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Anti-Obesity Property of Indian Tulsi Plant (*Ocimum sanctum*) using *in silico* Docking Techniques

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ABSTRACT: The complex disease of obesity is brought on by having too much body fat. Obesity is a health issue and a significant contributor to the emergence of major conditions such as insulin resistance, type 2 diabetes, cardiovascular disease, and several cancers. The sedentary lifestyle, dietary fluctuations, inactivity, stress, etc. are additional contributing factors. This study aims to screen the bioactive compound Eugenol from *Ocimum sanctum* which has anti-obesity properties and to provide scientific justification in terms of its active ingredient to the target protein for treating obesity using molecular drug docking. In the early stages of drug development, rational drug design is a key step for preclinical studies of therapeutic properties. Even with recognised biological targets, it is challenging to produce a lead chemical and an effective treatment. In this *in silico* research target identification was done by NCBI and sequence conversion was done using Emboss Seqrt for the mutated Obesity Gene LEP. Protein binding sites were carried out using Cavity Plus Server where six cavity pockets were identified for Binding. Molecular docking was done using HDOCK. Docking is done to check the stability of the binding of the Ligand with its Receptor. The results elucidated show how the anti-obesity compound Eugenol can help in minimising the obesity properties since it has direct binding affinities with mutated LEP Gene. Computer-aided systems biology techniques have reignited interest in harnessing medications' natural promiscuity to repurpose known pharmaceuticals, understand and generate drugs, focus on intricate paths, and discover links between distantly linked proteins; these initiatives will have far-reaching consequences.

Keywords: Obesity, LEP, HDock, Eugenol, *Ocimum sanctum*, *silico* Docking Techniques.

INTRODUCTION

Obesity is a condition in which there is an abnormal build up of body fat due to genetic, psychological, and socio-environmental variables and an imbalance between calorie intake and energy expenditure that favours the former (De Blasio *et al.*, 2021). Both the developed and developing worlds are seeing an increase in obesity rates. It was once believed that obesity only affected high-income nations (Tsigosa *et al.*, 2008). Chronic lifestyle-related disorders are the main cause of morbidity and mortality worldwide. One of the main risk factors for cardiovascular disease is having high plasma amounts of cholesterol, especially low-density lipoprotein (LDL) cholesterol (Ross, 1999). In 1994, Friedman and colleagues made the cytokine-like hormone LEPtin (also known as OB protein), which changed how people viewed obesity research and how to maintain their energy balance (Trayhurn, 2009). The LEP gene produces LEPtin, a 164-KDa hormone released by white adipose tissue. Obesity and type-2 diabetes are increased and LEPtin levels are decreased due to a mutation in the LEP gene (Mashmoul *et al.*, 2013).

Numerous illnesses, in particular diabetes, hypertension, osteoarthritis, and heart disease, are linked to obesity. There has been an increase in research into the potential use of herbal extracts and isolated components from natural products to treat obesity (Bhattacharyya *et al.*, 2008). *Ocimum sanctum* Linn (Holy basil) also known as tulsi or Tulasi is a revered plant in India (Suanarunsawat *et al.*, 2011) that is a member of the Lamiaceae family. It has been traditionally used to treat a variety of diseases and shows promise in the treatment of obesity and associated co-morbidities (Upadhyay, 2017). The herb can fight physical, chemical, metabolic, and psychological stress due to its unrivalled pharmacological activities. It is also protective of numerous organs and tissues. Tulsi has anti-oxidant, anti-depressant, and antibacterial qualities and because of its wide range of effects it undoubtedly has a very important medication value (Satapathy *et al.*, 2017). According to a study done by Bhattacharyya *et al.* (2008) a fixed dose regimen of the plant extract was administered to 35 individuals based on a baseline psychological assessment.

The results showed that *Ocimum sanctum* considerably reduced stress and depressive symptoms associated with generalised anxiety disorders (Qadir and Ahmed 2017). It has been demonstrated that the leaves of *Ocimum sanctum* L. (OS) may have a lipid-lowering effect.

While there was no discernible impact on HDL-C, the EO (essential oils) dramatically reduced serum total cholesterol, LDL-C, triglycerides, and atherogenic index. High levels of liver total cholesterol were reduced by EO and triglyceride. The EO isolated from OS leaves helps to decrease lipid levels n high-cholesterol laboratory mice (Rodrigues *et al*., 2022). A versatile naturally occurring chemical known as a phenolic monoterpenoid, eugenol (EUG) is frequently discovered in essential oils from a variety of plant species. Eugenol is advantageous in metabolic alterations and gut microbiota as a result of the rise in the prevalence of obesity and the emergence of novel medicines (Nisar *et al.,* 2021). EUG has been classified as a nonmutant, generally acknowledged as safe (GRAS), chemical by the World Health Organization (WHO). Despite having powerful antioxidant qualities, EUG protects neurons and lowers the risk of inflammatory and oxidative stress-related illnesses. Several types of research have shown that Eugenol plays a part in lipid metabolism (Jo *et al.,* 2014). In an experiment done by Jo HK ., et al Hepatic triglyceride (TG) levels and the steatosis score in mice treated with eugenol dropped by 45 and 72 percent respectively in vivo (Ashokan, 2011). A thorough literature review was done on human research that reported on clinical outcomes following tulsi consumption. A total of 24 studies that reported therapeutic benefits on metabolic diseases, cardiovascular disease, immunity, and neurocognition were identified. The research examined supports traditional applications and indicates that tulsi is a beneficial treatment for lifestyle-related chronic diseases such as diabetes, metabolic syndrome, and psychological stress (Jamshidi and Cohen 2017). Many therapeutic benefits of eugenol have been identified by researchers, including antibacterial, antiviral, and antioxidant activities. The key processes involved in its therapeutic activities are free radical scavenging activity, inhibition of reactive oxygen species creation, inhibition of reactive nitrogen species generation, enhancement of cytoantioxidant capacity, and disordering of DNA and proteins. Nutraceuticals have numerous benefits in the treatment of chronic diseases such as diabetes, obesity, cardiovascular disease, and cancer (Taleuzzaman *et al.,* 2022).

Important insights about a protein's function can be found by predicting the binding locations between two interacting proteins (Alodeani *et al.,* 2015). The association of proteins or the binding of other ligands to proteins is a common component of biological processes. Accurate prediction of putative binding sites on the protein surface can be very beneficial for rational drug design on target proteins with medical relevance, for predicting the geometry of protein-protein as well as protein-ligand complexes, and for assessing the propensity of proteins to aggregate or oligomerize (Yan *et al.,* 2020). In structural molecular biology and computer-assisted drug design, molecular docking is a crucial tool. Predicting the prevailing binding mode of a ligand with a protein having a known three-dimensional structure is the aim of ligand—protein docking. Effective docking methods use a scoring system that correctly ranks candidate dockings and efficiently explores high-dimensional spaces (Yadav *et al.,* 2017).

This study aimed to screen out the effective bioactive compound Eugenol from *Ocimum sanctum*, which has anti-obesity properties and with the help of in silico molecular docking techniques find out how it will act on the target Obesity gene LEP.

MATERIALS AND METHODS

Target identification. The amino acid sequences of the selected protein target LEP was retrieved from NCBI and UniPort database in FASTA format.

Sequence Conversion. Position changes were made in the mutated region of the selected LEP sequence and was converted into FASTA format using EMBOSS Seq.

Ligand Retrieval. PubChem was used to retrieve eugenol. The PubChem database is made to make the most current and complete source of information on the chemical structures of small organic compounds and their biological activity available within the scientific community (Leis *et al.,* 2010). For docking computation, this structure was utilised. The chosen 3D structure of the ligand was downloaded in SDF format from the PubChem Compound database before being converted to PDB format and optimised with Discovery Studio (Youjun., *et al.,* 2018).

Validation of Eugenol. According to Lipinski's rule of five, an orally active drug should typically have no more than five hydrogen bond donors (OH and NH groups), no more than ten hydrogen bond acceptors (notably N and O), a molecular weight under 500 g/mol, a partition coefficient log P of no more than five, and no more than four violations. Using a software programme called Molinspiration a Physicochemical Properties Calculator, the physical and chemical properties of the Eugenol was examined (Liu *et al.,* 2009). Lipinski rule of 5 helps to distinguish between drug like and non-drug like molecules. If predicted high probability of success or failure due to drug likeness for molecules complying with 2 or more of the five rule (Table 1).

Prominent binding site prediction. Prominent binding site prediction of LEP gene was done using Cavity Plus before docking studies. The web server CavityPlus provides functional evaluations and the discovery of protein cavities. CavityPlus applies CAVITY to protein three-dimensional structural data as an input to find possible binding sites on the surface of a particular protein and rank them according to ligandability and druggability scores (Moris and Lim-Wilby 2008).

Molecular Docking analysis. For molecular docking H DOCK server was used. For reliable and quick protein-protein docking, the HDOCK server offers a fully integrated set of homology search, template-based modelling, structure prediction, macromolecular docking, biological information incorporation, and task administration. The server automatically predicts the interaction between receptor and ligand molecules using input data for both molecules (amino acid sequences or Protein Data Bank structures). This is done using a hybrid method of template-based and template-free docking (Bray 2004). The PDB files of ligand and target receptor were uploaded to H Dock server for docking

analysis. Analysis on HDock yielded results for Receptor and Ligand surface residue.

RESULTS AND DISCUSSION

Protein sequence preparation: The Amino acid sequences of LEP gene was retrieved from NCBI in FASTA format as shown in Fig. 1. Position changes were made in the mutated region of the LEP gene using Emboss seqrt as shown in Fig. 2.

The above 3D images show the Normal LEPtin Gene with Valine at the 94th position, Aspartic acid at 100th position and Arginine at 105th Position

```
        10                  20                  30                  40                  50
MHWGTLCGFL  WLWPYLFYVQ  AVPIQKVQDD  TKTLIKTIVT  RINDISHTQS
        60                  70                  80                  90                 100
VSSKQKVTGL  DFIPGLHPIL  TLSKMDQTLA  VYQQILTSMP  SRNVIQISND
       110                 120                 130                 1 40                1 50
LENLRDLLHV  LAFSKSCHLP  WASGLETLDS  LGGVLEASGY  STEVVALSRL
                                   160
                        QGSLQDMLWQ  LDLSPGC
```

Fig. 1. Normal Protein sequence of LEP gene.

```
        10                  20                  30                  40                  50
MHWGTLCGFL  WLWPYLFYVQ  AVPIQKVQDD  TKTLIKTIVT  RINDISHTQS
        60                  70                  80                  90                 100
VSSKQKVTGL  DFIPGLHPIL  TLSKMDQTLA  VYQQILTSMP  SRNMIQISNY
       110                 120                 130                 1 40                1 50
LENLWDLLHV  LAFSKSCHLP  WASGLETLDS  LGGVLEASGY  STEVVALSRL
                                   160
                        QGSLQDMLWQ  LDLSPGC
```

Fig. 2. Mutated Protein sequence of LEP gene.

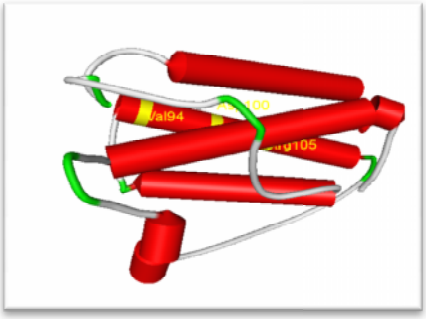

Fig. 3. 3D structure of Normal LEP as visualized by Discovery Studio 3.0.

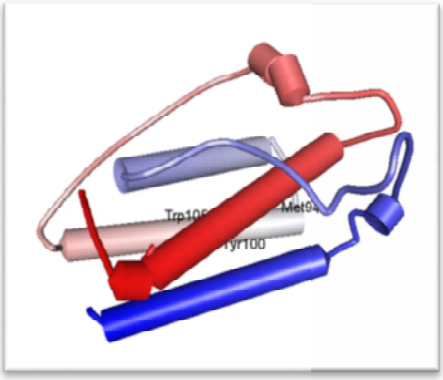

Fig. 4. 3D Structure of LEP gene Mutated as visualized by Discovery Studio 3.0.

Ligand Preparation:

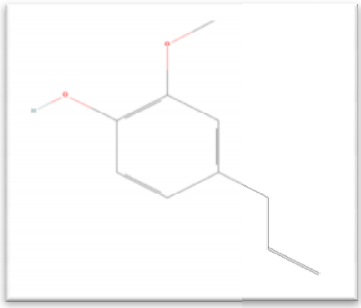

Fig. 5. 2D structure of Eugenol($C_{10}H_{12}O_2$.).

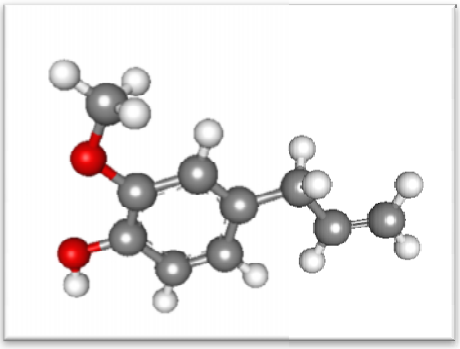

Fig. 6. 3D structure of Eugenol as visualized by Discovery Studio 3.0.

Table 1: Molecular Physicochemical Properties of Eugenol using Molinspiration.

Molinspiration property		
Physicochemical Properties	**Good Druglikeness Property Range**	**Drug likeness range for Eugenol**
Log P	>5	2.10
Molecular Weight	>500 Dalton	164.20
Total Polar Surface Area	>140 Å^2	29.46
Number of Hydrogen Bond Acceptors	>10	2
Number of Hydrogen Bond Donors	>5	1
Molar Refractivity	40-130	48.559792
Number of Violations		0

Drug likeness score of Eugenol shows Molecular weight as 164.20g/ mol as shown in Table 1, and the calculated LogP value is 2.10 which is an acceptable range for a good drug. The predicted value of our drug compound is 29.46 which show it has a good TPSA score. It also has zero violations and follows all Lipinski's rule of five.

Binding site prediction. To determine the protein binding sites Cavity Plus Server was used. Five Cavity pockets which are the active sites were detected on the mutated LEP Gene as shown in Fig. 7.

Binding cavities on protein surfaces are crucial for protein function because they are frequently the locations where a protein attaches to other biological macromolecules like nucleic acids and proteins or tiny molecules like metabolites and medicines, for functional protein annotation and structure-based drug design, computational protein cavity detection has been regarded as a crucial step (Morris and Lim-Wilby 2008).

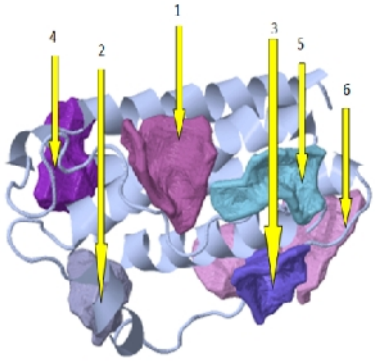

Fig. 7. Six(6) Cavity pockets detected on the Mutated LEP gene.

Table 2: Output of Cavity Results of the Mutated LEP gene.

Cavity Results				
Sr. No.	**Pred. Max pKd**	**Pred. Avg pKd**	**Drug Score**	**Druggability**
1.	8.14	5.41	-1299.00	Weak
2.	6.96	5.01	-897.00	Weak
3.	6.81	4.95	-715.00	Weak
4.	6.50	4.85	-1249.00	Weak
5.	6.42	4.82	-985.00	Weak
6.	6.14	4.72	-1389.00	Weak

Molecular docking: The predicted model of Eugenol as ligand was docked with the mutated LEP Gene from human as a receptor as shown in Fig. 8. The top 10 binding models generated by HDock were taken and the one with the highest docking score *i.e* -97.90 model 1 as shown in Table 1 was taken for molecular docking because it has more Binding affinity towards the Receptor.

As shown in the above Table 4 it shows the Receptor interface residue with Amino acids Aspartic acid, Tryptophan, Histidine, Histidine, Leucine, Proline, Tryptophan and Alanine on the A chain where the Ligand (Eugenol) can be binded in the positions 76, 105, 109, 118, 1198, 120,121 and 122 to slow down the obesity mutations.

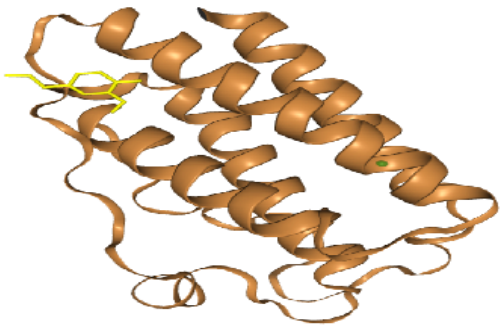

Fig. 8. Docking of the mutated LEP gene with Eugenol.

Table 3: A docking summary of the top 10 models is shown above. The top 10 binding models are the most significant models.

Complex Template Information										
Molecule	**PDB ID**		**Chain ID**	**Align_length**		**Coverage**		**Seq_ID(%)**		
Receptor	1AXB		A	144		1.000		97.2		
Summary of the Top 10 Models										
Rank	1	2	3	4	5	6	7	8	9	10
Docking Score	-97.90	-82.95	-77.84	-72.02	-71.15	-70.14	-66.73	-66.75	-64.90	-63.62

Table 4: Receptor Interface Residue of Model 1.

Receptor interface residue(s):		
ASP	76A	3.789
TRP	105A	3.081
HIS	109A	2.672
HIS	118A	3.809
LEU	119A	2.928
PRO	120A	3.040
TRP	121A	3.479
ALA	122A	3.808

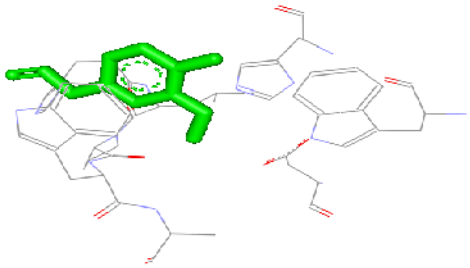

Fig. 9. Ligand Binding site atoms as visualized by Discovery Studio 3.0.

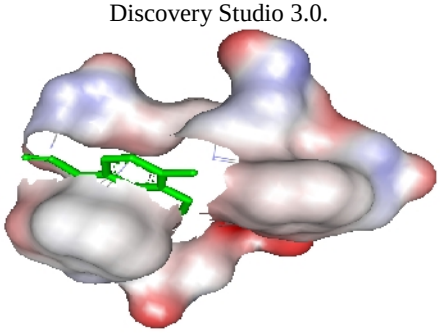

Fig. 10. Surface around Ligand as visualized by Discovery Studio 3.0.

Fig. 9 and 10 with the help of molecular visualisation tool Discovery studio shows Ligand binding site atoms and surface around Ligands which is a crucial step in understanding the drug binding affinity of the Ligand with the Receptor.

DISCUSSION

Obesity is a growing epidemic that threatens to deplete health-care resources by increasing the prevalence of diabetes, heart disease, hypertension, and cancer (Kang and Park 2012). Increased physical activity and calorie restriction are now recommended treatments for obese people. When behavioural therapy is ineffective, pharmaceutical medication is recommended (Mirzadeh *et al.*, 2022). Utilizing the Molinspiration tool, the chemical characteristics and bioactivity of Eugenol found in *Ocimum sanctum* in SMILES format from NCBI PubChem was studied. In this *in silico* investigation, Molinspiration was utilised to validate all predicted chemical compound and establish the structural drug affinity ratings. The drug was assessed to see if the chemical compound satisfied the requirements for drug likeness. Similarities between drugs Values like the number of hydrogen acceptors (10), the number of hydrogen donors (5), the molecular weight (500 Da), and the partition coefficient log P > 5 were validated using Lipinski's rule of 5 and Molinspiration. Polar surface area and lipophilicity (LogP value) are two crucial variables in estimating the oral bioavailability of pharmaceuticals (TPSA value) (Liu *et al.*, 2009). The Ligand Eugenol follows all Lipinki's rule of five as shown in Table 1 which is later used for docking with its receptor *i.e.* mutated LEP gene.

The docking process consists of two steps: determining the binding affinity and predicting the ligand's structure, position, and orientation with respect to its receptor. To understand the ligand discovery process, it is crucial to determine whether a protein structure is a good target before doing structure-based drug design on it. The term "druggability" or "ligandability assessment" is used to describe this, and interest in it has grown recently. The evaluation often entails finding ligand-binding sites on the protein surface and estimating how well they will bind tiny compounds that are similar to drugs. When it came to finding ligand-binding sites, Cavity performed well. It also worked well for predicting the ligandabilities and druggabilities of the binding sites that were found (Yuan *et al.*, 2013). With a docking score of -1389, Cavity 6 has more or less increased druggability as a result of the results of the altered LEP interface, which are displayed in Table 2. This is because the more negative energy, the simpler it is to attach to a ligand.

The 3D structure was then seen using Discovery Studio software, together with the surface surrounding the Ligand and the atoms that connect to it, as shown in Figs. 9 and 10.

CONCLUSION AND FUTURE SCOPE

The molecular docking *in silico* study done on Eugenol and mutated LEP Gene can be used to mimic the interaction between a small molecule and a protein at the atomic level, allowing us to characterise small molecule behaviour in target protein binding sites as well as elucidate key biochemical processes**.** With the findings of the binding sites using Cavity pockets the Ligand will be able to bind the Receptor and form a stable complex in minimising the mutational effect of the mutated LEP Gene since *Ocimum sanctum* has anti-obesity properties.

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