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ORIGINAL ARTICLE

Novel Phytochemical Inhibitors of Leptin: A Molecular Docking Approach for Potential Cardiovascular and Antidiabetic Therapies

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ABSTRACT

OBJECTIVE: To identify phytochemicals as potential leptin inhibitors, particularly in the context of immune and cardiovascular health, using molecular docking analysis.
METHODOLOGY: This study was conducted from 2023 to 2024 with the joint efforts of the Department of Bioinformatics and Biotechnology, Government College University Faisalabad, and the Center of Excellence and Molecular Biology, University of Punjab Lahore. The 3D structure of Leptin was retrieved from the Protein Data Bank and refined for docking analyses using PyRx. A ligand library consisting of 5,006 phytochemicals was prepared from various databases. The interactions were visualized using Discovery Studio. Compounds with high docking scores were further analyzed for their ADMET properties based on Lipinski's Rule of Five, ensuring their drug-like characteristics and predicting their safety profiles.
RESULTS: The compounds Peonidin, Diosmin, 7-[3-(3-hydroxy oct-1-enyl)-4,6-dioxabicyclo[3.1.1]heptan-2-yl]hept-5-exonic acid, Carnitine, Dihydrodaidzin, 4-dimethylpodophyllotoxin, 4-demethyldeoxypodophyllotoxin, (+)-sophorol and Tylophorinidine demonstrated successful binding to the Leptin. Thus, it potentially interferes with the leptin-leptin receptor interaction. Diosmin and Dihydrodaidzin showed the most vital binding score of -7.9 Kcal/mol of all the top-ranking compounds.
CONCLUSION: These compounds may act as effective inhibitors of Leptin, which may help develop natural, affordable therapies for cardiovascular disorders and diabetes. However, further research is required to validate their efficacy and safety.

KEYWORDS: Phytochemical Inhibitors, Leptin, Molecular Docking, Cardiovascular, Antidiabetic Therapies.

INTRODUCTION

Obesity has leading epidemic proportions and is the primary cause of cardiovascular diseases, diabetes, and comorbidity conditions like pulmonary hypertension. Leptin signaling has also been implicated in mood regulation[1]. Research shows a strong connection between obesity and the development of leptin resistance as a potential biomarker and therapeutic target. Leptin is vital in central energy metabolism and interacts significantly with the immune system[2]. Leptin receptors influence both innate and adaptive immune responses. Additionally, many factors are linked to obesity, leptin resistance, and bladder cancer[3].

The leptin protein, composed of 167 amino acids, is primarily expressed in tissues like the mammary gland, skeletal muscle, ovary, stomach, placenta, and lymphoid tissues, and predominantly in white adipose tissue. The concentration level of circulating Leptin is directly linked to body fat levels, reflecting its role in long-term energy storage. Caloric intake fluctuations significantly impact leptin levels, with marked decreases during starvation[4]. In mouse models, leptin receptor isoforms have the same ligand-binding site and show an alternative splicing pattern. The Ob-Re isoform is secreted into the bloodstream, while other Ob-R receptors are proteins located on the plasma membrane. The minor short forms of Ob-R receptors—Ob-Ra, c, d, and f—are involved in intracellular signaling specific effects ofLeptin[5]. Leptin binding to the Ob-Rb receptor activates several signaling pathways, including the JAK-STAT pathway, MAPK pathways and insulin receptor substrate[6].

The level of Leptin is measured usually high in obese patients. It plays a significant role in hypothalamic-pituitary function, regulating appetite, thermogenesis, and food intake. However, many obese individuals are resistant to elevated levels of circulating Leptin, with diet and body weight being critical factors in this resistance[7]. Leptin and insulin work together to regulate long-term energy balance, as Leptin directly affects the liver and has insulin signaling pathways[8]. Obesity treatments can modulate leptin action in the brain, especially in areas related to food intake. LERP therapy has been effective in treating congenital leptin deficiency[9].

The Ob-Ra isoform transports the Leptin through the blood-brain barrier, while the Ob-Rb isoform intervenes in signal transduction and is shown high expression in the hypothalamus. Leptin is essential for maintaining metabolism, neuroendocrine function, bone metabolism and immune function. Recent studies suggest that excess Leptin can lead to hepatic inflammation and fibrosis[10]. Ongoing research explores the potential of Leptin in treating non-alcoholic fatty liver disease, particularly concerning lipid handling, hepatic inflammation, and fibrosis. In contrast to mammals, Leptin does not affect food intake in non-mammals and may not be expressed in adipose tissue. Recent evidence suggests that increased expression of negative regulators in the hypothalamus is a crucial mechanism underlying leptin resistance[11]. Using in silico methods is highly important in drug design, enabling the screening of compound libraries through bioinformatics tools. Leptin is also associated with the progression of liver cancer, type II diabetes, and cardiac diseases, mainly myocardial infarction, ischemic stroke and peripheral vascular disease[12]. Leptin, a cytokine family member, is encoded by the OB gene and produces a 16kDa hormone named after the Greek word "leptos," which means lean. Due to its pro-inflammatory and anorexigenic functions, Leptin also shows many associated benefits to malnutrition and inflammation, which are common in chronic kidney disease (CKD) patients and associated with a high risk of cardiovascular morbidity and mortality[13].

Moreover, Leptin's pro-inflammatory effects may cause kidney damage. This study aimed to explore the role of leptin protein in obesity and related comorbid diseases, such as cardiovascular

disease and diabetes. Furthermore, it also examines the role of Leptin as a potential biomarker and therapeutic target for developing therapeutic strategies for treating obesity and related comorbid diseases, such as cardiovascular disease and diabetes.

METHODOLOGY

Study Location and Duration

This study was conducted from 2023 to 2024 with the joint efforts of the Department of Bioinformatics and Biotechnology, Government College University Faisalabad, and the Center of Excellence and Molecular Biology, University of Punjab Lahore.

Protein Structure Retrieval and Preparation

The 3D structure of Leptin was retrieved from the Protein Data bank (PDB) using PDB ID: 3v6o. This structure was selected based on its high resolution and close resemblance with the native structural confirmation of Leptin. The structure was refined by removing solvent residues and non-standardized ligands, and an accurate protonation state was assigned, followed by energy minimization using ChimeraX. This optimized structure was loaded into PyRx as a receptor for subsequent docking analyses.

Ligand Library Preparation

A library of 5006 phytochemicals was compiled from PubChem, MPD3, and MAPS databases. These phytochemicals were selected because of their drug-like properties. This library was subsequently prepared for molecular docking.

Molecular Docking

The prepared directory of phytochemicals was docked with Leptin's residues using the PyRx docking algorithm. Aicar (PubChem ID: 4), a known leptin inhibitor, was used as a reference ligand to validate docking results. The PyRx program ensured the perfect conformation of the ligands to achieve the lowest energy structure. The top docked compounds were selected based on their RMSD and Binding energy values. The Discovery Studio generated 2D receptor-ligand interaction plots, providing detailed visualizations of the interactions with docked complexes.

In-Silico Analysis of ADMET Properties

Compounds with the highest docking scores were further evaluated based on Lipinski's Rule of Five (Ro5), excluding any compounds that violated Ro5. The molinspiration server was used to calculate the physicochemical properties. The drug-like features of the phytochemicals were then evaluated using the SwissADME web tool. ADMET properties were assessed to predict the behaviour of the drug candidates, including potential toxicity, ability to cross the blood-brain barrier, intestinal absorption, subcellular distribution, and overall safety profile.

RESULTS

Molecular Docking Analysis

From 5006 docked molecules, ten top-most docking poses were selected. These compounds were chosen based on binding site occupancy, minimum binding free energy, and lower RMSD values, exhibiting binding packet energies ranging from -7.9 Kcal/mol to -4.0 Kcal/mol (**Table I, Figure I**). Diosmin and Dihydrodaidzin emerged as the highest-ranking compounds with the strongest binding affinity among the selected binding sites. Two of the top ten phytochemicals, Diosmin and Dihydrodaidzin
showed a strong binding affinity toward residues, TYR466, LYS486, GLN491, GLN501, PRO 502, ASP 532 and SER 541 respectively.

ADMET Analysis

All the selected candidates exhibited no violations of Lipinski's Rule of Five, demonstrating favorable drug-like features, including appropriate molar weight (**Table II**). These findings were further validated using the SwissADME server, with detailed results in **Table III**.

Table I: Top ten phytochemicals with docking scores and RMSD values

PubChem ID	Phytochemical name	Score	Value of RMSD
5281613	Diosmin	-7.9	3.3062
441773	Peonidin	-6	2.702
213	7-[3-(3-Hydroxyoct-1-enyl)-4,6-dioxabicyclo[3.1.1]heptan-2-yl]hept-5-enoic acid	-5.7	1.1910
288	Carnitine	-4.2	1.2157
10341941	Dihydrodaidzin	-7.8	2.0428
2116	DL-alpha-Tocopherol	-5.8	2.8407
122667	4'-Demethylpodophyllotoxin	-6.6	1.1309
23724667	(+)-Sophorol	-6.8	1.7353
161749	Tylophorinidine	-6.6	1.5286
160705	4'-Demethyldeoxypodophyllotoxin	-6.4	3.3220

Table II: Lipinski rule parameters for screened phytochemicals

Compounds	M. weight (g/mol) (<500)	Number of HBA (<10)	Number of HBD (<5)	MLogP (<5)
Diosmin	608.54g/mol	15	8	-3.23
Peonidin	301.27g/mol	6	4	0.57
7-[3-(3-Hydroxyoct-1-enyl)-4,6-dioxabicyclo[3.1.1]heptan-2-yl]hept-5-enoic acid	352.47g/mol	5	2	2.38
Carnitine	161.20g/mol	3	1	-3.79
Dihydrodaidzin	418.39g/mol	9	5	-0.92
DL-alpha-Tocopherol	430.71	2	1	6.14
4'-Demethylpodophyllotoxin	400.38g/mol	8	2	1.21
(+)-Sophorol	300.26g/mol	6	2	0.82
Tylophorinidine	365.42g/mol	5	2	2.00
4'-Demethyldeoxypodophyllotoxin	384.38g/mol	7	1	2.00

Table III: ADMET analysis parameters of active compounds

Compounds	Diosmin	Peonidin	7-[3-(3-Hydroxyoct-1-enyl)-4,6-dioxabicyclo[3.1.1]heptan-2-yl]hept-5-enoic acid	Carnitine	Dihydrodaidzin	DL-alpha-Tocopherol
1. Absorption						
Blood-brain barrier (BBB)	No	No	Yes	No	No	No
GI Absorption	Low	High	High	High	Low	Low
P-glycoprotein substrate	Yes	Yes	No	No	Yes	Yes
2. Metabolism						
CYP450 1A2 Inhibitor	No	Yes	No	No	No	No
CYP450 2C9 Inhibitor	No	No	Yes	No	No	No
CYP450 2D6 Inhibitor	No	No	Yes	No	No	No
CYP450 2C19 Inhibitor	No	No	No	No	No	No
CYP450 3A4 Inhibitor	No	No	Yes	No	No	No

DISCUSSION

The advent of in silico pharmacology has revolutionized drug design, significantly reducing the costs and time associated with traditional methods[14,15]. Advanced bioinformatics tools and cheminformatics have enabled the screening of extensive compound libraries for drug-likeness properties[16]. In this study, 5006 phytochemicals were docked to recognize the potent leptin receptor inhibitors. Ten phytochemicals were identified as potential inhibitors, including Peonidin, Diosmin, 7-[3-(3-hydroxy oct-1-enyl)-4,6-dioxabicyclo[3.1.1]heptan-2-yl]hept-5-exonic acid, Carnitine, Dihydrodaidzin, 4-dimethylpodophyllotoxin, 4-demethyldeoxypodophyllotoxin, (+)-Sophorol, DL-alpha-Tocopherol, and Tylophorinidine, depending on their docking scores and binding affinity. Key residues such as TYR466, LYS486, GLN491, GLN501, PRO 502, ASP 532 and SER 541 were identified as critical for binding affinity in best-docked compounds, Diosmin and Dihydrodaidzin (**Table I**)[17].

Leptin deficiency was mainly associated with insulin resistance, obesity, impaired glucose tolerance, and cardiovascular diseases[18]. Obese individuals often exhibit higher leptin concentrations, highlighting the importance of developing leptin inhibitors as potential antidiabetic and anti-cancer agents. Previous studies have suggested various leptin inhibitors, but many have limitations[19,20]. In contrast, our docking study identified ten potent phytochemicals with no ADMET violations, the highest docking scores, and the lowest RMSD values, providing a solid foundation for developing novel leptin inhibitors[21].

These ten phytochemicals revealed the ideal docking scores out of a large library of 5006 phytochemicals. They were chosen through non-covalent bonding, strong hydrogen bonds, and other thermodynamic factors, including binding energy. The top chemicals have binding energies ranging from -7.9 Kcal/mol to -4.0 Kcal/mol. Diosmin and Dihydrodaidzin showed the strongest binding score and the highest affinity of all the top-ranking compounds, positioning them as a possible leptin antagonist (**Table I**)[22]. After docking analysis, the selected inhibitors' physiochemical properties and drug-likeness (DL) were assessed using the Molinspiration and SwissADME servers. The ADMET study showed that the chosen inhibitors had good pharmacokinetic profiles and did not break Lipinski's Rule of Five, suggesting potential inhibitors against Leptin (**Table II & III**)[23,24]. Finding possible leptin antagonists can be done efficiently and economically using a molecular docking methodology and a phytochemical library. Particularly in the early phases of drug discovery, this computational screening eliminates the need for costly and time-consuming wet-lab studies. Compounds Diosmin and Dihydrodaidzin had a high binding affinity, indicating they could be potent leptin inhibitors. These results demonstrate that reported phytochemicals can interact with Leptin and may alter its action.

Since all of the chosen compounds followed Lipinski's Rule of Five, they could be effective therapeutic agents for managing diseases linked to Leptin[25]. The findings of this study will significantly impact the field of metabolic illnesses and obesity treatment.

Compared to synthetic drugs, the phytochemicals found in this work may provide a platform for developing new leptin inhibitors with greater bioavailability and fewer side effects. Furthermore, the discovery of distinct binding interactions between Leptin and essential amino acid residues (TYR466, LYS486, GLN491, GLN501, PRO 502, ASP 532 and SER 541) may direct the development of more effective and focused leptin antagonists (**Table I**)[25]. Additionally, the increased interest in plant-based therapies is consistent with the possible application of natural substances as leptin inhibitors. Because they are typically considered safer and the body can

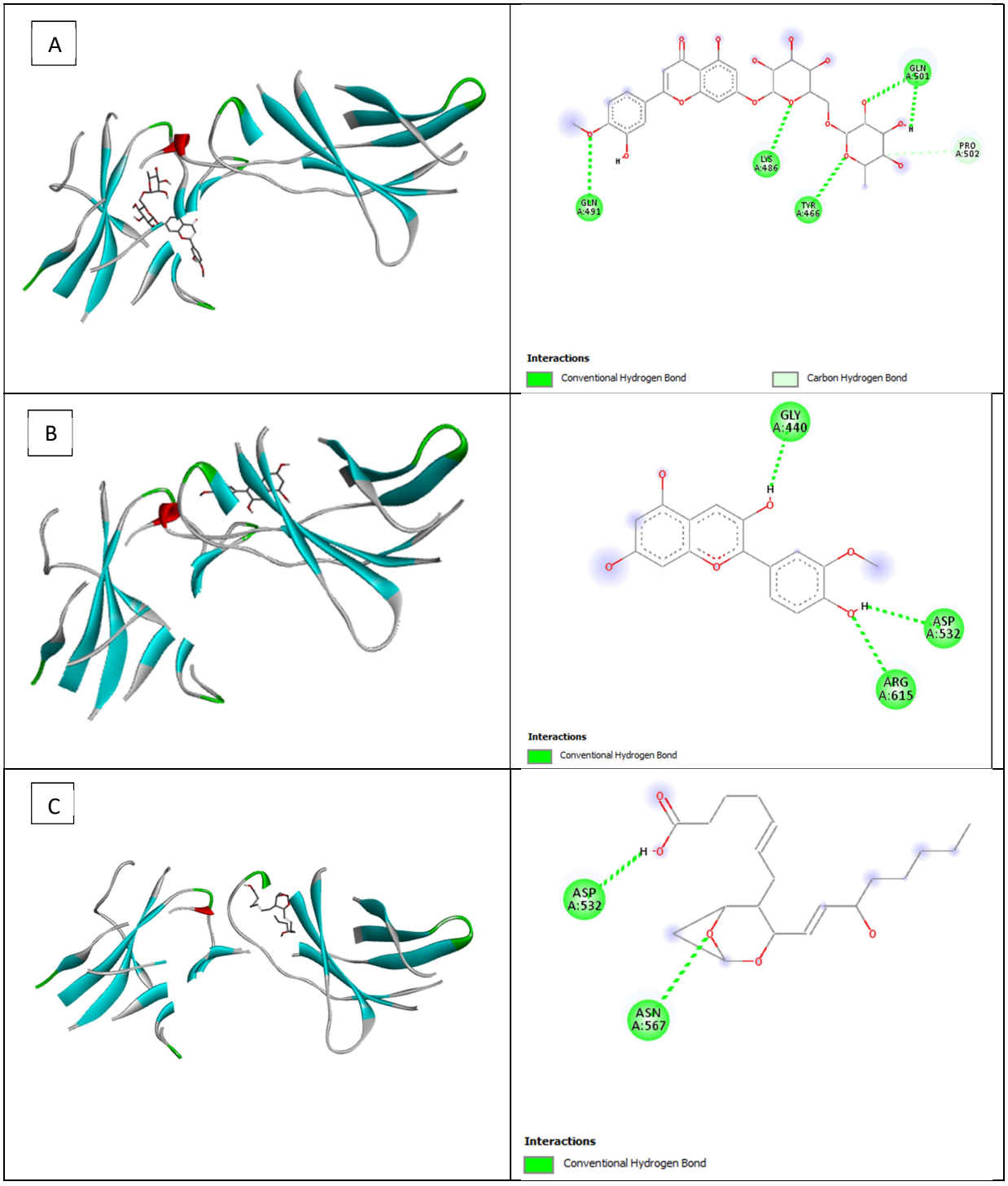
handle them better, phytochemicals are appealing options for long-term therapy plans. The results of this investigation contribute to the growing data on the use of natural ingredients in therapeutic development, especially in metabolic disorders[17].

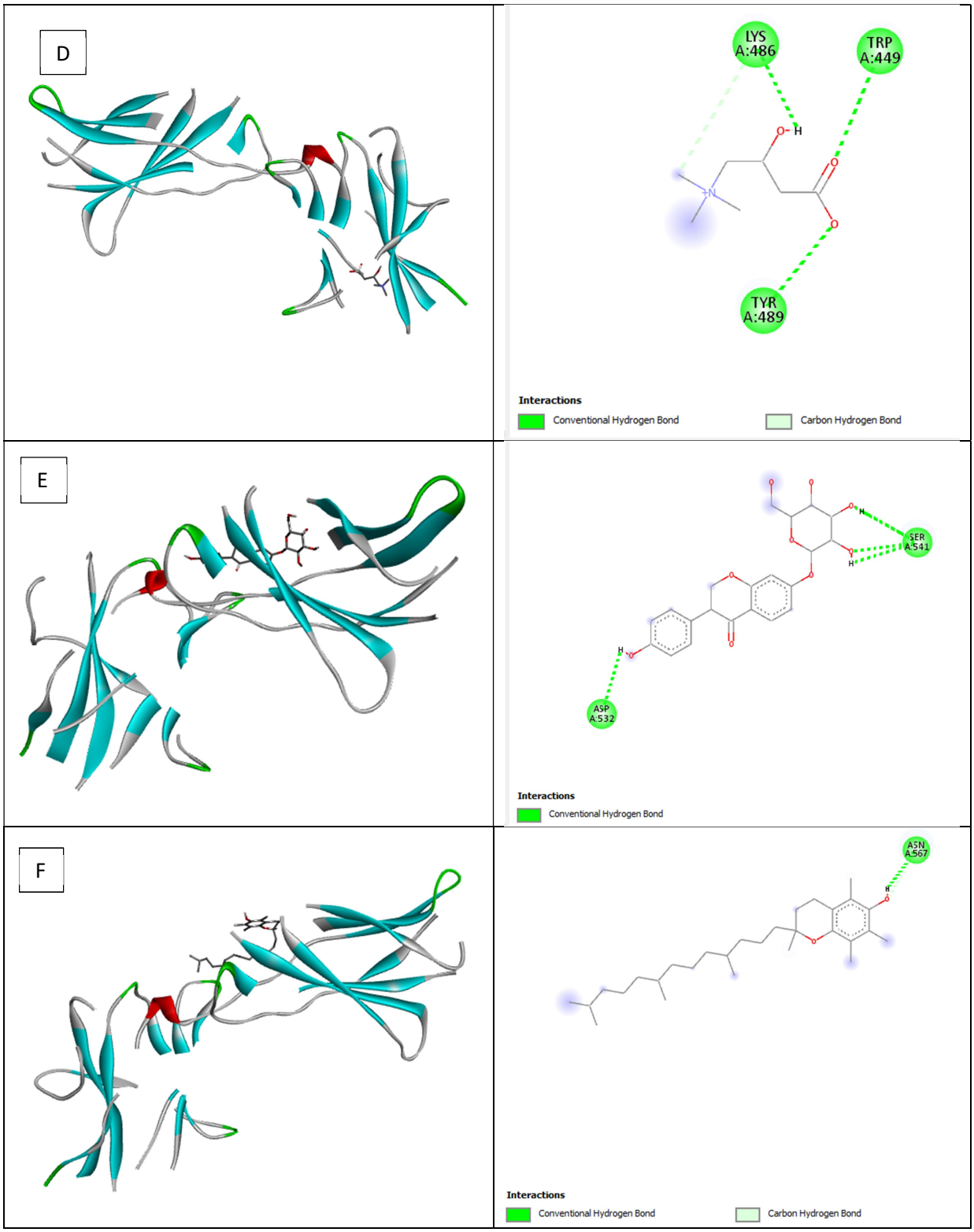
The study includes several limitations that should be noted despite the encouraging results. However, a handy tool, molecular docking is intrinsically constrained by its dependence on theoretical models and assumptions. The binding affinities and interactions seen in silico in a biological system might not always correspond to the same effectiveness. Confirming the discovered drugs' binding potential and therapeutic efficacy requires experimental validation, such as *in vitro* and *in vivo* testing[18,19]. The leptin structure found in the PDB (3v6o) was the only one that could be used for optimization and docking. Molecular docking simulations employ static structures, but protein structures undergo dynamic changes *in vivo*, including conformational shifts. A more profound knowledge of how these phytochemicals interact with Leptin in a dynamic biological environment may be possible through a more thorough investigation using molecular dynamics simulations.

Furthermore, although the ADMET study shows beneficial pharmacokinetic features, it is still predicated on computational projections[20,26]. More experimental investigations are needed, such as studies of absorption, distribution, metabolism, and excretion, to validate the pharmacokinetic characteristics of these substances. This study did not investigate other possible sources of leptin inhibitors, such as synthetic small molecules or peptides, and instead concentrated only on phytochemicals. While phytochemicals have many benefits, investigating additional chemical classes might produce more effective inhibitors or enhance the potential of discovered candidates as drugs[27].

Future study directions should include validating identified leptin inhibitors through experiments[28-30]. *In vitro,* tests should be performed to determine the binding affinity and inhibitory potential of the chosen compounds, and *in vivo* experiments should be conducted to assess the effectiveness of the compounds in animal models of sickness associated with Leptin. Research utilizing site-directed mutagenesis for TYR466, LYS486, GLN491, GLN501, PRO 502, ASP 532 and SER 541 residues may validate the significance of these residues in leptin-phytochemical interactions, offering a more in-depth understanding of the inhibitory mechanism[31]. To better understand how the phytochemicals interact with Leptin over time[32,33], molecular dynamics simulations may be used in conjunction with docking investigations[34-37].

Figure I: Docking complexes of Leptin with most potent phytochemicals. 3D structures show the spatial interaction of Leptin protein and phytochemicals. While 2D structures showed an interaction of phytochemicals with amino acid residues in the active site of Leptin

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TRP A:449
TYR A:489
Interactions
Conventional Hydrogen Bond
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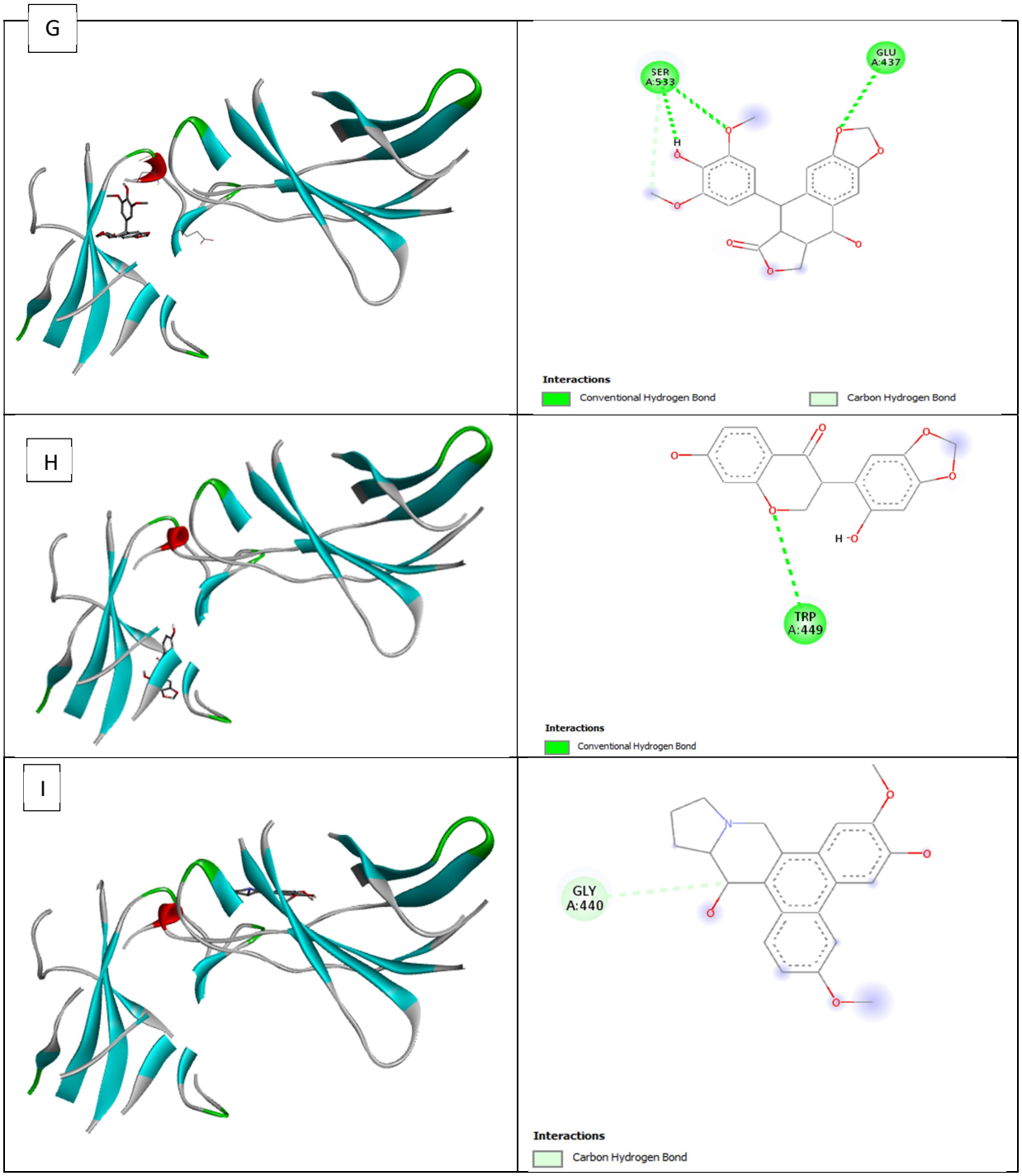
G
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GLY A:440
Interactions
Carbon Hydrogen Bond

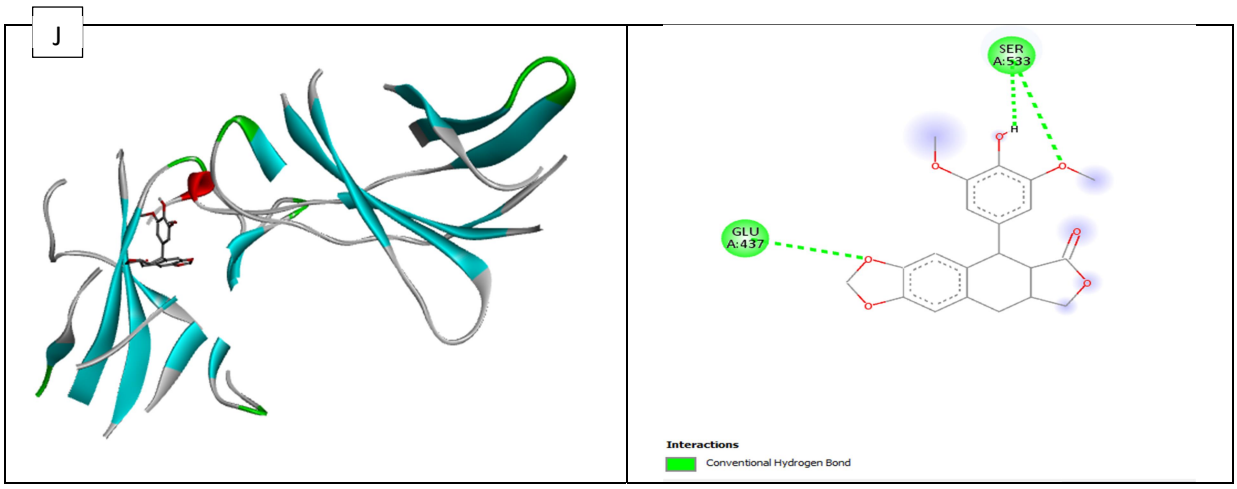

Figure I:

A: Docking of Diosmin with Leptin displays hydrogen bonds formed by the side chain atoms of TYR466, LYS486, GLN491 and GLN501, amino acids indicated in green colour. Carbon-hydrogen interaction with PRO 502 is shown in a light green colour. The 2D interaction emphasizes the active residues within the receptor's binding pocket.

B: Docking of Peonidin shows that hydrogen bonds are formed by the side chain atoms of GLN 440, ASP 532, and ARG 615, amino acids (indicated in green). The 2D interaction depicts the active residues within Leptin's binding pocket.

C: Docking of 7-[3-(3-Hydroxyoct-1-enyl)-4,6-dioxabicyclo[3.1.1]heptan-2-yl]hept-5-enoic acid illustrates the formation of hydrogen bonds by the side chain atoms of ASP 532, ASN 567 amino acids (indicated in green). The 2D interaction diagram shows the active residues within the binding pocket of Leptin.

D: Docking of the Carnitine compound with Leptin displays hydrogen bonds formed by the side chain atoms of TRP 449, LYS 486, and TYR 489437 amino acids. Carbon-hydrogen interaction with LYS 486 amino acid is indicated in green. The 2D interaction diagram emphasizes the active residues within the binding pocket.

E: Docking of the Dihydrodaidzin compound with Leptin shows the formation of hydrogen bonds by the side chains of ASP 532 and SER 541 amino acids (indicated in green). The 2D interaction diagram displays the active residues within the binding pocket of Leptin.

F: DL-alpha-Tocopherol docked complex illustrates the hydrogen bond and carbon-hydron interaction with side chain atoms of ASN 567 amino acid (indicated in green). The 2D interaction highlights the active residues within Leptin's binding pocket.

G: 4'-Demethylpodophyllotoxin docked complex with Leptin displays the formation of hydrogen bonds by the side chain atoms of ILE 489, ASP 489, GLU 457 and LYS 486 (indicated in green). The 2D interaction emphasizes the active residues within Leptin's pocket.

H: (+)-Sophorol docked complex with Leptin depicts the formation of hydrogen bonds by side chain atoms of TRP 449 (indicated in green). The 2D interaction shows the active residues within Leptin's binding pocket.

I: Tylophorinidine docked complex with Leptin shows the formation of carbon-hydrogen bonds by the side chain atoms of GLY 440 amino acid (indicated in light green). The 2D interaction highlights the active residues within Leptin's binding pocket.

J:4'-Demethyldeoxypodophyllotoxin docked complex shows the formation of hydrogen bonds by the side chain atoms of GLU 437 and SER 533 (indicated in green). The 2D interaction emphasizes the active residues within the binding pocket.

CONCLUSION

A comprehensive phytochemical database of 5006 compounds was screened, leading to the identification of the top 10 leptin inhibitors, including Peonidin, Diosmin, 7-[3-(3-hydroxy oct-1-enyl)-4,6-dioxabicyclo[3.1.1]heptan-2-yl]hept-5-exonic acid, Carnitine, Dihydrodaidzin, 4-dimethylpodophyllotoxin, 4-demethyldeoxypodophyllotoxin, (+)-Sophorol, DL-alpha-Tocopherol, and Tylophorinidine. These compounds demonstrated the highest binding affinity and met the criteria for drug-like properties. Diosmin and Dihydrodaidzin exhibited the strongest binding affinity, making them promising candidates for drug development. Further, *in-vitro* and *in-vivo* studies are necessary to evaluate their potential side effects and benefits for human use.

Ethical permission: This paper required no ethical permission because it is based on *in silico* studies.
Conflict of Interest: No conflicts of interest, as stated by authors.
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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

Rauf A: Writing original draft, methodology and formal Analysis
Qazi S: Formal Analysis
Malik BA: Methodology
Aslam S: Writing original draft
Anwar U: Writing original draft
Ashfaq UA: Conceptualization, supervision and validation
Bhatti R: Conceptualization and validation

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