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Research Article

In silico Interaction of Rhamnus' Flavonoids With Fat Mass And Obesity Associated Protein

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Abstract

The anti-obesity potential of various plant extracts and their associated bioactive compounds is well known. Molecular docking studies of FTO with flavonoids, using Orlistat (an anti-obesity drug) as a control, were performed to identify the effects of *Rhamnus*' flavonoids with FTO (Obesity and obesity associated protein). Prior to molecular docking simulation, Rhamnus flavanoids were analysed using AutoDockTools (version 1.5.6). Docking simulations of the interaction of Rhamnus flavanoids with FTO were performed using AutoDock Vina version 1.1.2. Their binding affinities were obtained. BIOVIA Discovery Studio software was used to visualise the interaction between receptor and ligand. Our study approved the binding ability to FTO protein, and the affinity was as Aloe Emodin Dimer>Emodin>6-Methoxysorigenin. As a results, Rhamnus flavonoids have the remarkable ability to FTO protein, which means they are potent molecules as a potent FTO-inhibitor. Interestingly, Orlistat has lower affinity than Aloe-emodin dimer (-8.7 vs -10.8), which means aloe-emodin dimer more potent to bound the active site. In contrast, two other Rhamnnus flavonoids were shown lower binding affinity when compared to Orlistat.In conclution, Rhmanus phytomolecules able to bind to the catalytic site of FTO as well as "Orlistat" has been demonstrated by molecular docking. Thus, Rhamnus flavonoids especially "Aloe-emodin dimer" is a potent molecule to develop "anti-obesity drug".

Keywords: Rhamnus, emodin, FTO, fat mass and obesity, molecular docking.

Rhamnus Flavonoidlerinin Yağ Kütlesi Ve Obezite İle İlişkili Protein ile İn Silico Etkileşimi

Öz

Çeşitli bitki özlerinin ve bunların içeriğindeki biyoaktif bileşiklerin obeziteye karşı potansiyeli iyi bilinmektedir. Flavonoidlerin obezite ve obezite ile ilişkili protein (FTO) ile ilişkisinin mekanizmasını keşfetmek için kontrol olarak Orlistat (obezite karşıtı bir ilaç) kullanılarak FTO'nun flavonoidlerle moleküler kenetlenme çalışmaları yapılmıştır. Moleküler kenetlenme simülasyonundan önce, Rhamnus flavanoidleri AutoDockTools (sürüm 1.5.6) kullanılarak analiz edilmiştir. Rhamnus flavanoidlerinin FTO ile etkileşiminin docking simülasyonları AutoDock Vina versiyon 1.1.2 kullanılarak gerçekleştirilmiştir. Bağlanma afiniteleri elde edilmiştir. Reseptör ve ligand arasındaki etkileşimi görselleştirmek için BIOVIA Discovery Studio yazılımı kullanılmıştır. Çalışmamız FTO proteinine bağlanma yeteneğini çalışılan Rhamnus'a özgü flavanoidler için Aloe Emodin Dimer>Emodin>6-Methoxysorigenin olarak bulundu. Sonuç olarak, Rhamnus flavonoidleri FTO proteinine dikkat çekici bir bağlanma affinitesine sahiptir, bu da güçlü bir FTO inhibitörü olarak aday moleküller olabilecekleri anlamına gelir. İlginç bir şekilde, Orlistat, Aloe-emodin dimerinden daha düşük afiniteye sahiptir

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(-8.7 vs -10.8), bu da aloe-emodin dimerinin aktif bölgeye bağlanmak için daha güçlü olduğu anlamına gelir. Buna karşılık, diğer iki Rhamnnus flavonoidi Orlistat ile karşılaştırıldığında daha düşük bağlanma afinitesi göstermiştir. Sonuç olarak, Rhmanus fitomoleküllerinin FTO'nun katalitik bölgesine "Orlistat" kadar bağlanabildiği moleküler yerleştirme ile gösterilmiştir. Bu nedenle, Rhamnus flavonoidleri, özellikle "Aloe-emodin dimer", "obezite karşıtı ilaç" geliştirmek için güçlü bir moleküldür.

Anahtar Kelimeler: Rhamnus, emodin, FTO, fat mass and obesity, molecular kenetleme

1. Introduction

It is currently proven that, without affecting the human genome, the mother's nutrition or the father's eating habits, especially during pregnancy, affect the newborn's health. Many herbs are used for the purpose of slimming among the public (van Dijk et al., 2015) If gene-environment interactions have an effect on gene regulation, can we interfere with the regulatory mechanisms of the gene with a food we consume, or can we trigger or treat obesity by manipulating proteins via food? In Turkey, particularly among the public, many herbs are used for weight loss. Could these extracts with traditional use be effective in the regulation of obesity?

Rhamnus sp. is popularly known as buckthorn or bitter buckthorn and grows naturally in the Mediterranean basin, Europe, southwest and central Asia. Rhamnus sp, leaves and fruit used for years for their diuretic, and depurative action upon the organism (Chen et al., 2018). The antioxidant and antimicrobial activities of the plant, with the cytotoxic action of the plant possibly accounting for the antimicrobial activity (Chen et al., 2018; Khuda et. Al., 2022). Rhamnus species are known to be rich in anthraquinones, flavanol triheterosides, anthracenes, anthrones, naphthalenes (Bouhlel Chatti et. al. 2022).

Despite Rhamnus sp.'s unknown side effects and toxicity, the extract is still used as a laxative by people who want to lose weight without a prescription.

The first obesity susceptibility gene identified by GWAS was FTO (fat mass and obesity associated gene), which consists of 9 exons with a cytogenetic location in the 12.2nd region (16q12.2) on the long (q) arm of chromosome 16 (Scuteri et.al., 2007; Peters el. al. 2013). The FTO gene was given this name because of the fused toes (FT) phenotype characteristic that occurs with the deletion of 1.6 megabases of sequence in the genomic sequence of the mouse by insertional mutation. Fto is a nuclear protein and regulates energy balance by establishing a functional relationship between nutrition and genes encoding peptides as a transcription co-factor.

In that frame, due to its obesity-related popularity, we want to evaluate the molecular affinity of Rhamnus' flavonoids to "FTO" in silico. This is the first report that evaluate the possible action of mechanism of Rhamnus' flavonoids with FTO protein interaction. Rhamnus sp. is a plant that is popularly used for weight loss and has a cathartic effect. Therefore, we compared the plant extract with "orlistat", a commercially available drug for weight control of obese individuals, which also has a cathartic side effect.

2. Material and Method

To obtain detailed binding information, analyses were performed for Rhamnus flavonoids such as Emodin (PubChem CID: 3220), Aloe-emodin dimer (PubChem CID: 437987), and 6-Methoxysorigenin (PuBChem ID: 12442904), using AutoDockTools (version 1.5.6) prior to molecular docking simulation. The anti-obesity drug Orlistat (PubChem CID: 3034010).

Docking simulations of the interaction of Rhamnus flavonoids on the FTO protein (PDB ID: 3LFM) in a box with x × y × z dimensions 40 40 40 were performed.

We removed all water molecules and ions in the protein (receptor) and added polar hydrogen atoms to the receptor molecule. To predict the docking interaction energy, a 3D grid box where the protein molecule was surrounded. The input 'grid parametres' files were set to X = 29.043, Y = -6.644 and Z = -29.329 (Hardinsyah et.al., 2023), the others were set to the default values given by the software.

The docking simulations of Rhamnus flavanoids interaction on FTO protein were done using version 1.1.2 of AutoDock Vina (Trott et. Al., 2010). Their affinity for binding and RMSD values were obtained.

The interaction between receptor with ligand were visualized with the BIOVIA Discovery Studio software (BIOVIA).

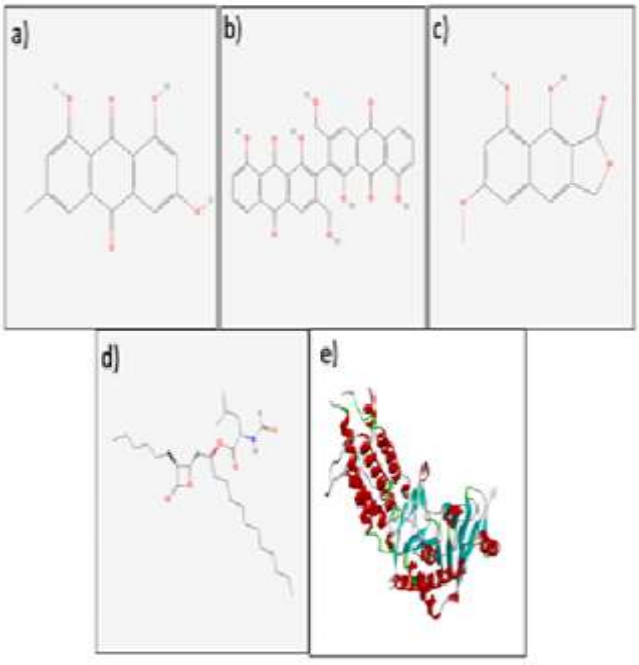

Figure 1. Structure of ligands used for the study. a) Emodin b) Aloe-Emodin Dimer c) 6-Methoxysorigenin, d) Orlistat, e) FTO protein (PDB ID: 3LFM)

3. Results and Discussion

In that study, we evaluated the interaction between FTO protein and Emodin, Aloe-Emodin Dimer, and 6-Methoxysorigenin in silico. The result of our study, we found that binding affinity of flavonoids was in the order of Aloe-emodin Dimer >Emodin> 6-Methoxysorigenin.

In the analysis of the interaction of each ligand with the FTO protein, interactions with different sites on the receptor have been observed. We compared the ligand binding site with anti-obesity

drug "Orlistat" and found x and y has similar binding site. (or none of the ligand has similar binding site as anti-obesity drug "Orlistat").

The minimum bond length was found between the receptor and Orlistat, and flavonoids had relatively long binding distances even though they bind to similar amino acid as Orlistat. (Table 1). Interestingly, both Orlistat, which is used to combat obesity, and the common flavonoids of Rhamnus sp, which is used for weight loss, bind to the FTO protein at ARG 96.All the flavonoids have strong binding capacity to FTO. The molecular interactions were recognized by FTO through hydrogen bonds and all molecules bind FTO (Table 1).

We have found four hydrogen bonds between 3LFM and Emodin with ARG(A:96), TYR(A:106), HIS(A:232) and ARG(A:322) with a distance of 2.80, 2.26, 2.93 and 2.88, respectively (Figure 2).

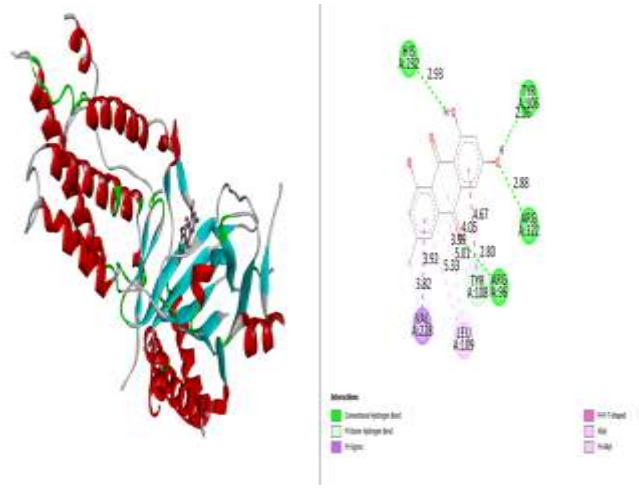

Figure 2. Emodin docked onto FTO receptor (Left) and molecular interactions between Emodin (PubChem CID: 3220) and 3LFM.

The Aloe-emodin dimer shown four different hydrogen binding ability with 3LFM by ARG(A:96), TYR(A:108), SER(A:229) and MET(A:226) with a distance of 2.81, 3.0, 3.36 and 2.47 (Figure 3).

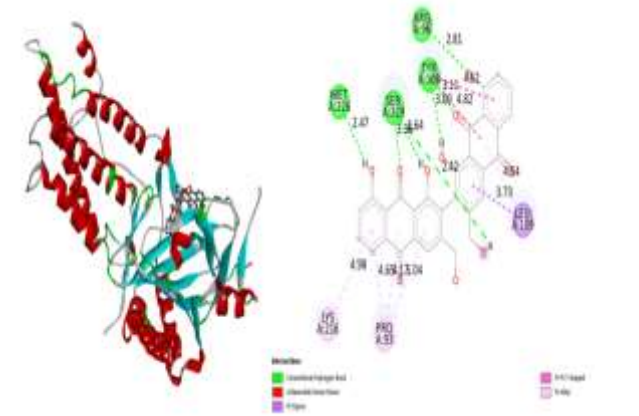

Figure 3. Aloe-emodin dimer docked onto FTO receptor (Left) and molecular interactions between Aloe-emodin dimer (PubChem CID: 437987) and 3LFM.

6-Methoxysorigenin similarly had three hydrogen bonds between 3LFM, two of them TYR(A:106) and ARG(A:322) was the same with Emodin, still one more with GLU (A:234) with 3.01 distance (Figure 4).

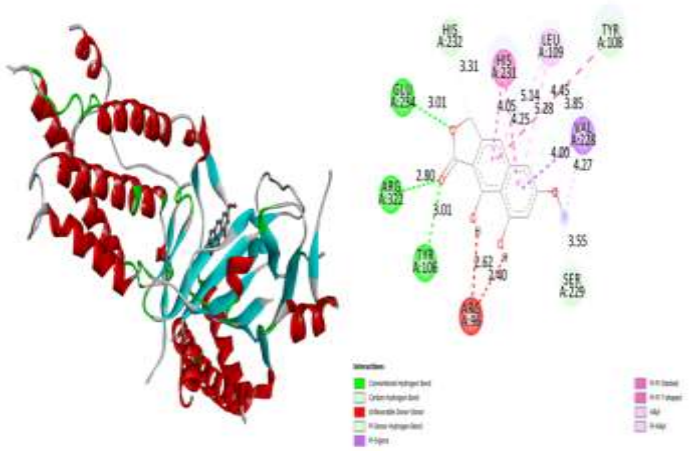

Figure 4. 6-Methoxysorigenin docked onto FTO receptor (Left) and molecular interactions between 6-Methoxysorigenin (PubChem CID: 12442904) and 3LFM.

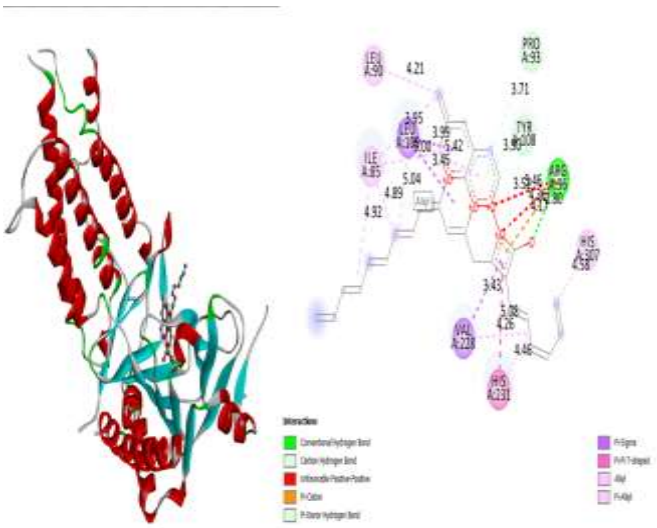

Figure 5. Orlistat docked onto FTO receptor (Left) and molecular interactions between Orlistat (PubChem CID: 3034010) and 3LFM.

Also, all flavonoids shown interaction via Pi-pi bounds and pi-sigma bounds with FTO. All three flavonoids were shown ARG (A:96) bounds; but this interaction was an unfavourable donor-donor bound for 6-Methoxysorigenin.

Table 1. The binding affinities and the molecular interactions between Emodin, Aloe-emodin dimer, 6-Methoxysorigenin and Orlistat target DNA, obtained by molecular docking simulations.

FTO (PDB ID: 3LFM)	**Docking molecules**			
	Emodin	**Aloe-emodindimer**	**6Methoxysorigenin**	**Orlistat**
Binding Affinity (kcal/mol)	-8.6	-10.8	-7.4	-8.7
H.Bonding interaction (Distance; Å)	ARG-A:96 (2.80) ARG- A:322 (2.88) TYR-A:106 (2.26) HIS-A:232 (2.93)	ARG-A:96 (2.81) MET-A:226 (2.47) TYR-A:108 (3.00) SER-A:229 (3.36)	ARG-A:322 (2.80) TYR-A:106 (3.01) GLU-A:234 (3.01)	ARG-A:96 (2.80)
Carbon-Hdrogen bond (Distance; Å)	NA	NA	TYR-A:108 (3.85) SER-A:229 (3.55) HIS-A:232 (3.31)	PRO-A:93 (3.71) TYR-A:108 (3.9)
Pi-Sigma (Distance; Å)	VAL-A:228 (3.92)	LEU-A:109 (3.73)	VAL-A: 228 (4.00)	LEU-A:109 (3.45) VAL-A: 228 (3.43)
Pi-Donor Hydrogen bond (Distance; Å)	TYR-A:108 (4.67)	NA	SER-A:229 (3.55) HIS-A:232(3.31) TYR-A:108 (4.45)	NA
Unfavorable donor-donor (Distance; Å)	NA	NA	ARG-A:96 (2.40)	NA

3.2. Discussion

The European Food Safety Authority (EFSA) has approved the supposedly safe use of plants that contain hydroxyanthracene (rheum, rhamnus, and aloe), even though they are used by the public, especially as laxatives, in 2018. In contrast, its widely used for loose weight; none of the studies was performed to evaluate the molecular interactions of these hydroxyanthracene with FTO.

Previously, Mohammed et al. have been studied molecular docking of flavonoids (Exemestane >Kaempherol >Letrozole >Rutin) and found quercetin has significant binding capacity and establish physical interaction with FTO. Similarly, Hardinsyahd and collauges shown that, the flavonoids of Clitoria ternatea kombucha exhibited better binding affinity to 3LFM compared to orlistat. Their results were as Quercetin (ΔG-7.78), Dibenzylamine> Trifolin(ΔG-7.72)> Quercetin-3β-D-glucoside(ΔG-7.66)> and α-Pyrrolidinopropiophenone (ΔG-5.67), while orlistat was found -3.71 kcal/mol (Hardinsyah et.al., 2023).

Our study approved the binding ability to FTO protein, and the affinity was as Aloe-Emodin Dimer>Emodin>6-Methoxysorigenin.

Similar to the previous research, we have found Rhamnus flavonoids have the remarkable ability to FTO protein, which means they are potent molecules as a potent FTO-inhibitor. Interestingly, Orlistat has lower affinity than Aloe-emodin dimer (-8.7 vs -10.8), which means Aloe-emodin dimer more potent to bound the active site. In contrast, two other Rhamnnus flavonoids were shown lower binding affinity when compared to Orlistat.

4. Conclusions and Recommendations

Flavanoids from Rhamnus sp. have promising potential as FTO inhibitors. However, it does not bind as tightly to the molecule as the commercially available active form 'Orlistat', except that Aloe-emodin dimer binds with higher energy than the commercially available form.

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