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Exploration of Mechanism of *Withania somnifera* (L.) Dunal in the Treatment of Huntington disease: A Network Pharmacology Approaches Integrated with Molecular docking and dynamics

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ABSTRACT

Withania somnifera (L.) Dunal (Ashwagandha) is a traditional medicine that has several health-promoting and therapeutic benefits, including neuroprotective, sedative, and adaptogenic effects. The objective of current study is to investigate the mechanism of action of *W. somnifera* in the management of Huntington's disease by combining network pharmacology techniques with molecular docking and dynamics. The literature was searched to identify the main phytoconstituents of *W. somnifera*. The Swiss Target perdition database and SEA database were used to identify the targets of various phytoconstituents of *W. somnifera*, whereas targets associated with Huntington's disease were identified using GeneCards and DisGeNet database. A Venn diagram was used to identify overlapping targets and interaction among targets was checked using the STRING database. Cytoscape 3.10.1 was used to construct and analyse the network. The enrichment studies of the Kyoto Encyclopaedia of Genes and Genomes and gene ontology pathways were also performed. The molecular docking and molecular dynamic studies were performed using Schrodinger software. A total of 948 targets were identified which can be hit by *W. somnifera* and 513 targets were identified in Huntington's disease. A total of 111 targets were identified. Network Pharmacology results have shown that the phytoconstituents of *W. somnifera* can be useful in Huntington disease through the modulation of inflammatory and apoptotic signalling pathways. The selected phytoconstituents of *W. somnifera* have also shown favoured interactions in the active site of targets involved in inflammation and apoptosis as indicated by molecular docking and dynamics results. Overall, it can be concluded that *W. somnifera* plays an important role in Huntington disease through the modulation of inflammatory and apoptogenic signalling pathways.

Keywords: Huntington's disease, Network analysis, Molecular docking, Molecular dynamics, *Withania somnifera* (L.) Dunal.

INTRODUCTION

The therapeutic properties of *Withania somnifera* (L.) Dunal, often known as Indian ginseng (Ashwagandha in Sanskrit), or Indian winter cherry, have drawn a lot of attention in recent years.[1-3] Over thousands of years, Indian medical systems have utilised it extensively as a nerve tonic, adaptogen, memory enhancer, anti-stress, and to treat gout, rheumatoid arthritis, infertility, infectious infections, sleeplessness, anxiety, and cognitive deficiencies. The primary medical systems that use its compositions are Ayurvedic and Unani.[4,5] A wonderful herbal Rasayana that has been utilised for generations to treat neurological disorders is called "Sattvic Kapha Rasayana."[6] Ashwagandha's potential health benefits have drawn more attention in recent years, especially in relation to stress management, cognitive function, and physical performance. Supplementing with ashwagandha may have neuroprotective effects, assist treat obsessive-compulsive disorder, and have anti-inflammatory, immunomodulatory, anxiolytic, and anti-convulsive properties, according to a number of studies.[7-10]

Studies have shown that because of the brain's high oxygen consumption and the high amount of polyunsaturated fatty acids in their membranes, neuronal cells are susceptible to oxidative damage.[11] By controlling various antioxidant enzymes, amyloid beta clearance, calcium influx, neurite outgrowth, lipid peroxidation, inflammation, and other debilitating processes implicated in Alzheimer's, Parkinson's, Amyotrophic lateral sclerosis (ALS), and Huntington's disease [HD], W. somnifera extract and its active constituents have been shown to have anti-oxidant qualities and protect neuronal cells from toxins.[12,13]

The degeneration of neurones in the basal ganglia causes HD, a fatal neurodegenerative illness that is incurable. Present-day drugs simply treat the symptoms and slow the disease's progression.[14,15] According to statistics, half of the children will inherit the allele that causes the disease because it is inherited in an autosomal dominant fashion. Huntingtin undergoes a conformational change into its insoluble form when the IT15 gene, which codes for the huntingtin (htt) protein on chromosome 4, is mutated. Rapid neuronal death results from the accumulation of the mutant huntingtin protein's N-terminal region, which contains enlarged polyglutamine repeats. Acetylcholine, serotonin, GABA, and dopamine become unbalanced as a result. It is widely acknowledged that the GABAergic system plays a part in the pathophysiology of HD and that *W. somnifera* operates via this system.[16]

Though its potential health benefits are encouraging, further study is required to completely understand ashwagandha's mechanisms of action and assess how well it treats Huntington disease. Therefore, employing network pharmacological techniques in combination with molecular docking and dynamics studies, we have investigated the potential mechanism of *W. somnifera* in the treatment of Huntington disease in the current study.

MATERIALS AND METHODS

Exploring potential targets and active ingredients

The Web of Science and PubMed databases were used to find the main phytoconstituents of W. somnifera. To get the CAS number, each of the gathered chemical components was separately put into the PubChem database.[17] Some of the substances were included even though they did not meet the aforementioned requirements because a literature review verified their good pharmacological qualities.[18] The appropriate target genes were then obtained by entering the canonical SMILES of the gathered active components into the SwissTargetPrediction database.[19] A probability higher than 0.1 served as the threshold for target screening. The targets were extracted in the MS Excel file.

Identifying targets with different expression

The genecard database provided the microarray platform and the expression profile data (relevance score>20).[20] In the search box of the gene card, Huntington disease was added and search for the targets involved in HD. Further, DisGeNet database was also used to search the targets involved in HD after creating account in

the database. The targets from Gene card and DisGeNet were extracted in the excel file. The duplicates were detected and excluded using MS Excel. The common targets between compound targets and targets involved in HD were identified using VennDiagram (both targets were added in the separate columns).[21]

Building networks of protein-protein interactions (ppi) and identifying important genes

The interaction among common targets obtained from VennDiagram was checked using STRING database with Default settings.[22] After that, Cytoscape 3.10.1 was used to import the PPI network from the STRING database to investigate the crucial genes. Cytoscape plugins to guarantee the accuracy of the results.[23]

Constructing the component-target-pathway network

A network of connections between active components, common targets for Huntington disease and *W. somnifera*, and pathways were constructed using Cytoscape. "Nodes" stand for components, targets, and pathways in the component-target-pathway network, while "edges" show the connections between them. The number of connections between nodes in the network is represented by the "degree" parameter, which is used to evaluate the key elements and objectives.[24]

Molecular docking

The RCSB PDB database provided the target protein's three-dimensional (3D) structure, while the PubChem database provided the molecular ligand's two-dimensional structure.[25] The target was selected based on literature studies and the X-ray crystallographic structure of MAPK1 PDB ID- 4QTA, NOS2 PDB ID-3E7G, PDE10A PDB ID-4LM3, MAP2K1 PDB ID-7B7R, BRAF PDB ID-8C7X, GSK3B PDB ID-7SXJ, AKT1, PDB ID- 4GV1, PTGS2 PDB ID-5F19, HMGCR PDB ID-2R4F were retrieved from the protein data bank.

Ligand and protein preparation

After the extraction of phytoconstituents and protein, they underwent additional minimization and preparation steps to ensure their suitability for subsequent molecular modelling studies. "Ligprep" module was used to minimize and prepare the sdf formatted ligands. To confirm the cleanliness and appropriateness of the target crystal structure for further analysis, co-crystallized solvents and ions were removed from the original structure. Chain A was selected and chosen whereas chain B was removed from the crystal structure of targets before proceeding the protein preparation. Therefore, protein structure underwent preparation through a series of steps, including the addition of hydrogen atoms, adjustment of bond orders, elimination of water molecules, substitution of missing atoms, and incorporation of side chains followed by energy minimization using the "Protein Preparation wizard" module of Schrodinger suite. Following this, the structure of both the phytoconstituents and protein was ready for further investigations.

Receptor grid

After optimizing and preparing the protein, a critical procedure ensued, wherein a receptor grid was generated to delineate the precise space for the subsequent docking of the ligand. The generation of the grid involved the selection of the co-crystallized ligand, which served to illuminate the active site of the protein, pinpointed by the coordinates (X= -6.86, Y= -21.08, Z= 7.33). The Site Map module was used to create grid in targets where internal ligand was not available.

Molecular docking study

The investigation involved a docking study using the standard precision (SP) parameters aiming to identify the energetically preferred binding conformation of the phytoconstituents within the active site of targets. The docking study utilized the Ligand Docking module from Schrödinger.

The doc score was computed by the Maestro module of Schrödinger. Lower the doc score, more energetically favoured binding conformation of ligand in the active site of target. Doc score less than -0.7 considered as cut off vale for further molecular dynamics studies.

Molecular dynamics (MD) simulation

As previously mentioned, molecular dynamics (MD) simulation was utilised to ascertain the docked complex's stability. DESMOND was used for the 100 ns MD simulation of the protein-ligand combination with the lowest MM-GBSA binding energy. The orthorhombic simulation box

was constructed using a TIP3P explicit water model and a system builder panel. The distance of 10 Å was maintained between the edge of the simulation box and the protein surface. To maintain a steady isosmotic salt environment, 150 mM NaCl was administered after the system had been neutralised. The system was minimised through 2000 iterations. Using the NPT (normal pressure and temperature) ensemble at 300 K and 1.01 bars, the minimised system was run through a 100 ns MD simulation using the default relaxation prior to simulation. The Nose-Hoover Chain thermostat and the Martyna-Tobias-Klein barostat were used to maintain the temperature and pressure, respectively. The energy and structure were recorded and saved in the trajectory file at 10-ps intervals during the simulation, which was run with a time step of 2 fs. Trajectories and three-dimensional structures were inspected using MAESTRO.

RESULTS

Selection of phytoconstituents

A total of 30 main phytoconstituents of *W. somnifera* were selected based on the literature review which have neuroprotective potential (Table 1).

Table 1: Phytoconstituents of *W. somnifera*

Sr. No	Phytoconstituents of *W. somnifera*	PubChem CID
1	2,4-methylene-cholesterol	157009865
2	2,3-Didehydro-somnifericin	70684083
3	Withanolide A	11294368
4	Solasodine	442985
5	Withasomnine	442877
6	Withasomniferolide B	155548693
7	Somnifericin	101687980
8	Withanolide D	161671
9	Withaferin A	265237
10	Withanone	21679027
11	Sominone	44249449
12	Withasomniferanolide A	155531810
13	Somniferawithanolide	102066416
14	Visosalactone B	57403080
15	Anaferine	443143
16	Choline	305
17	Beta-sitosterol	222284
18	Ashwagandhanolide	16099532
19	Tetracosanoic acid	11197
20	Alpha-Amyrin	73170
21	Withanolide D	161671
22	Withanolide B	14236711
23	Withanolide C	101559583
24	17beta-hydroxy withanolide k/withanolide f	44562998
25	4beta-Hydroxywithanolide E	73621
26	Linoleic acid	5280450
27	Oleic acid	445639
28	Aspartic acid	5960
29	Palmitic acid	985
30	Elaidic acid	637517

Network pharmacology

Targets of selected phytoconstituents

The Swiss Target Prediction database and SEA database identified 1059 targets of W. somnifera.

Target involved in Huntington's disease

624 targets associated with Huntington's disease have been identified using GeneCards, (relevance score >20) and DisGeNet database.

Common targets between phytoconstituents and huntington's disease

Using the Venny 2.1.0 tool, we have identified 111 common targets between the phytoconstituents of W. somnifera and Huntington's disease. The common targets were compiled in Table 2 and shown in Figure 1.

Table 2: List of common targets between *W. somnifera* and Huntington's disease

Phytoconstituent	Target of each phytoconstituent	Common targets
2,4-Methylene cholesterol	PSEN2, NPC1, AR, EGFR, CSF1R, PPARG, ACHE, G6PD, ESR1, AGTR1, F11, CCR5, CRYAB, CD4, KCNH2, DRD2, HMGCR, SLC6A4	AR, HMGCR, ESR1, SLC6A4, G6PD, DRD2, ACHE, KCNH2, PPARG, AGTR1, CCR5, EGFR, CSF1R, PSEN2, F11, CD4, CRYAB, NPC1, PTGS2, TERT, NR3C1, GSK3B, LRRK2, GYS1, PDE10A, CASP3, PYGL, ABCB1, MAOB, BCL2, CASP8, CASP1, TBK1, MMP3, NOS2, MMP1, CTSD, F2, MAP3K5, NTRK1, GRIN2A, ELANE, MAPK1, KIT, CREBBP, CFTR, CCND1, MAP2K1, MTOR, ADA, ICAM1, CDK5, PDGFRB, IL1B, IL6, PSENEN, PSEN1, SIGMAR1, AKT1, MAOA, CCR2, INSR, FAAH, NOS3, APP, IL2, BRAF, MPO, NQO1, MIF, ACE, SIRT1, HMOX1, RAF1, CNR1, GLUL, BACE1, NPY2R, TNF, MME, PGK1, SLC6A3, HTT, SCN4A, ABAT, ADRB2, PTPN11, RBP4, CBS, GNAO1, TTR, NDUFA10, CHAT, NDUFS1, ASS1, NDUFS4, ND1, NDUFV1, MT- MT-ND2, MT-ND5, SPTLC1, TLR2, GRIK2, CPT2, YARS1, TH, MMP2, HIF1A, CYP2D6, SLC5A7, VCP
Withanolide D	LRRK2, TERT, GYS1, AR, PYGL, CASP3, GSK3B, NR3C1, PDE10A, HMGCR, PTGS2	
Withanolide A	MAOB, TERT, GYS1, AR, PYGL, CSF1R, ACHE, CASP8, MMP1, TBK1, CASP3, BCL2, NOS2, ABCB1, CCR5, MMP3, NR3C1, PDE10A, HMGCR, PTGS2, CASP1	
Withanolide B	MAP3K5, GRIN2A, LRRK2, AR, PYGL, F2, CTSD, MMP1, NTRK1, ABCB1, ELANE, GSK3B, MMP3, NR3C1, PDE10A, HMGCR, PTGS2	
Withanolide C	LRRK2, TERT, GYS1, AR, PYGL, CFTR, CSF1R, KIT, MTOR, CASP8, MMP1, NTRK1, CREBBP, CASP3, ABCB1, MMP3, MAPK1, KCNH2, MAP2K1, CCND1, HMGCR, CASP1	
Withanolide F	LRRK2, AR, PYGL, CASP8, MMP1, NTRK1, ADA, ICAM1, PDGFRB, ELANE, MMP3, CDK5, MAPK1, MAP2K1, PDE10A, DRD2, HMGCR, PTGS2, CASP1	
4beta-Hydroxywithanolide E	TERT, AR, IL1B, F2, CTSD, MMP1, NTRK1, AGTR1, ABCB1, GSK3B, MMP3, CDK5, MAPK1, NR3C1, KCNH2, MAP2K1, CCND1, HMGCR, PTGS2	
2,3-Didehydrosomnifericin	IL6, PSEN2, NPC1, PSEN1, LRRK2, TERT, AR, IL1B, CASP8, NTRK1, CREBBP, NOS2, ABCB1, GSK3B, NR3C1, CCND1, HMGCR, PTGS2, CASP1, PSENEN	
Solasodine	MAOA, FAAH, NPC1, APP, AR, NOS3, EGFR, CSF1R, AKT1, F2, KIT, SIGMAR1, G6PD, ESR1, IL2, ABCB1, INSR, CRYAB, CCND1, PDE10A, CCR2, HMGCR	
Somnifericin	PSEN2, NPC1, PSEN1, TERT, AR, CTSD, BRAF, G6PD, MTOR, MMP1, NOS2, ABCB1, GSK3B, MMP3, CDK5, MAPK1, CCND1, HMGCR, PTGS2, PSENEN	
Withasomnine	SIRT1, MAOA, FAAH, MAOB, HMOX1, NQO1, PSEN2, PSEN1, LRRK2, ACE, AR, EGFR, MPO, F2, ACHE, NOS2, GSK3B, HMGCR, MIF, PSENEN	
Withaferin A	TERT, AR, CSF1R, KIT, BRAF, PDGFRB, GSK3B, MAPK1, RAF1, NR3C1, HMGCR, PTGS2	
Withanone	LRRK2, TERT, AR, PYGL, CSF1R, CTSD, KIT, BRAF, MMP1, CREBBP, PDGFRB, NOS2, ABCB1, INSR, GSK3B, MAPK1, NR3C1, PDE10A, HMGCR, PTGS2	
Sominone	NPC1, AR, IL1B, NOS3, PYGL, CSF1R, BRAF, G6PD, MTOR, ESR1, NOS2, ABCB1, GSK3B, CRYAB, PDE10A, HMGCR	
Withasomniferolide A	CNR1, PSEN2, APP, PSEN1, AR, PYGL, CSF1R, KIT, BRAF, SIGMAR1, G6PD, ICAM1, BCL2, MAPK1, NR3C1, KCNH2, PDE10A, DRD2, HMGCR, PSENEN	
Withasomniferolide B	GLUL, NPY2R, PSEN2, APP, PSEN1, AR, PYGL, CASP8, MMP1, NTRK1, BACE1, GTR1, CASP3, BCL2, CCR5, ELANE, MMP3, MAPK1, NR3C1, KCNH2, PDE10A, HMGCR, CASP1, PSENEN	
Somniferawithanolide	PSEN2, PSEN1, TNF, AR, PYGL, CFTR, CTSD, MMP1, NTRK1, ADA, CREBBP, CCR5, INSR, MAPK1, KCNH2, MAP2K1, PDE10A, PTGS2, PSENEN	
Viscosalactone B	MAOB, AR, PYGL, MME, CSF1R, BRAF, MTOR, NTRK1, PGK1, AGTR1, CDK5, MAPK1, CRYAB, MAP2K1, PDE10A, PTGS2	
(-)-Anaferine	HTT, ABAT, FAAH, MAOB, AR, SLC6A3, EGFR, AKT1, CHE, SIGMAR1, CCR5, MAPK1, KCNH2, PDE10A, DRD2, SCN4A, ADRB2	
Ashwagandhanolide	-	
Linoleic acid	IL6, CNR1, GLUL, FAAH, PSEN2, PSEN1, TERT, AR, CBS, MT-ND1, MT-ND5, PPARG, SIGMAR1, G6PD, MT-ND2, NDUFS4, PTPN11, ESR1, TLR2, ICAM1, SPTLC1, NOS2, MAPK1, NDUFV1, NR3C1, NDUFA10, GNAO1, DRD2, RBP4, HMGCR, PTGS2, NDUFS1, PSENEN	

Oleic acid	CNR1, FAAH, PSEN1, TERT, AR, SLC6A3, MT-ND1, PPARG, ACHE, SIGMAR1, G6PD, BACE1, PTPN11, ESR1, TLR2, SPTLC1, NOS2, NR3C1, GNAO1, HMGCR, PTGS2, SLC6A4
Aspartic acid	GRIK2, GLUL, YARS1, NOS3, CBS, TH, NOS2, CPT2, RAF1, ASS1
Palmitic acid	ABAT, FAAH, PSEN2, PSEN1, TERT, AR, MT-ND1, MME, PPARG, ACHE, G6PD, TLR2, ABCB1, CPT2, MMP2, MAPK1, GNAO1, RBP4, HMGCR, PTGS2, PSENEN
Alpha-Amyrin	CNR1, FAAH, TERT, AR, IL1B, PYGL, PPARG, ACHE, SIGMAR1, G6PD, BACE1, PTPN11, ESR1, NOS2, NR3C1, HIF1A, DRD2, HMGCR, PTGS2, SLC6A4
Beta-sitosterol	NPC1, TERT, TNF, AR, PYGL, SLC6A3, F2, PPARG, ACHE, SIGMAR1, G6PD, PTPN11, ESR1, NOS2, CRYAB, NR3C1, CD4, HIF1A, CYP2D6, DRD2, HMGCR, SLC6A4
Choline	TERT, ACE, TTR, MME, PPARG, CHAT, ACHE, CCR2, RBP4, SLC5A7, PTGS2, MIF
Tetracosanoic acid	GRIK2, FAAH, PSEN2, PSEN1, TERT, ACE, AR, G6PD, MMP1, ESR1, CPT2, MMP2, MMP3, HMGCR, PTGS2, PSENEN
Elaidic Acid	CNR1, FAAH, VCP, TERT, AR, SLC6A3, MT-ND1, PPARG, CTSD, ACHE, SIGMAR1, G6PD, BACE1, PTPN11, TLR2, SPTLC1, NOS2, MMP2, NR3C1, GNAO1, HMGCR, PTGS2, SLC6A4

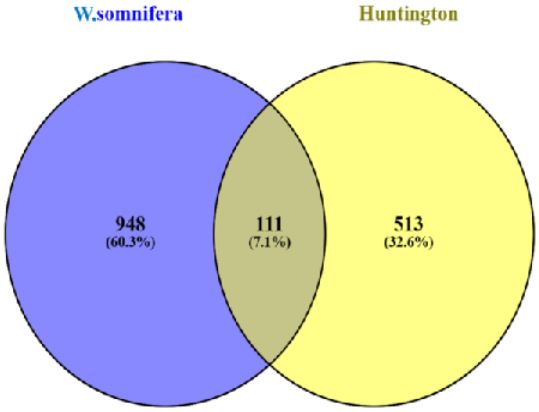

Fig. 1. Common targets between *W. somnifera* and Huntington's disease

PPI, network construction, and pathway analysis

The STRING database investigated the protein-protein interactions of 111 possible therapeutic targets of W. somnifera with Huntington disease and regulated 181 pathways. After a peer review of the literature, 16 pathways associated with Huntington disease were selected. Alzheimer's disease (hsa05010) scored the lowest false discover rate of 6.48E-25 by triggering 31 genes in Huntington disease. Likewise, the HIF-1 signaling pathway (hsa04066) scored a low false discover rate of 2.73E-13 by triggering 14 genes in Huntington disease. The neurotrophin signalling route, PIK3-AKT, MAPK, and NF-Kappa B signalling pathways had the lowest FDR and were thought to be involved in Huntington disease (Table 3).

Table 3: 16 KEGG pathways involved in Huntington disease ranked by FDR (p-value)

Term ID	Term description	Targets	Observed gene count
hsa05010	Alzheimer disease	MAPK1, NDUFA10, IL1B, APP, NDUFS4, MAP2K1, INSR, CASP3, BACE1, GSK3B, PSEN1, NOS2, GRIN2A, CASP8, MAP3K5, MTOR, MT-ND1, MT-ND5, MT-ND2, PSEN2, PTGS2IL6, NDUFS1, TNF, RAF1, MMEBRAF, CDK5, AKT1, PSENEN, NDUFV1	31
hsa04066	HIF-1 signaling pathway	MAPK1, HMOX1, CREBBP, EGFR, NOS3, MAP2K1, INSR, NOS2, MTOR, PGK1, BCL2, IL6HIF1A, AKT1	14
hsa04151	PI3K-Akt signaling pathway	MAPK1, IL2, CCND1, TLR2, PDGFRB, EGFR, CSF1R, KIT, NOS3, MAP2K1, INSR, GYS1, GSK3B, MTOR, BCL2, IL6, RAF1, NTRK1, AKT1	19
hsa04933	AGE-RAGE signaling pathway in diabetic complications	MAPK1, MMP2, CCND1, IL1B, ICAM1, NOS3, CASP3, BCL2, IL6, TNF, AGTR1, AKT1	12
hsa01521	EGFR tyrosine kinase inhibitor resistance	MAPK1, PDGFRB, EGFR, MAP2K1, GSK3B, MTOR, BCL2, IL6, RAF1, BRAF, AKT1	11
hsa04722	Neurotrophin signaling pathway	MAPK1, MAP2K1, GSK3B, PSEN1, MAP3K5, PSEN2, BCL2, RAF1, BRAF, NTRK1, AKT1, PTPN11	12
hsa04010	MAPK signaling pathway	MAPK1, PDGFRB, IL1B, EGFR, CSF1R, KIT, MAP2K1, INSR, CASP3, MAP3K5, TNF, RAF1, BRAF, NTRK1, AKT1	15

hsa04068	FoxO signaling pathway	SIRT1, MAPK1, CCND1, CREBBP, EGFR, MAP2K1, INSR,IL6, RAF1, BRAF, AKT1	11
hsa04630	JAK-STAT signaling pathway	IL2, CCND1, PDGFRB, CREBBP, EGFR, MTOR, BCL2, IL6, RAF1, AKT1, PTPN11	11
hsa04024	cAMP signaling pathway	CFTR, MAPK1, CREBBP, MAP2K1, ADRB2, GRIN2A, DRD2, PDE10A, RAF1, BRAF, AKT1	11
hsa04152	AMPK signaling pathway	CFTR, SIRT1, CCND1, PPARG, HMGCR, INSR, GYS1, MTOR, AKT1	9
hsa04150	mTOR signaling pathway	MAPK1, MAP2K1, INSR, GSK3B, MTOR, TNF, RAF1, BRAF, AKT1	9
hsa04022	cGMP-PKG signaling pathway	MAPK1, NOS3, MAP2K1, INSR, ADRB2, AGTR1, RAF1, AKT1	8
hsa04064	NF-kappa B signaling pathway	IL1B, ICAM1, PTGS2, BCL2, TNF	5
hsa05016	Huntington disease	NDUFA10, CREBBP, PPARG, NDUFS4, CASP3, HTT, CASP8, MAP3K5, MTOR, MT-ND1, MT-ND5, MT-ND2, NDUFS1, NDUFV1	14
hsa04728	Dopaminergic synapse	SLC6A3, GSK3B, GRIN2A, MAOA, DRD2, MAOB, TH, AKT1	8

The network between phytoconstituents -targets- pathways (Fig. 2) was constructed by treating edge count topological parameters using Cytoscape ver 3.10.1.

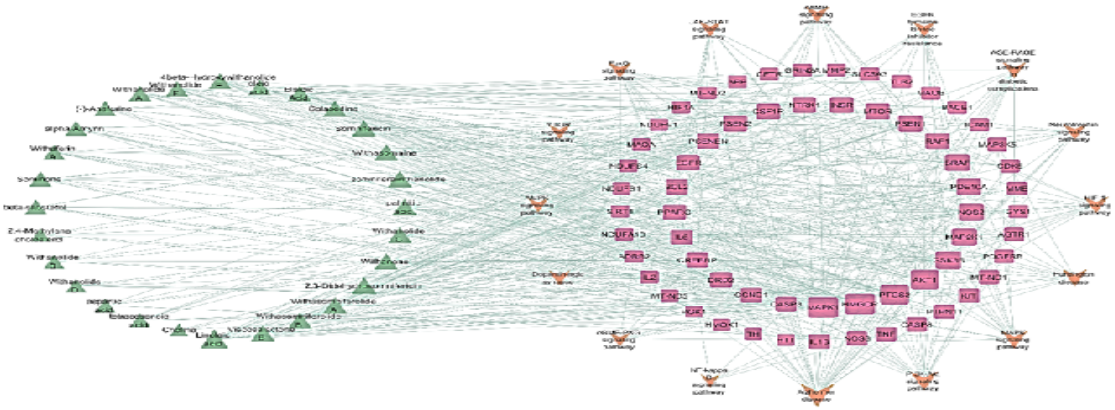

Fig. 2. *W. somnifera* bioactives' interactions with their targets and altered pathways. The green triangle represents the phytoconstituents, the pink square represents the targets, and the orang arrow represents the pathways

The top 10 genes implicated in pathways were determined by network analysis to be MAPK1, HMGCR, PTGS2, AKT1, MAP2K1, GSK3B, NOS2, BRAF, PDE10A, and RAF1 (Table 4).

Table 4: Network analysis of *W. somnifera*

Targets	Degree	Betweenness	Closeness
MAPK1	24.0	794.3	0.5
HMGCR	24.0	810.0	0.4861111
PTGS2	21.0	707.5	0.47297296
AKT1	20.0	774.0	0.4906542
MAP2K1	15.0	263.3	0.44491526
GSK3B	15.0	319.7	0.4375
NOS2	14.0	365.5	0.44491526
BRAF	13.0	164.5	0.42
PDE10A	13.0	216.4	0.40076336
RAF1	12.0	205.8	0.42

KEGG pathway analysis

The Metascape platform was used to conduct GO enrichment analysis on core targets. The best 16 significant items were then chosen for visual analysis on the LogP ($p<0.01$) value shown in bubble diagrams (Figure 3).

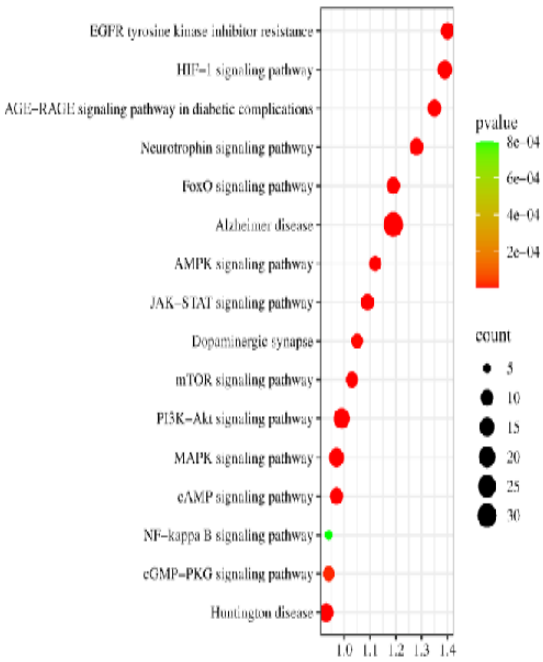

Fig. 3. GO enrichment analysis of GG target. Bubble plot representing top 16 pathways that were involved

The findings demonstrated that the biological processes involved were primarily neurotrophine signaling, Alzheimer disease, chemical synaptic transmission, synaptic signalling, and cellular responses to organonitrogen compounds. These results implied that W. somnifera mode of action for treating Huntington disease was the consequence of several molecular biological mechanisms working in concert.

Molecular docking findings

To validate the findings, Schrodinger was used to perform molecular docking on ten core target proteins with top GG compounds (Table 5). Molecular docking studies showed good docking score between ligands and phytoconstituents.

Table 5: Docking score of *W. somnifera*

Target name and PDB IDs	Phytoconstituents	Docking score (XP)	Numbers of Hydrogen bonds	Hydrogen bonds	Hydrophobic bondsscore
MAPK1 PDB ID-4QTA	Tetracosanoic acid	-7.5	2	MET-108 MET-108	LEU-156, ILE-84, LEU-107, MET-108, ALA-52, ILE-31, CYS-166, VAL-39, PHE-168, TYR-36, ALA-35, ILE-56, PRO-58, TYR-64
	4beta-Hydroxywithanolide E	-7.2	2	SER-153 ALA-35	ILE-31, LEU-156, MET-108, ILE-84, LEU-107, VAL-39, TYR-36, ALA-35, ALA-52, CYS-166
	Withanolide f	-6.8	2	SER-153 ALA-35	ILE-31, LEU-156, MET-108, ILE-84, LEU-107, VAL-39, TYR-36, ALA-35, ALA-52, CYS-166
	Somniferawithanolide	-6.6	3	GLU-33 TYR-36 ASN-154	TYR-113, LEU-107, MET-108, ILE-84, ALA-52, CYS-166, VAL-39, LEU-156, TYR-36, TYR-113
	Withasomnine	-6.6	1	MET-108	ILE-31, LEU-156, VAL-39, MET-108, LEU-107, ALA-52, ILE-84, CYS-166
	Somnifericin	-6.4	1	MET-108	ILE-31, LEU-156, TYR-113, MET-108, ILE-84, LEU-107, VAL-39, TYR-36, ALA-35, ALA-52, CYS-166
	2,3-Didehydro-somnifericin	-5.9	2	ASP-106 LYS-151	ILE-31, LEU-156, TYR-113, MET-108, ILE-84, LEU-107, VAL-39, TYR-36, ALA-35, ALA-52, CYS-166
NOS2 PDB ID-3E7G	Withanolide f	-7.2	3	GLU-377 TRP-463 ARG-199	ILE-201, CYS-200, ALA-197, PHE-369, TRP-194, TYR-489, MET-355, VAL-352, PRO-467, PRO-466, LEU-464, TRP-463, ILE-462, MET-374, MET-120
	Withasomniferolide B	-6.6	1	TRP-463	ILE-201, CYS-200, ALA-197, PHE-369, TRP-194, TYR-489, MET-355, VAL-352, PRO-467, LEU-464, TRP-463, ILE-462, MET-374, MET-120, TYR-491
	Visosalactone B	-6	4	ASN-354 ASN-354 ARG-199 ILE-201	ILE-201, CYS-200, MET-355, VAL-352, LEU-464, TRP-463, ALA-262, MET-374, MET-120, TYR-491
	Somniferawithanolide	-5.5	2	GLU-377 GLU-377	CYS-200, ALA-197, TRP-194, TYR-489, MET-355, VAL-352, TRP-463, TYR-373, TYR-491, PHE-369, PRO-350

PDE10A PDB ID-4LM3	2,3-Didehydro-somnifericin	-11.3	3	SER-677 SER-571 SER-573	LEU-675, VAL-678, TYR-524, ALA-689, ILE-692, PHE-729, TYR-693, TYR-730, LEU-635, VAL-733, PHE-696, MET-714, PHE-639, MET-713, ILE-711, TYR-574,
	Visosalactone B	-9.8	1	SER-677	LEU-675, VAL-678, TYR-524, ALA-689, ILE-692, PHE-729, TYR-693, LEU-635, VAL-733, PHE-696, MET-714, PHE-639, MET-713, ILE-711, ALA-636
	Beta-sitosterol	-7.7	1	SER-677	LEU-675, VAL-678, TYR-524, ALA-689, ILE-692, PHE-729, TYR-693, TYR-730, LEU-635, VAL-733, PHE-696, MET-714, PHE-639, MET-713,
	Withasomnine	-7.6	1	GLN-726	PHE-729, TYR-693, ILE-692, PHE-696, MET-713, LEU-635, TYR-524, VAL-678, ALA-689, LEU-675
	2,4-methylene-cholesterol	-7.2	2	ASN-572	MET-714, MET-713, ILE-711, ILE-692, TYR-693, PHE-696, PHE-729, VAL-733, ALA-636, LEU-635
				HIE-567	PHE-570, MET-591
	Somniferawithanolide	-6.9	3	TYR-524 HIP-525 HIE-567	TYR-524, LEU-675, ILE-692, PHE-729, PHE-696, MET-714, MET-713, ILE-711, LEU-635, ALA-636, MET-591
	4beta-Hydroxywithanolide E	-6.9	2	HIE-567	MET-714, MET-713, ILE-711, ILE-692, TYR-693, PHE-696, PHE-729, VAL-733, ALA-636, LEU-635
				ASN-572	PHE-570, MET-591
	Withanolide D	-6.8	3	HIP-525	MET-714, MET-713, ILE-711, ILE-692, TYR-693, PHE-696, PHE-729, VAL-733, ALA-636, LEU-635
				HIE-567	PHE-570, MET-591, PHE-639
	Withanolide A	-6.7	2	TYR-524 SER-573	TYR-524, LEU-675, ILE-692 ,PHE-729, LEU-635, PHE-696, ALA-363, PHE-639
	Somniferawithanolide	-6.4	3	TYR-524 HIP-525 HIE-567	MET-714, MET-713, ILE-711, ILE-692, PHE-696, PHE-729, ALA-636, LEU-635, LEU-675 PHE, MET-591,
MAP2K1 PDB ID-7B7R	Withanolide A	-7.2	5	LYS-156 GLN-153 ASP-152 SER-150 SER-194	MET-146, MET-143, LEU-197, VAL-127, LEU-74, ALA-95, ALA-76, VAL-82, CYS-207
	Tetracosanoic acid	-6.5	2	LEU-74 SER-150	ILE-99, MET-219, LEU-215, VAL-211, PHE-209, LEU-115, CYS-207, LEU-118, PHE-129, VAL-127, ILE-126, MET-143, ILE-141, ALA-76, LEU-74, LEU-197
	Withanolide C	-6	2	SER-194 SER-150	ALA-76, LEU-74, ALA-95, MET-143, MET-146, CYS-207, LEU-197, VAL-82

	Anaferine	-5.9	2	SER-150 SER-194	ALA-76, VAL-81, VAL-82, LEU-74, ALA-95, MET-146, MET-143, LEU-197
	Beta-sitosterol	-5.9	1		TYR-229, ALA-76, LEU-74, VAL-82, LEU-197, CYS-207, VAL-127, MET-146, MET-143, ALA-95
	Withanolide B	-5.9	4	LYS-156 GLN-153 ASP-152 SER-150	MET-146, VAL-82, MET-143, ALA-95, LEU-74, LEU-197, CYS-207, ALA-76
BRAF PDB ID-8C7X	Sominone	-8.124	1	GLU-501	LEU-505, ILE-527, VAL-528, TRP-531, CYS-532, ILE-463, PHE-583, ALA-481, VAL-482, PHE-595, LEU-514, VAL-471
	Somnifericin	-7.76	3	SER-465 THR-526 ASN-580	ILE-463, PHE-595, ALA-481, PHE-468, VAL-471, TRP-531, CYS-532, LEU-514, PHE-583
	2,3-Didehydro-somnifericin	-7.757	3	SER-465 THR-526 ASN-580	ILE-463, PHE-595, ALA-481, PHE-468, VAL-471, TRP-531, CYS-532, LEU-514, PHE-583
	Withasomnine	-7.615	1	CYS-532	ALA-481, LEU-514, PHE-595, TRP-531, CYS-532, PHE-583, ILE-463, VAL-471
	Withanolide B	-7.391	2	ASN-580 CYS-532	PHE-468, PHE-595, ILE-463, VAL-471, ALA-481, LEU-514, TRP-531, CYS-532, PHE-583
	Withaferin A	-7.111	2	THR-529, ASN-580	PHE-595, ALA-481, LEU-514, PHE-583, TRP-531, CYS-532, VAL-471, PHE-468, ILE-463
	Beta-sitosterol	-6.459	1	GLU-501	PHE-595, LEU-505, ILE-527, ALA-481, LEU-514, TRP-531, CYS-532, PHE-583, VAL-471
	Visosalactone B	-6.209	2	THR-529 ASN-580	PHE-468, PHE-595, VAL-471, ALA-481, TRP-531, CYS-532, LEU-514, PHE-583, ILE-463
	Linoleic acid	-6.008	1	ASN-580	PHE-595, PHE-583, LEU-505, LEU-514, ILE-527, VAL-471, ALA-481, TRP-531, CYS-532
GSK3B PDB ID-7SXJ	Somnifericin	-6.1	3	PHE-67 PHE-67 LYS-85	PHE-67, VAL-70, ALA-83, ILE-62, LEU-132, TYR-134, VAL-135, LEU-188, CYS-199
	Withasomnine	-5.9	1	VAL-135	ILE-62, PRO-136, VAL-135, TYR-134, LEU-132, CYS-199, VAL-70, LEU-188, ALA-83, VAL-110
	Withanolide C	-5.9	1	ARG-141	TYR-140, ILE-62, PRO-136, TYR-71, VAL-70, VAL-135, TYR-134, VAL-110, LEU-132, LEU-188, CYS-199, ALA-83
AKT1 PDB ID- 4GV1	2,3-Didehydro-somnifericin	-6.2	4	GLU-228 ASN-279 LYS-276 LYS-276	VAL-164, PHE-161, LEU-156, PHE-438, ALA-230, TYR-229, MET-227, ALA-177, MET-281,
	Withaferin A	-6.1	3	THR-160 LYS-276 GLU-228	LEU-156, PHE-161, VAL-164, MET-281, PHE-438, ALA-230, TYR-229, MET-227, ALA-177

	Somniferawithanolide	-5.5	1	ASN-279	VAL-164, PHE-161, LEU-156, PHE-438, ALA-230, TYR-229, MET-227, ALA-177, MET-281
PTGS2 PDB ID-5F19	Tetracosanoic acid	-6.9	3	ASN-382 PHE-210 THR-212	PHE-395, LEU-294, VAL-295, ILE-408, PHE-407, TYR-404, VAL-444, VAL-447, ALA-199, LEU-391, PHE-200, LEU-390, ALA-202, TRP-387, TYR-385, PHE-210, TYR-148
	Sominone	-6.3	4	THR-212 THR-212 ASN-382 ALA-443	ALA-443, VAL-444, VAL-447, TYR-404, ILE-408, VAL-295, LEU-294, TYR-148, PHE-210
	Somniferawithanolide	-6.3	2	GLN-203 GLN-289	ILE-274, VAL-447, VAL-444, ILE-408, TYR-404, LEU-391, VAL-295, LEU-294, VAL-291
	Aspartic acid	-5.9	6	HIS-386 GLN-454 HIP-214 ASN-382 THR-212 PHE-210	VAL-447, PHE-210, TYR-148
HMGCR PDB ID-2R4F	2,3-Didehydro-somnifericin	-4.5	1	GLY-808	MET-657, MET-655, ALA-768, CYS-526, ALA-525

Molecular simulation findings

Molecular dynamics parameters (RMSD, RMSF, Target-ligand contacts histogram, PL-Contacts, Ligand-target contacts and Target-ligand contacts) have shown the stability of selected phytoconstituents of *W. somnifera* in the active site of the targets.

DISCUSSION

The coding region of the Huntington's disease gene, which is found on the short arm of chromosome 4, repeats a cytosine-adenine-guanine (CAG) trinucleotide, which causes Huntington disease, an autosomal dominant neurological illness. When the number of CAG repeats rises to more than 35, HTT is more likely to misfold and form insoluble aggregates in the cytoplasm and nucleus of neurones. The accumulated aggregates cause apoptosis and cell malfunction, which ultimately results in the affected brain regions atrophying severely. The FDA has only approved tetrabenazine (TBZ) as a treatment for chorea in Huntington disease. The central nervous system's vesicular monoamine transporter 2 (VMAT-2) is reversibly inhibited by TBZ.[26] Therefore, it is anticipated that the clinical use of ashwagandha and its ingredients will benefit neurodegenerative illnesses in light of their anti-degenerative properties. Ashwagandha and its components are safe by several groups in the past. The behavioural, biochemical, and enzymatic alterations brought on by 3-NP were ameliorated by the long-term administration of *W. somnifera* root extracts. According to biochemical analysis, systemic 3-NP administration markedly raised levels of lipid peroxidation, nitrite, and lactate dehydrogenase enzymes, decreased levels of antioxidant enzymes, and prevented ATP synthesis by preventing mitochondrial complex activity in various brain regions. There have also been reports of the GABAergic system's role in Huntington disease aetiology. Ashwagandha is a viable option for treating Huntington disease since it works through the GABAergic system, restores acetylcholinesterase and glutathione enzyme levels, and enhances cognitive function. These findings suggest that the neuroprotective effects of *W. somnifera* are mediated via its antioxidant properties.[27,7]

Withaferin A, which is extracted from ashwagandha, has been shown to have positive benefits in mice in another study. A hallmark of several neurodegenerative illnesses, including Huntington disease, and an indication of ageing is the incapacity of cells to maintain proteostasis. To improve compromised proteostasis and slow the progression of the disease, it has been demonstrated that low doses of withaferin A treatment in the R6/2 transgenic mouse model of Huntington disease and HD150Q cells strongly activates the heat shock

response (HSR) by activating heat shock factor 1 (HSF1) by thiol oxidation. 2,3 Withaferin A has been demonstrated to suppress proteasomal malfunction and autophagy induction at higher levels; these actions may be connected to its impact on thiol modification within the cell. Understanding the conformational dynamics, stability, and hydrogen bond interactions of these complexes is made possible by the molecular dynamics simulations used to investigate the interaction withanolide derivatives. Understanding the possible therapeutic effects of withanolides on biological targets such as 8DNS requires knowledge of these discoveries.[28]

When Huntington's disease mice were given withaferin A, their body weight decreased, their behavioural and motor abnormalities were rectified, and they lived noticeably longer. Heat shock activation decreased mutant huntingtin aggregates, and enhanced striatal function in the mouse brain were all validated by biochemical investigations. Additionally, as seen by decreased microglial activity, withaferin A dramatically decreased inflammatory processes. It has also been demonstrated that W. somnifera root extract and its component withanolide. A greatly enhances motor activity and cognitive function. This improvement has been ascribed to the effects of *W. somnifera* supplementation on acetylcholinesterase enzyme activity, antioxidant status restoration, and oxidative stress inhibition. Therefore, extracts from *W. somnifera* or their refined form, withaferin A, may be useful as a treatment for Huntington disease.[2,3,29]

Therefore, our goal in this study was to find more phytoconstituents and then use computational analysis to find assuming from W. somnifera as a potential GABA-A receptor agonist for the treatment of Huntington's disease. This work offers a viable substitute for synthetic medications by utilising natural chemicals derived from *W. somnifera*, utilising historic medical expertise to promote contemporary therapeutics. These drugs' promising clinical translation is further supported by their favourable pharmacokinetic and safety profiles, which provide a more secure and convenient solution for insomnia sufferers.

It is important to recognise several limitations even if this computational analysis offers insightful information about the potential of hygrine, tropine, and withasomnine as GABA-A receptor agonists. First off, the results are predicated on molecular simulations and in silico predictions, which require experimental verification to verify the drugs' true safety and effectiveness. Furthermore, the study ignored possible interactions with other molecular targets implicated in the pathogenesis of insomnia by concentrating only on the GABA-A receptor. To investigate the wider pharmacological effects and methods of action of these phytochemicals, more investigation is necessary. The study has following limitations: safety and pharmacokinetic profile of phytoconstituents were not predicted.

CONCLUSION

Utilising natural chemicals from *W. somnifera*, this study offers a viable substitute for manufactured medications, utilising ancient medical expertise to promote contemporary therapeutics. *W. somnifera* could plays an important role in Huntington's disease through modulation of inflammatory and apoptogenic signalling pathways as indicated by network pharmacological and molecular docking along with molecular dynamics studies. However, further experimental studies are required to confirm the safety and efficacy of various phytoconstituents of *W. somnifera* in Huntington's disease.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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