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Molecular docking studies of various bioactive molecules from *Withania somnifera* against bronchial asthma

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ABSTRACT

Asthma is a complex respiratory disorder characterized by chronic inflammation and hyperresponsiveness of the bronchial airways. Despite the availability of effective treatments and diagnostic methods, some patients remain symptomatic and exhibit a poor response to conventional asthma therapies. Herbal drugs are considered safe and effective alternative approaches that may target multiple pathogenic pathways involved in asthma. *Withania somnifera* (WS) is a well-known medicinal plant containing several bioactive compounds and has been extensively studied for its anti-inflammatory and immunomodulatory properties.

The present study aimed to explore the potential mechanisms of WS bioactive molecules in the treatment of asthma using an in silico screening approach.

Six active phytoconstituents (ligands) of WS were docked against two target receptor proteins, namely the glucocorticoid receptor and the β_2-adrenergic receptor, to predict binding energies and binding patterns. The obtained results were compared with those of known ligands of the glucocorticoid and β_2-adrenergic receptors (fluticasone and salbutamol, respectively).

Among all docked ligands, withaferin A, withanolide D, and withanolide B exhibited the highest binding affinities (lowest estimated free binding energy of -8.4 kcal/mol) toward the glucocorticoid receptor (GR). In contrast, the WS alkaloids tropine and anaferine showed lower affinities, as evidenced by higher binding energy values. Furthermore, withanolide D and withanolide B demonstrated the highest affinity for the β_2-adrenergic receptor among the screened compounds, whereas salbutamol and the plant alkaloids tropine and anaferine exhibited lower affinity toward the β_2-adrenergic receptor compared with the plant withanolides.

WS bioactive compounds, particularly withanolides, are predicted to possess multitarget anti-inflammatory and bronchodilator potential. Further experimental validation is warranted.

INTRODUCTION

Asthma is a complex respiratory disorder characterized by chronic inflammation and hyperresponsiveness of the bronchial airways. Common clinical symptoms include cough, wheezing, chest tightness, and dyspnea, which are usually reversible with appropriate asthma therapy. The disease represents a significant global health burden, with more than 300 million people affected worldwide [1,2]. Despite the availability of effective treatments and diagnostic methods, a substantial proportion of patients remain symptomatic and exhibit a poor response to conventional asthma therapies.

Current pharmacological approaches to asthma management include combination therapy with inhaled corticosteroids (ICSs), anticholinergic drugs, and bronchodilators such as β_2-agonists [3]. In humans, glucocorticoids regulate a broad range of physiological functions essential for life

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and play a crucial role in maintaining basal and stress-related homeostasis [4]. Corticosteroids diffuse across the cell membrane and activate glucocorticoid receptors (GRs) in the cytoplasm. Activated GRs subsequently translocate to the nucleus, where they suppress the expression of multiple inflammatory genes involved in inflammatory pathways [5]. In addition to their anti-inflammatory effects, corticosteroids increase pulmonary β-receptor expression and responsiveness [6]. However, the risk of local and systemic adverse effects limits their long-term use and remains a major clinical challenge [7].

Because of corticosteroid resistance, adverse effects, and the complex cellular and immunological interactions involved in asthma pathophysiology, there is an unmet need to develop safer and more effective therapeutic agents. In recent years, increasing attention has been directed toward traditional medicine owing to its perceived safety, affordability, and widespread acceptance. Herbal drugs are considered promising alternative approaches, as they may target multiple pathogenic pathways involved in asthma and associated airway remodeling disorders [8].

Withania somnifera (WS) is a well-known medicinal plant used in Indian traditional systems of medicine, including Ayurveda and Unani. It possesses considerable therapeutic potential for the treatment of various diseases due to its anti-inflammatory, immunomodulatory, and antioxidant properties [9]. However, its anti-asthmatic effects have not yet been fully elucidated. Steroidal lactones such as glycowithanolides, along with flavonoids and alkaloids, constitute the major bioactive components of WS roots and are believed to contribute significantly to its pharmacological effects [10].

A better understanding of ligand-receptor binding modes has led to important advances in drug design and the discovery of novel therapeutic agents [11]. Molecular docking is a valuable computational chemistry tool used for virtual screening and the analysis of protein-ligand interactions. Therefore, the present study aimed to investigate the potential mechanisms of WS bioactive molecules in the treatment of asthma using an *in silico* molecular docking approach.

MATERIALS AND METHODS

Molecular Docking Study

Molecular docking was performed using AutoDock Vina (version 1.2.3). The crystal structures of the glucocorticoid receptor (GR; PDB ID: 2V95) and the β_2-adrenergic receptor (PDB ID: 2R4R) were retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). The three-dimensional (3D) structures of the selected ligands-withaferin A (PubChem ID: 265237), withanolide D (PubChem ID: 161671), withanolide B (PubChem ID: 14236711), tropine (PubChem ID: 449293), anaferine (PubChem ID: 443143), withasomnine (PubChem ID: 442877) – as well as the reference drugs fluticasone (PubChem ID: 444036) and salbutamol (PubChem ID: 2083), were downloaded from the PubChem database in SDF format.

The SDF files were converted to PDB format using Open Babel GUI (version 2.3.1) and subsequently subjected to molecular docking against the target receptors (2V95 and 2R4R). Protein preparation was carried out using AutoDock Tools [11] by removing heteroatoms and co-crystallized water molecules, followed by the addition of polar hydrogens and Kollman charges. Blind docking was performed by defining the grid box to encompass the entire protein structure, with the exhaustiveness parameter set to 16.

Protein-ligand binding interactions were visualized using BIOVIA Discovery Studio Visualizer 2021 software [12].

RESULTS

In this study, the anti-inflammatory, immunomodulatory, and bronchodilator potential of WS bioactive molecules (four withanolides and two alkaloids) was evaluated and compared with standard ligands (fluticasone and salbutamol) of the glucocorticoid and β_2-adrenergic receptors. Molecular docking was performed using the AutoDock Vina program to assess protein-ligand binding affinity (binding energy) and binding modes. The *in silico* results, including binding energies and amino acid residues involved in hydrogen bond formation with the screened ligands (WS phytochemicals and standard drugs), are summarized in Tables 1 and 2.

Table 1. Protein-ligand binding affinities (kcal/mol), binding pocket residues, and H-bonds of WS bioactive molecules and fluticasone against glucocorticoid receptor

Ligand	Dock score (kcal/mole)	Binding pocket residues	No. of H-bonds
Withaferin-A	-8.4	PHE49, ARG26, GLU287	3
Withanolide-D	-8.4	ASP160, THR270, ASP278, LYS34	3
Withanolide-B	-8.4	SER161	1
Tropine	-5.0	LEU30, THR36, LYS34	2
Anaferine	-5.4	VAL285, LEU23, ASN22	1
Withasomnine	-6.6	MET243, GLN240, LYS350	0
Fluticasone	-7.1	GLU287, VAL285, ASN22, ARG26	1

Table 2. Protein-ligand binding affinities, binding pocket residues, and H-bonds of WS bioactive molecules and salbutamol against the β_2-adrenergic receptor

Ligand	Dock score (kcal/mole)	Binding interaction with amino acids	No. of H-bonds
Withaferin-A	-8.6	GLY320, PRO323, LEU324	1
Withanolide-D	-9.2	VAL126, VAL210, SER161, MET165	1
Withanolide-B	-9.1	PRO323	0
Tropine	-5.3	VAL160, PRO211, SER207	1
Anaferine	-5.8	PRO330, ILE72	0
Withasomnine	-6.6	GLU122, VAL160, TRP158, VAL157	0
Salbutamol	-5.8	THR164, VAL166, GLU122, THR118	2

The results indicated that all selected WS constituents exhibited appreciable binding affinity toward both the glucocorticoid and β_2-adrenergic receptors. Binding energies of the ligands interacting with the glucocorticoid receptor ranged from -8.4 to -5.0 kcal/mol. Among all docked ligands, three withanolides-withaferin A, withanolide D, and withanolide B – showed the highest binding affinities, with the lowest estimated free binding energy (-8.4 kcal/mol), exceeding that of the standard drug fluticasone (-7.1 kcal/mol). In contrast, the WS alkaloids tropine and

anaferine exhibited lower affinities, as indicated by higher binding energy values (-5.0 and -5.4 kcal/mol, respectively), compared with WS withanolides and fluticasone.

Similarly, the plant withanolides demonstrated strong affinity for the β2-adrenergic receptor. Withanolide D and withanolide B were the highest-affinity β2-adrenergic receptor ligands among the compounds screened, with binding energies of -9.2 and -9.1 kcal/mol, respectively. In comparison, the standard drug salbutamol showed a lower binding affinity, with a binding energy of -5.8 kcal/mol. Figures 1-14 illustrate the binding modes, number of hydrogen bonds, and key interacting amino acid residues of the receptors with the respective ligands.

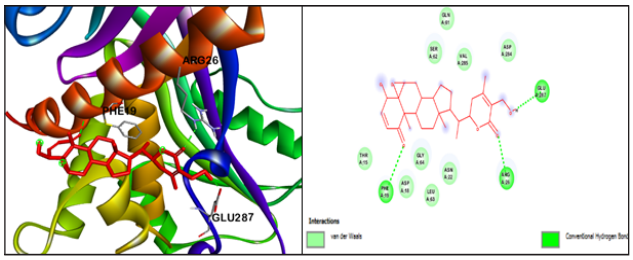

Figure 1. 3D and 2D docking interaction of Withaferin A with protein interacting residues of glucocorticoid receptor

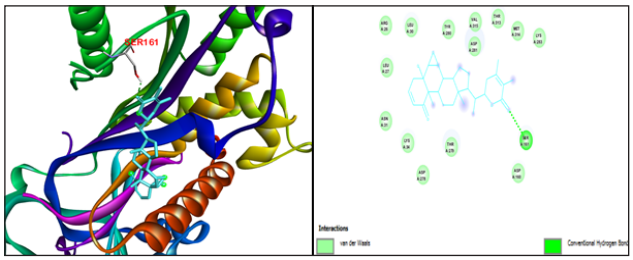

Figure 2. 3D and 2D docking interaction of Withanolide B with protein interacting residues of glucocorticoid receptor

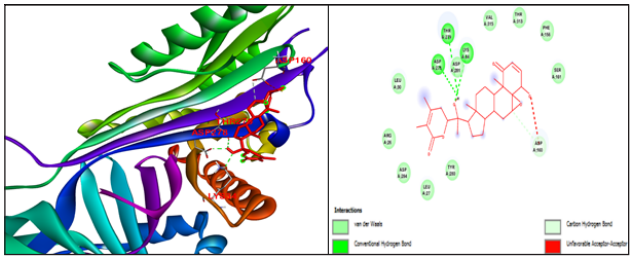

Figure 3. 3D and 2D docking interaction of Withanoide D with protein interacting residues of glucocorticoid receptor

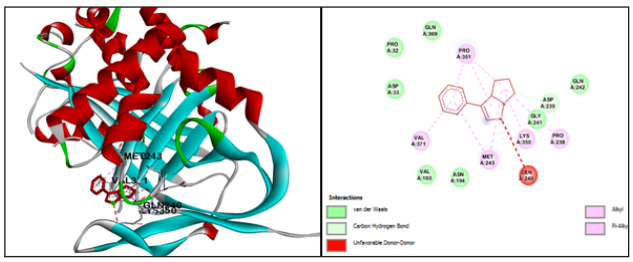

Figure 4. 3D and 2D docking interaction of Withasomnine with protein interacting residues of glucocorticoid receptor

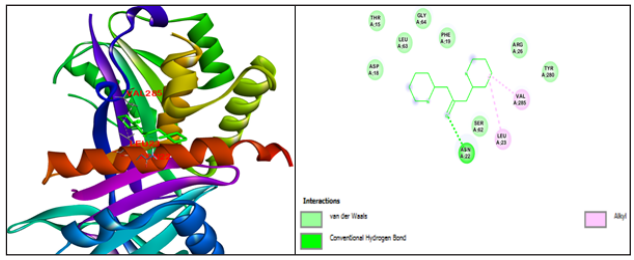

Figure 5. 3D and 2D docking interaction of Anaferine with protein interacting residues of glucocorticoid receptor

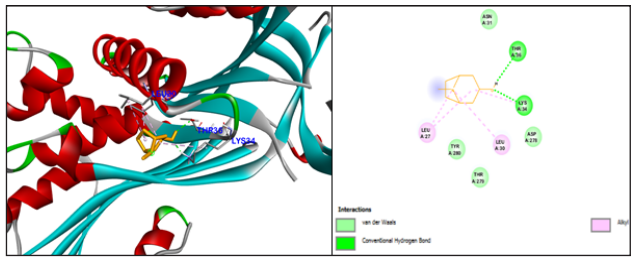

Figure 6. 3D and 2D docking interaction of Tropine with protein interacting residues of glucocorticoid receptor

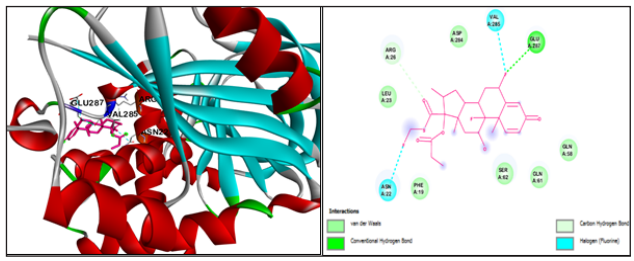

Figure 7. 3D and 2D docking interaction of Fluticasone with protein interacting residues of glucocorticoid receptor

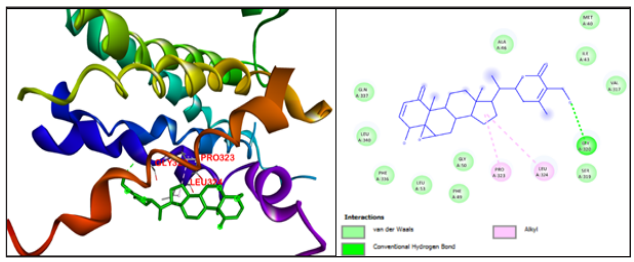

Figure 8. 3D and 2D docking interaction of Withaferin A with protein interacting residues of β2 receptors

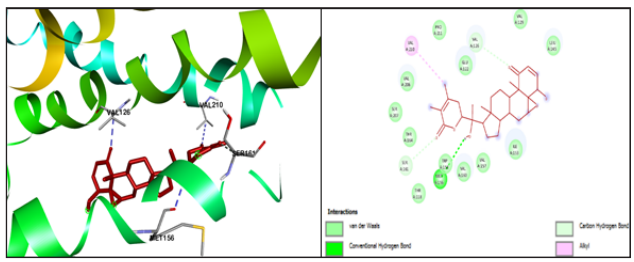

Figure 9. 3D and 2D docking interaction of Withanolide D with protein interacting residues of β2 receptors

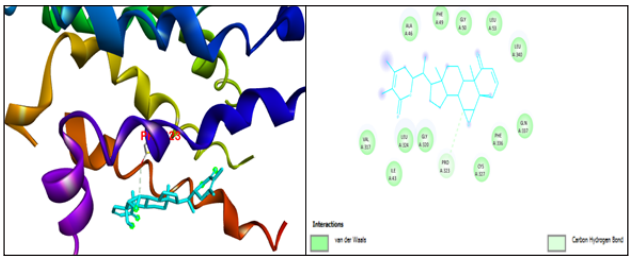

Figure 10. 3D and 2D docking interaction of Withanolide B with protein interacting residues of β2 receptors

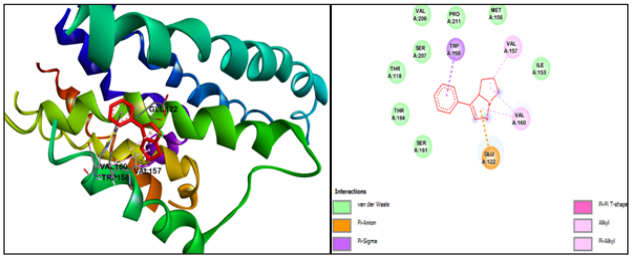

Figure 11. 3D and 2D docking interaction of Withasomnine with protein interacting residues of β2 receptors

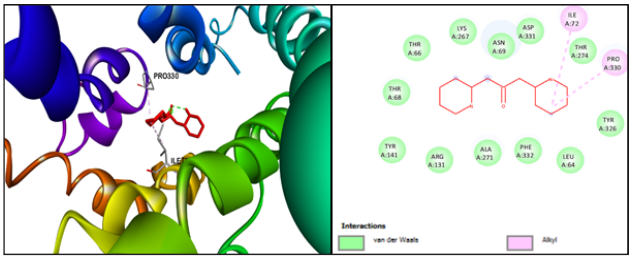

Figure 12. 3D and 2D docking interaction of Anaferine with protein interacting residues of β2 receptors

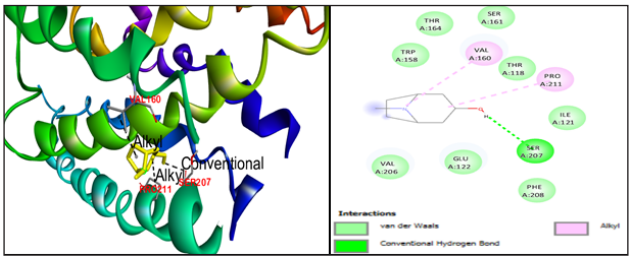

Figure 13. 3D and 2D docking interaction of Tropine with protein interacting residues of β2 receptors

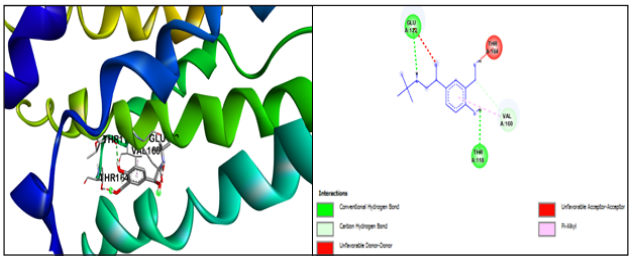

Figure 14. 3D and 2D docking interaction of Salbutamol with protein interacting residues of β2 receptors

DISCUSSION

Current asthma therapies are primarily aimed at controlling airway inflammation, with limited effects on airway remodeling. Moreover, adverse effects and poor therapeutic responses to conventional allopathic drugs complicate long-term asthma management. Consequently, interest in medicinal plants as complementary or alternative therapeutic strategies has increased worldwide in recent years. Molecular docking is a widely used computational tool for the virtual screening of large compound libraries and has facilitated the identification of novel bioactive lead molecules. Numerous studies have reported the successful application of molecular docking and virtual screening in the discovery of ligands targeting various receptors, including G protein-coupled receptors and glucocorticoid receptors (GRs) [13,14].

Withania somnifera (WS) is a well-established medicinal plant containing multiple bioactive compounds and has been extensively investigated for its anti-inflammatory and adaptogenic properties in both *in vitro* and *in vivo* studies [15,16]. Several molecular docking studies have explored the binding affinity of WS bioactive constituents toward the glucocorticoid receptor [17,18]. To the best of our knowledge, the present study is the first *in silico* investigation to simultaneously evaluate the anti-inflammatory and bronchodilator potential of WS-derived ligands through comparative binding analysis against both the glucocorticoid and β_2-adrenergic receptors.

Molecular docking results are commonly interpreted based on ligand-receptor interactions, where lower binding energy reflects higher binding affinity and better fitness of the ligand toward the target protein. Considerable efforts have been directed toward the discovery of novel GR agonists that are structurally similar to glucocorticoids or capable of eliciting comparable therapeutic effects with fewer adverse reactions than currently available glucocorticoids [19]. Increasing evidence indicates that WS withanolides exert multitarget effects by modulating various inflammatory mediators, including myeloperoxidase (MPO) and interleukin-6 (IL-6) [20,21].

In the present study, WS withanolides exhibited strong binding affinity toward the glucocorticoid receptor, exceeding that of the reference drug fluticasone, thereby suggesting significant anti-inflammatory and immunomodulatory potential. In contrast, the WS alkaloids tropine and anaferine demonstrated lower affinity toward GR amino acid residues than both the withanolides and fluticasone, as evidenced by higher binding energy values and fewer hydrogen bond interactions. Detailed analysis of ligand-receptor interaction diagrams revealed that withanolide B formed a hydrogen bond with only one GR residue (Ser161) despite its low binding energy, whereas withanolide D and withaferin A interacted with multiple residues within the binding pocket. Notably, withaferin A interacted with key active-site residues Glu287 and Arg26, similar to fluticasone, and formed three hydrogen bonds – more than those formed by fluticasone – indicating a highly favorable binding mode among the screened ligands.

Although the well-established β_2-adrenergic receptor agonist salbutamol formed a greater number of hydrogen bonds and interacted with multiple active-site residues, WS withanolides demonstrated higher binding affinity, as reflected by lower binding energy values. In comparison, the plant alkaloids tropine and anaferine showed weaker binding toward the β_2-adrenergic receptor. Collectively,

these findings suggest that WS bioactive compounds possess dual anti-inflammatory and bronchodilator potential and may serve as promising complementary or alternative therapeutic agents for the management of asthma and other inflammatory airway disorders.

CONCLUSION

The present study investigated the anti-inflammatory and bronchodilator potential of WS bioactive constituents using a molecular docking approach against the glucocorticoid and β_2-adrenergic receptors. Six bioactive compounds were screened to evaluate their binding affinity and interaction patterns with these target receptors, and the results were compared with those of established ligands, fluticasone and salbutamol. Analysis of protein-ligand interactions demonstrated that WS withanolides exhibited superior docking performance, characterized by lower free binding energy and higher binding affinity toward both the glucocorticoid and β_2-adrenergic receptors, compared with WS alkaloids and the reference drugs.

Overall, these findings suggest that WS bioactive compounds may possess multitarget activity with considerable therapeutic potential for the management of bronchial asthma. However, further *in vitro* and *in vivo* studies are warranted to validate these *in silico* observations and to elucidate their clinical relevance.

FUTURE PERSPECTIVES

Further experimental and clinical studies are therefore warranted to facilitate the development of WS-derived bioactive compounds with improved potency and selectivity for asthma pharmacotherapy.

AUTHORSHIP CONTRIBUTION STATEMENT

N.H.A. and K.H.S. contributed equally to the methodology, data curation, and writing of the original draft. S.R. and M.N. were responsible for reviewing and editing the manuscript. A.R. and K.G. supervised the study and critically reviewed the manuscript. All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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