Docking studies on the interaction of flavonoids with fat mass and obesity associated protein

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Abstract: Obesity is the excessive fat accumulation in human body leading to increases a risk of various chronic diseases such as diabetes, cardiovascular diseases, cancer and osteoarthritis. Several flavonoids are known to have lipolytic activity influencing lipolysis and adipogenesis in adipose cells. To explore mechanism of the association of flavonoids in obesity and obesity associated protein (FTO), molecular docking studies were done for FTO with flavonoids, with orlistat (antiobesity drug) as a control. Autodock tools were used for docking flavonoids and orlistat with FTO. The results were visualized by PyMol and Discovery studio visualizer. Upon docking simulation, it was observed that flavonoid quercetin showed highest binding affinity (most negative ΔG), whereas daidzein was least affinity towards FTO. The binding affinity of other flavonoids was in the order of Exemestane >Kaempherol >Letrozole >Rutin. This study concludes that flavonoids primarily, quercetin ameliorates obesity by establishing a physical interaction with FTO. Interactions were also observed between FTO and other flavonoids and were of not greater inhibition compared to quercetin.

Keywords: Molecular docking, Anti-obesity effects, Orlistat, Quercetin.

INTRODUCTION

Obesity is a common health problem globally, affecting >1 billion overweight adults and at least 300 million people all over the world (Cao, 2010). Obesity is characterized by excess adipose tissue and it contributes to numerous chronic diseases such as type 2 diabetes, hypertension, cardiovascular diseases, stroke and cancers of the breast, endometrium, prostate and colon (Cao, 2007). According to World Health Organization, behavioral and environmental factors such as sedentary lifestyles combined with excess energy intake are mainly responsible for the dramatic increase in obesity during the past 2 decades (Cao, 2010). Various plant extracts and their corresponding bioactive components are well known for their potential to exert anti-obesity effects (Rayolam *et al.,* 2008). Several natural phytochemicals affecting the adipocyte life cycle have been predicted. Flavonoids are a broad class of low molecular weight, characterized by the flavon nucleus present mostly in plant based foods; over 4000 flavonoids have been identified. Flavonoids are benzo-γ-pyrone derivatives consisting of phenolic and pyrane rings and are classified according to substitutions. Dietary flavonoids differ in the arrangements of hydroxyl, methoxy, and glycosidic side groups and in the conjugation between the A- and B-rings. During metabolism, hydroxyl groups are added, methylated, sulfated or glucuronidated (Masoodi and Alhamdan, 2010). Intake of dietary flavonoids has shown to have a numerous biological activities, includes inhibiting cell proliferation, triggers apoptosis, and its beneficial health effects are mostly attributed to their antioxidant and chelating abilities (Middleton and Kandaswami, 1986; Cook and Samman, 1996; Havsteen, 2002). Additionally, flavonoids have been reported to possessed other biological effects such as antiatherosclerotic, anti-inflammatory, antitumor, antithrombogenic, anti-osteoporotic and antiviral (Havsteen, 2002).

In recent times, a considerable research interest has been focused on plant flavonoids that might ameliorate the risk of obesity. Flavonoids could activate neutral lipid hydrolysis from lipid stores in adipose tissues and in liver via elevating cyclic AMP levels (Peluso, 2006). Moreover, it has been reported to inhibit intestinal glucose and fructose transport by glucose transporter 2 (Kwon *et al.,* 2007). Indeed, some flavonoids reduce the mass of intraperitoneal adipose tissues (Murase *et al.,* 2002; Tsuda, 2008). Epidemiological and laboratory studies have mentioned that tea and tea polyphenols have defensive activity against a number of chronic diseases, such as neurodegenerative disease, heart disease, diabetes, cancer and obesity (Grove and Lambert, 2010). Previously, by molecular docking simulation, the interaction of drugs with target proteins have been extensively studied (Kand *et al.,* 2006; Ryuichiro *et al.,* 2008; Mine, 2001; Takashi *et al.,* 2008). This study aims to investigate the physical interaction of flavonoids with FTO, through molecular docking simulation. This will provide information on bioactive conformation of protein through simulation studies, affinity binding orientation of ligands and it further pave the way for strategies of flavonoids for the treatment of obesity and obesity-related metabolic disorders.

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MATERIALS AND METHOD

Software and datasets

Softwares used in this study are freely accessible for academic use. The Protein Data Bank (PDB) is a worldwide source for handling and distribution of three dimensional biological macromolecular structure data (Berman *et al.,* 2000). The protein structure of FTO (3LFM) was downloaded from Protein Data Bank. The Drug Bank database (Wishart *et al.,* 2006) is unique bioinformatics and cheminformatics resource that combines detailed drug (i.e., chemical, pharmacological and pharmaceutical) data with comprehensive drug target (i.e., sequence, structure and pathway) information. Each Drug Card entry contains more than 80 data fields with half of the information being devoted to drug/chemical data and the other half devoted to drug target or protein data (Wishart *et al.,* 2006). Orlistat and all the flavonoid molecules were downloaded from this database (fig. 1). The PDB file of FTO obtained from the Protein Data Bank was visualized using PyMol (version 1.2), a Python-based visualization software [http://www.pymol.org.]. Docking results were also analyzed and visualized using Pymol. The docking was achieved using the docking software AutoDock 4.2 (The Scripps Research Institute, "www.scripps.edu") (Morris *et al.,* 1998), with the support of AutoDock Tools (ADT) an accessory program allowing the user to interact with AutoDock from a Graphic User Interface (GUI). AutoDock is a suite of automated docking tools designed to predict how small molecules/ligands such as substrates or drug molecules bind to a receptor/protein of known 3D structure.

Receptor ligand docking

For getting the drug-receptor binding energy and inhibition constant, molecular docking was performed. The detailed procedure followed is:

Preparation of ligand and receptor files

The PDB files deposited in Protein Data Bank are often far perfect for docking analysis and are present with potential problems like missing hydrogen atoms, multiple molecules, added waters etc. The downloaded PDB file of FTO (PDB ID 3LFM) was first read in ADT, added waters removed and polar hydrogens were added. Kollman charges were added. Finally file was saved with. pdbq extension (q=charge). In a similar procedure, the ligand files were read in ADT, all hydrogens added, charges added and non-polar hydrogens merged and saved with pdbqt extension. ADT then automatically predicted the best root. The ligand files were then saved with. pdbqt extension (q=charge).

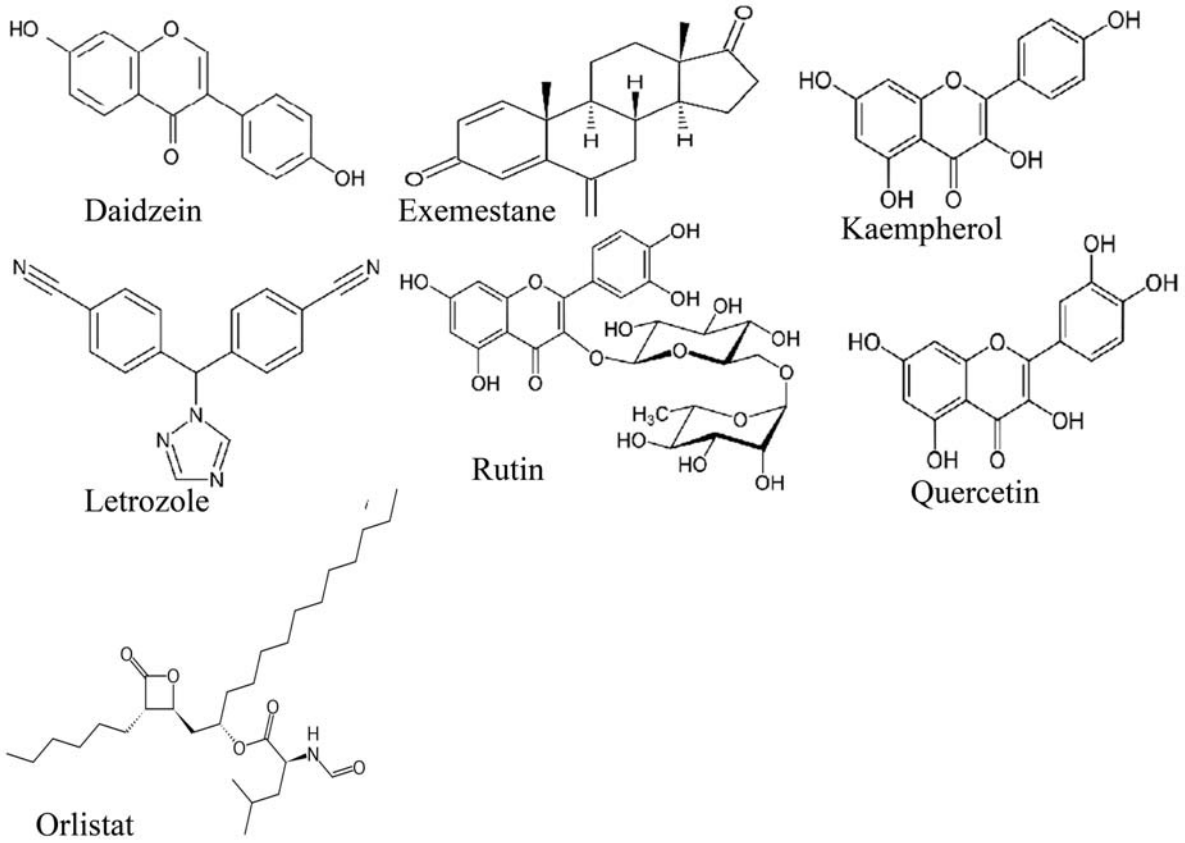

Fig. 1: Structure of ligands used for the study.

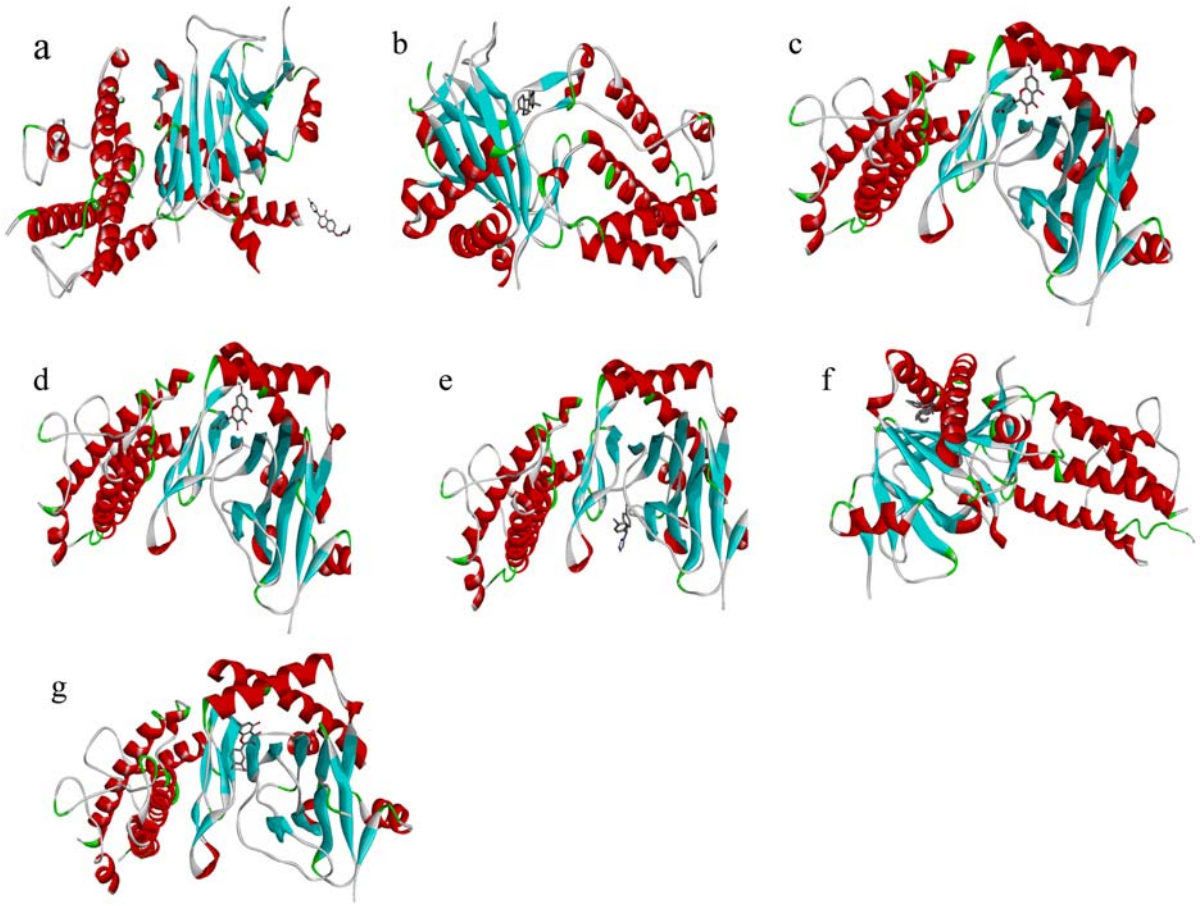

Fig. 2: Ligand molecules docked onto FTO receptor (red and sky blue) in their lowest energy docked confirmation. Ligands are shown in stick model a. Daidzein. b. Exemestane. c. Kaempherol. d. Letrozole. e. Rutin. f. Quercetin. g. Orlistat.

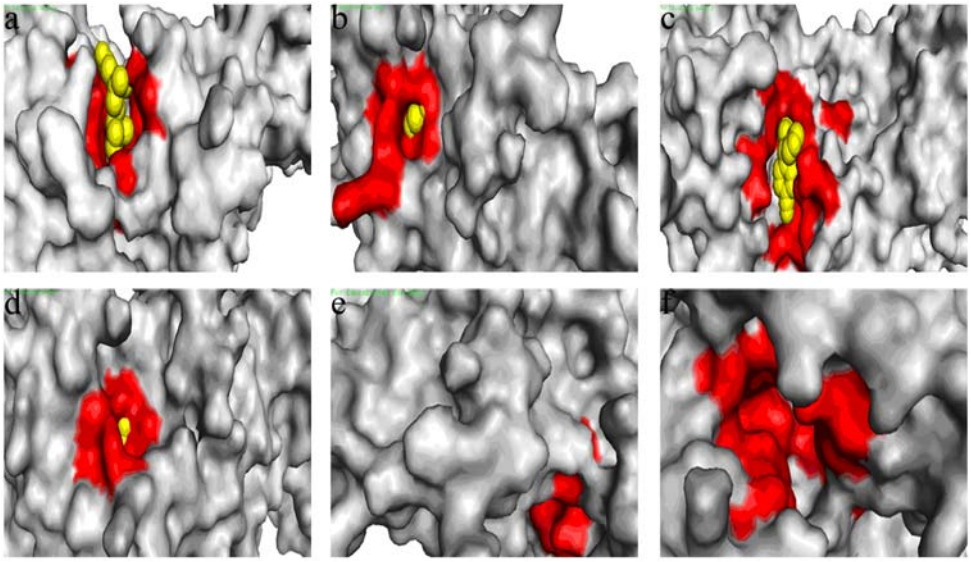

Fig. 3: Ligand molecules in the active site of FTO. a. Exemestane. b. Kaempherol. c. Letrozole. d. Rutin. e. Quercetin. f. Orlistat. Ligands are shown in yellow color and the residues of receptor involved in bonding are red colored. Note quercetin and orlisat are completely inside the receptor indicating strong binding.

Table 1: Amino acid residues involved in binding of flavonoids with 3LFM

Daidzein	Exemestane	Kaempherol	Letrozole	Quercetin	Orlistat	Rutin
No interactions found	85 ILE	68 ALA	85 ILE	69 PHE	85 ILE	85 ILE
	90 LEU	69 PHE	86 GLN	72 LEU	92 THR	92 THR
	92 THR	72 LEU	90 LEU	73 HIS	93 PRO	93 PRO
	93 PRO	73 HIS	91 LEU	78 LEU	94 VAL	94 VAL
	94 VAL	78 LEU	92 THR	94 VAL	96 ARG	96 ARG
	96 ARG	95 SER	93 PRO	95 SER	106 TYR	108 TYR
	108 TYR	96 ARG	106 TYR	97 ILE	108 TYR	109 LEU
	109 LEU	135 ALA	107 LYS	99 ILE	109 LEU	227 ALA
	227 ALA	139 PHE	108 TYR	139 PHE	203 LEU	228 VAL
	228 VAL	204 LEU	109 LEU	204 LEU	227 ALA	229 SER
	229 SER	205 ASN	228 VAL	205 ASN	228 VAL	230 TRP
	230 TRP	206 PHE	229 SER	206 PHE	229 SER	231 HIS
	231 HIS	317 PHE	230 TRP	317 PHE	230 TRP	233 ASP
		318 SER	231 HIS		231 HIS	322 ARG
			232 HIS		233 ASP	
			233 ASP		234 GLU	
			234 GLU		322 ARG	
			322 ARG			

Grid parameter file preparation

For the prediction of docking interaction energy, a three-dimensional box (grid) was created in which the protein molecule is enclosed. The grid volume was kept large in order to permit the ligand to rotate freely, even with its most fully extended conformation. The parameters essential to create such a grid were stored in the Grid Parameter File with .gpf extension.

Running autogrid

Autogrid4 generates one map for each kind of atom in the ligand. For instance, a molecule containing carbon, nitrogen, oxygen, hydrogen, maps will be generated as molecule. C. map molecule. N. map, molecule. O. map, molecule. H. map. These are grid maps in ASCII format and are readable by Auto Dock. Auto Grid also creates corresponding output of the macromolecular file with the extension .glg.

Docking parameter file preparation

The docking parameter file, trains AutoDock about the map files to use, the ligand to move, and other properties used for the ligand. AutoDock's search approaches comprise the Monte Carlo simulated annealing (SA), local search (LS), the Genetic Algorithm (GA) and the hybrid genetic algorithm with local search (GALS). The latter one also referred to as the Lamarckian genetic algorithm (LGA) was the chosen algorithm for this analysis.

Running auto dock

Lastly, Auto Dock was run from the GUI of ADT and docked ligand files were used for the analysis. The dlg files were read in ADT and PyMol for the binding energies prediction in the docked ligand protein complexes.

RESULTS

Table 1 shows the binding residues that directly interact with the ligands used in this work. Each ligand corresponds to different locations on the receptor. Though the binding sites are slightly different, the locations of each ligand on the receptor show large differences. Orlistat and quercetin lie completely inside the binding pocket of the receptor (fig. 3) whereas daidzein lies completely outside the binding site.

Table 1 summarizes the amino acid residues involved in the binding of flavonoids and orlistat with FTO. Two hydrogen bonds were formed between exemestane and FTO through TYR 108 and ARG 96 residues with varying bond length and surface area. Three hydrogen bonds between kaempherol and FTO, six with letrozole, eight with quercetin, seven with orlistat and six with rutin were formed (table 2). The smallest bond length was observed between receptor and ligands, orlistat and quercetin (table 2).

The Auto Dock program computes the binding and docking energies. The energy terms include intermolecular energy (comprised of van der Waals energy, hydrogen bonding energy, desolvation energy and electrostatic energy), internal energy and torsional energy. The first two energies make up docking energy, the first and the third items constitute the binding energy. Table 3 shows the energy information of the flavonoids and the anti-obesity drug orlistat. Quercetin corresponds to the lower binding energy (-1.78 e+32 kcal/mol), while the other flavonoids including orlistat showed higher values (table 3). The docking energies show the same propensity

as the binding energy. Inhibition constant was also calculated with the order of Quercetin >Orlistat > Exemestane >Kaempherol >Letrozole >Rutin >Daidzein. fig. 2 shows the surface view of receptor with different ligands. The binding site of the receptor is colored in red and the ligands are in yellow color.

Table 2: Hydrogen bonds between 3LFM and flavonoids

Flavonoid	Residue	Distance (Å)	Surface area ($Å^2$)
Exemestane	TYR 108	4.0	4.2
	ARG 96	4.5	8.5
Kaempherol	SER 95	1.1	1.4
	ASN 205	1.2	15.1
	GLU 234	2.9	21.8
Letrozole	GLN 86	6.1	1.2
	LEU 91	5.4	1.0
	TYR 106	2.6	30.5
	GLU 234	3.5	4.9
	LYS 107	4.6	0.2
	ASP 233	4.8	1.4
Quercetin	SER 318	3.4	0.7
	SER 95	1.0	44.4
	SER 95	1.2	4.0
	PHE 206	1.4	12.5
	GLU 234	3.5	4.8
	SER 318	2.4	0.7
	ARG 322	4.9	0.8
	ARG 322	5.0	2.0
Orlistat	ARG 96	1.1	33.7
	ARG 96	1.5	42.3
	ASP 233	1.9	0.2
	GLU 234	2.9	21.8
	TYR 106	3.9	1.7
	TYR 108	3.6	3.6
	ALA 227	5.1	0.2
Rutin	SER 95	1.8	44.4
	ARG 96	4.8	3.3
	TYR 106	3.9	1.7
	TYR 108	3.6	3.6
	ALA 227	4.1	0.2
	ARG 322	2.0	22.0

DISCUSSION

In this study, a structure based rational design was used to identify the best flavonoid molecule as a novel inhibitor of FTO. FTO has been found as the best target for obesity therapy in a number of recent studies. Studies revealed that a single nucleotide polymorphism in the FTO gene is associated with body mass index and obesity risk (Frayling *et al.,* 2007; Dina *et al.,* 2007). Studies in animals validated that FTO function is responsible for energy homeostasis (Fischer and Koch, 2009) and metabolic disturbances (Church *et al.,* 2009), confirms association with obesity. An association of FTO demethylase activity and fat mass was proposed by the view that the single mutation in mouse FTO protein resulted in a slim type mouse (Church *et al.,* 2009). This has prompted considerable interest in the development of FTO antagonists for clinical use.

Table 3: Binding and docking energies of flavonoids and 3LFM calculated by Auto Dock 4.2

Flavonoids	Binding energy (kcal/mol)	Docking energy (kcal/mol)	Inhibition constant (μM; 298.15 K)
Daidzein	+2.60	+2.60	0.00
Exemestane	-3.96	-3.92	1.24
Kaempherol	-3.75	-3.98	1.06
Letrozole	-3.55	-4.13	2.49
Rutin	-1.11	-1.11	2.43
Quercetin	-1.78 e+32	-1.99 e+32	8.67
Orlistat	-4.86	-5.03	2.26

A number of clinical studies have reported the potential inhibitory effect of flavonoids against adipocytes. Flavonoids affect lipolysis and adipogenesis in adipocytes. Numerous flavonoids have been shown to possessed lipolytic actions in primary rat adipose cells (Kuppusamy and Das, 1992; Kuppusamy and Das, 1994). Quercetin, a phenolic compound, presents mostly in fruits and vegetables and it prevents insulin-mediated lipogenesis by inhibiting insulin receptor tyrosine kinase (Shisheva and Shechter, 1992). Several herbal extracts, like pycnogenol (Hasegawa, 2000) and Ginkgo biloba (Dell'Agli and Bosisio, 2002), have been shown to prevent lipogenesis in adipose cells. However, it is unclear that quercetin, kaempferol, catechin (major compounds present in pycnogenol (Packer *et al.,* 1999), and Ginkgo biloba (Van Beek, 2002), help to modulate adipogenesis in adipose cells. Studies have also revealed that flavonoids blocks the proliferation and differentiation of both pre and post confluent preadipocytes (Harmon and Harp, 2001) by elevating homologous protein expression in the CCAAT/ enhancer-binding protein (C/EBP) in 3T3-L1 adipocytes (Harmon *et al.,* 2002). Flavonoids such as quercetin, kaempferol and catechin were inhibited markedly the adipogenesis of preadipocytes by blocking adipogenesis linked transcription factor expression (Chien *et al.,* 2005).

CONCLUSION

The results obtained from the study are confirms the hypothesis that flavonoids (particularly quercetin) interact with fat mass and obesity associated protein to bring about their actions in obesity and its associated metabolic disorder. Antiobesity drug, orlistat was also incorporated in the study to prove that quercetin could be a potent inhibitor for FTO.

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