cereal grain that was used as sustenance for humans and animals by ancient civilizations. Phenolic acid is the most abundant phytochemical in the outer layer of barley kernels. It is available in three main forms: free, conjugated, and bound, with the bound form having the highest concentration, followed by conjugated and free forms. The predominant phenolic acids in barley are p-hydroxy benzoic, p-coumaric and ferulic acids (3). Ferulic acid (4-hydroxy-3-methoxy-cinnamic acid) is a hydroxycinnamic acid derivative with many potential health benefits because of its antioxidant and anti-inflammatory activity (4). Many investigations were carried out on experimental animals to prove the influence of ferulic acid on lipid metabolism causing a decrease in the blood lipids (5). Furthermore, our previous study on the HPLC-UV spectra analysis of hulled barley grains revealed that ferulic acid was the dominant phytochemical present at a concentration of 9.11mg/g of hulled barley grains when compared with other phenolic compounds.

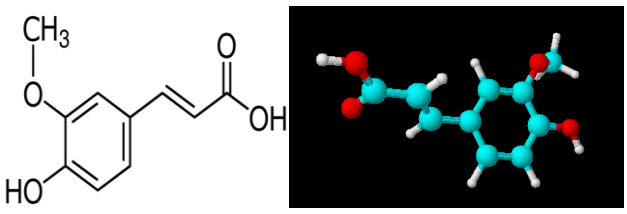

Fig. 1:2D and 3D Structure of ferulic acid

SIRT1 is a member of sirtuin family and it is a nicotinamide adenosine dinucleotide (NAD)-dependent deacetylase that removes acetyl groups from various histone and non-histone proteins. SIRT1 has the ability to deacetylate variety of substrates and is thus implicated in different biochemical function, such as gene regulation, metabolism, and ageing (6). SIRT1 catalyses the reaction that produces nicotinamide and transmits the acetylation of the substrate to cleaved NAD, resulting in a unique metabolite known as O-acetyl-ADP ribose. SIRT1 substrates include the tumour suppressor protein p53, members of the FoxO family (forkhead box factors controlled by insulin/Akt), PPAR-γ, p300, PGC-1 alpha (PPAR gamma coactivator), and NF-kappa B (nuclear factor kappa B).

PPAR-γ is a nuclear receptor transcription factor that is activated by ligands and greatly influences lipid metabolism. PPAR-γ is mostly expressed in target tissues of insulin, in which it supports adipogenesis and the production of adipogenesis-related genes (7). FOXO1 acts like a transcriptional regulator and attaches to the PPAR-γ promoter. Activated SIRT1 deacetylates FOXO1which in turn interacts with PPAR-γ as well. In order to bind to the target region of DNA, PPAR-γ establishes a duplex well with the retinoic acid receptor (RXR), and FOXO1 binding to PPAR-γ is thought to disrupt this PPAR-γ /RXR complex, resulting in PPAR-γ inability to bind DNA. FOXO1 signalling is antagonised by PPAR-γ, suggesting a reciprocal antagonistic connection between FOXO1 and PPAR-γ. FOXO1 functions are anti-adipogenic in adipocytes, whereas insulin and PPAR-γ functions are pro-adipogenic. In preadipocytes, FOXO1 activation suppresses adipocyte proliferation, whereas PPAR-γ has the opposite effect (8).

Liver X receptor (LXR) consists of two members, LXR-α and LXR-β. Both α and β LXRs are expressed in murine and human adipocytes but LXR-α is predominantly upregulated during fat cell differentiation. Numerous studies suggest that LXR-α null mice when fed a hypercaloric diet produces liver fat deposition, whereas wild-type mice are highly resistant to fat accumulation. Therefore, LXR-α are found to positively regulate cholesterol excretion by regulating CYP7A as well as cholesterol efflux regulatory protein (9).

In this perspective, the goal of this study is to evaluate the binding affinity of ferulic acid by performing in vitro autodock on PPAR-γ, SIRT1, FOXO1 and LXR-α target proteins.

MATERIALS AND METHODS

Preparation of ligand

The 2D structure of ferulic acid was drawn using ACD chemsketch (http://www.acdlabs.com/resources/freeware/chemsketch/) and saved in 3D MDL MOL Format and converted to PDB (http://www.rcsb.org/pdb/home/home.do)format Open Babel Molecular Converter (http://openbabel.org/docs/current/Introduction/93goals.html). The 3D structure of ferulic acid (PubChemCID:445858.) was retrieved from PubChem(http://pubchem.ncbi.nlm.nih.gov/) database.

Preparation of target protein

Structures of PPAR-γ, FOXO1, SIRT1 and LXRα were retrieved from UniProt (http://www.uniprot.org/). The crystal structures of PPAR-γ, FOXO1 and LXR were downloaded from PDB database and their PDB ids were 3ADX – A chain, 4LG0– A chain, 4ZZI– A chain, and 3IPQ– A chain respectively. Further, the structures were visualized using Biovia Discovery studio visualizer (http://accelrys.com/products/discovery-studio/visualization.html).

Molecular Descriptor calculation

Molinspiration online database (http://www.molinspiration.com/) was used to calculate the descriptors of ferulic acid, such as log P, polar surface area (PSA), molar mass, no. of atoms, no. of O or N, no. of OH or NH, no.of revolving bonds, volume, drug likeness includes G protein coupled receptors (GPCR) ionotropic receptors and number of violations to Lipinski‘s rule of Five.

Lipinski’s rule of five

Lipinski‘s rule of five [10] defines drug-ability properties of the lead compounds particularly absorption and permeation. The general guidelines are as follows: LogP<= 5, molecular weight <= 500 g/mol, Hydrogen bond donors (sum of amino and hydroxyl group) <= 5, no of revolving bonds<= 15 Hydrogen bond acceptors (sum of nitrogen and oxygen) <= 10.

Autodock

AutoDock is a set of docking technologies that may be used automatically. This system can be used to model adaptable micro compounds, such as drugs interacting to known three-dimensional receptor proteins. It is a good method for docking investigations since it uses optimization methods for structural exploration. The binding energy was calculated using Auto Dock (http://autodock.scripps.edu/resources/tools).

Molecular interaction studies

The chemical and hydrophobic interactions were examined using Acceryls Discovery Studio Visualizer http://accelrys.com/products/discovery-

studio/visualization.html. Default ligand–receptor interaction tool was used to determine hydrogen and hydrophobic interactions. This application runs on Windows and Linux.

RESULTS

Molecular docking analysis

Molecular docking tests were conducted using Auto dock 4.2 in order to assess the binding affinity of the ferulic acid with the target proteins.

Table 1: Molecular docking studies of PPAR-γ with ferulic acid

PPAR-γ		**Ferulic acid**	**Distance**	**Binding energy (Kcal/mol)**
Residue	**Atom**			
LEU465	N	O	2.87	**-5.79**
VAL450	O	H	2.04	
GLN454	NE2	H	2.80	
LYS457	-	-	4.10	
LEU465	CD2	-	3.53	
LYS457	NZ	-	4.98	

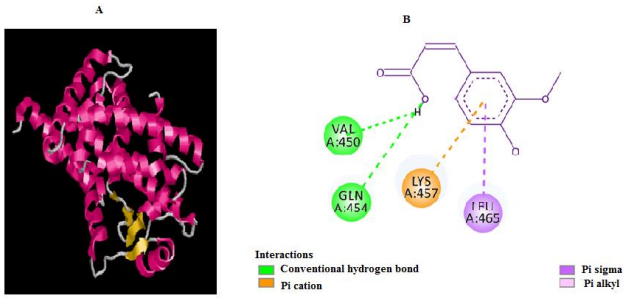

Fig.2: Residual interaction of ferulic acid in binding pocket of PPAR-γ. (A) Structural visualization of PPAR-γ using Rasmol. (B) 2D interaction between ferulic acid and PPAR-γ protein sequences.

Table 2: Molecular docking studies of SIRT1 with ferulic acid

SIRT 1		**Ferulic acid**	**Distance**	**Binding energy (Kcal/mol)**
Residue	**Atom**			
SER442	N	O	2.93	**-6.18**
SER441	OG	H	2.20	
GLN345	OE1	H	2.79	
HIS363	-	-	5.82	
ALA262	-	-	4.78	

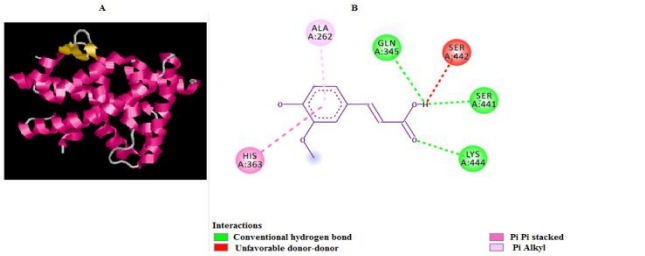

Fig. 3: Residual interaction of ferulic acid in binding pocket of SIRT1. (A) Structural visualization of SIRT1 using Rasmol.(B) 2D interaction between ferulic acid and SIRT1 protein sequence.

Table 3: Molecular docking studies of FOXO1 with ferulic acid

FOXO1		Ferulic acid	Distance	Binding energy (Kcal/mol)
Residue	Atom			
ASN204	O	H	2.01	-8.63
GLY208	N	O	2.94	
GLY208	N	O	3.00	
TYR165	OH	O	2.74	
TYR165	OH	H	1.86	
TYR165	OH	O	2.72	
TRP209	N	-	3.82	
TRP209	-	-	4.39	
TRP209	CB	-	3.9	

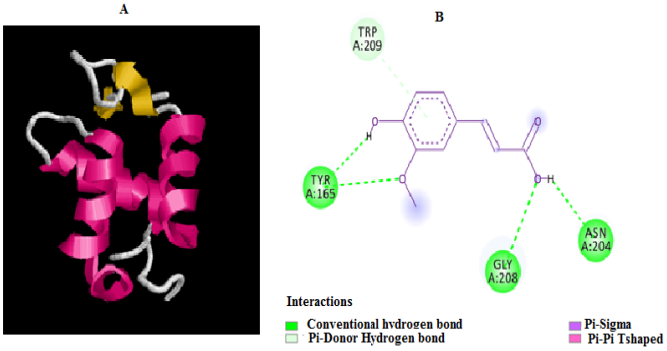

Fig.4: Residual interaction of ferulic acid in binding pocket of FOXO1. (A) Structural visualization of FOXO1 using Rasmol. (B) 2D interaction between ferulic acid and FOXO1 protein sequences.

Table 4: Molecular docking studies of LXR-α with ferulic acid

LXR-α		**Ferulic acid**	**Distance**	**Binding energy (Kcal/mol)**
Residue	**Atom**			
ASP444	O	H	1.85	-5.68
LYS291	NZ	O	2.62	
HIS421	ND1	O	3.04	
SER418	OG	H	1.98	
LYS291	O	C	3.06	
THR292	O	C	3.18	
TRP443	-	-	4.76	

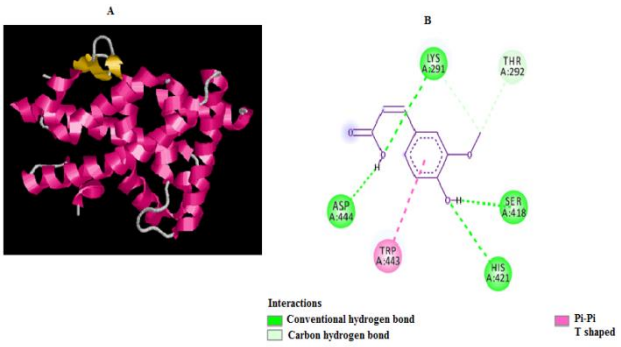

Fig.5: Residual interaction of ferulic acid in binding

pocket of LXR-α.(A) Structural visualization of LXR-α using Rasmol (B) 2D interaction between ferulic acid and LXR-α protein sequence.

Table 5: Molecular properties of ferulic acid

Description	Value
Molecular weight	194.186
Log P	1.4986
Rotatable bonds	3
Hydrogen bond donor	2
Hydrogen bond acceptor	3
Surface area	81.065

DISCUSSION

In silico docking tests were conducted to assess the binding affinity of the bioactive compounds with the target protein. Docking has been shown to be a useful method for predicting ligand-target protein interactions. It also allows for the visualisation of putative atomic interactions between a ligand's functional group and the dynamic domain of a protein. The most effective strategy to control hyperlipidemia disorders is modulating the activity of PPAR-γ, SIRT 1, FOXO1and LXR-α target proteins.

In this study, two hydrogen bond interactions were found between ferulic acid and PPAR-γ, at residues LYS81, THR85 (Fig.2) with the docking energy of -5.78 kcal/mol displayed in Table 1. A good binding affinity was observed in the molecular docking study**,** between ferulic acid and PPAR-γ. PPAR-γ is predominantly produced in white adipose tissue and it is down regulated in hyperlipidemic condition resulting in decreased levels of adiponectin levels. Also, the in silico outcomes clearly demonstrated increased levels of adiponectin in ferulic acid co-administered rats when compared with high fat diet fed rats. Similarly, compounds like caffeic acid and chlorogenic acid were reported to be effective against hyperlipidemia by regulating PPAR-γ (11).

SIRT1 activity is tightly regulated in response to many environmental signals, which is not surprising. One feeding regimen known to enhance sirtuin activation is calorie restriction (CR), which is a 20-40% reduction in calories taken below supplemented feeding without starving. Many of the diseases linked with obesity and metabolic syndrome in animals can be improved by CR, including body fat reduction, decreased serum triglycerides and LDL cholesterol, increased HDL cholesterol, and improved insulin sensitivity. CR and prolonged fasting causes the stimulation of SIRT 1 which in turn deacetylates PGC 1α. PGC 1α binds and activates FOXO1, leading to activation of gluconeogenesis thus improving glucose homeostasis in liver. Also, FOXO 1 regulates oxidative stress by activating genetic traits engaged in the oxidative detoxification such as catalase, constitutive NOS and manganese superoxide dismutase (MnSOD). In this study, two hydrogen bond interactions were found between ferulic acid and SIRT 1 at residues SER 441 and GLN 345 with docking energy of -6.18 kcal/mol displayed in Table 2 and Fig.3. Also, residual interaction of ferulic acid with FOXO 1 at residues ASN204, TYR165 (Fig.4) with the docking energy of -8.63 kcal/mol is shown in Table 3. These results run in parallel with the findings of Sin *et al.,* who proved that modulation of SIRT-1 and FOXO1 signalling axis by resveratrol has antihyperglycemic effect on skeletal muscle (12).

SIRT1 has been demonstrated to control cholesterol metabolism by deacetylation of LXRs, in addition to fatty acid metabolism. LXR activation is advantageous because it not only reduces intestinal cholesterol uptake but also increases reverse cholesterol transport. SIRT1 can deactivate LXR-α directly, resulting in higher LXR-α turnover and target gene expression. In the absence of hepatic SIRT1, another LXR-α target gene, CYP7A1, is lowered, according to an independent investigation by Rodgers *et al.,* (13). This result implies that SIRT1 improves bile acid production in addition to cholesterol efflux. Interestingly, the coregulator PGC-1α was found to be needed for CYP7A1 regulation. In our current study, two hydrogen bond interactions were found between ferulic acid and LXR-α at residues ASP444 and SER418 with the docking energy of -5.68 kcal/mol depicted in Table 4 and Fig.5. Also, Betaine a natural compound found in wheat product, spinach and shrimp, has shown to activate the expression of LXR-α (14).

The current *in silico* docking analysis found that the HFD-mediated down regulation of the PPAR-γ pathway was critical in the deactivation of the SIRT 1 pathway. Since Ferulic acid shows a good binding affinity towards PPAR-γ, SIRT 1, FOXO1 and LXR-α it could hold a great promise for use in the treatment of hyperlipidaemia and obesity in future after thorough clinical study. Table 5represents molecular properties of ferulic acid described through docking analysis. During drug discovery, the drug-likeness and rule of 5 proposed by Lipinski (10) predicts that, poor absorption is more likely to happen if the drug does not exceeds 5 hydrogen bond (2 in ferulic acid), 10 H-bond acceptors (3 in ferulic acid), the molecular weight is greater than 500 (194.186 in ferulic acid), the calibrated logarithm P value is greater than 5 (1.4986 in ferulic acid) and no of revolving bonds exceed 15 (3 in ferulic acid). However, it is highly favourable for ferulic acid to follow Lipinski‘s rule of five. All these facts propose ferulic acid as a good therapeutic drug for hyperlipidemia, and can be considered for further drug development.

CONCLUSION

To conclude, the results of *in silico* studies showed that ferulic acid exhibited maximum binding affinity with the target proteins and can act as lead compound for the formulation and production of hypolipidemic drugs in future.

CONFLICT OF INTEREST

There is no conflict of interest among authors.

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