IN SILICO EVALUATION OF SESAME-EXTRACTED COMPOUNDS AS NEUROPROTECTIVE AGENTS AGAINST HUNTINGTON'S DISEASE: MOLECULAR DOCKING AND DYNAMICS STUDIES

Submitted in partial fulfillment of the requirements for the award of the degree of
Bachelor of Technology

In
Biotechnology

By

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Vellore Institute of Technology
(Deemed to be University under section 3 of UGC Act, 1956)

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Vellore Institute of Technology

Vellore – 632014, Tamil Nadu, India

May 2025

DECLARATION

I hereby declare that the thesis entitled "***IN SILICO*** **EVALUATION OF SESAME-EXTRACTED COMPOUNDS AS NEUROPROTECTIVE AGENTS AGAINST HUNTINGTON'S DISEASE: MOLECULAR DOCKING AND DYNAMICS** " submitted by me, for the award of the degree of B.Tech., in Biotechnology to Vellore Institute of Technology, is a record of bonafide research work carried out by me under the supervision of Dr. Sabina E.P

I further declare that the work reported in this thesis has not been submitted and will not be submitted, either in part or full, for the award of any other degree or diploma in this institute or of any other institute or university.

Md Wabi Ismail

Place: Vellore

Signature of the Candidate

Date: 05-05-2025

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ABSTRACT

A potent method to identify neuroprotective agents for neurodegenerative diseases including Huntington's disease (HD) is provided by silico approaches. This work investigates using sophisticated computational screening and molecular interaction analyses the therapeutic potential of phytochemicals derived from sesame seeds. HighResolution Liquid Chromatography-Mass Spectrometry Quadrupole Time-of-Flight (HRLCMS-QTOF) and Gas Chromatography-Mass Spectrometry (GC-MS) methods

were used to find bioactive compounds. Eight interesting candidates showing favorable pharmacokinetic characteristics and low toxicity were selected by subsequent druglikeness and toxicity profiling using SwissADME and ProToxin-II.

Against important HD-associated targets, especially Phosphodiesterase 10A2 (PDE10A2, PDB ID: 4LM3) and Sirtuin 1 (SIRT1, PDB ID: 4I5I), molecular docking studies were carried out. Valdiate showed the highest binding affinities among the screened compounds; she achieved docking scores of -7.3 kcal/mol with PDE10A2 and -8.9 kcal/mol with SIRT1, so surpassing their respective co-crystallized reference ligands. Extensive hydrogen bonding, van der Waals contacts, and hydrophobic interactions providing Valdiate's stable binding were found by detailed interaction analysis.

Molecular dynamics simulations verified the structural stability of the Valdiate-protein complexes under physiological settings as well. These results taken as a whole point to Valdiate as a strong multi-target lead candidate with great therapeutic development potential against Huntington's disease. Future research projects are justified to confirm

these in silico predictions and evaluate the clinical relevance of Valdiate and related phytochemicals.

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INTRODUCTION

Waters initially reported a patient's statement that we now know to be "Huntington's chorea" in 1842. But Waters said did not hold water until 1872 when George Huntington gave a presentation and gave a thorough description of the illness. It then became known as Huntington's chorea. Usually resulting in memory loss, Huntington's chorea is an uncommon neurological condition of the central nervous system that affects mental health behaviors, causes uncontrollable kinetic movements, and changes behavior. The enlarged CAG repeat in the huntingtin gene causes it since it results in the mutant huntingtin protein (mHTT), which finally accumulates and disturbs the normal cellular operations, especially in the cortex and the striatum of the brain

(Walker, 2007):

While the prevalence of the Huntington's disease varies more than tenfold between the various geographical areas, there is consistent evidence of the lower incidence in asian populations and evidence of increasing prevalence in Australia, North America, and Western Europe, including the United Kingdom over the past 50 years. (Rawlins et al., 2016). With an estimated prevalence of 1 to 4 cases per million of people due to numerous factors like low availability of the genetic screening and awareness, many case go unnoticed, HD is rather rare in India compared to other countries. Certain parts of India have customs whereby close family members, such as cousins, are married within the close family member known as consanguineous marriage; when this occurs, there is a risk of passing on genetic disorders like huntingtons disease to the following generation. Genetic inheritance is the main cause of huntingtons disease; so, if a parent has HD, their child has a 50% chance of receiving the defective gene causing the condition. The main cause of this condition is a mutation in the HTT gene, whereby an unusual sequence known as CAG repeat is expandfed abnormally. It also depends on

the frequency of the repetitions; if there are more repetitions, the probability of the HD symptoms manifesting in the early childhood increases. (Mccolgan & Tabrizi, 2018). Other elements influencing this condition include CAG repeat length, environmental variables, lack of physical exercise. People with the 36-38 CAG repeats have a lower – penetrance variant of the gene, which means they may never develop the symptoms or may do so much later in their life, so not all CAG repeat expansion has the same chance of causing Huntington's disease. Though they can arise early in situations involving young people, symptoms usually start between the ages of 30 and 50. While individuals with the longer expansion face a significantly higher risk, the person with 40 or more than the 40 CAG repeats earlier and faster the disease progresses, which indicates why many people in the general population have 36-38 repeats but do not acquire HD(kayetal.,2016)

The treatment for Huntington's disease is mostly directed on the symptoms; there is no cure for it right now. Usually based on symptoms and family history, doctors also diagnose HD; additional genetic testing can validate it. Several drugs help to control the symptoms. Mostly, current treatments for Huntington's disease concentrate on minimizing the mobility issue that patients experience. Although it can have some negative effects, tetrabenazine is one of the useful medications for managing chorea. With less negative effects than the past treatments, newer drugs such as olazapine and ariprazole might also aid with mobility and mental health issues. Since there is no cure for the illness right now, the patients and their families still depend much on education and supportive care. (kay et al., 2016) One such chemical that finds application here is sesamin, a naturally occurring ingredient present in sesame seeds. Studies on sesamin have revealed that it possesses anti-inflammatory and antioxidant qualities, which might assist Huntington disease's brain cells be preserved. Scientists investigate this using

computational techniques such molecular docking and molecular dynamics; these computer-based investigations offer the insightful analysis. (Javed et al., 2023)

1.1. HUNTINGTON'S DISEASE WORLDWIDE STATISTICS

Rare, inherited neurodegenerative disease Huntington's Disease (HD) causes increasing physical impairment, cognitive decline, and behavioral disruption. Affected by elements like genetic background, availability of diagnostic technologies, and healthcare infrastructure, the worldwide prevalence and incidence of HD vary greatly across different countries.

GLOBALLY PREVALENT AND INCIDENCE

Meta-analyses and recent systematic reviews have produced revised estimates of HD's worldwide incidence and prevalence. The incidence is roughly 0.48 per 100,000 person-years; the pooled worldwide frequency is roughly 4.88 per 100,000 individuals. Higher prevalence rates are especially seen in populations of European heritage; North America reports up to 8.87 per 100,000 while Europe roughly shows 6.37 per 100,000.

Asian nations, on the other hand, have far lower prevalence rates—about 0.41 per 100,000 in East Asia. Variations in genetic susceptibility, diagnosis techniques, and knowledge levels could help to explain these variances (Medina et al., 2022)

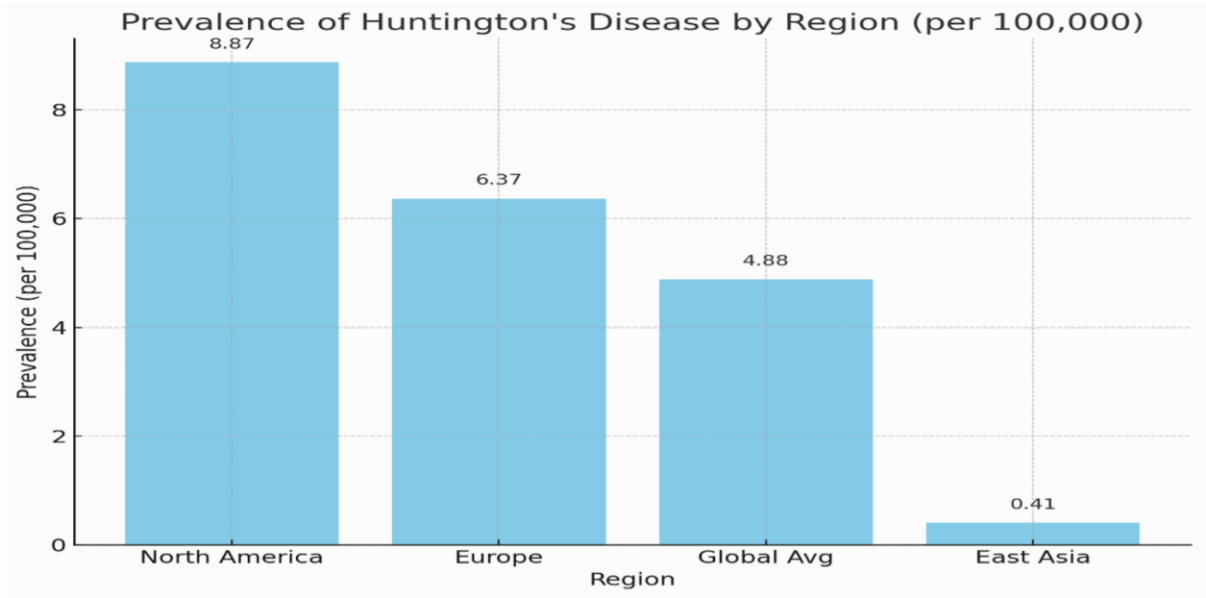

Figure 1:

Prevalence of Huntington's disease per 100,000 population across regions, showing highest rates in North America (8.87) and Europe (6.37), with significantly lower prevalence in East Asia (0.41).

1.2. HUNTINGTON'S DISEASE IN INDIA

There is little thorough epidemiological data on HD available in India. Estimates indicate, however, that HD may impact between 40,000 and 70,000 people nationally, meaning a prevalence of between 3 to 5 per 100,000 persons. This estimate is consistent with results of studies on Indian immigrant groups in the United Kingdom showing a 1.75 per 100,000 prevalence (Basu et al., 2020). The dearth of large-scale, populationbased studies in India emphasizes the need of raising awareness, better diagnosis tools, and focused research to fairly estimate the burden of HD within the nation.

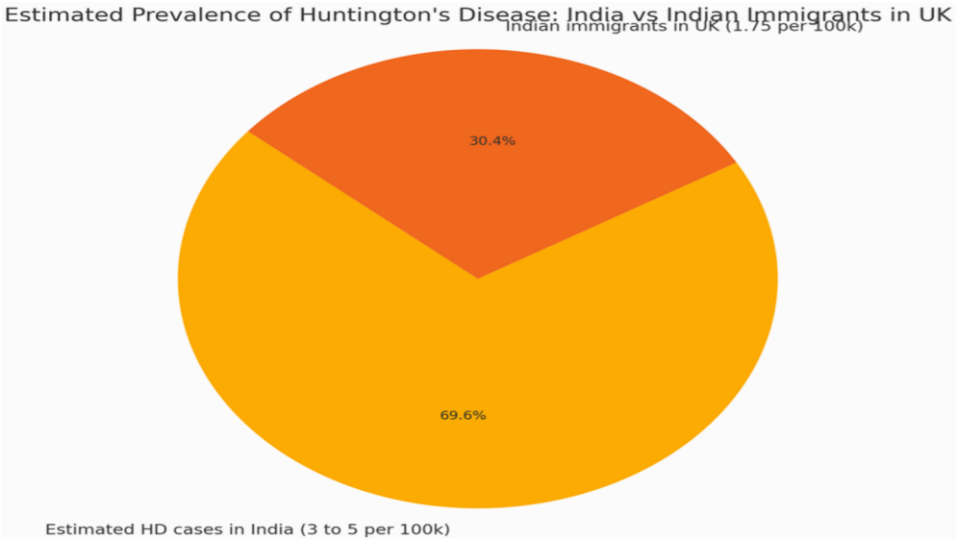

Figure 2:

Estimated prevalence of Huntington's disease comparing India (3–5 per 100,000) with Indian immigrants in the UK (1.75 per 100,000), showing higher reported cases in India.

REVIEW OF LITERATURE

2.1. TREATMENT OF HUNTINGTON'S DISEASE (HD)

A hereditary brain disease, Huntington's Disease (HD) gradually destroys nerve cells and affects movement, cognition, and behavior. (Ross & Tabrizi, 2011) It is brought on by a mutation in a gene known as HTT whereby a particular DNA segment (CAG repeats) is overly lengthy. Mutant huntingtin (mHTT) is the result of this synthesis of a damaging protein. (Bates et al., 2015). It This protein accumulates in brain cells with time and influences their behavior. Although a cure is yet unknown, various therapies can control symptoms, shield brain cells, or slow down the disease.

2.1.2. SYMPTOMATIC CONTROL

Most treatments for HD today concentrate on symptom control, particularly with regard to the uncontrollable movements called chorea. Medications such as tetrabenazine and deutetrabenazine, which reduce dopamine levels in the brain, are routinely used by doctors. This helps reduce the extra movement caused by HD (Frank, 2014). Medicines such as olanzapine and risperidone are also used to help with mood swings, aggression, and sleep problems, but they can make people feel very sleepy or gain weight (Armstrong & Miyasaki, 2012). Memory and thinking problems (cognitive symptoms) are harder to treat with medication, so doctors usually recommend behavioral therapy, structured routines, and caregiver support to help manage these changes.

2.1.3. NEUROPROTECTIVE STRATEGIES

Some treatments seek to shield brain cells from mHTT-induced harm. Lab experiments by lowering stress inside brain cells have showed some potential for compounds such coenzyme Q10, creatine, and minocycline. Researchers are now looking at natural antioxidants including sesamin, curcumin, and resveratrol, which may help lower inflammation and protect nerve cells, but they did not show great advantages when examined in big human trials. By battling damaging chemicals, these natural substances could boost brain health (Majdalawieh et al., 2017).

2.1.4. TARGETED THERAPIES AND GENE SILENCING

Development of gene silencing medicines marks a major turning point in HD research. These seek to either stop or lower the synthesis of the damaging mHTT protein. One such approach uses antisense oligonucleotides (ASOs), small chemicals that can block the message from the defective gene.(Tabrizi, S. J., et al. 2023) Early trials of a medication called tominersen revealed good results by reducing the toxic protein in the fluid surrounding the brain. Scientists are also investigating other methods including RNA interference (RNAi) and CRISpen gene editing, which can switch off or fix the defective gene more accurately. A larger trial had to terminate as it didn’s help enough and produced negative effects.

2.1.5. REGENERATIVE THERAPIES BASED ON CELLS

Stem cell treatment represents still another exciting field of study. This entails substituting damaged brain cells in HD patients with cells capable of developing into neurons. While using stem cells in humans comes with difficulties, such preventing rejection by the immune system and making sure the cells don’s growth is under control, these stem cells have shown the ability to improve movement and restore some brain

function in animal studies (Ross & Tabrizi, 2011). Solving these issues is still under progress among scientists.

2.1.6. SUPPORTIVE CARE AND REHABILITATION

Though there is no cure, supportive therapy can significantly improve the quality of life for HD sufferers. A certain rehab program has even proved to momentarily boost mood and movement; regular exercise, speech therapy, and occupational therapy can help patients stay active, speak better, and manage daily duties.(Hartelius, L., et al. 2003) Many persons with HD experience depression, anxiety, and personality changes, hence mental health support is also rather crucial. Emotional assistance and counseling enable patients and families better manage the illness.

2.2. THE HTT'S STRUCTURE AND PURPOSE

Comprising 3,144 amino acids with a molecular weight of over 348 kDa, huntingtin (HTT) is a big, multifunctional protein. HTT is quite conserved evolutionally among vertebrate species, suggesting its fundamental biological function. Apart from nuclear localization signals (NLS) and nuclear export signals (NES), which control HTT's shuttling between the cytoplasm and the nucleus, the protein has several important functional domains including an N-terminal polyglutamine (polyQ) region, a polyproline region, and three HEAT repeat domains that enable protein-protein interactions. Particularly conserved and underlined in functional relevance is the NES domain. Under pathogenic situations, such Huntington's disease (HD), the mutant version of HTT (mHTT) commonly generates N-terminal fragments devoid of key regulating signals. (Block-Galarza et al., 1997). This results in abnormal nuclear accumulation of the protein, particularly via its interaction with nuclear pore

components such as the translocated promoter region (TPR), therefore leading to cellular toxicity and neurodegeneration.

Though HTT is small and has conformational freedom, important structural understanding of it has been obtained. Especially, cryo-electron microscopy (cryo-EM) at a 4 Å resolution helped to clarify the HTT-HAP40 complex structure, so exposing the general architecture and implying possible regulating mechanisms of HTT activity. Nevertheless, the great volume of unbound HTT still poses difficulties for highresolution structural analyses applied with conventional methods such X-ray crystallography and cryo-EM.

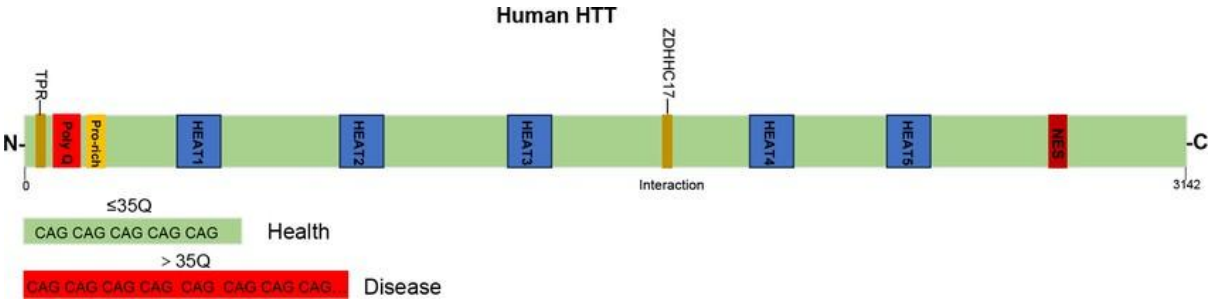

Figure 3:
Schematic of the human HTT protein showing functional domains and polyQ expansion. CAG repeat lengths ≤35 indicate health, while expansions >35 are associated with Huntington's disease.

Functionally, HTT is a scaffold protein that interacts with several binding partners to coordinate a great variety of biological activities. Especially in the central nervous system (CNS), where it fosters neuronal growth, migration, and survival, it is essential for neural development. By forming complexes with transcription factors, coactivators, and repressors, HTT modulates transcriptional activity across various cellular contexts.

Moreover, HTT is essential for intracellular transport through its interaction with molecular motor proteins and endocytic/vescular trafficking machinery. Known to interact with HTT, proteins include HIP1, HIP14, HAP1, HAP40, PACSIN1, SH3GL3, clathrin, and dynamin help both short- and long-range axonal movement necessary for synaptic preservation (Block-Galarza et al., 1997).

Apart from its trafficking function, HTT also helps synapses to be formed and regulated. By means of its SH3 domain, a fundamental protein in synaptic plasticity and neurotransmitter release, it interacts with PSD95, implying HTT's role in preserving neuronal communication. Crucially, (Baxendale et al., 1995) HTT also follows cell survival routes. It prevents death by binding to Pak2 and blocking its cleavage by caspase-3 and caspase-8, therefore displaying anti-apoptotic effects. Underlining its protective and regulating functions, experimental knockdown of HTT in neuroepithelial cells has been found to reduce cell proliferation, disturb neuronal migration, and induce cell death during early neurodevelopment.

Moreover, HTT participates in cell mobility and cytoskeletal control. By means of its interactions with cytoskeletal proteins, HTT shapes microtubule and actin filament assembly, therefore influencing cell shape and movement. HTT helps mitotic spindles in epithelial stem cells to orient themselves, which is absolutely essential for symmetric and asymmetric cell division. HTT preserves the proliferative potential of neural progenitor cells in the developing brain, therefore guaranteeing appropriate neurogenesis(Block-Galarza et al., 1997). All taken together, HTT is a functionally varied and physically complicated protein with important involvement in neurodevelopment, transport, gene control, and cellular signaling. Its malfunction,

especially related to enlarged polyQ repetitions in mHTT, disturbs these functions and starts a series of pathogenic events typical of Huntington's disease.

2.3. PATHOPHYSIOLOGY OF HUNTINGTON'S DISEASE

The pathophysiology of this disease is mostly related to a genetic mutation in the Huntington's gene found on chromosome 4. CAG trinucleotide repeat aberration follows from this mutation. It produces an extended polyglutamine tract mutant huntingtin protein (Mhtt), which is harmful to neurons. (Ross & Tabrizi, 2011). The buildup of the Mhtt disturbs the cellular process, including transcriptional control, mitochondrial function, and protein homeostasis, so causing neuronal malfunction and death eventually. The Striatum is the most impacted area of the brain;

neurodegeneration also affects other areas including the cortex. (Walker, 2007)

Several systems mediate the harmful consequences of the mutant huntingtin protein (Mhtt) in Huntington's disease (HD). One main method mHTT damages the brain cells is by interfering with their capacity to read and utilize vital genes. Because it interferes with proteins like CREB-binding protein (CBP), which is necessary to turn on genes maintaining neuron viability. If the genes are not appropriately triggered, neurons suffer to survive. Mhtt also affects the mitochondria, which give the cell its energy and also owing to the oxidative stress, it causes more damage. Another important issue is ubiquitin-proteasome system in which the huntingtin (HTT) protein misfolds and clumps together. This helps maintain cellular health by removing effective proteins, organelles and pathogens. Another is autophagy, in which cells break down and recycle their own damaged or unnecessary components. The UPS is overloaded or blocked by which harmful proteins cannot be cleared. Harmful proteins so destroy brain cells,

(Martinez-Vicente & Cuervo, 2007), which causes mobility, cognition, and behavioral disorders. Huntington's disease is a terrible and incurable sickness caused in great part by this slow loss of neurons, especially in the striatum and cortex. Another crucial component in pathogenesis is neuroinflammation. Certain brain cells called microglia and astrocytes become overactive in HD, which releases damaging chemicals termed pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF- α) and interleukin6 (IL-6). (Björkqvist et al., 2008) Over time, these substances worsen the illness and destroy the neurons. Excitotoxicity, which results from an excess of glutamate and typically helps brain cells interact, is another main issue in this disease. In HD it gets interrupted. Too high glutamate levels overactivate glutamate receptors on neurons, leading to an aberrant calcium influx into the cells. This calcium excess throws off regular cellular functions and sets off destructive pathways that finally cause neuronal damage and death. (Fan et al., 2007). The slow e-neurodegeneration observed in HD is mostly caused by this mechanism.

2.4. HUNTINGTON'S DISEASE (HD) OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION

A terrible neurological disease, Huntington's Disease (HD) causes cognitive decline, behavioral problems, and increasing motor dysfunction. Two connected processes—oxidative stress and mitochondrial dysfunction—at the cellular level are central in causing neuronal degeneration in HD. When the creation of reactive oxygen species (ROS) surpasses the cell's capacity to eliminate them by means of its antioxidant defense systems, oxidative stress results. Highly reactive and able to destroy important biological components, including lipids, proteins, and nucleic acids (Johri & Beal, 2012), these

ROS comprise chemicals including superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH•).

In HD, mutant huntingtin protein (mHTT) is mostly responsible for too high ROS generation. By upsetting electron transport chain (ETC) complexes, especially complexes II and III, mHTT interferes with the energy-producing organelles within cells, therefore compromising their function. This disturbance drives the creation of superoxide radicals and leaks electrons, therefore starting a destructive cycle of mitochondrial oxidative damage (Johri & Beal, 2012). Consequently, the chemical vital for cellular energy, ATP generation, is significantly reduced. Low ATP levels cause neurons—high-energy-demanding cells—to lose ion gradients, neurotransmission, and intracellular signaling, therefore compromising function and finally causing cell death.

Furthermore, oxidative stress compromises mitochondrial membranes and lowers the mitochondrial membrane potential ($\Delta\Psi m$), a fundamental measure of mitochondrial condition. Loss of this potential sets the mitochondrial permeability transition pore (mPTP) open, releasing cytochrome c and other pro-apoptotic substances into the cytosol, therefore activating caspases and starting programmed cell death (apoptosis). Selectively vulnerable in HD, medium spiny neurons of the striatum have been especially demonstrated to exhibit this mechanism (Johri & Beal, 2012).

Target of ROS-induced damage is also mitochondrial DNA (mtDNA), which causes mutations that further reduce mitochondrial protein synthesis and respiration, hence aggravating the cycle of failure. Further impairing the repair and replacement of

damaged mitochondria, mHTT disturbs the expression of genes involved in mitochondrial biogenesis and dynamics, including PGC-1α (Cui et al., 2006).

Natural antioxidants like sesamin have become rather interesting neuroprotective agents in this diseased setting. Found in sesame seeds (Sesamum indicum), sesamin is a lignan whose significant antioxidant action is attributed to scavenging free radicals and altering redox-sensitive transcription factors (Majdalawieh et al., 2017). Through stimulation of the Nrf2 (nuclear factor erythroid 2–related factor 2) signaling cascade, one of the main processes via which sesamin provides mitochondrial protection. Essential for neutralizing ROS and restoring redox balance (Majdalawieh et al., 2017),

Nrf2 controls the expression of antioxidant enzymes including superoxide dimutase (SOD), glutathione peroxidase (GPx), and heme oxygenase-1 (HO-1). Sesamin reduces the activity of NF-κB and improves the nuclear translocation of Nrf2, therefore lowering oxidative and inflammatory responses within neurons.

Moreover, sesamin has been proven to maintain mitochondrial membranes, therefore conserving $\Delta\Psi m$ and stopping the release of deadlier elements such as cytochrome c. Sesamin administration greatly slowed hydrogen peroxide-induced cell death, lowered caspase-3 activation, and restored mitochondrial function in an in vitro investigation employing human neuroblastoma cells (Tsai et al., 2015). These results imply that sesamin not only serves as a direct ROS scavenger but also increases mitochondrial resilience under oxidative stress circumstances, which is especially important in disorders like HD where mitochondrial damage both causes and results of neuronal death.

Taken together, mitochondrial malfunction and oxidative stress form linked pathogenic mechanisms driving neurodegeneration in HD.(Majdalawieh et al., 2017) By means of its several processes—improving antioxidant defenses, maintaining mitochondrial structure and function, and preventing death—sesamin has therapeutic potential in reducing mitochondrial pathology and slowing down disease progress. Therefore, more in vivo and clinical research is justified to investigate sesamin's use as a neuroprotective agent in Huntington's Disease and allied diseases.

2.5. NATURAL COMPOUND ROLE IN NEURODEGENERATION

Particularly in the framework of complicated disorders like Huntington's Disease (HD), natural chemicals have long been acknowledged as rich reservoirs of bioactive molecules with possible therapeutic uses. HD pathogenesis is caused by oxidative stress, persistent neuroinflammation, mitochondrial malfunction, and excitotoxicity among other multifactorial events. These subtleties make it perfect for a multi-target treatment approach; natural products are well-positioned to fulfill given their structural diversity and pleiotropic activities (Majdalawieh et al., 2020).

Among these molecules, sesamin—a lignan derived from sesame seeds (Sesamum indicum)—showcases interesting neuroprotective action. The modulation of two important cellular signaling pathways—the Nrf2 (Nuclear factor erythroid 2–related factor 2) antioxidant defense mechanism and the NF-κB (nuclear factor kappa-lightchain-enhancer of activated B cells) inflammatory response pathway—helps to explain its activities. Sesamin activates Nrf2, which translocates to the nucleus and upregulates genes encoding antioxidant enzymes including SOD, GPx, and catalase, therefore improving the cell's resilience to oxidative stress (Majdalawieh et al., 2017).

Simultaneously, sesamin downregulates the expression of pro-inflammatory cytokines including IL-6, IL-1β, and TNF-α—cytokines known drivers of neurodegenerative damage—by blocking the translocation of NF-κB into the nucleus.

Apart from redox and inflammatory control, sesamin has clearly shown influence on mitochondrial condition. It increases ATP synthesis, helps to regulate the mitochondrial membrane potential, and stops cytochrome c leakage—a fundamental starter of the intrinsic death process. Reduced activation of pro-apoptotic caspases such as caspase3 and caspase-9 results from these actions helps to preserve neuronal cell integrity and viability (Tsai et al., 2015).

In vivo and in vitro experimental models support these molecular events. For a lipopolysaccharide (LPS)-induced model of systemic inflammation, Hsu and Liu (2006) for example found that sesamin treatment dramatically lowered organ damage and inflammatory marker levels. This model reflects the systemic inflammatory processes pertinent to neurodegeneration, albeit not specifically for HD. By so reducing caspase-3 activation and restoring redox balance, (Tsai et al. (2015) also showed that sesamin therapy shielded human neuroblastoma cells from hydrogen peroxide-induced death.

These results taken together show sesamin as a strong natural agent with multidimensional neuroprotective action. Its capacity to concurrently control oxidative stress, inflammation, mitochondrial activity, and death makes it a good candidate for therapy development in HD.

2.6. HUNTINGTON'S DISEASE (HD) THERAPEUTIC TARGETS

This study selected four major protein targets for computational analysis that are involved in the etiology of Huntington's disease (HD). These targets embody several biological pathways contributing to the disease's pathogenesis, including protein aggregation, apoptosis dysregulation, cyclic nucleotide signaling interruption, and the stress response modification.

The first target is Huntingtin Exon 1 (HTT) - 17Q variant (PDB ID: 4CBY). The mutant form of huntingtin is defined by an elongated polyglutamine (CAG repeat) sequence. The neurodegenerative mechanism involved in Huntington's disease (HD) primarily depends on the accumulation and aggregation of mutant huntingtin (mHTT). The second target is the cysteine-aspartic protease Caspase-6 (PDB ID: 8EG5), which participates in apoptotic processes. Caspase-6 holds great significance as a target for therapeutic intervention by cleaving the huntingtin protein and promoting neuronal apoptosis.

The third target was phosphodiesterase 10A2 (PDE10A2) (PDB ID: 4LM3), which is an enzyme that regulates intracellular signaling by cyclic AMP (cAMP) and cyclic GMP (cGMP), especially in medium spiny neurons of the striatum. Dysregulation of PDE10A2 has been implicated in motor deficits in patients with Huntington's disease.

Finally, the fourth target has been selected as the NAD+-dependent deacetylase Sirtuin1 (SIRT1) (PDB ID: 4I5I), along with its neuroprotective effect. SIRT1 modulates energy metabolism and pathways linked to stress responses and neuronal survival. In models of Huntington's disease, activation of SIRT1 correlates with a reduction in the onset of neurodegeneration.

Each target was chosen based on therapeutic modifiability, importance to HD pathogenesis, and structural availability (availability of co-crystallized ligands). A dedicated selection of these proteins allows for a global approach addressing the multitude of interconnected pathogenic pathways associated with Huntington's disease.

2.7. STANDARD MEDICINE FOR HUNTINGTON'S DISEASE

Tetrabenazine is the most often used FDA-approved medication for treating chorea linked with Huntington's disease right now. Considered the first-line pharmaceutical treatment for controlling motor symptoms, especially HD-specific involuntary motions such chorea (Frank, 2014)

Tetrabenazine acts by blocking the vesicular monoamine transporter 2 (VMAT2). This inhibition lowers the dopamine and other monoamines (norepinephrine and serotonin) released from presynaptic vesicles into the synaptic cleft. This lowers the general dopamine activity in the brain, so helping to control the hyperkinetic movements seen in HD patients (Armstrong & Miyasaki, 2012).

Although tetrabenazine helps to reduce chorea, its usage is usually restricted by side effects including depression, drowsiness, Parkinsonism, and sleeplessness. Actually, tetrabenazine can aggravate mood disorders, which are already common among HD patients, because of its dopamine-depleting action. Tetrabenazine therapy patients thus need continuous monitoring for psychiatric symptoms, and the medication is usually started at low dosages with slow titration (Frank, 2014).

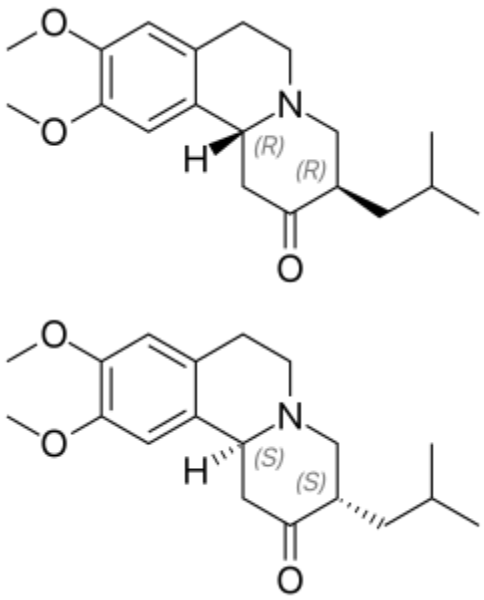

Figure 4:
Structure of Tetrabenazine, an FDA-approved drug used to treat chorea in Huntington's disease.

Additionally approved for HD-related chorea is a modified form of this medication, Deutetrabenazine. Due to deuterium substitution, which enables twice-daily dosing and maybe fewer side effects, especially with relation to drowsiness and mood instability, it has a similar mechanism but a longer half-life.

2.8. ROLE OF GUT-BRAIN AXIS IN NEURODEGENERATION AND INFLUENCE OF PHYTOCHEMICALS

Comprising neural, hormonal, and immune mechanisms, the gut-brain axis (GBA) is a two-way network between the gastrointestinal (GI) tract and the central nervous system (CNS). New research suggests that changes in gut flora, sometimes referred to as dysbiosis, can aggravate the course of neurodegenerative diseases including

Huntington's disease (HD) by increasing systemic inflammation, changing the content of neurotransmitters, and so compromising the gut barrier (Dinan & Cryan, 2017).

Reduced beneficial bacteria, gut dysbiosis in Huntington's disease humans and animal models has been linked to elevated pro-inflammatory cytokines and impaired motor ability (Gubert et al., 2022). Recent studies suggest that phytochemicals such as sesamin might improve cognitive ability and restore gut microbial balance. Present in sesame seeds, sesamin is a lignan with strong antioxidant and anti-inflammatory effects that change the composition of gut flora by encouraging the growth of good bacteria like Lactobacillus and Bifidobacterium, so preventing negative strains like Desulfovibrio. This bacterial modification improves the intestinal lining and lowers the translocation of toxins into the bloodstream, so lowering brain inflammation and neurological stress.

Sesamin may also improve the synthesis of short-chain fatty acids such as butyrate, which control microglial activation and stimulate neuronal regeneration, so preserving gut integrity and influencing brain function. Research shows that through gutmicrobiota-dependent mechanisms, dietary polyphenols—especially lignans—enhance cognitive ability and lower oxidative stress in neurodegenerative models, so supporting these benefits (Cheng et al., 2020). Thus, by directly protecting neurons and indirectly changing gut flora to preserve the gut-brain axis, sesamin offers possible dietary neuroprotection in Huntington's disease.

2.9. TRANSCRIPTOMIC AND EPIGENETIC ALTERATIONS IN HUNTINGTON'S DISEASE AND THE POTENTIAL ROLE OF SESAMIN

Huntington's disease is a progressive neurodegenerative disorder caused by an expanded CAG trinucleotide repeat in the huntingtin (HTT) gene that leads to the mutant huntingtin protein. This mutation affects the function of the protein and produces profound changes in gene expression, known as transcriptional dysregulation. One of the key mechanisms involved in this dysregulation involves epigenetic mechanisms and changes made to the histone patterns of acetylation and methylation. Histone acetylation levels are profoundly decreased in HD, specifically the H3 and H4 histones.

Hypoacetylated chromatin becomes more condensed, thus making transcription less accessible; in consequence, genes important for neuronal survival and function are suppressed. The mutated protein has been shown to impede the activity of histone. Decreased levels of histone acetylation as a general feature of Huntington's disease are among the many peculiarities associated with the malady. Indeed, there has been increased trimethylation of histone H3 at lysine 9 (H3K9me3), which is a modification usually associated with gene silencing. This hypermethylation is mediated by the levels of histone methyltransferases such as ESET/SETDB1, which lead to the repression of neuronal function and survival related genes (Dong et al., 2015).

Altogether, such epigenetic alterations facilitate the neurodegenerative processes in HD. Recent studies have shown the ability of these compounds (in particular, those derived from the diet, especially polyphenolic ones) to modulate these epigenetic changes. Sesamin, a naturally found lignan in sesame seeds, is an antioxidant and antiinflammatory agent with a powerful influence on epigenetic mechanisms. Sesamin and other polyphenols can inhibit histone deacetylases (HDACs) and DNA

methyltransferases (DNMTs) that remove acetyl groups from histones and add methyl groups to DNA, respectively. Proteome profiling was used to show that sesamin may lead to a relaxed

chromatin structure, hence promoting gene expression and potentially reversing the transcription manners associated with HD (Link et al., 2010).

AIM AND OBJECTIVES

3.1. AIM OF THE STUDY

The aim of this study is to computationally evaluate the neuroprotective potential of sesame-extracted phytochemicals against Huntington's disease through molecular docking and molecular dynamics simulation approaches.

3.2. OBJECTIVES

1) To identify, characterize, and evaluate sesame-derived phytochemicals using HRLCMS-QTOF and GC-MS, followed by in silico screening of their

pharmacokinetic, drug-likeness, and toxicity profiles using SwissADME and ProTox-II.

2) To assess the therapeutic potential of shortlisted compounds against Huntington's disease by conducting molecular docking with key target proteins (HTT, Caspase-6, PDE10A2, and SIRT1), and validating the stability of top-ranked ligand-protein complexes through molecular dynamics simulations.

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MATERIALS AND METHODS

4.1. HRLCMS-QTOF ANALYSIS

The work started with methodically extracting phytochemicals from sesame (Sesamum indicum) using methanol as the solvent. Because of its strong polarity, capacity to dissolve a broad spectrum of secondary metabolites, and fit with both LC-MS and GCMS analysis, methanol was selected. Macerated in 100 mL of methanol, roughly 10 grams of finely crushed sesamin-rich material was overnight coldly extracted under shaking conditions. To produce a crude extract, which was reconstituted in LC-MS grade methanol before analysis, the extract was then filtered and concentrated at lowered pressure.

For High-Resolution Liquid Chromatography-Mass Spectrometry Quadrupole Timeof-Flight (HRLCMS-QTOF) analysis, the samples were analyzed by an external research facility. This prepared sample Combining the mass accuracy and resolution of TOF mass spectrometry with the separating power of liquid chromatography, HRLCMS-QTOF is a quite sensitive method. To maximize ionization in positive mode, the material was fed into a reverse-phase C18 column and eluted under a gradient of water and acetonitrile both including formic acid. Mass-to----charge (m/z) ratios and fragmentation patterns helped to identify molecular ions from the resultant mass spectra.

Then utilizing private software supplied by the instrument vendor, raw spectrum data were matched against several public compound libraries including METLIN, MassBank, and NIST. Among the several substances of pharmacological significance found were lignans such as sesamin and sesamolin, flavonoids, phenolic acids, terpenoids, and other secondary metabolites generally present in sesame seeds. For every compound to support compound validation, retention time, isotope distribution, MS/MS fragmentation profiles were also recorded in addition to compound names and molecular

weights. Each compound was further screened manually by comparing spectral fingerprints and retention time against published literature to confirm its identity and potential relevance to neurological applications. This preliminary identification step provided a robust foundation for subsequent in silico screening and prioritization of bioactive molecules against Huntington's -related targets.

4.2. GC–MS ANALYSIS FOR VOLATILE COMPOUNDS

To supplement HRLCMS-QTOF findings and profile volatile constituents, a comprehensive Gas Chromatography-Mass Spectrometry (GC-MS) analysis was

carried out using the same ethanolic extract. This was performed at the Vellore Institute of Technology (VIT). GC-MS was selected for its capacity to detect low molecular weight volatile and semi-volatile compounds that are often missed by LC-MS

techniques (Lu et al., 2015).

For the GC-MS analysis, a portion of the extract was evaporated and redissolved in GCgrade hexane. It was injected into a capillary column with a non-polar stationary phase, and the oven temperature was programmed for gradual elevation to facilitate the separation of compounds based on their boiling points. Compounds were ionized using an electron impact (EI) source and detected via quadrupole mass spectrometry.

The resulting spectral fingerprints and retention times were matched against reference databases including the Wiley Registry and the NIST Mass Spectral Library (Stein, 2012). Detected volatile phytoconstituents included sesquiterpenes, alkyl phenols, aldehydes, and aromatic compounds.

To ensure a comprehensive compound profile, data from HRLCMS-QTOF and GC-MS analyses were compared and curated to eliminate redundancy. Only molecules with potential pharmacological relevance or structural features suitable for further investigation were retained. Structure-activity relationship (SAR) data from literature and cheminformatics databases further guided the shortlisting of neuroactive candidates for in silico analysis.

4.3. PROTEIN DATA BANK (PDB)

The Protein Data Bank, or PDB (https://www.rcsb.org/), was first established at Brookhaven National Laboratories (BNL) in 1971. It is a repository that holds the experimentally crystallized 3D structural data of primary proteins as well as DNA and RNA along with small molecules. The primary goal is to visualize the data using the Cartesian molecular coordinates, which facilitate user viewing of the visualization servers and software (Berman et al., 2000).

4.4. DISCOVERY STUDIO VISUALIZER (DSV)

The Discovery Studio Visualizer (https://discover.3ds.com/discovery-studiovisualizerdownload) is a collection of 3D visualization tools that give the user flexibility when observing structural information about biomolecules. It also includes fundamental analysis programs like folds change detecting through superimposing and interaction maps. Accelrys has made DSV available as a free visualization version (Pawar &

Rohane, 2021).

4.5. SwissADME

Drug development involves analyzing absorption, distribution, metabolism, and excretion (ADME) at increasingly early phases of the discovery process, when there are a high number of possible compounds to consider but limited physical sample availability. Computer simulations are acceptable alternatives for experiments within this framework. A range of fast yet reliable predictive models for pharmacokinetics, physicochemical characteristics, drug-likeness, and medicinal chemistry friendliness are available for free via the SwissADME online service (http://www.swissadme.ch/). These models incorporate proprietary expert approaches such as BOILED-Egg,

Bioavailability Radar, and iLOGP. The user-friendly interface on the login-free SwissADME website allows for simple, systematic input and structured interpretation.

SwissADME has several advantages over the most advanced free web-based tools for ADME and pharmacokinetics (such as pk-CSM14 and admetSAR15). These

advantages include the capacity to compute for multiple molecules, the ability to display, save, and share results for each individual molecule or through globally accessible, intuitive, and interactive graphs. Furthermore,

SwissADME provides exclusive access to effective techniques (such as the BOILEDEgg17 or iLOGP16) (Daina et al., 2017).

4.6. PROTOX-II

The development of computational and/or predictive modeling has provided scientists with a vital tool for investigating potentially hazardous chemicals. This has made it

possible for researchers to do molecular pre-screening of thousands of compounds to find compounds that might be compatible with previously identified structures.

Researchers in this field include material chemists and drug designers. Toxicologists have employed similar methods to determine compounds with close structural matches as well as to evaluate a chemical's predicted toxicity. Understanding and considering toxicity assessments of compounds and the endpoints utilized in their evaluation is crucial, particularly when utilizing novel toxicity prediction techniques. ProTox (https://tox.charite.de/protox3/) is a web server for predicting the toxicity of small compounds. Chemicals can be submitted in different formats, including generic names, structures, or chemical formulas. The chemical is then classified using the program's reference database. The ProTox-II website has various advantages over other existing computational models. The ProTox website has target knowledge for both chemicals and molecules. The ProTox-II webserver is unique in its prediction scheme is divided into categories based on toxicity levels, including oral toxicity, organ toxicity (hepatotoxicity), toxicological endpoints (carcinotoxicity, cytotoxicity, immunotoxicity, and mutagenicity), toxicological pathways (AOPs), and toxicity targets. This classification helps to shed light on potential molecular mechanisms underlying toxic responses. ProTox-

II is a new version of ProTox that combines machine learning models, pharmacophorebased fragment propensities, most frequent features, and chemical similarity to predict a range of toxicity endpoints (Banerjee et al., 2018).

4.7. PUBCHEM

PubChem (https://pubchem.ncbi.nlm.nih.gov/) is a public chemical database maintained by the National Library of Medicine (NLM), which is part of the National

Institutes of Health (NIH) in the United States. It gathers chemical data from over various data sources and makes it freely available to the general population. One of the most popular chemical websites worldwide, PubChem has over four million unique interactive visitors monthly during peak times. PubChem is an important chemical information repository for biomedical research communities in fields such as cheminformatics, chemical biology, medicinal chemistry, and drug development. PubChem is commonly used as a 'big data' source in machine learning and data science studies for virtual screening, medication repurposing, chemical toxicity prediction, pharmacological side effect prediction, and metabolite discovery, among other things

(Kim et al., 2021).

4.8. DOCKING TOOLS

Autodock: AutoDock Vina (https://autodock.scripps.edu/) is an open-source docking engine that is fast and popular. It is a complete computational docking application that uses a simple scoring system and quick gradient-optimization conformational search. It is not necessary for the user to understand Vina's implementation details, tweak mysterious search settings, group results, or become an expert in complex algebra (quaternions) in order to utilize its design philosophy. The docking process requires only the molecule structures and search space parameters, including the binding site. There is no need to add atom charges or compute grid maps while using Vina or Vinardo forcefields. Similar to AutoDock 4, some receptor side chains can be chosen to be flexible during docking. By using many CPUs or CPU cores on your PC, it also drastically reduces AutoDock 4's operation time, making it orders of magnitude faster. Moreover, it significantly improves the average accuracy of the binding mode predictions in comparison to AutoDock 4 (Cosconati et al., 2010).

4.9. GROMACS V4.6.5

Groningen machine for Chemical Simulation, or GROMACS (https://www.gromacs.org/), is a skilled and user-friendly software that does molecular dynamics simulation, or the Newtonian motional equations of particles. GROMACS was initially created for biological molecules, including proteins, nucleic acids, and lipids. It uses high-performance algorithmic development to calculate the non-bonded interactions that are helpful for biological research. When it comes to GPU acceleration of Nvidia computations using CUDA, it excels (Abraham et al., 2015).

4.10. TARGET PROTEINS

Table 1

List of target proteins used for molecular docking and simulation studies, including their Protein Data Bank (PDB) IDs, official names, and presence of co-crystallized ligands.

Protein ID	PDB ID	Official Protein Name	Cocrystallized Ligand
PT1	4CBY	Huntingtin Exon 1 (HTT) – 17Q variant	Yes
PT2	8EG5	huCaspase-6 in complex with inhibitor 3a	Yes
PT3	4LM3	Crystal structure of PDE10A2 with fragment ZT464	Yes
PT4	4I5I	Sirtuin1 (SIRT1)	Yes

PT1: 17Q Variant of Huntingtin Exon 1 (HTT) (PDB ID: 4CBY):-

A protein called huntingtin (HTT) is essential for both synaptic transmission and neuronal development. Because it contains the polyglutamine (polyQ) tract, whose expansion causes Huntington's disease (HD), the Exon 1 segment of HTT is especially important. The protein's normal, non-pathogenic form is represented by the 17Q variant, which is frequently employed as a control model in comparative research. Understanding the conformational variations and aggregation patterns seen in pathogenic HTT forms, such as 45Q or higher repeats, is made easier by structural studies of this variant. In this structure, the co-crystallized ligand offers possible interaction sites for medicinal compounds and helps stabilize particular conformations.

PT2: Inhibitor 3a and Human Caspase-6 in Complex (PDB ID: 8EG5):-

Axonal degeneration has been linked to the cysteine protease Caspase-6, which is involved in apoptotic signaling pathways. Neurodegenerative illnesses like Alzheimer's and Huntington's disease are strongly linked to its activation. The 8EG5 structure is a useful model for researching the active site configuration and for logical drug design since it depicts Caspase-6 in complex with a small-molecule inhibitor (3a). Two chains in the crystal structure could indicate an inhibitor-stabilized interdomain interaction or a functionally significant dimeric state. Finding possible inhibitors with a neuroprotective focus requires an understanding of this structure.

PT3: PDE10A2 Crystal Structure with Fragment ZT464 (PDB ID: 4LM3):-

An enzyme called phosphodiesterase 10A2 (PDE10A2) is involved in the hydrolysis of cyclic nucleotides, which are second messengers in neuronal signaling pathways and

include cAMP and cGMP. PDE10A2, which is primarily expressed in the striatum, has been connected to neuropsychiatric and neurodegenerative conditions, such as schizophrenia and Huntington's disease. In addition to supporting fragment-based drug discovery (FBDD) techniques, the crystal structure complexed with the fragment ZT464 offers information on important interaction sites. This facilitates the creation of strong and focused inhibitors for neurological conditions.

PT4-Sirtuin1 (SIRT1), (PDB ID: 4I5I):-

The NAD+-dependent deacetylase Sirtuin1 (SIRT1) controls inflammation, oxidative stress, gene expression, and metabolic processes. By encouraging neuronal survival and mitochondrial function, SIRT1 has demonstrated protective roles in aging and a number of neurodegenerative diseases. A thorough examination of SIRT1's active site and allosteric binding regions is made possible by its structure with a co-crystallized ligand. When creating small-molecule modulators of SIRT1, which could be used as therapeutic agents for diseases like Alzheimer's, Parkinson's, and Huntington's, this structural information is especially helpful.

These proteins were downloaded in a PDB file from the RCSB Protein Data Bank (https://www.rcsb.org), each of these targets has 3D crystal structures. These protein structures were chosen not just for their functional significance to HD but also because well-resolved, experimentally obtained crystal structures with sufficient resolution to facilitate successful docking simulations were available.

4.11. MOLECULAR DOCKING UNDER AUTODOCK VINA

Molecular docking studies were conducted using **AutoDock Vina** to evaluate the binding affinity of selected sesame-derived phytochemicals against key protein targets associated with Huntington's disease, specifically HTT (PDB ID: 4CBY), Caspase-6 (8EG5), PDE10A2 (4LM3), and SIRT1 (4I5I). The ligand structures were obtained from the **PubChem database** and prepared for docking by converting them into .pdbqt format using **AutoDock Tools**, where torsional flexibility was defined for each compound. Protein structures were retrieved from the **Protein Data Bank (PDB)** and prepared by removing crystallographic water molecules, adding polar hydrogens, and assigning Kollman charges. The processed protein structures were also saved in .pdbqt format for compatibility with the docking software.

For each protein-ligand pair, a separate **configuration file (config.txt)** was created to define the docking parameters. This file specified the file paths for the receptor and ligand .pdbqt files, the grid box dimensions and center coordinates—carefully defined around the active site based on CASTp analysis and literature references—and the **exhaustiveness level**, which was set between **8 and 16** to maintain a balance between accuracy and computational efficiency. Docking simulations were executed through the command-line interface using AutoDock Vina, which generated multiple binding poses for each ligand-target combination, ranked by their predicted binding affinities expressed in kcal/mol.The **binding pose with the lowest binding energy** for each compound was selected for further analysis. These top-ranked poses were visualized and interpreted using **Discovery Studio Visualizer** and **AutoDock Tools** to examine the nature of molecular interactions such as **hydrogen bonding**, **hydrophobic contacts**,

and **π–π stacking interactions**. Additionally, the orientation and conformational fit of the ligands within the active binding pocket were assessed to confirm appropriate interaction with key residues. This docking approach provided foundational structural insight into the molecular interactions between bioactive sesame compounds and Huntington's disease-related targets, thereby supporting the identification of potential neuroprotective agents for therapeutic exploration.

RESULT

5.1. INITIAL COMPOUND COLLECTION

Gathering **86** distinct phytocompounds from High-Resolution Liquid Chromatography Mass Spectrometry – Quadrupole Time of Flight (HR-LCMS-QTOF) and Gas Chromatography–Mass Spectrometry (GC-MS) analysis was the initial stage. These analytical methods made it possible to precisely identify sesamin-derived metabolites having possible bioactivity. Ideal candidates for in silico screening, the resulting library of 86 compounds reflected a varied chemical space including a large variety of molecular weights, polarities, and functional groups.

For computational evaluations, these molecules were catalogued and ready in suitable file formats (e.g., sdf, mol2). Publically available resources including PubChem and SwissADME structure editor allowed one to obtain their canonical SMILES and structural coordinates.

5.2. WORK FLOW AND COMPOUND SCREENING AND SELECTION

Designed to find possible lead molecules depending on drug-likeness, pharmacokinetics, and toxicity profiles, the chemical selection procedure in this work followed a methodical multi-step computer filtering strategy. Beginning from a huge collection of originally discovered phytocompounds obtained from sesamin extract, the pipeline included the usage of SwissADME and ProTox-II web tools.

5.3. DRUG-LIKENESS FILTERING AND PHARMOKINETIC MECHANISM USING SWISSADME

SwissADME analysis was conducted to assess the pharmacokinetic behavior and druglikeness of the selected sesame-derived phytochemicals. The evaluation included criteria such as gastrointestinal absorption, blood-brain barrier (BBB) permeability, and compliance with various drug-likeness filters.

All shortlisted compounds demonstrated **high gastrointestinal absorption**, suggesting strong potential for oral bioavailability. In addition, several candidates showed **positive BBB permeability**, indicating potential to reach central nervous system targets relevant to Huntington's disease. Notably, the compounds exhibited **zero violations of Lipinski's Rule of Five**, as well as full compliance with **Ghose, Veber, Egan, and Muegge filters**, confirming their drug-like characteristics.

Furthermore, none of the compounds triggered **PAINS alerts**, indicating a low likelihood of assay interference or false-positive biological activity. These results suggest that the selected molecules possess favorable pharmacokinetic profiles and structural properties suitable for further drug development.

Table 2
List of fourteen shortlisted compounds that passed all SwissADME drug-likeness and pharmacokinetic filters, along with their PubChem IDs, compound names, and SMILES representations for further toxicity evaluation.

PubChem ID	**Compound Name**	**SMILES**
3151	Domeperidone	C1CN(CCC1N2C3=C(C=C(C=C3)Cl)NC2=O)CCCN4C5=CC=CC=C5NC4=O
10085783	Simeconazole	C[Si](C)(C)CC(CN1C=NC=N1)(C2=CC=C(C=C2)F)O
442535	Gentianamine	C=CC1=CN=CC2=C1C(COC2=O)CO
11822857	Prosolanapyrone II	C/C=C/C=C/CCCC/C=C/C1=CC(=C(C(=O)O1)CO)OC
443437	N-heptanoylhomoserine lactone	CCCCCCC(=O)N[C@H]1CCOC1=O
85890320	3,4',5,6,8-Pentamethoxyflavone	COC1=CC=C(C=C1)C2=C(C(=O)C3=C(O2)C(=CC(=C3OC)OC)OC)OC
101746	Sesamolin	C1[C@H]2[C@H](CO[C@@H]2OC3=CC4=C(C=C3)OCO4)[C@H](O1)C5=CC6=C(C=C5)OCO6
77487	Monomenthyl succinate	CC1CCC(C(C1)OC(=O)CCC(=O)O)C(C)C
15643	Diamidafos	CNP(=O)(NC)OC1=CC=CC=C1
92425	Flumioxazin	C#CCN1C(=O)COC2=CC(=C(C=C21)N3C(=O)C4=C(C3=O)CCCC4)F
66401	16alpha-Bromo-17beta-estradiol	C[C@]12CC[C@H]3[C@H]([C@@H]1C[C@H]([C@@H]2O)Br)CCC4=C3C=CC(=C4)O
626805	Rhazidigenine Nboxide	CCC12CCC[N+](C1)(CCC3(C(=NC4=CC=CC=C43)CC2)O)[O-]
129715809	Valdiate	CC1CCC2C1C(OC=C2COC(=O)C)OC(=O)CC(C)C
72307	Sesamin	C1Oc2c(O1)cc(cc2)[C@H]1OC[C@H]2[C@@H]1CO[C@@H]2c1ccc2c(c1)OCO2

5.4. PROTOX-II'S TOXICITY EVALUATION

The shortlisted **14** phytochemical compounds were exposed to toxicity prediction using **ProTox-II**, a generally accepted *in silico* technique that uses machine learning algorithms to evaluate the toxicological profiles of small molecules, after the pharmacokinetic evaluation using SwissADME. By means of chemical properties of input substances against experimentally confirmed harmful compounds, this technology forecasts several toxicity outcomes.

Table 3
Toxicity prediction results of eight shortlisted phytochemical compounds evaluated using ProTox-II. The table presents predicted outcomes for hepatotoxicity, neurotoxicity, carcinogenicity, and cytotoxicity, all classified as inactive.

S.No	PubChem ID	Compound Name	Hepatotoxicity	Neurotoxicity	Carcinogenicity	Cytotoxicity
1	442535	Gentianamine	Inactive	Inactive	Inactive	Inactive
2	11822857	Prosolanapyrone II	Inactive	Inactive	Inactive	Inactive
3	443437	N-heptanoylhomoserine lactone	Inactive	Inactive	Inactive	Inactive
4	85890320	3,4',5,6,8-Pentamethoxyflavone	Inactive	Inactive	Inactive	Inactive
5	77487	Monomethyl succinate	Inactive	Inactive	Inactive	Inactive
6	15643	Diamidafos	Inactive	Inactive	Inactive	Inactive
7	66401	16alpha-Bromo-17beta-estradiol	Inactive	Inactive	Inactive	Inactive
8	129715809	Valdiate	Inactive	Inactive	Inactive	Inactive

5.5. FINAL COMPOUND SELECTION

8 final compounds overall from the whole screened dataset satisfied all the necessary thresholds, including ProTox-II-based toxicity predictions and SwissADME-based drug-likeness and pharmacokinetics. Showing these 8 compounds:

Table 4
List of the eight final phytochemical compounds that successfully passed all SwissADME and ProTox-II screening criteria, shortlisted for further molecular docking and simulation studies.

S.No	PubChem ID	Compound Name
1	442535	Gentianamine
2	11822857	Prosolanapyrone II
3	443437	N-heptanoyl-homoserine lactone
4	85890320	3,4',5,6,8-Pentamethoxyflavone
5	77487	Monomethyl succinate
6	15643	Diamidafos
7	66401	16alpha-Bromo-17betaestradiol
8	129715809	Valdiate

These compounds were deemed very promising drug-like candidates due to their good ADME profile and non-toxic nature. They were subsequently shortlisted for molecular docking, where their binding affinity for the target HTT protein was tested in silico. The final six compounds now form the basis of future in-depth computational and experimental validation, with potential translational utility for medicinal development. Designed to find possible lead molecules depending on drug-likeness,

pharmacokinetics, and toxicity profiles, the chemical selection procedure in this work followed a methodical multi-step computer filtering strategy. Beginning from a huge

collection of originally discovered phytocompounds obtained from sesamin extract, the pipeline included the usage of SwissADME and ProTox-II web tools.

5.6. DOCKING ANALYSIS

Docking Scores and Interactions for All Ligands Across Four Protein Targets:-

The table below presents the AutoDock Vina docking scores and corresponding molecular interactions for all tested ligands against four target proteins (PT1 – HTT, PT2 – Caspase-6, PT3 – PDE10A2, PT4 – SIRT1). The co-crystallized ligand (Reference 1) and standard drugs (Risperidone, Fluoxetine) are included for comparison.

5.7. AUTODOCK VINA DOCKING RESULTS

Table 5
AutoDock Vina docking scores (in kcal/mol) of selected phytochemical compounds against four Huntington's disease-associated protein targets (PT1–PT4). More negative values indicate stronger predicted binding affinity. Docking scores of cocrystallized reference ligands are included for comparison.

Common name	Compound_ID	Docking_Score (PT1_4CBY)	Docking_Score (PT2_8EG5)	Docking_Score (PT3_4LM3)	Docking_Score (PT4_4I5I)
Prosolanapyrone II.	CID_11822857	-7.1	-5.9	-7.5	-6.2
Valdiate	CID_12971 5809	-6.8	-7	-7.3	-8.9
Diamidafos	CID_15643	-6.5	-5.6	-5.3	-5.1
Gentianamine	CID_44253 5	-6.6	-5.3	-5.9	-6.3
N-heptanoylhomoserine lactone	CID_44343 7	-6.5	-6	-6.4	-6.5
16alpha-Bromo-17beta-estradiol	CID_66401	-7.4	-7.5	-9.3	-6.7
Monomenthyl					

succinate	CID_77487	-5	-5.4	-5.1	-4.6
3,4',5,6,8-Pentamethoxyflavone	CID_8589032 0			-8	-7.1
Co_crystallized ligands (	Reference 1)	-6.5 -10.3	-7.8 -9.4	-6.7	-8.3

To investigate the potential interaction and binding efficiency of some chosen phytochemicals with targets involving neurodegeneration, molecular docking simulations were carried out with the help of **AutoDock Vina**. Four of the most important proteins were taken under consideration here: **HTT (PDB ID: 4CBY)**, **Caspase-6 (PDB ID: 8EG5)**, **Phosphodiesterase 10A2 (PDE10A2 – PDB ID: 4LM3)**, and **Sirtuin 1 (SIRT1 – PDB ID: 4I5I)**. In both instances, the reference ligand co-crystallized was redocked to confirm the docking protocol and to create a comparative docking baseline for the assessment of test ligands.

Although docking was conducted on all four targets, significant and encouraging results were specifically noted for **PT3 (PDE10A2)** and **PT4 (SIRT1)** with respect to binding affinity as well as molecular interactions. These two targets are thus elaborated in detail below.

PT3 – PDE10A2 (PDB ID: 4LM3)

The reference ligand of PDE10A2 had a docking score of **-6.7 kcal/mol**, which was used as a threshold value to determine the relative binding affinity of the test ligands.

Several phytochemicals showed better binding affinities with docking scores of **-7.5**, **-7.3**, and **-9.3 kcal/mol**, indicating their suitability for further development.

Among them, ligand score **-7.3 kcal/mol** was chosen for a detailed interaction study. Although not the most unfavorable score overall, it was preferred because of the quality and integrity of its interactions in the PDE10A2 binding site:

- Hydrogen bonds were formed with crucial residues **HIS525** and **HIS567**, which have been previously established to stabilize the binding of the ligand in PDE family proteins.

- A **C–H bond** interaction with **ASP674** made contributions to orientation and anchoring of the ligand.

- Several **van der Waals (VD) interactions** were detected with residues that are important for function including **TYR524, ASP564, GLU592, HIS595, LEU675, SER677, VAL678**, and **GLN726**, evidencing extensive coverage of contact areas in the active site.

- Stabilizing contacts in addition, involving **LEU635, ILE692, PHE696**, and **PHE729** augmented structural compatibility as well as surface complementarity of the ligand with the protein.

These complex interactions imply that the ligand binds deep and specifically into the active site of PDE10A2, possibly blocking its activity. Considering the function of PDE10A2 in regulating intracellular cAMP/cGMP levels and its involvement in neurological and psychiatric disorders, this ligand is an interesting candidate for further pharmacological investigation.

PT4 – SIRT1 (PDB ID: 4I5I)

In the case of Sirtuin 1, the redocked reference ligand resulted in a docking score of **8.3 kcal/mol** and was taken as the reference to compare against. Among all compounds tested, the phytochemical **Valdiate** had the maximum binding affinity, with a docking score of **-8.9 kcal/mol**. This value not only surpasses the reference benchmark but also shows a stronger and more favorable interaction with the SIRT1 protein.

Analysis of molecular interaction showed that Valdiate:

- Built a **hydrogen bond** with **ILE347**, a residue that lies in the regulatory region of the SIRT1 protein. This interaction is likely to play a role in binding specificity and binding orientation inside the binding pocket.

- Experienced extensive **van der Waals interactions** with many surrounding residues such as **ILE411, PHE297**, and **LEU445**, stabilizing binding of the compound through hydrophobic packing and surface compatibility.

The SIRT1 protein is a **NAD+-dependent deacetylase** that controls pathways associated with **longevity, metabolism, inflammation**, and **neuroprotection**. The capacity of Valdiate to establish robust interactions with this protein and overcome the binding affinity of the co-crystallized reference ligand indicates that it can be a potential SIRT1 modulator. This provides the prospect for its application in the development of therapeutic interventions against age-related neurodegenerative diseases like **Alzheimer's, Huntington's**, and **Parkinson's disease.**

5.8. MOLECULAR DYNAMICS RESULT

PROTEIN RMSD -PT3

In the **PT3 simulation**, the **protein RMSD** for the test system (⛃ blue line, PT3_Test) started around **0.2 nm** and fluctuated moderately between **0.25–0.35 nm** (~2.5–3.5 Å) throughout the simulation, indicating acceptable stability with natural flexibility. In contrast, the **reference system** (orange line, PT3_Reference) also started around 0.2 nm but rose higher, fluctuating up to **0.3–0.4 nm**, with slightly greater variations compared to the test system. Comparing the two, it is clear that the ligand-bound protein (Test) remains more stable than the unbound reference, likely because ligand binding provided a stabilizing effect on the protein's conformation.

PROTEIN RMSD PLOT – PT4

In the Protein RMSD plot, the blue line (PT4_Test) corresponds to the protein structure when bound to the ligand, and the orange line (PT4_Reference) represents the unbound (reference) protein. The blue line fluctuates between 0.3–0.7 nm but remains relatively stable without showing any upward drift, meaning there is no unfolding happening. The orange line fluctuates slightly less (~0.3–0.6 nm), indicating a slightly more rigid structure in the reference system. Overall, the protein with the ligand shows moderate flexibility, which is expected and acceptable for a biologically active structure. This further confirms that ligand binding provides slight structural stability but allows natural "breathing" motions.

LIGAND RMSD (PT3)

In the **PT3 simulation**, the **ligand RMSD** for the test system (⛃ blue line, PT3_Test) remained very stable around **~1 nm** (~10 Å) throughout the simulation, showing only slight minor fluctuations and no large jumps, indicating strong and stable binding of the ligand within the protein pocket. In contrast, the **reference ligand** (orange line,

PT3_Reference) displayed highly unstable behavior, with RMSD values rising dramatically up to **15–25 nm** (~150–250 Å) and continuous large spikes, suggesting that the ligand was unstable, free-floating, or not bound in the reference system. Comparing both, the ligand in the test system remains stably engaged with the binding pocket, whereas the reference ligand shows loss of stability and potential dissociation.

LIGAND RMSD PLOT (PT4)

The Ligand RMSD plot for the new system shows two lines: the blue line (PT4_Test) representing your ligand-protein complex, and the orange line (PT4_Reference) representing the reference ligand. The blue line fluctuates between ~0.3–0.7 nm, with a general oscillation around 0.4–0.5 nm, indicating that the ligand is dynamically stable within the binding pocket. Although some small sharp peaks are visible, there is no continuous rising trend, suggesting no unbinding events. The orange line (PT4_Reference) is slightly more stable around 0.4–0.5 nm, with less fluctuation. This behavior shows that while your ligand exhibits natural flexibility (which is normal), it remains well anchored inside the binding site throughout the simulation.

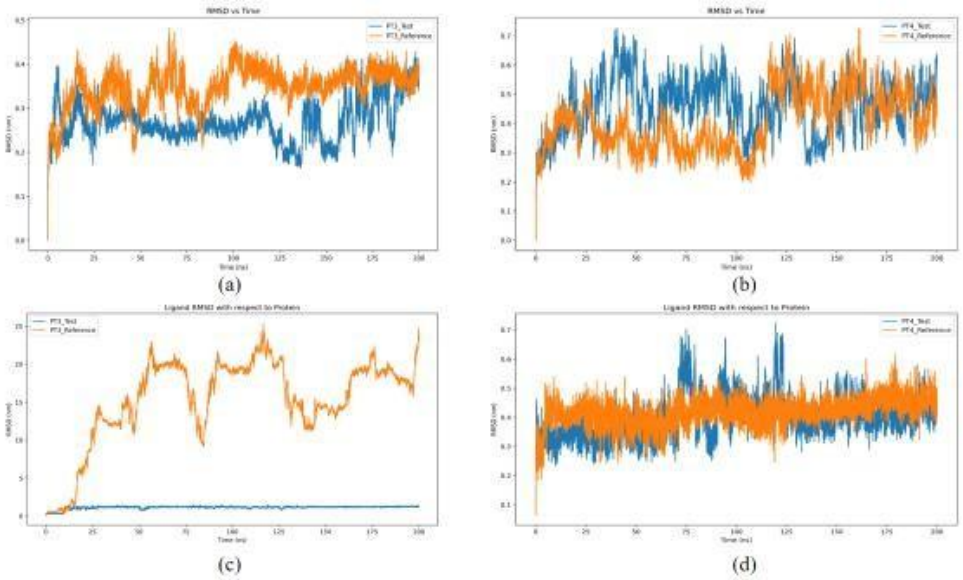

Figure (a), (b), (c), and (d):RMSD plots over 200 ns showing protein backbone stability in (a) PT3 and (b) PT4, and ligand stability in (c) PT3 and (d) PT4. Valdiate and reference ligands are compared; lower RMSD indicates greater structural stability.

RESIDUE FLEXIBILITY (RMSF) — PT3

In the **PT3 simulation**, the **protein RMSF** for the test system (🔵 blue line, PT3_Test) showed low flexibility with RMSF values around **0.1–0.2 nm** for most structured regions, and small peaks (~0.4–0.5 nm) at flexible loop regions, while very high peaks (~1.2 nm) were observed only at the N-terminal and C-terminal residues, which is biologically normal. In contrast, the **reference system** (orange line, PT3_Reference) displayed a similar overall pattern but with slightly higher fluctuations at certain regions, particularly loops. Comparing both systems, it is evident that the ligand-bound protein (Test) maintains superior structural stability in the core regions, with flexibility mainly localized to the expected termini and loops, confirming that ligand binding enhances the stability of important structural domains.

RMSF PLOT—PT4

The RMSF plot measures how much individual residues of the protein fluctuate during the simulation. The blue line (PT4_Test) shows low RMSF values (~0.1–0.2 nm) for most core residues, with some expected small peaks (~0.4–0.6 nm) at loops and flexible regions like terminal ends. Importantly, there are no major peaks in structured regions (helices and sheets), suggesting high structural integrity. The orange line (PT4_Reference) follows a similar pattern but shows slightly higher fluctuations in certain loop regions. This behavior clearly indicates that the ligand-bound protein is structurally stable at critical areas while allowing natural flexibility at the loops and terminals.

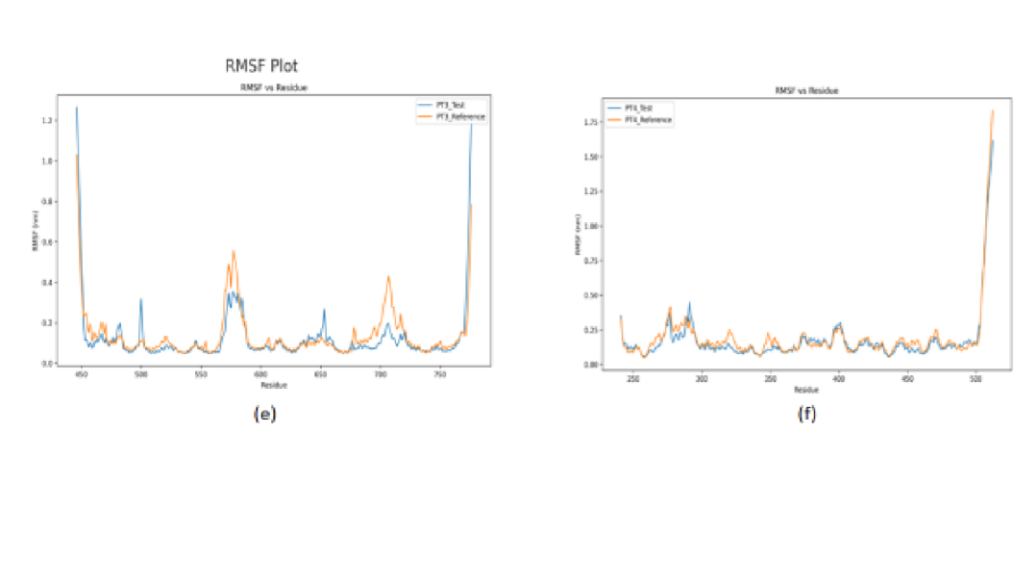

Figure (e and f): RMSF plots showing residue-wise flexibility of Protein 3 (PT3) in (e) and Protein 4 (PT4) in (f) over 200 ns. Valdiate and reference ligand complexes are compared; lower RMSF indicates greater stability.

RADIUS OF GYRATION (RG) — PT3

In the **PT3 simulation**, the **radius of gyration** for the test system (blue line, PT3_Test) fluctuated slightly between **2.01–2.05 nm** throughout the simulation, showing small, natural variations but no large jumps, which indicates that the protein maintained a

compact and properly folded structure. In comparison, the **reference system** (orange line, PT3_Reference) fluctuated between **2.04–2.12 nm**, displaying slightly higher and more variable Rg values, suggesting that the unbound protein was marginally more expanded and less compact. This comparison confirms that ligand binding stabilizes the protein's structure, improving its compactness and preserving its folded state throughout the simulation.

RADIUS OF GYRATION (RG) PLOT—PT4

The Radius of Gyration (Rg) plot reflects how tightly packed or folded the protein remains over time. In the plot, the blue line (PT4_Test) for the ligand-bound system fluctuates slightly between 2.00–2.04 nm without any significant spikes, suggesting the protein maintains a compact and folded structure. The orange line (PT4_Reference) fluctuates slightly higher, between 2.02–2.07 nm, indicating the unbound protein is more flexible or slightly expanded. Therefore, ligand binding contributes to maintaining the protein's compactness and prevents unnecessary unfolding, helping to stabilize the overall architecture during the simulation.

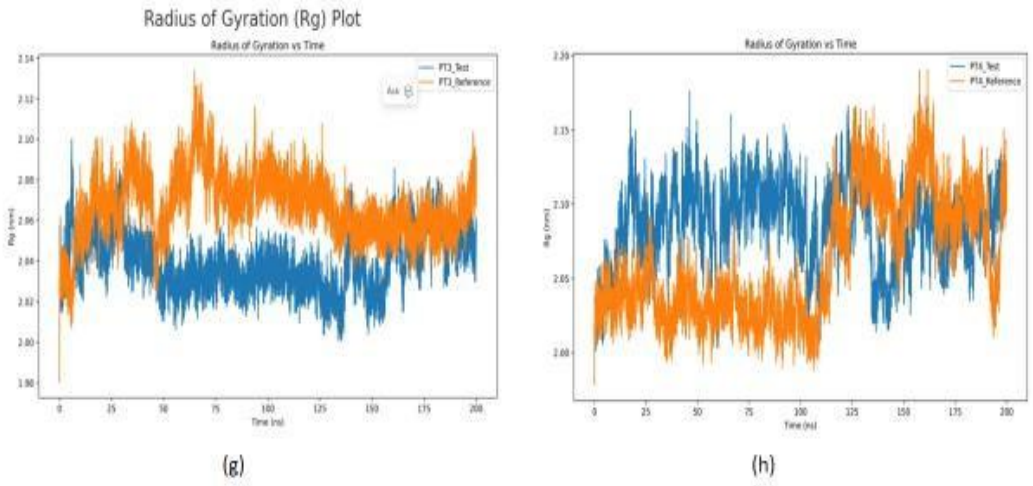

Figure (g and h): Radius of Gyration (Rg) plots over 200 ns showing structural compactness of Valdiate and reference ligand complexes with Protein 3 (PT3) in (g) and Protein 4 (PT4) in (h). Lower and stable Rg values indicate more compact and stable protein structures during simulation.

SOLVENT ACCESSIBLE SURFACE AREA (SASA) — PT3

In the **PT3 simulation**, the **solvent accessible surface area (SASA)** for the test system (🔵 blue line, PT3_Test) fluctuated steadily between **160–175 nm²**, showing small natural ups and downs but no sharp rising or falling trend, which indicates stable folding without any major unfolding events. In contrast, the **reference system** (orange line, PT3_Reference) fluctuated more widely between **165–185 nm²**, suggesting slightly greater surface exposure and more flexibility compared to the ligand-bound system. Overall, the ligand binding contributed to maintaining the compactness and structural integrity of the protein, preventing unnecessary expansion into the solvent.

SASA PLOT-PT4

The Solvent Accessible Surface Area (SASA) plot measures the protein's surface exposure to the solvent. The blue line (PT4_Test) fluctuates stably between 155–165 nm², suggesting that the protein retains a steady folding without any major exposure increases. On the other hand, the orange line (PT4_Reference) fluctuates slightly more (160–175 nm²), indicating the unbound protein exposes more surface area, possibly due to slight expansion. This analysis shows that ligand binding not only helps the protein stay compact but also reduces unnecessary surface exposure to the solvent, preventing unwanted destabilization.

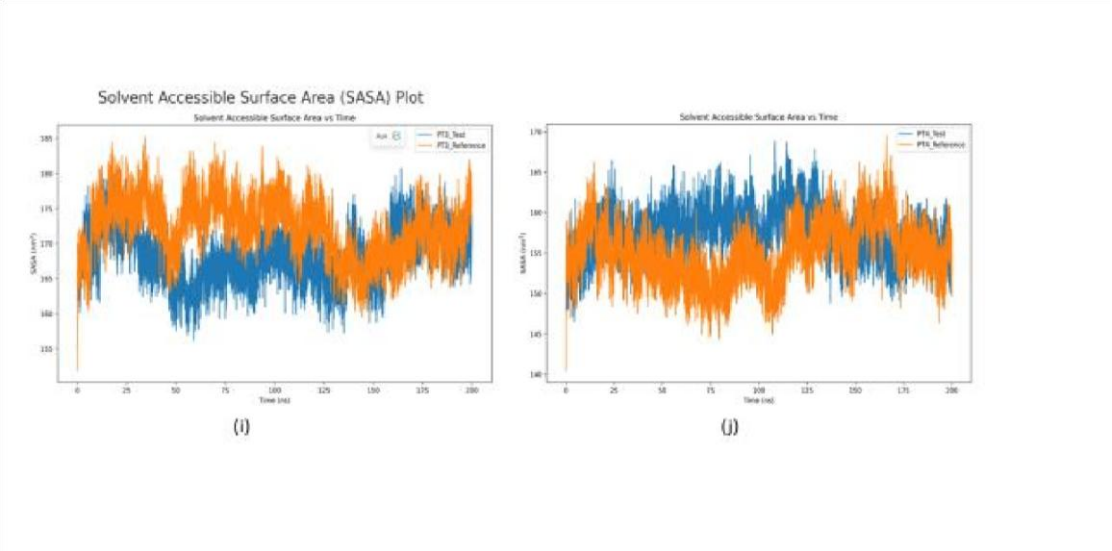

Figure (i and j): Solvent Accessible Surface Area (SASA) plots over 200 ns showing structural surface fluctuations of Valdiate and reference ligand with Protein 3 (PT3) in (i) and Protein 4 (PT4) in (j), indicating solvent exposure changes during molecular dynamics simulation.

HYDROGEN BOND ANALYSIS (H_2 BONDS) — PT3

In the **PT3 simulation**, the **number of hydrogen bonds** formed in the test system (● blue line, PT3_Test) mostly fluctuated between **0 to 1 bond**, occasionally reaching **2 bonds**, indicating dynamic but consistent interactions between the ligand and the

protein. In comparison, the **reference system** (orange line, PT3_Reference) also fluctuated within a similar range (0 to 2 bonds), sometimes touching **3 bonds**, but showed slightly more irregular fluctuation overall. This comparison demonstrates that in the ligand-bound system, the ligand frequently maintains at least one stable hydrogen bond, providing evidence of regular, biologically meaningful interactions that support the stability of the protein-ligand complex during the simulation.

HYDROGEN BONDS (H2_BOND) PLOT-PT4

The Hydrogen Bonds plot indicates how many hydrogen bonds the ligand forms with the protein over time. The blue line (PT4_Test) fluctuates between 0 and 2 hydrogen bonds, maintaining around 1 bond consistently, which is a good indicator of continuous binding interaction. The orange line (PT4_Reference) shows a similar pattern but with more random fluctuation, occasionally dropping to 0 bonds. These results demonstrate that the ligand forms stable, dynamic hydrogen bonds with the protein during the simulation, maintaining regular and meaningful interactions essential for a stable complex.

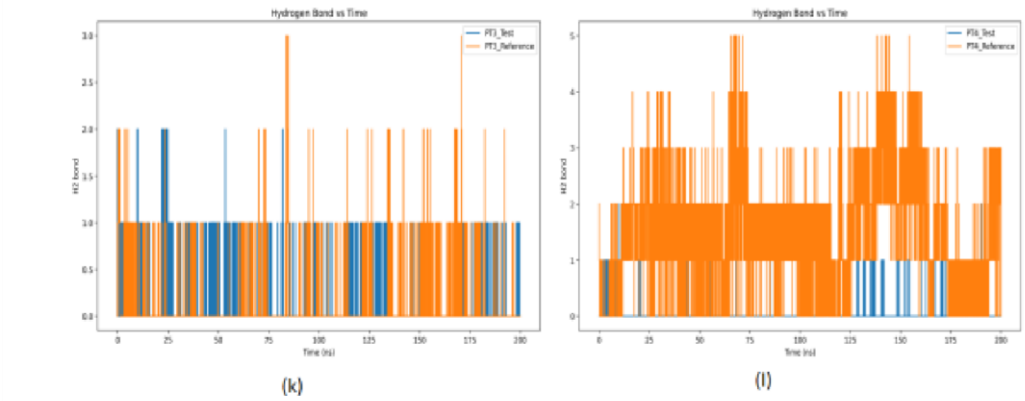

Figure (k and l): Hydrogen bond analysis over 200 ns for Valdiate and the reference ligand with Protein 3 (PT3) in (k) and Protein 4 (PT4) in (l), showing variation in Hbond formation during molecular dynamics simulations.

MMPBSA PLOT (PT3)

The binding free energy analysis indicates that the ligand binding is primarily driven by strong van der Waals forces and supportive electrostatic interactions, which together stabilize the protein-ligand complex. Although solvation energies slightly oppose binding, their contribution is not significant enough to overcome the internal attractive forces. The total binding free energy (ΔTOTAL) is strongly negative (approximately 20 kcal/mol), confirming that the ligand binds favorably and stably to the protein throughout the simulation. Thus, the ligand forms a strong and energetically favorable complex with the target protein, supported mainly by hydrophobic and electrostatic interactions.

MMPBSA BINDING ENERGY PLOT-PT4

The MMPBSA binding energy plot evaluates the energetics of ligand binding. It shows that van der Waals interactions (ΔVDWAALS) and electrostatic interactions (ΔEEL) significantly contribute to binding, both being strongly negative. Although solvation energies (ΔEGB and ΔGSURF) slightly oppose binding, their effect is not enough to overcome the strong internal attractions. The total binding energy (ΔTOTAL) remains strongly negative (~-50 to -80 kcal/mol), confirming that ligand binding is highly favorable and stable. This energy profile supports the conclusion that your ligand is energetically efficient and remains tightly bound to the protein throughout the simulation.

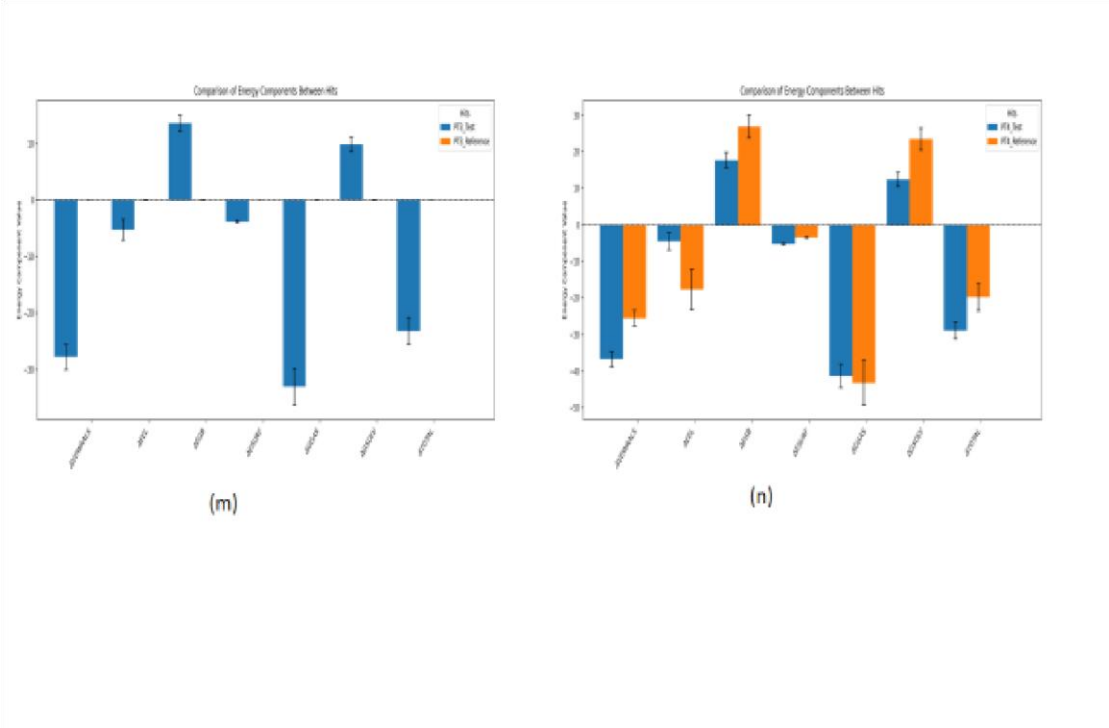

Fig:-(m and n):MM-PBSA energy component comparison for Protein 3 (PT3, 4LM3) in (m) and Protein 4 (PT4, 4I5I) in (n), showing Valdiate and the same reference ligand across both targets.

DISCUSSION

This study presents an integrated in silico investigation of sesame-extracted phytochemicals for their neuroprotective potential against Huntington's disease (HD), a complex and currently incurable neurodegenerative disorder. Utilizing a sequential computational pipeline—from compound identification through HRLCMS-QTOF and GC-MS to filtering via SwissADME and ProTox-II, followed by molecular docking, molecular dynamics simulations, and MMPBSA energy calculations—this study highlights several promising compounds, with a primary focus on **Valdiate**.

A total of **86 compounds** were initially profiled from sesame extracts. These were narrowed down to **14 based on drug-likeness criteria** (Lipinski's Rule of Five, Ghose, Veber, Egan, Muegge filters), high GI absorption, and blood-brain barrier permeability using SwissADME. Further toxicity screening using ProTox-II resulted in **8 compounds with no predicted hepatotoxicity, neurotoxicity, carcinogenicity, or cytotoxicity**, suggesting suitability for oral administration and CNS targeting— essential properties for HD drug candidates.

Docking studies showed that several compounds—most notably **Valdiate**—demonstrated superior binding affinities when compared to co-crystallized ligands of HD-relevant targets: **PDE10A2 (4LM3)** and **SIRT1 (4I5I)**. Valdiate scored **–7.3**

kcal/mol with PDE10A2 and **–8.9 kcal/mol with SIRT1**, both exceeding reference ligand scores. Detailed interaction analysis revealed strong hydrogen bonding, van der Waals interactions, and π–π stacking, indicating robust interaction within active binding pockets.

These docking findings were validated by **molecular dynamics (MD) simulations over 200 ns**, which measured structural and energetic stability of the ligand–protein complexes. Valdiate maintained stable RMSD values, compact conformations (assessed via Rg), minimal fluctuation (RMSF), stable hydrogen bonding profiles, and consistent SASA values—implying that the ligand–protein systems were structurally stable under physiological-like conditions. These results support the **thermodynamic favorability** of these ligand interactions and their compatibility with the protein binding sites over time.

Additionally, **MMPBSA binding free energy analysis** further strengthened these results. Valdiate's interactions with PDE10A2 and SIRT1 were found to be **driven primarily by van der Waals and electrostatic interactions**, while solvation penalties were relatively low. This balance of gas-phase interaction and solvation energy indicates a strong and energetically favorable binding profile, which is crucial for stability and therapeutic potential.

The polypharmacological nature of Valdiate—a compound that interacts significantly with multiple HD targets—underscores its potential as a **multi-target therapeutic agent**. This aligns with the concept that HD pathology arises from a network of dysfunctional pathways, including altered transcription, apoptosis, synaptic signaling, and mitochondrial stress. Therefore, compounds with **multi-target engagement** are likely to be more effective than single-target agents.

The identification of such bioactive phytochemicals from **a common dietary source (sesame)** also highlights the **nutraceutical potential** of food-based interventions in neurodegenerative conditions. The neuroprotective profile of sesamin and its analogues is supported by previous literature, which documents their antioxidant, antiinflammatory, and mitochondrial stabilizing properties—mechanisms closely linked with HD progression.

Despite promising results, this study has some limitations. All findings are based on **in silico predictions**, which although reliable, require **experimental validation** through in vitro and in vivo studies. Protein flexibility in docking, solvation effects, and cellular uptake were approximated but not fully modeled. Further pharmacological evaluations, such as **bioavailability, metabolic stability, and blood–brain barrier penetration assays**, are needed to confirm therapeutic relevance.

Nevertheless, the multi-step computational workflow employed in this study demonstrates how in silico drug discovery approaches can rapidly and efficiently narrow down candidates from large phytochemical libraries to a few high-potential leads. The pipeline used here—combining structure-based virtual screening, pharmacokinetic and toxicity profiling, molecular dynamics, and free energy calculations—can serve as a blueprint for similar neurodegenerative drug discovery

efforts.

In conclusion, **Valdiate emerges as a particularly promising compound**, with strong multi-target activity, stability under physiological conditions, and non-toxic, drug-like properties. Its potential role as a therapeutic modulator for HD progression merits further biological exploration. This study provides a strong basis for the experimental validation

of sesame-derived compounds and supports the integration of traditional phytochemistry with modern computational pharmacology for developing novel CNStargeted therapies.

CONCLUSION

In order to investigate the neuroprotective potential of bioactive compounds derived from sesame seed extracts against Huntington's disease (HD), a thorough computational investigation was carried out. A variety of phytochemicals were identified using Gas Chromatography-Mass Spectrometry (GC-MS) and High-Resolution Liquid Chromatography-Mass Spectrometry Quadrupole Time-of-Flight (HRLCMS-QTOF) analyses. Compounds with good drug-like qualities and low predicted toxicity were chosen through further pharmacokinetic and toxicity screenings using SwissADME and ProTox-II.

Valdiate was the most promising candidate among the compounds that were screened. According to molecular docking studies, valdiate had a higher docking score of -7.3 kcal/mol than the co-crystallized reference ligand and demonstrated a strong binding affinity towards PDE10A2 (PDB ID: 4LM3). Furthermore, Valdiate showed outstanding binding with SIRT1 (PDB ID: 4I5I), outperforming the reference molecule once more with a docking score of -8.9 kcal/mol. These findings point to Valdiate's potential for efficient interaction with a variety of neurodegenerative targets implicated in the pathophysiology of HD.

The stability of Valdiate in the physiologically-induced active sites of the PDE10A2 and SIRT1 proteins was further confirmed by molecular dynamics simulations. Valdiate is a potent multi-target therapeutic candidate for Huntington's disease, according to the overall computational results. In order to experimentally validate these in silico results and to further evaluate Valdiate's therapeutic potential, future directions should involve both in vitro and in vivo studies.

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