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Computational study on the interaction of flavonoids with fat mass and obesity associated protein

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Abstract

Obesity is a complex metabolic disorder linked to an increased risk of the most common and severe human diseases. Several flavonoids are known to have lipolytic activity influencing lipolysis and adipogenesis in adipose cells. In the current study, the inhibitory effect of various flavonoid compounds on fat mass and obesity associated protein (FTO) was assessed. The protein structure of FTO (3LFM) was downloaded from Protein Data Bank. The inhibitory effect of flavonoids was compared with a known clinical anti obesity drug. Autodock tools were used for docking flavonoids and antiobesity drug orlistat with FTO. It was examined that flavonoid quercetin proved maximum affinity (most negative ΔG), while daidzein showed no affinity towards FTO. The empathy of other flavonoids was in the order of Exemestane > Kaempherol > Letrozole > Rutin. It was concluded that flavonoids (particularly quercetin) may act as an effective drug against fat mass and obesity associated protein. Anti obesity drug, orlistat was also incorporated in the study to prove that quercetin could be a potent inhibitor for FTO.

Key words

Flavonoids, Obesity, Obesity associated protein, Orlistat, Quercetin

Introduction

Obesity and related disorders constitute the highest threat to global human health. According to a current statement by the World Health Organization, there are >1 billion chunky adults worldwide, and at most the 300 million of these individuals are clinically obese (Cao, 2010). Obesity is a complex metabolic disorder linked to an increased risk of the most common and severe human diseases, including hypertension, type 2 diabetes, stroke ,cardiovascular diseases, and some types of cancer such as colorectal, breast and prostate cancers (Cao, 2007). Additionally, obese individuals of all ages often suffer with social and psychological difficulties such as depression and low self-esteem.Various plant extracts and their corresponding bioactive components are well known for their potential to exert anti-obesity effects (Rayalam *et al .,* 2008). Several natural phytochemicals affecting the adipocyte life cycle have been predicted. Dietary flavonoids are secondary products of plants which humans eat daily in fruit, vegetables, tea, wine and other foods. Research on dietary flavonoids has shown them to have a broad spectrum of biological activities, including prevention of proliferation in cell cultures, triggering apoptosis, changing the activity of certain intracellular enzymes and function as antioxidants (Ogunbayo *et al*., 2008). Additionally, various studies have shown that flavonoids may deliver a number of clinical effects such as anti atherosclerotic, anti-inflammatory, antitumor, antithrombogenic, antiosteoporotic and antiviral (Havsteen, 2002). It has been reported that flavonoids have various biological activities such as, antibacterial, antioxidant and anticancer effect and play roles in prevention of obesity. Hence, flavonoids may act as novel drug candidates (Kamisoyama *et al* ., 2008).

Recently, much attention has been focused on plant flavonoids that might be beneficial in decreasing the risk of obesity and obesity associated metabolic disorders. It has been shown that several flavonoids could trigger neutral lipid hydrolysis from lipid stores in adipose tissues and liver via a mechanism by which the elevation of cyclic AMP levels is induced by direct inhibition of phosphodiesterase (Peluso, 2006). Moreover, several flavonoids inhibit intestinal glucose and fructose transport

by glucose transporter 2 (Kwon *et al*., 2007) . Indeed, some flavonoids significantly decrease the weight of intra peritoneal adipose tissues (Murase *et al*., 2006; 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). Investigation on the effects of flavonoids on FTO might lead to more effective strategies for the treatment of obesity and obesity-associated metabolic disorders. In recent years, molecular docking has been widely used in studying the interaction of drugs with target proteins (Kang *et al*., 2006; Ryuichiro *et al* ., 2008; Mine, 2001). Protein Data Bank (PDB) is a worldwide source for handling and distribution of three dimensional biological macromolecular structure data (Berman *et al.,* 2008) . 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. AutoDock is a suite of software for predicting the optimal bound conformations of ligands to proteins (Morris *et al*., 2009 and 1998). The present study was carried out to discover how flavonoids interact with FTO, and docking simulation was performed based on the three dimensional structure of FTO.

Materials and Methods

Softwares and datasets : Orlistat and all the flavonoid molecules were downloaded from the database. The PDB file of FTO obtained from 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. Docking was achieved using docking software AutoDock 4.2 (www.scripps.edu, The Scripps Research Institute,), with the support of AutoDock Tools (ADT) an accomplice program user to interact with AutoDock from a Graphic User Interface (GUI).

Receptor ligand docking and receptor files : For getting the drug-receptor binding energy and inhibition constant, molecular docking was performed. The downloaded PDB file of FTO (PDB ID 3LFM) was first read in ADT and Kollman charges were added, lastly file was saved with pdbq extension (q=charge). In a parallel procedure, the ligand files were studied in ADT, all hydrogens added, charges added and non-polar hydrogens merged and saved with .pdbqt extension. ADT then automatically predicted the best root. Ligand files were then saved with .pdbqt extension (q = charge).

Grid parameter file preparation : For prediction of docking interaction energy, a three-dimensional box (grid) was created in which protein molecule was enclosed. The grid volume was kept large in order to permit the ligand to revolve freely, yet with its most fully extended conformation. The parameters essential to create such a grid were stored in the Grid Parameter File with gpf extension. Autogrid 4 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 grid maps in ASCII format are readable by AutoDock. AutoGrid 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, ligand to move, and other properties used for ligand. AutoDock's search approaches comprise the Monte Carlo simulated annealing (SA), local search (LS), Genetic Algorithm (GA) and hybrid genetic algorithm with local search (GALS). The latter, also referred to as Lamarckian genetic algorithm (LGA), was the chosen algorithm for this analysis. Lastly, Auto Dock was run from the GUI of ADT and docked ligand files were used for analysis. The dlg files were read in ADT and PyMol for binding energies prediction in docked ligand protein complexes.

Results and Discussion

The Auto Dock program computes binding and docking energies. The energy terms include intermolecular energy (comprised of van der Waals energy, desolvation energy, hydrogen bonding energy and electrostatic energy), torsional energy and internal energy. The first two energies make up docking energy, the first and the third items constitute binding energy. Table 3 shows energy information of flavonoids and anti-obesity drug orlistat. Quercetin corresponded to lower binding energy (-1.78 e+32 kcal mol^{-1}), while the other flavonoids including orlistat showed higher values (Table 1). Docking energies showed the same propensity as binding energy. Inhibition constant was also calculated with the order of Quercetin > Orlistat > Exemestane > Kaempherol > Letrozole > Rutin> Daidzein. Fig. 1 shows the surface view of receptor with different ligands. The binding site of the receptor was coloured red and the ligands were yellow colored. In this study, a structure based rational design was used to identify the best flavonoid molecule as a novel inhibitor of FTO, which was found as the best target for obesity therapy in a number of recent studies. Genome-wide association studies have revealed a significant association of a single nucleotide polymorphism (SNP) in the *FTO* gene with increased body mass index and obesity risk (Frayling, 2007; Dina *et al*., 2007) . Animal model studies validated that FTO is functionally responsible for energy homeostasis (Fischer *et al.* 2009), and affects the whole body metabolism (Church *et al* ., 2009), confirming relationship

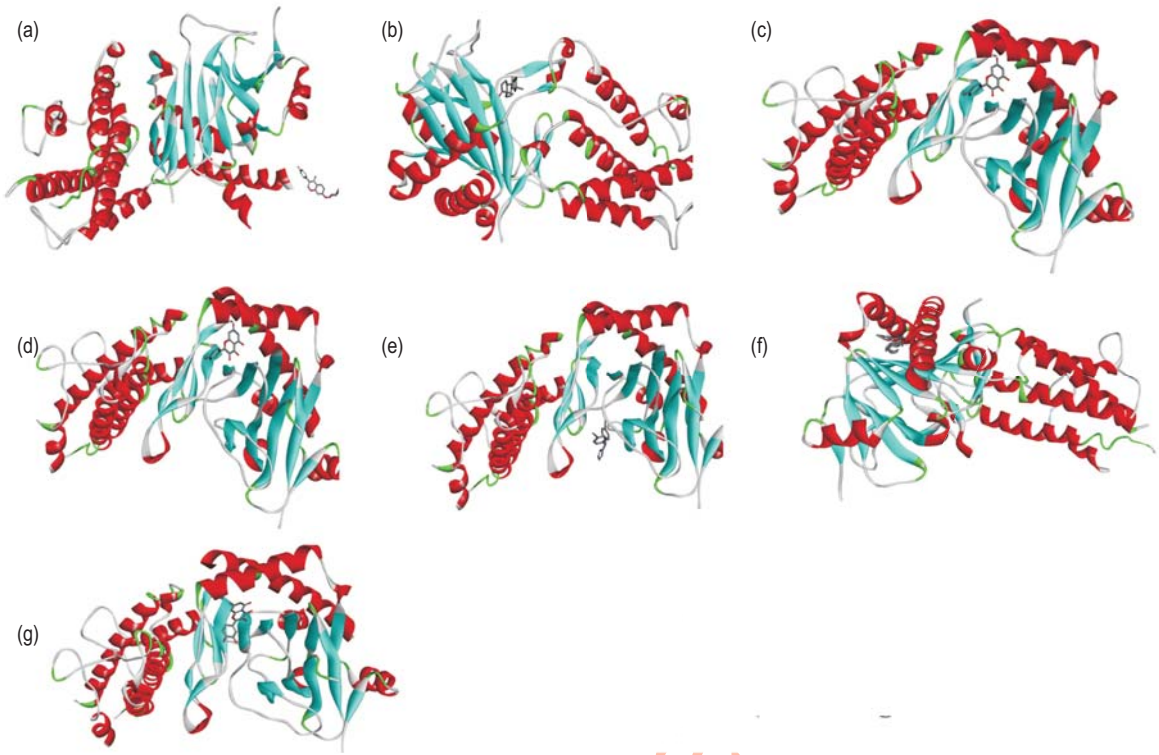

Fig. 1 : Showing Ligand molecules docking onto FTO Receptor (red and sky blue) in their lowest energy docked confirmation. Ligands are shown in stick model (a) Diadzein, (b) Exemestane, (c) Kaempherol, (d) Letrozele, (e) Rutin, (f) Quercetin and (g) Orlistat

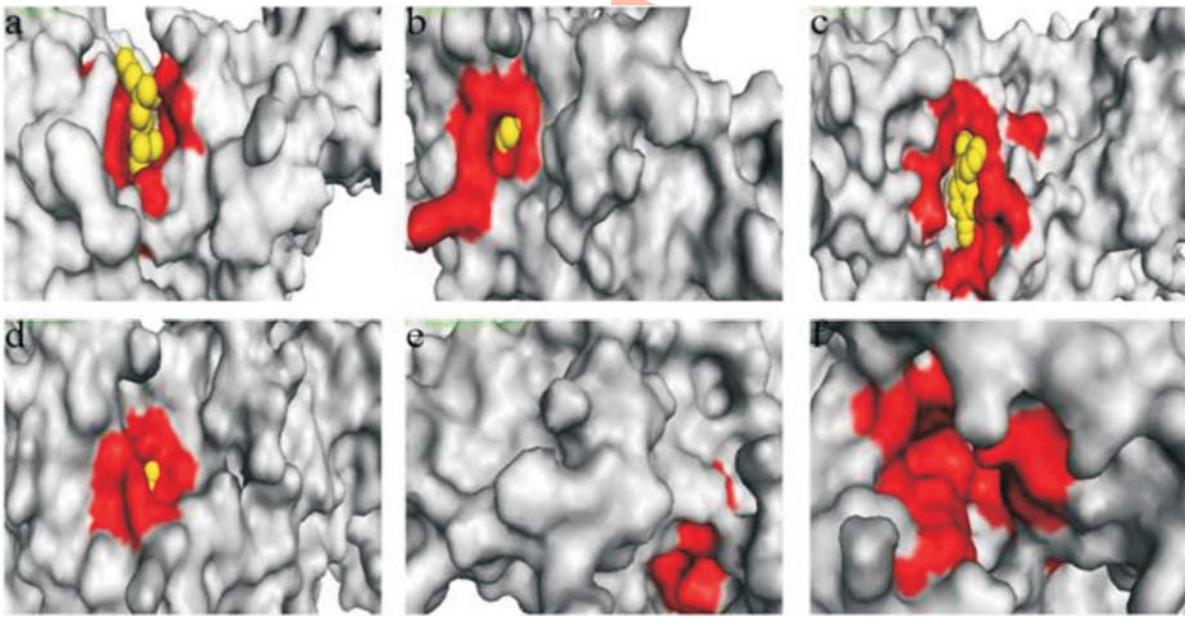

Fig. 2 : Ligand molecules at the active site of FTO. (a) Exemestane Diadzein, (b) Kaempherol, (c) Letrozele, (d) Rutin, (e) Quercetin and (f) Orlistat. Ligands are shown in yellow colour and residues of receptors involved in the bonding are in red color. Quercetin and Orlistat are completely inside the receptor indicating strong binding

with obesity. An association between FTO demethylase activity and elevated fat mass was proposed by the observation that the single mutation in mouse FTO protein resulted in a slim type mouse. This has prompted considerable interest in the development of FTO antagonists for clinical use.

In the present study each ligand corresponded to different locations on the receptor. Though the binding sites were slightly different, the location of each ligand on the receptor showed large differences. Orlistat and quercetin lay completely inside the binding pocket of the receptor (Fig. 2) whereas daidzein lay completely outside the binding site. Table 2 summarizes the amino acid residues involved in 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 3). The smallest bond length was observed between receptor and ligands, orlistat and quercetin. A number of clinical studies have reported the potential inhibitory effect of flavonoids against adipocytes. Flavonoids affect lipolysis and adipogenesis in adipocytes. Woehning *et al* . (2013) concluded that common FTO gene variant complicates weight maintenance in severe obese patients. Quercetin, dominantly present in fruits and vegetables, has shown antilipogenic properties in preventing insulin-mediated lipogenesis by inhibiting insulin receptor tyrosine kinase from phosphorylating substrates (Shisheva and Shechter, 1992). Several herbal extracts, like pycnogenol (Hasegawa, 2000) and Ginkgo (Dell'Agli and Bosisio, 2002) have been found to prevent lipogenesis in adipose cells. Though, it is not clear if are flavonoids, such as quercetin, kaempferol, catechin (known important components of pycnogenol (Packer *et al*., 1999: Van Beek, 2002), help to modulate adipogenesis in adipose cells. Recently, Harmon and Harp (2001) revealed that isoflavone genistein blocks proliferation and terminal differentiation of both pre and postconfluent preadipocytes by elevating homologous protein expression in CCAAT/ enhancer-binding protein (C/EBP) in 3T3-L1 adipocytes (Harmon *et al*., 2002). Flavonoids quercetin, kaempferol and catechin were found in a study inhibiting markedly the adipogenesis of 3T3-L1 preadipocytes by blocking adipogenesis linked transcription factor expression (Chien *et al*., 2005). Flavonoid supplementation as a therapeutic method for reducing disease risk factors is receiving increasing attention. *In vitro* and animal studies support the notion that quercetin, the major flavonoid consumed by human beings, reduces disease risk factors (Boots *et al.,* 2008). For example, mice fed with high-fat diet, quercetin lowered circulating plasma cytokines (interferon-, interleukin [IL]-1, and IL-4), without change in body composition or

Table 1 : Binding and docking energies of flavonoids and 3LFM calculated by AutoDock 4.2

Flavonoids	Binding energy (kcal mol^{-1})	Docking energy (kcal mol^{-1})	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

Table 2 : Showing 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			

Table 3 : 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

weight (Stewart *et al.,* 2008).In addition, quercetin significantly decreased total serum cholesterol and phospholipid levels, and liver enzyme activity involved with fatty acid synthesis in mice fed quercetin for 15 days (Odbayar *et al.*, 2006).

From this study, it was concluded that flavonoids (particularly quercetin) may act as an effective drug against fat mass and obesity associated protein. Anti obesity drug, orlistat was also incorporated in the study to prove that quercetin could be a potent inhibitor for FTO.

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Conflict of Interest: There is no financial or personal conflicting interests rose from this study.

References

Berman, H.M., J. Westbrook and Z. Feng: The protein data bank. *Nucleic Acids Res*., **28**, 235-42 (2008).

Boots, A.W., G.R., Haenen and A. Bast: Health effects of quercetin: From antioxidant to nutraceutical. *Eur. J. Pharmacol.*, **585**, 325-337 (2008).

Cao, Y.: Adipose tissue angiogenesis as a therapeutic target for obesity and metabolic diseases. *Nat. Rev. Drug. Discov.*, **9**, 107-15 (2010).

Cao, Y.: Angiogenesis modulates adipogenesis and obesity. *J. Clin. Invest.*, **117**, 2362-8 (2007).

Chien, P.J., Y.C., Chen and S.C. Lu: Dietary flavonoids suppress adipogenesis in 3T3-L1 preadipocytes. *Journal of Food and Drug Analysis.*, **13**, 168-175 (2005).

Church, C., S. Lee and E.A. Bagg: A mouse model for the metabolic effects of the human fat mass and obesity associated *FTO* gene. *PLoS Gene,t*, **5**, 1000599 (2009).

Cook, N.C., and S. Samman: Flavonoids -chemistry, metabolism, cardioprotective effects and dietary resources. *J. Nutr. Biochem.*, **7**, 66-76 (1996).

Dell'Agli, M. and E. Bosisio: Biflavones of *Ginkgo biloba* stimulate lipolysis in 3T3-L1 adipocytes. *Planta Medica.*, **68**, 76-79 (2002).

Dina, C., D. Meyre and S. Gallina: Variation in *FTO* contributes to childhood obesity and severe adult obesity. *Nature Genet.*, **39**, 724-726 (2007).

Fischer J., L. Koch and C. Emmerling: Inactivation of the *FTO* gene protects from obesity. *Nature*., **458**, 894-898 (2009).

Frayling, T.M., N.J. Timpson and M.N. Weedon: A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. *Science*, **316**, 889-894 (2007).

Grove, K.A. and J.D. Lambert: Laboratory, epidemiological, and human intervention studies show that tea (Camellia sinensis) may be useful in the prevention of obesity. *J. Nutr.,* **140**, 446–453 (2010).

Havsteen, B.H.: The biochemistry and medical significance of the flavonoids. *Pharmacol. Ther* ., **96**, 67-202 (2002).

Hasegawa, N.: Inhibition of lipogenesis by pycnogenol. *Phytother Res.,* **14**, 472-473 (2000).

Harmon, A.W. and J.B Harp: Differential effects of flavonoids on 3T3-L1 adipogenesis and lipolysis. *Am. J. Physiol. Cell Physiol*., **280**, 807-813 (2001).

Harmon, A.W., Y.M. Patel and J.B. Harp: Genistein inhibits CCAAT/enhancer-binding protein beta (C/EBPβ) activity and 3T3-L1 adipogenesis by increasing C/EBP homologous protein expression. *Biochem J.*, **367**, 203-208 (2002).

Kamisoyama H., K. Honda, Y. Tominaga, S. Yokota, S. Hasegawa: Homology modelling of CB1 receptor and selection of potential inhibitor against obesity Biosci. *Biotechnol. Biochem.*, 72, 3225 (2008).

Kwon, O., P. Eck and S. Chen : Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. *FASEB J*., **21**, 366–77 (2007).

Kang, N.S., C.H. Chae and S.E. Yoo : Study on the hydrolysis mechanism of phosphodiesterase 4 using molecular dynamics simulations. *Mol. Simul.*, **32,** 369–374 (2006).

Murase, T., A. Nagasawa and J. Suzuki: Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. *Int. J. Obes. Relat. Metab. Disord.*, **26**, 1459–64 (2006).

Mine, Y.: Molecular dynamic simulation of model lipid membranes: structural effects of impurities. *Mol. Simul*., **27**, 187–197 (2001).

Morris, G.M., R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S.

Goodsell and A.J. Olson: AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.*, **30**, 2785–2791 (2009).

Morris, G.M., D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew and A.J. Olson: Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comp. Chem* ., **19**, 1639–1662 (1998).

Odbayar, T.O., T. Kimurat, D. Badamhand, Y. Takahashi, I. Takashi and T. Tsushida: Comparative studies of some phenolic compounds (quercetin, rutin, and ferulicacid) affecting hepaticfatty acid synthesis in mice. *J. Agric. Food Chem.,* **54**, 8261-8265 (2006).

Ogunbayo, O.A., R.M. Harris, R.H. Waring, C.J. Kirk and F. Michelangeli: Inhibition of the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase by flavonoids: a quantitative structure-activity relationship study. *IUBMB Life*., **60**,853–858 (2008).

Packer, L., G. Rimbach and F. Virgili: Antioxidant activity and biological properties of a procyanidin- rich extract from pine *(Pinus maritima)* bark, pycnogenol. *Free Radic. Biol. Med.*, **27**, 704-724 (1999).

Peluso, M.R.: Flavonoids attenuate cardiovascular disease, inhibits phosphodiesterase, and modulate lipid homeostasis in adipose tissue and liver. *Exp. Biol. Med* ., **231**,1287-99 (2006).

Ryuichiro. I., T. Tohru and S. Kentaro: Refinement of comparative models of protein structure by using multicanonical molecular dynamic simulation. *Mol. Simul.,* 2008, **34**, 327–336

Rayalam, S., M.A. Della-Fera and C.A. Baile: Phytochemicals and regulation of the adipocyte life cycle. *J. Nutr. Biochem*., **19**, 717-26 (2008).

Stewart, L.K., J.L. Soileau, D. Ribnicky, Z.Q. Wang, I. Raskin, A. Poulev, M. Majewski, W.T .Cefalu and T.W. Gettys: Quercetin transiently increases energy expenditure but persistently decreases circulating markers of inflammation in C57BL/6Jmice fed a high-fat diet. *Metabolism*., **57**, 39-46 (2008).

Shisheva, A. and Y. Shechter: Quercetin selectively inhibits insulin receptor function *in vitro* and the bioresponses of insulin and insulinomimetic agents in rat adipocytes. *Biochemistry*., **31**, 8059-8063 (1992).

Tsuda, T. : Regulation of adipocyte function by anthocyanins; possibility of preventing the metabolic syndrome. *J. Agric. Food Chem*., **56**, 642–6 (2008).

Van Beek, T.A.: Chemical analysis of *Ginkgo biloba* leaves and extracts. *J. Chromatogr.,* **967**, 21- 55 (2002).

Wishart, D.S., C. Knox, A.C. Guo, S. Shrivastava, M. Hassanali, P. Stothard, Z. Chang and J. Woolsey*:* Drug Bank: A comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res.*, **34**, D668-72 (2006).

Woehning, A., J.H. Schultz , E. Roeder , A. Moeltner, B. Isermann, P.P. Nawroth, C. Wolfrum and G. Rudofsky: The A-allele of the common FTO gene variant rs9939609 complicates weight maintenance in severe obese patients. *Inter. J. Obesity.*, **37**, 135 (2013).