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In Silico Molecular Docking Analysis of Limonene with The Fat Mass and Obesity-Associated Protein by Using Autodock Vina

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Abstract
Purpose: This study aimed to predict the binding affinity, orientation, and physical interaction between limonene and fat mass and obesity-associated protein.
Methods: The mechanism of limonene and protein association was explored by molecular docking, a bioinformatic tool. The association results were compared with the reported results of the anti-obesity drug such as orlistat and with the flavonoids. AutoDock Vina tools were used for the molecular docking of limonene with fat mass and obesity-associated protein. PyMol and Discovery Studio Visualizer was used to visualize the results of this docking.
Result: The binding affinity of limonene was higher (Least negative G) than the orlistat and flavonoids such as Daidzein, Exemestane, Kaempherol, Letrozole, And Rutin.
Novelty: In this study, the limonene can alleviate obesity by interacting with the fat mass and obesity-associated protein. This inhibitory interaction was more significant as compared to other reported phytochemicals and drugs.

Keywords: AutoDock Vina, Binding Affinity, Limonene, Molecular Docking
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INTRODUCTION

Obesity in adolescents, children, and adults is rapidly increasing due to high dietary fatty food intake is most common. The risk of other diseases such as cardiovascular problems, hyperlipidemia, type 2 diabetes, and certain types of cancers are high in an obese person [1]. Now, obesity is considered a social problem, and a significant expenditure for the treatment of obesity is set in the budgets of national healthcare of developed countries. Side effects and low efficacy of drugs divert the attention of researchers towards the healthy diet and therapies for obesity [2], [3].

Limonene is chemically a monocyclic monoterpene with molecular formula $C_{10}H_{16}$, and IUPAC's name is 1-methyl-4-(1-methylethenyl) cyclohexane. It is present in more than 300 plants, mainly in citrus essential oils, with a lemon-like odor [4-5]. It is rich in isomeric forms such as d-limonene (R-(+)-limonene) and l-limonene and is considered a safe flavoring agent to be used in baked foods, desserts, fruits juices, ice cream, and soft drinks. Plants produce these terpenes as secondary metabolites as a defense for pathogens and pests repelling, attract the insects for the control of herbivore, signaling hormone, dispersal of seeds, and pollination. d-limonene has been reported as a low toxic causing compound in humans with the repeated dose for one year [5]. The antioxidant, chemotherapeutic, and anti-inflammatory properties of d-limonene have been reported [6]. Different studies of d-limonene also have reported the inhibition activity of lipid peroxidation, prevention from the damage caused by free radicals, hypertension induced by stress, and psychological and physical stress [7].

Citrus acid consists of 95% R- (+)-limonene extracted from the orange peel of Citrus *sinensis* by steam distillation [8]. In a study, the chemical composition of essential oil of three citrus species, C. *paradisii*, C. *reticulata*, and C. *sinensis* was investigated. The highest content of limonene was reported in C. *sinensis*.

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37.63–69.71% in Lemon (Citrus *limon*), 67.90–90.95% in bitter orange (Citrus *aurantium*), 81.52–86.43% in orange maltaise (Citrus *sinensis*), and 51.81–69.00% in mandarin (Citrus *reticulate*) have reported the presence of limonene in the extract of essential oil from their peel [9]. Shu (2010) has reported a significant effect of d-limonene in reducing the blood glucose level of streptozotocin (STZ)-induced diabetic rats [10]. Recently, it was reported in different studies that the derivatives of many citrus compounds target the transcriptional factors of the nuclear receptor such as liver X receptors (LXRs) and peroxisome proliferator-activated receptors (PPARs) showing treatment effects of metabolic disorders [11], [12]. Kumar Sharma *et al*. (2011) reported the reason behind the decrease of serum lipid by the citrus *naringin*. The inhibitory effect in the expression of LXR and elevation of expression of PPARγ in the liver of diabetic type 2 by the citrus *naringin* was found. There is no computation study done to identify the interaction of d-limonene with the receptors of PPAR and LXR [13].

The fat mass and obesity-associated protein (FTO) is a dioxygenase enzyme that brings a most frequent internal modification by acting on N6-methyladenosine (m^6A) and N^6,2′-O-dimethyladenosine (m^6A_m) in the eukaryotic mRNA. It also catalyzes the demethylation in the DNA bases of uracil and thymidine. The regulation of FTO-dependent methyladenosine as a physiological role is partially defined. This biochemical modification has appealed to significant attention. The association of *FTO* gene variation and adiposity with increased body mass has been reported in many studies [14]. Fischer *et al*. (2009) compared the reduction of adiposity and protection from induced diet obesity by the ubiquitous inactivation of *Fto* and increased body mass and fat with the overexpression of FTO in mice [15].

Molecular docking is a recent bioinformatics technique widely studied to analyze the molecular interaction of drugs with the target protein (receptor) [16]. Protein Data Bank (PDB) is a global source of a database of the dimensional structure of the biological structure of macromolecules. PubChem is an open-source chemistry database having molecules of drugs. AutoDock Vina is a suite of software to predict the conformations of optimal bound between the ligands and proteins [17]. This study aims to predict the possible physical interaction, binding affinity, and orientation of ligands between the limonene and FTO.

METHODS

Software and Databases

All the software and databases used in this study are freely available for the use in academic purposes. The PDB (https://www.rcsb.org/) is used to download the 3D structure of the protein. PubChem (https://pubchem.ncbi.nlm.nih.gov/) is used to download the 3D structure of ligands. Python 3.9.1 (https://www.python.org/downloads/) was downloaded and used for language purposes. Discover Studio (https://discover.3ds.com/discovery-studio-visualizer-download) was downloaded and used for molecular visualization, sharing, and analyzing the protein and ligand in modeling studies. MGLTools (https://ccsb.scripps.edu/projects/visualization/) was downloaded and used for the analysis and visualization of biomolecular systems. AutoDock Vina Suite (https://ccsb.scripps.edu/projects/docking/) was downloaded and used for the virtual screening of proteins and ligand interaction. PyMol (https://pymol.org/2/) was downloaded and used for the visualization of docking results.

Preparation of Receptor File

The 3D structure of the FTO (3LFM) protein (receptor) was downloaded from the PDB. Open the protein file in the Discovery Studio and removed all the molecules other than protein, such as water molecules and ligands, and then saved the file in the .pdb extension.

Preparation of Ligand File

The 3D structure of limonene (CID=440917) was downloaded from the open-source chemistry database PubChem. Ligand file was opened in Discovery Studio and then saved the file in the .pdb extension

Grid Setting and File Preparation

The MGLTools were used for setting the grid parameter on the receptor. The protein file with .pdb extension was opened into the MGLTools, polar hydrogen atoms were added in the protein molecule, and then selected the protein as a macromolecule was saved in. pdbqt file. Then added the ligand, set the torsion angle, and saved the ligand in the. pdbqt file.

Preparation of Conf .txt File

The conf .txt file was prepared in such a way that all the center axes and size axes of the set grid were written with the receptor, ligand, and output files extension.

Docking

The AutoDock Vina performed the molecular docking between receptor and ligand according to the procedure described by Trott *et al*. (2009) and Vina *et al*. (2020) [16], [17]. The results were visualized in PyMol and Discovery Studio.

RESULTS AND DISCUSSION

Molecular docking is a computational bioinformatics tool used to predict the non-covalent interaction among the macromolecules. Most frequently, a receptor (protein) molecule and a ligand, other small protein or nucleic acids are used for this procedure. Unbound simulated structures of molecules are used with the ultimate goal is to produce binding affinity and bound conformations [16]. For the virtual screening and molecular docking, AutoDock Vina, a new program, is used. The software developed for the speed-up of the orders of magnitude and improvement in-accurate prediction of binding modes. AutoDock Vina itself adjusts the grid maps and transparently clusters the results to the users [17]. The scoring function of the dependent part of conformation in AutoDock Vina is designed for working by using equation 1.

$$c = \sum_{i<j} f_{titj}(r_{ij}), \quad (1)$$

where i is each atom assigned to t_i type and f_{titj} is an interactive function of the symmetric set of r_{ij} interatomic distance, the overall atoms summation can move in relative pairs to each other, excluding the interaction 1-4.

Figures 1 and 2 are showing the structure of the FTO protein receptor and limonene used for the study of molecular docking. Figure 3 is showing the results of molecular docking between the FTO protein receptor and limonene. The limonene was fit into the place where the active site was present. The binding affinity of limonene with FTO was -5.0 kcal/mol. The chemical interactions between the limonene and amino acid residues such as Val 228, Val 94, His 231, Leu 109, and Tyr 108 were present, as shown in figure 4. The bond numbers, types, and sizes between limonene and amino acid residues are written in table 1. Figures 5, 6, and 7 are showing the hydrogen bond, hydrophobic bond, and solvent accessibility surface areas of the active site of FTO protein around the limonene (ligand).

Several recent studies reported the best target site is to inhibit the FTO in obesity therapy [18]. The risk of obesity and body mass index depends on the SNP (single nucleotide polymorphism) in the *FTO* gene [19]. Studies on animal models revealed the homeostasis energy and metabolic disturbances in obesity were associated with the functionality of FTO [20]. Church *et al*. (2009) reported mutation in FTO protein effect the fat mass and demethylase activity with the slim type of mice. Such types of studies promoted the interest of research to develop antagonists against the FTO. Jing *et al*. (2013) reported the D-limonene therapeutic effects on the mice having obesity with metabolic disorders. The use of d-limonene confirmed the increase in serum high-density lipoprotein cholesterol (HDL-c), glucose tolerance, fasting blood glucose levels, decrease in brown and white adipocytes, decrease in low-density lipoprotein cholesterol (LDL-c), and serum triglyceride in the mice [21].

The binding affinity of limonene with the FTO protein to act as an inhibitor was found better than the studies related to the flavonoids. Mohammed *et al*. (2015) reported the binding energy of Daidzein (+2.60 kcal/mol), Exemestane (-3.96 kcal/mol), Kaempherol (-3.75 kcal/mol), Letrozole (-3.55 kcal/mol), Rutin (-1.11kcal/mol), Quercetin (-1.78e+32 kcal/mol) and Orlistat (-4.86 kcal/mol) [18] that was quite high as compared to the limonene (-5.00 kcal/mol) with the FTO protein receptor. The result of molecular docking of limonene was found better than the anti-obesity drug (Orlistat) reported by Mohammed *et al*. (2015). Due to the less binding energies, limonene is found a good competitive inhibitor to block the expression of adipogenesis-linked transcription factors [22].

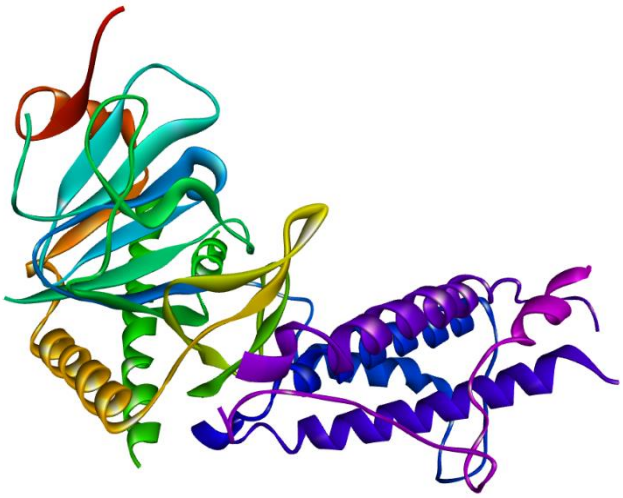

Figure 1. 3D structure of the FTO protein used as a receptor for the molecular docking

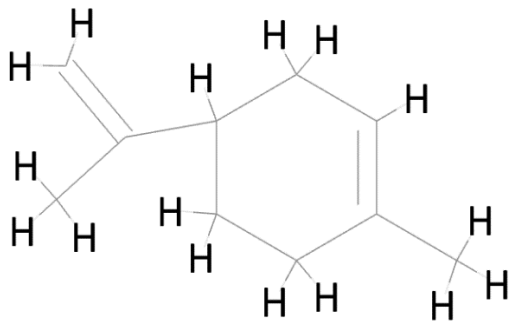

Figure 2. The structural formula of limonene used as a ligand for the molecular docking

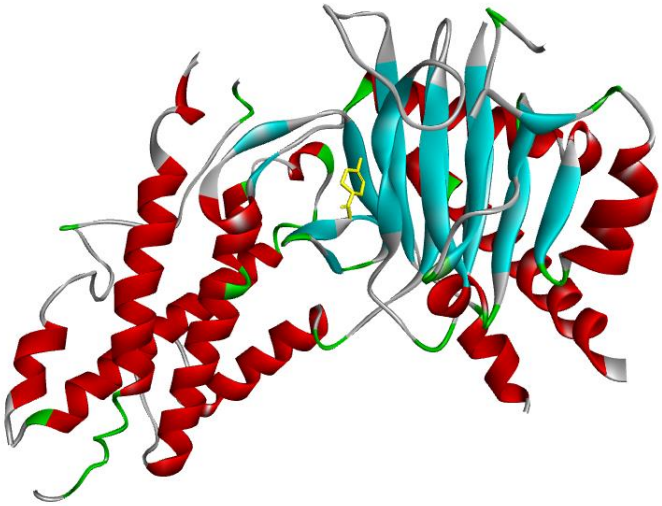

Figure 3. Showing the molecular docking between the FTO protein (receptor) in blue, white, red, and green colors and limonene (ligand) in yellow color.

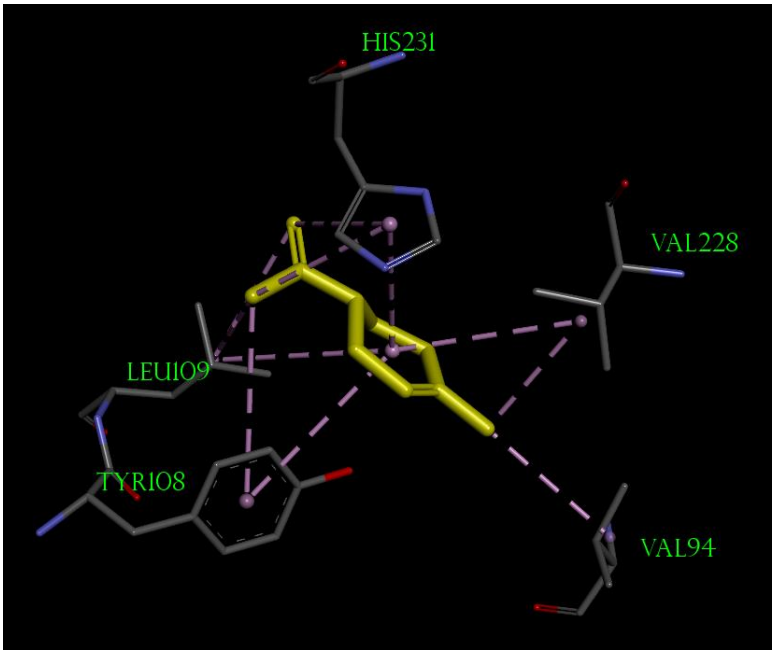

Figure 4. Showing the molecular interaction between the limonene (yellow) and amino acid residues (labeled) of the active site of FTO protein

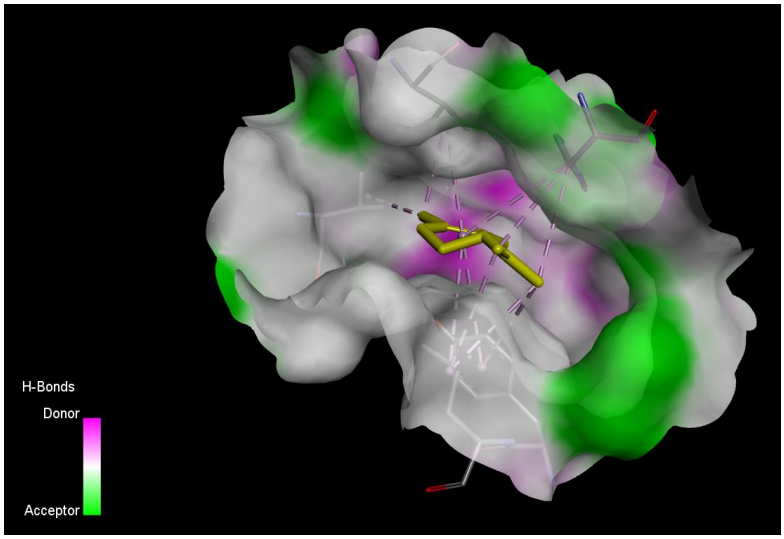

Figure 5. Showing the hydrogen bond surface of the active site of FTO protein around the limonene

Green representing the acceptor side, pink representing the donor side, and white representing the neutral area of the H-bonds surface of the active site.

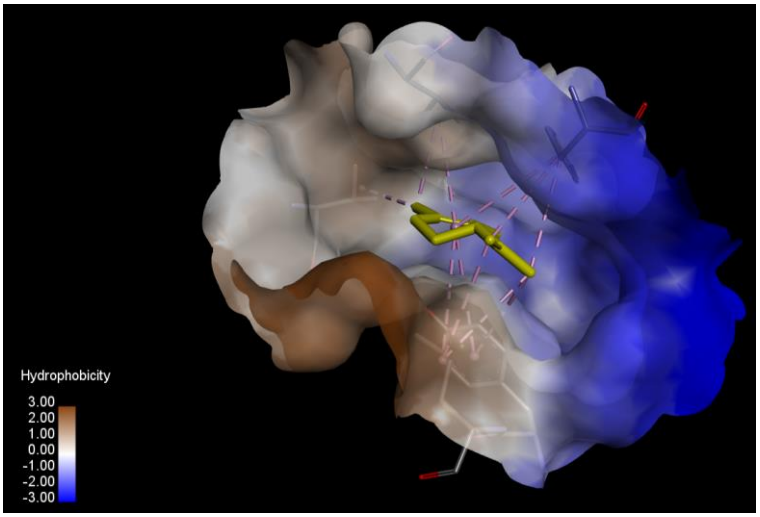

Figure 6. Showing the hydrophobic bond surface of the active site of FTO protein around the limonene.

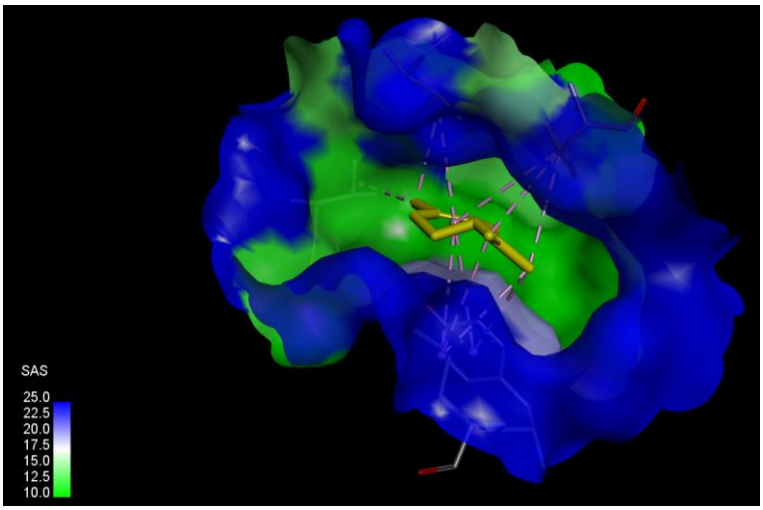

Figure 7. Showing the solvent accessibility surface area of the active site of FTO protein around the limonene

Table 1. Showing the number, type, and size of bonds between the amino acid residues of FTO protein with the limonene

Amino acid residues	Number of bonds	Type of bond interaction	Size of bond length (A°)
Val 228	2	Alkyl	3.69
		Alkyl	4.65
Val 94	1	Alky	4.01
His 231	3	Pi-alkyl bonds	4.49
		Pi-alkyl bonds	4.72
		Pi-alkyl bonds	4.85
Leu 109	3	Alkyl	4.34
		Alkyl	4.58
		Alkyl	5.32
Tyr 108	2	Pi-alkyl bonds	5.04
		Pi-alkyl bonds	5.10

CONCLUSION

The study confirmed the inhibitory effect of limonene with FTO protein. The inhibition of protein is associated with a decrease in obesity and other associated metabolic disorders. Moreover, the docking results of limonene were also compared with phytochemical inhibitors for FTO to prove the better results of docking. The interaction between the limonene and FTO should be confirmed through *in vitro* studies and better understand the mechanism.

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