STRUCTURAL INSIGHTS INTO NATURAL COMPOUNDS AS INHIBITORS OF HUNTINGTON'S DISEASE.

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Abstract.
Huntington's disease is an autosomal dominant condition with a progressive neurological phenotype, chorea, dystonia, disorganized cognitive decline, and behavioural problems. Huntington disease is a rare neurological illness with a prevalence of 2.7 per 100,000 persons globally. Usually, a parent with Huntington's disease who possesses a mutation in the Huntingtin gene passes it on to their children (HTT). However, new mutations account for up to 10% of instances. Before the age of 20, up to 10% of those with the gene mutation experience symptoms, while between 4 and 11% experience them beyond the age of 60. The progression of HD cannot be slowed down or stopped by treatment. Effective disease progression biomarkers to evaluate therapeutic interventions are required because there are presently no commercially accessible medications that can successfully prevent the illness, decrease its development, or delay its start. However, disease-modifying treatments may show promise for the future. The urgent need is to discover HD inhibitors. In this review article we have tried to find some natural compounds those maybe potential inhibitors for this rare neurodegenerative disease using molecular docking software Autodock Vina; and the natural substance *Withania somnifera* exhibited promising molecular docking studies, making it a viable inhibitor for Huntington's disease.

Keywords: Huntington's disease, Neurodegenerative, Mutation, Biomarkers, Inhibitors, Docking.

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INTRODUCTION.

An autosomal dominant disorder with a progressive neurodegenerative phenotype that includes chorea, dystonia, uncoordinated cognitive decline, and behavioural issues is known as Huntington's disease[1]. Typically, the symptoms don't appear until the middle ages. It is known that, it can manifest at any time between infancy and senescence. The mutant protein, referred to as modified Huntingtin, has an expanded CAG repeat with a polyglutamine strand of variable length at the N-terminus[1]. The assumption that this tail delivers a detrimental increase in function is supported by evidence[1].Although research in transgenic animal models of the ailment is shedding light on potential causes and treatments, the precise pathophysical mechanisms of Huntington's disease are still not well known[1]. Unwanted erratic movements, behavioral and psychological disorders, and dementia are all symptoms of the rare neurological condition known as Huntington's disease[2]. Symptoms typically appear between the ages of 35 and 50[2]. Juvenile Huntington's disease is a condition in which symptoms first appear before the age of 20 and are accompanied by behavioural problems in the educational setting. The movement, thinking, intellectual, and psychological problems that Huntington's disease patients frequently encounter have a profound impact on their ability to function. Huntington's disease was the first

autosomal disorder; genetic linkage analysis and repetitive DNA polymorphisms were the only methods used to pinpoint the genetic defect's location on a human chromosome[3].

With an incidence of 2.7 per 100,000 people worldwide, Huntington's disease is an uncommon neurological condition. Due to the differences in ascertainment and diagnostic criteria, the prevalence varies geographically by more than ten times. Patients may have enlarged CAG repeats as a result of the disease's adult-onset. The lower end of the CAG repeats likewise only partially penetrates. As a result, the population as a whole may see expanded repeats more frequently. Asia exhibits a lesser prevalence, whereas Europe, North America, and Australia exhibit a higher prevalence. This can be a result of HTT gene halotypes. In the United States, experts believe that one in every 10,000 people, or approximately 30.000 people overall, has Huntington disease, with 16% of all instances being juvenile Huntington disease. No single population is more prone to having Huntington's disease than another. Sexes, all races, and ethnicities are impacted by it.
According to current estimates, there are 10 cases of Huntington disease per 100,000 persons, with incidence rates being greater in Australia, North America, North-western Europe, and the Middle East. Depending on the source of the information, the estimate ranges from 5.96 to 13.70 cases per 100,000 persons. An estimated 0.41 to 0.70 incidents per 100,000 people occur in Asia, according to estimates. But due to some undiagnosed individuals, the exact prevalence might perhaps be higher.

HISTORY.

Huntington's chorea is a different term for HD. Regardless of the fact that dance mania was becoming more and more popular in 1374; it was Paracelsus (1493–1541) who first used the name chorea to describe this movement disorder and proposed that the central nervous system (CNS) could be responsible for it. In the years that followed, up until the 17th century, the illness's exact nature remained a mystery, and it remained perplexing. The words "that illness" and "San Vitus dance" were terms used by English colonists to describe HD in the early 1600s. Victims with chorea were typically thought to be under the devil's spell since HD is marked by uncontrollable muscular jerks and twitches. HD supposedly had an effect on one of the suspected witches who was hanged in Salem, Massachusetts, throughout the 1690s.

Two decades later, in the 1840s, doctors in the US, UK, and Norway made the first attempt to define HD as "chronic hereditary chorea." The illness was first precisely described in 1872 by a 22-year-old American physician named George Huntington who practised in Long Island, New York. In the Medical and Surgical Reporter of Philadelphia, he provided a brief, coherent, anecdotal article on chorea. Researchers have spent more than a century attempting to identify the enormous populations of individuals who are most prone to get HD because of the genetic makeup of the disease [4].

CAUSE.

The HTT gene, which produces the huntingtin protein, contains an extended CAG trinucleotide repeat of variable length. Huntingtin is synthesised in unusually long polyglutamine sequences in mutation carriers, which confer harmful gains in function and make the protein more prone to fragmentation, which leads to neuronal malfunction and death[5]. A CAG repeat expansion that results in an unusually long polyglutamine (polyQ) tract in the huntingtin protein is the root cause of Huntington disease (HD)[1].Usually, a parent with Huntington's disease who inherits a mutation in the Huntingtin gene passes it on to their children (HTT). However, new mutations account for up to 10% of instances. The Huntingtin protein (Htt) is genetically encoded by this gene. Huntington's disease is brought on by a gene mutation on chromosome 4. Cytosine, Adenine, and Guanine (CAG) repeat expansion is the primary culprit. This is known as the Huntingtin gene's trinucleotide repeat expansion, which produces an aberrant mutant protein (mHtt). The mutated gene is bigger than it should be. The mutation causes an excessive amount of the DNA's cytosine, adenine, and guanine building components to be produced (CAG)[6]. CAG repeats in a healthy set are no more than 36 times, but in Hungtinton's disease, they exceed 36 times. A person may or may not acquire Hungtinton's disease if the repetition is between 36 and 39, but if it is between 40 and 50, there is a good chance that they will. A more hazardous form of the HTT protein is produced as a result of this alteration. The build up of the harmful protein in the brain damages some brain cells, causing severe

symptoms such problems with behaviour, speech, coordination, and mental well-being.

In the event of juvenile Huntington's disease, the symptoms might start considerably earlier or much later than the typical age range of 30 to 50[7]. After they first appear, the symptoms often get worse over time. Up to 10% of people with the gene mutation develop symptoms before age 20, and around 4-11% develop them after the age of 60[7].

GENE INVOLVED.

The Huntingtin gene (HTT), which generates the Huntingtin protein (Htt), is duplicated twice. A brief repeat known as a trinucleotide repeat expansion, which varies in length across individuals and may alter length between generations, is a created segment of the HTT gene, also known as the HD gene and the intriguing transcript 15 (IT15)[8]. A dynamic mutation may raise the number of repeats if the repeat is intact in the functional gene, leading to a faulty gene. A mutant Huntingtin protein (mHtt) is created when the length of this repeatedly occurring segment exceeds a predetermined threshold. These protein's diverse functions result in pathological alterations, which therefore lead to illness symptoms. A mutation in either of a person's HTT alleles causes the condition, which is genetically dominant. The extent of the repeating part of the gene, rather than sex, determines inheritance, therefore the parent with the disorder's severity can be the determinant.

Trinucleotide repeat illnesses, such as Hungtinton's disease, are brought on when the span of the recurring part of a gene is more than what is considered normal. At 4p16.3, on chromosome 4, the HTT gene is located. The trinucleotide repeat, or C, A, G repetition, is a sequence of three DNA bases that is present in the HTT gene. As the three-letter genetic code for the amino acid glutamine, CAG, is repeated across the polyglutamic tract, a section of the gene, it produces a chain of glutamine defined as a polyglutamic tract[8]. The polyQ region of humans normally has less than 36 repeating glutamines, which results in the synthesis of the cytoplasmic protein huntingtin. A combination of 36 or more glutamines, however, causes the emergence of a protein with unique features. Under the impact of this altered form, known as mutant huntingtin (mHtt), certain types of neurons deteriorate more quickly. Different areas of the brain are impacted differently because they depend on distinct types of neurons in varying amounts. The number of CAG repeats, which also is frequently associated with how significantly this process is altered, accounts for around 60% of the variance in the age at which symptoms first appear. Other genetic and environmental factors that have an impact on HD's underlying process account for the remaining heterogeneity. A diminished type of the sickness is created after 36 to 39 repetitions, with a noticeably later beginning and a more progressive escalation of symptoms. Quite often the onset is so late that no one notices the symptoms. Juvenile HD is a category of HD with incredibly high repeat counts that can present even before age of 20 (more than 60). Rigidity, convulsions, and slow movements are features of the Westphal form of juvenile HD. This accounts for around 7% of HD carriers.

Source: **RCSB PDB: Homepage** https://www.rcsb.org

INHERITANCE.

Affected individuals normally inherit one copy of the gene with an enlarged trinucleotide repeat from an affected parent, which means that the condition is inherited in an autosomal dominant manner. Those who have a mutant copy of the gene will develop the disease due to the high penetrance of the mutation[9]. Each affected person's offspring has a 50% chance of receiving the mutant allele and developing the condition under this type of inheritance pattern. This probability is gender-neutral.

Oogenesis has less instability than spermatogenesis. Alleles passed down through the maternal line often have repetition lengths that are similar, whereas those passed down through the male line are more likely to lengthen over time. If neither parent possesses more than 36 CAG repeats, rarely is a novel mutation responsible for Huntington's disease [5].

When either parent possesses two larger copies of the HD gene, that is exceptional, the likelihood increases to 75% and approaches 100%. It is unusual for someone to be affected by both genes. It was once thought that HD was the only disease in which the presence of a second mutant gene had no influence on symptoms or advancement; however, it has since been found that this is rarely the case and that it can affect both the phenotype and the pace of advancement[10].

MOLECULAR MECHANISM.

The genetic cause of HD is an increase of the CAG tri-nucleotide repeat sequence in exon 1 of the HTT gene. Affected individuals have between 16 and 20 more CAG repeats (> 35 repetitions) compared to the general population. This results in the translation of the huntingtin (Htt) protein, which is connected to protein aggregation and gain-of-function toxicity[11].

It is known that the exon-1 region of the Huntingtin (HTT) gene, which is found on chromosome 4, has undergone a mutation that has caused a CAG repeat region to grow there. The polyglutamine (polyQ) portion of the HTT protein's N-terminus increases in HD patients as a result. Therefore, although the HTT gene ordinarily contains roughly 35 CAG repeats, mutations that raise this to about 40 CAGs allow HD to develop with complete penetrance, whereas individuals with 36–39 CAG repeats within their HTT gene exhibit variance in the expression of HD[12]. Even though HD can be inherited in an autosomal dominant manner, it can vary between a parent and child with a tendency to increase in the generation after. This is because the amount of CAG repeats in the HTT gene is unstable. Also discovered to exhibit mosaicism-causing CAG repeat instability were the patient's brain and sperm cells. These extra CAG repeats may cause "gain-of-function" or "loss of function" in the wild-type HTT, as well as cardiac failure associated with HD and skeletal muscle atrophy. The majority of mHTT mutations disrupt normal protein function and promote a multitude of aberrant protein-protein interactions, which result in neuronal death and dysfunction in the cortex, striatum, and other regions of the brain[13]. The outcome is neuronal malfunction and cell death because mHTT interferes with a number of intracellular functions, including protein breakdown, mitochondrial respiration, and transcription. This is accomplished by abnormal interactions and the build up of mHTT aggregates, especially in the cell nucleus and neurophil of the afflicted neurons. Evidently, while the increase in CAG repeats is far less in other brain areas, it can exceed 1,000 in some subgroups of striatal neurons. Latest genomewide single nucleotide polymorphism (SNP) association research suggests that the DNA mismatch repair gene MutL homolog 1 (MLH1) and an SNP in a nuclear factor-B binding site in the HTT promoter may be involved in the altered onset of HD[14] .

Given the recent advancements in our understanding of the creation, processing, aggregation, and toxicity of mHTT, several therapeutic approaches have been put forth, some of which have demonstrated some promise outcomes against HD[15].

RESEARCH GAP.

There are two important areas of HD research: comprehending the neurotoxicity of mutant huntingtin protein to brain cells, and developing novel drugs to counter-act it. Due to the identification of the HD gene, which was made possible by NINDS-funded research, researchers are now able to enrol HD gene carriers in clinical studies before patients experience any symptoms. ln order to comprehend the effects of the broken gene on various sections of the brain as well as the chemistry and metabolism of the body. Several clinical signs of neurodegenerative diseases may be caused by the ultimate stage of neural circuit breakdown. Researchers are utilising cutting-edge methods like optogenetics, in which neurons in the brains of living animals are activated or silenced using light beams, to investigate such circuit issues in HD. In order to investigate the causes of diseases and test potential novel medicinal interventions, scientists are also using stem cells.

The development of HD cannot be slowed down or stopped by treatment. Treatment options for chorea associated with HD include tetrabenazine and deuterabenazine. Antipsychotic drugs can help control violent outbursts, delusions, and hallucinations while also reducing chorea. Medication can be used to treat depression and anxiety. Unwanted side effects of medications used to treat HD symptoms include drowsiness, lethargy, decreased attention, restlessness, or hyperexcitability. Only when an individual's symptoms are bothering them should these drugs be taken.

An international clinical study has recently shown that a potential treatment for Huntington disease is safe and successfully lowers levels of the abnormal protein that causes the condition in patients.

Known as HD, this fatal neurological illness is inherited. It often appears in adulthood and causes psychological illnesses, dementia, and strange involuntary movements. There are presently no known effective therapies to halt the progression

of this illness. The sole genetic mutation that has been identified as the cause of HD has a 50% risk of being passed on to each child of a carrier. Nine trial sites in Canada, the UK, and Germany each enrolled 46 early HD patients. 34 of the 46 patients were given the medicine at random, while 12 were given a placebo. Each participant received four doses of the drug, and all subjects continued to receive the drug as part of an ongoing open-label inquiry after the trial was over. The medicine was injected directly into the cerebrospinal fluid of the patients once each month. The researchers, led by Dr. Sarah Tabrizi, director of the University College London Huntington Disease Centre and global chief investigator of the IONIS-HTTRX clinical trial, found that the medication significantly reduced the levels of mutant huntingtin protein in the patients' cerebrospinal fluid. None of the patients experienced any serious adverse side effects, demonstrating the safety and favourable patient acceptance of the medicine.

The drug is presently being evaluated in a big phase three multi-centre clinical study at the UBC Centre for Huntington Disease and other HD facilities around the world. Finding out whether the drug prevents or delays the onset of disease symptoms is the aim of this investigation. I want to explore other options for the treatment and prevention of Huntington's disease through this review study.

BIOMARKERS FOR HUNTINGTON DISEASE.

Biological processes, pathological situations, or pharmacologic reactions to therapeutic interventions can all be predicted using a feature known as a biomarker, which can be repeatedly studied and assessed. Therefore, a biomarker may be related to the illness itself or a side effect of treatment. A disease biomarker needs to be consistent, accurate, sensitive, specific, repeatable, affordable, non-invasive, and patient acceptable. It needs to be closely related to pathophysiology as well[16].

Considering that there are currently no commercially available drugs that may successfully prevent the condition, slow down its development, or delay its onset, effective disease progression biomarkers as well as indicators to assess treatment interventions are necessary. Drugs that alter the course of disease, however, appear to have potential. HD has a premanifest period before any motor symptoms appear, even though the underlying pathogenic processes are still in operation throughout this time. Since HD can be diagnosed genetically, disease-modifying therapies can be assessed even before symptoms manifest. Therefore, to track changes in clinically asymptomatic persons, biomarkers that reveal underlying condition processes or development are required. In addition to assisting with the therapeutic treatment of current HD patients, indicators that offer an impartial benchmark for assessing HD severity may be particularly helpful in a clinical trial setting, possibly acting as surrogate endpoints. Since then, biomarkers have been used more often at every stage of the pharmaceutical development. Numerous biomarker studies have been conducted, and they all show how these clinical, imaging, and biofluid processes interact. The aetiology of HD is known to include a number of critical components, including impaired proteasome activity, dysregulated transcription, oxidative stress, mitochondrial and metabolic dysfunction, neuroinflammation, and microglial activation[17]. For therapeutic clinical studies to assess the efficacy of purported disease-modifying drugs during pre-manifest HD, biomarkers must serve as outcome indicators.

CLINICAL BIOMARKERS.

In therapeutic research for HD, the United Huntington's Disease Rating Scale (UHDRS) is currently used to assess therapy response and monitor disease progression. It is a collection of scales designed to detect overt clinical HD changes[17]. Because of this, the UHDRS might not be sensitive enough to detect the subtle traits present in certain pre-manifest people, especially those who are still a few years away from expressing the disorder. The Diagnostic Confidence Level Criteria of the UHDRS are utilised in research to pinpoint the clinical onset of HD. Whenever a physical examination reveals sufficient motor deficits to provide the examiner with 99% assurance that the subject has HD, this dichotomous time point is reached. Furthermore, HD patients' motor traits typically decrease under stress, making them an unsatisfactory continuous assessment[18]. The subjective classification of illness onset might also appear to be quite arbitrary given that the disease has a prodrome that lasts for years and involves a variety of mild motor and cognitive deficits. Consequently, it would be quite implausible to utilise illness

manifestation as an end goal in clinical studies without long-term follow-up of a significant pre-manifest research group. An objectively observable condition biomarker that can also be used to gauge the severity of the disease in patients who have not yet shown symptoms must take the place of this arbitrary, motor-centric time-point. Pre-manifest trial participants are now staged depending on how much time will pass before their anticipated disease emerges; this is calculated using a patient's age and the non-standardized estimate of the CAG repeat length[17].

As an alternative to the UHDRS's binary concept of "disease onset," some academics have recommended include continuous measures, such as clinical features. It is possible to objectively quantify some UHDRS motor abnormalities, which improves accuracy and reduces inter- and intra-rater variability. There are deficiencies in this task even in HD that isn't yet evident, and they get worse over time and get worse along with other clinical features. Subtle cognitive impairment can also be seen in pre-manifest patients. As a result, their usefulness as outcome markers for use in clinical studies for preventative medicine is limited. These clinical indications, however, haven't shown to be sufficiently sensitive to gradual changes during the course of the pre-manifest period.Along with the ceiling effect in pre-manifest HD, clinical biomarkers are also susceptible to floor effects in advanced illness. Additionally, they are subject to vary based on the emotional states of the respondents, and they may be made more difficult by elements like the retest effect, educational level, and potential language barriers among non-native speakers. Finally, relying on clinical signs has the added challenge of attempting to distinguish a rise in symptoms from a change in the root condition[17].

WET BIOMARKERS

Wet biomarkers, particularly if they reflect the pathobiology of the disease, are yet another potential source of useful outcome measurements. Multiple potential molecular markers have been discovered as a result of the connection between HD and several pathogenic pathways[19].

One potential wet biomarker being researched that is probably HD-specific is the mutant Huntingtin protein (mHtt). The fact that it is the primary pathogenic molecule in HD makes this an appealing option. Along with being detectable in CSF and blood, it may also be measured in saliva, making it possible to have easy access to a protein whose effects are mostly harmful to the central nervous system. Peripheral immune cells from HD gene carriers contain higher levels of mHTT[20], had differences in mean mHTT amongst pre-manifest and early-stage HD patients, but none between early-stage and moderate-stage HD individuals[19].

Nonetheless, MHtt has proven useful as a pharmacologic marker of nucleotide-based gene silencing treatments meant to lower mHtt in animal studies. A human efficacy biomarker test for MHtt is still pending. A significant phenotypic improvement was seen when antisense oligonucleotides (ASO) and RNA interference (RNAi) both reduced the concentration of mHtt in the striatum and CSF. Gene silencing for HD is now undergoing its first human study. It is evaluating an antisense oligonucleotide that specifically targets mutant Huntingtin RNA and is projected to have a negative effect on mHtt production and, as a result, decrease the neuronal degeneration associated with HD. One of the study's outcome variables is undoubtedly mHtt, which acts as an effectiveness biomarker[19].

REVIEW OF LITERATURE.

An uncommon hereditary disorder called Huntington's disease causes the gradual death of brain nerve cells. Huntington's disease, which frequently results in issues with movement, thinking, cognitive abilities, and psychological abilities, has a significant influence on a person's functional capacity[21].

Huntington's disease symptoms often appear in individuals in their 30s or 40s, however they can appear at any age. When the ailment initially appears before the age of 20, it is known as juvenile Huntington's disease. Huntington's disease in its early stages has slightly different symptoms and could progress more swiftly[21].

Huntington's disease commonly results in mobility, intellectual, and psychological deficits, and its signs and symptoms can vary widely. Which symptoms appear first varies significantly from person to person. While certain symptoms may be more noticeable than others or have a greater impact on functionality, this might change during the course of the disease. Movement abnormalities linked to Huntington's disease can

impair both deliberate and involuntary movements, leading to symptoms like:
- Chorea
- Dystonia
- Unusual Or Slow Eye Movements
- Gait, Posture, And Balance Issues
- Difficulty Swallowing Or Speaking

Comorbidities in voluntary movements may have a greater detrimental effect on a person's ability to work, do daily tasks, communicate, and preserve independence than on involuntary motions.[3]. The psychological disorder most typically associated with Huntington's disease is depression. This isn't only a response to the discovery of Huntington's disease. Instead, depression appears to be caused by brain injury and subsequent changes in brain function[9]. Some warning signs and symptoms include:
- Feeling Irritable, Depressed, Or Uninterested
- Social Isolation
- Insomnia
- Fatigue And Energy Decline
- Frequently Having Thoughts Of Suicide Or Death

Some other psychiatric conditions may include:
- Obsessive-compulsive disorder.
- Mania, which can result in hyperactivity, impulsive conduct, high moods, and exaggerated self-esteem, A illness known as bipolar disorder.

Patients with Huntington's disease commonly have weight loss in addition to the previously mentioned issues, particularly as the disease progresses.

Over 100 distinct proteins are interacted with by the huntingtin protein, which appears to have a range of functions. Although the action of the altered protein (mHtt) is not entirely known, various cell types, particularly brain cells, are adversely affected[6]. As the illness worsens, other areas of the brain, including the cerebral cortex, also suffer damage. The striatum's subcortical basal ganglia are the first to suffer damage.

If physical HD symptoms begin to manifest, the sickness can be recognised. If HD is not inherited, genetic testing can be done to validate a physical diagnosis. Genetic testing can establish the presence of the disease-causing trinucleotide repeat (CAG) in the HTT gene in an individual or developing embryo even before symptoms appear. Prior, during, and after the evaluation process, as well as regarding the repercussions of a positive diagnosis, genetic counselling is offered. These effects can have an impact on a person's mental health, their decision to create a family, and their relationships with family and friends, among other things. Although presymptomatic testing was available, only 5% of people at risk of inheriting HD chose to undergo it[3].

METHODOLOGY.

We will first lookout for a target protein that we would like to work on.

➢ ***Target protein.***

Crystal structure of scFvC4 in complex with the first 17 AA of huntingtin
PDB DOI:10.2210/pdb4RAV/pdb
Classification:IMMUNE SYSTEM/Apoptosis
Organism(s): Homo sapiens
Expression System: Escherichia coli
Mutation(s): No Source:
RCSB PDB: Homepage
https://www.rcsb.org

Huntington's disease is brought on by the misfolding of fragments of the mutant huntingtin protein (mHTT), which have aberrant polyglutamine expansions. The huntingtin HTT (1-17), first 17 residues are where the C4 singlechain Fv antibody (scFv) attaches, providing both in situ and in vivo considerable protection against a range of phenotypic diseases. By studying the crystal structure of the complex between C4 scFv and HTT, we further investigate the structural basis for this inhibition and protection (1-17). As the peptide binds to residues 3–11, an amphipathic helix is created, establishing contact with the antibody fragment while shielding the hydrophobic face from solvent. The peptide's residues 12–17 are in an extended conformation and connect with the same region of another C4 scFv:HTT(1–17) complex in the asymmetric unit to form a dimeric C4 scFv:HTT(1–17) complex with a -sheet interface[22]. High-resolution NMR and physic-chemical characterization of species in solution were used to further investigate the nature of this scFv-peptide combination in solution. The findings provide a structural foundation for the early interactions that cause the production of disease-associated amyloid fibrils by HTT and shed light on how C4 scFv inhibits the

aggregation of HTT and, consequently, its therapeutic potential[23].

➢ ***Natural compounds that can be used to form compounds with the target protein.***

- ***Withania somnifera***

Withania somnifera, an indigenous plant found in the country India, the Middle East and various parts of Africa. This is commonly known as Ashwagandha or winter berry. Short and delicate, this species grows to a height of 35–75 cm. Radially branching from a central stem are the tomentose branches[24]. The leaves are oval, dull green, and often 10–12 cm long. Small, green, and bell-shaped, the blooms are. Fruit that is fully ripe is orange-red. *Withaniasomnifera* is vulnerable to a number of illnesses and pests. One of the most valuable medicinal plants,
Withaniasomnifera is thought to have anti-inflammatory, immunomodulatory, and antioxidant effects[25].

Table 1. Withania somnifera: Taxonomical characteristics (Source: Wikipedia)

Organism Name:	*Withaniasomnifera*
Matched Taxonomy Level:	Species
Genus:	*Withania*
Family:	Solanaceae
Kingdom:	Viridiplantae
SuperKingdom:	Eukaryota

Source: Wikipedia
https://en.wikipedia.org/wiki/Withania_somnifera

- ***Curcuma longa***

The rhizomes of the blooming *Curcuma longa* plant, which belongs to the Zingiberaceae family that also includes ginger, are what are utilized to make turmeric. The plant is a perennial, rhizomatous, herbaceous plant that is native to the Indian subcontinent and Southeast Asia. Since ancient times, curcuma longa has been widely used in traditional medicine for its antiinflammatory properties, which have received substantial scientific support[26].

Although turmeric and curcumin have been used for many years in Ayurvedic treatment, there is no reliable clinical proof that these substances are useful in curing any diseases. Numerous clinical experiments have investigated the effects of turmeric and curcumin on a range of human diseases and disorders, but no strong evidence of a disease-preventing or health-improving effect has been found[27]. As of 2020, there is no proof from science that Curcumin lowers inflammation. Weak data suggests that turmeric extracts may be helpful for easing knee osteoarthritis symptoms as well as for minimizing pain and muscle damage after physical activity[28].

Table 2: Curcuma longa: Taxonomical characteristics (Source: Wikipedia)

Organism Name:	*Curcuma longa*
Matched Taxonomy Level:	Species
Genus:	*Curcuma*
Family:	Zingiberaceae
Kingdom:	Viridiplantae
SuperKingdom:	Eukaryota

Source: Wikipedia
https://en.wikipedia.org/wiki/Turmeric

- ***Flavonoids***

Fruits, vegetables, grains, bark, roots, shoots, flowers, tea, and wine are just a few of the foods that include flavonoids, a class of chemical compounds with diverse phenolic structures. Flavonoids may also be found in some beverages, such as chai tea and wine. Attempts are being made to separate the purported flavonoids from the other components since these organic chemicals have several beneficial health benefits. In a range of nutraceutical, pharmacological, therapeutic, and cosmetic purposes, flavonoids are now widely acknowledged to be essential. This is accounted for by their anti-oxidative, antiinflammatory, anti-mutagenic, and anticarcinogenic qualities as well as their ability to influence the operation of crucial cellular enzymes. In plants, they serve as antioxidants, bacterial and anti-inflammatory agents[29.] Numerous local and systemic diseases, including cancer, heart disease, diabetes, and celiac disease, have inflammation as a potential root cause. There is no scientific proof that any of these disorders are impacted by dietary flavonoids. According to a recent assessment, eating foods high in flavonoids may lower your chance of developing some cancers, such as colorectal, breast, prostate, and stomach cancer[30].

➢ **Tools that are used.**

- ***Pymol.***

PyMOL is a cross-platform molecular graphics programme that allows users to view electron densities, surfaces, trajectories, proteins, nucleic acids, and small molecules in three dimensions (3D)[31]. Additionally, it can create movies, change

molecules, and ray tracing. To enhance its capabilities and make the construction of PyMOL drugs simpler, this Python-based programme and a variety of Python plugin tools have been developed[31] .PyMOL is a proprietary, opensource molecular visualization programme created by Warren Lyford DeLano[31]. It was initially made commercially available by DeLano Scientific LLC, a private software company focused to creating useful tools that are widely available to scientific and educational groups[31].

- ***PubChem.***

PubChem is a database that contains information on chemical substances and how they react in biological investigations[32].

The system is maintained by the US National Institutes of Health's National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine (NIH). PubChem is available for free viewing via a web interface. In PubChem, there are numerous descriptions of different compounds as well as small molecules with less than 100 atoms and 1,000 bonds. Numerous database providers—more than 80 in total— support the growing PubChem database [33].

- ***Auto Dock Vina.***

Auto Dock Vina is a widely used and one of the quickest open-source docking engines[34]. According to tests on the training set used to develop AutoDock 4, AutoDockVina significantly improves binding mode predictions while speeding up molecular docking software by a factor of two. The whole computational docking method is based on a fast conformational search with gradient optimization and a simple scoring mechanism. With a two-orders-of-magnitude increase in speed, Auto Dock Vina not only significantly increases the binding mode predictions' accuracy but also their precision[34].

Auto Dock Vina generates grid maps automatically, clusters the results in a way that is transparent to the user, and then displays the results[35].

We will use these tools to create natural compounds that have the potential to inhibit Huntington disease by acting on the target protein

STEP-WISE PROCEDURE FOR MOLECULAR DOCKING

STEP 1.Preparation

Among the preparatory steps, at first we will download our target protein structure from PDB. The next action in this step would be visualizing the downloaded structure in PyMol and mainly perform these three steps:

1. Addition of polar hydrogen to the molecule.
2. Computation of the charge of the molecule.
3. Save the newly prepared molecule in PDB format.
4. The second step is to download the sdf file of the ligands required, namely *Withania somnifera*, *Curcuma longa* and Flavanoids. We can convert the sdf file to pdb by the online software known to be Open Babel.
5. After converting we can open it in pymol and make the necessary change that is:
6. Addition of hydrogen to the molecule. And save the file in PDB format.

STEP 2. Docking.

Molecular docking is a vital technique in computer-aided drug design and structural molecular biology. The goal of ligand-protein docking is to predict how a ligand will primarily interact to a protein with a recognised threedimensional structure[36]. Effective docking algorithms analyse high-dimensional spaces and employ a scoring scheme that correctly ranks probable dockings. Due to its ability to virtually screen large chemical libraries, rate the results, and provide structural hypotheses for how the ligands block the target, docking is a potent tool for lead optimization [37].

There are two distinct docking types.

- Rigid docking
- Flexible docking

- **Rigid docking**

Assuming the compounds are rigid, we are looking for a three-dimensional rearrangement of one of the compounds that produces the best match to the other compounds in terms of a scoring system. It is possible for the conformation of the ligand to occur with or without receptor binding activity[38].

- **Flexible Docking**

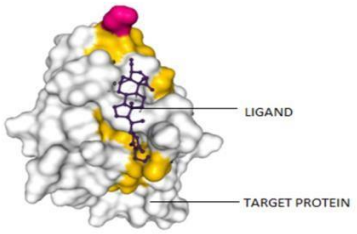
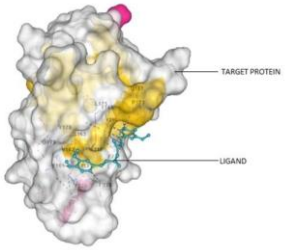
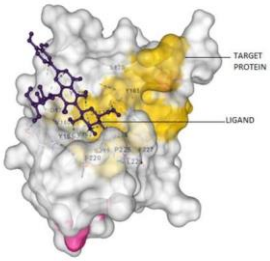
To find confirmations for the receptor and ligand molecules as they are present in the complex, we analyze molecular flexibility along with transformation[39].

The ability to virtually screen large chemical libraries, score the results, and provide structural suggestions for how the ligands block the target make docking a potent tool for lead optimization[40]. The molecular docking approach may model the atomic-level interaction between a small molecule and a protein, enabling us to define how small molecules behave at the binding site of target proteins and to better comprehend fundamental biological processes[41].

RESULTS AND DISCUSSION.

Molecular docking has shown us the clear results of the natural compounds that can be considered to be a future potential inhibitor of Huntington's disease. According to our results, *Withania somnifera* is the most suitable natural compound to act as an inhibitor of Huntington's disease due to its higher negative value against the other two ligands i.e *Curcuma longa* and *Flavanoids.* The results of the molecular docking done using Autodock Vina is tabulated below, TABLE 3:

Table 3. Protein and ligand docking score with their respective cavity size, Results are obtained from molecular docking completed with the help of AutoDockVina.

TARGET PROTEIN	LIGAND	VINA SCORE	CAVITY SIZE	THE COMPLEX OF LIGAND AND PROTEIN.
Crystal structure of scFvC4 in complex with the first 17 AA of huntingtin Source: **RCSB PDB:** **Homepage**https://www.rcsb.org	*Withania somnifera*	**-7.5**	**71**	 **Fig 1.tiff.** Target protein and *Withania somnifera complex.*
Crystal structure of scFvC4 in complex with the first 17 AA of huntingtin Source: **RCSB PDB:** **Homepage**https://www.rcsb.org	*Curcuma longa*	**-6.8**	**32**	 **Fig 2.tiff.** Target protein and *Curcuma longa complex.*
Crystal structure of scFvC4 in complex with the first 17 AA of huntingtin Source: **RCSB PDB:** **Homepage**https://www.rcsb.org	*Flavonoids*	**-6.6**	**77**	 **Fig 3.tiff.**Target protein and *Flavonoidscomplex.*

CONCLUSION.

The gradual death of brain nerve cells is a symptom of the uncommon hereditary disorder Huntington's disease. The symptoms of Huntington's disease, an uncommon neurological ailment, include uncontrollable movements, behavioral and psychiatric issues, and dementia. The movement, thinking, cognitive, and psychological problems that Hungtinton's disease patients frequently encounter have a considerable impact on their ability to function. Using genetic linkage analysis and widespread DNA polymorphisms, the genetic defect for the first autosomal disease, Hungtinton's disease, was discovered on a human chromosome.

Huntington's disease is brought on by a gene mutation on chromosome 4. Cytosine, Adenine, and Guanine (CAG) repeat expansion is the primary culprit. This is known as the huntingtin gene's trinucleotide repeat expansion, which produces an aberrant mutant protein (mHtt). The mutation causes an excessive amount of the DNA's cytosine, adenine, and guanine building components to be produced (CAG). CAG repeats occur 36 times or fewer in the normal set, but are more than 36 times in Huntington's disease. A person may or may not get Hungtinton's disease if the repetitions range from 36 to 39, but if they reach about 40, they almost surely will. The research gap was seen to be the lack of cure to this condition. The progression of HD cannot be slowed down or stopped by treatment. Treatment options for chorea associated with HD include tetrabenazine and deuterabenazine. Antipsychotic drugs can help control violent outbursts, delusions, and hallucinations while also reducing chorea. Medication can be used to treat depression and anxiety. Unwanted side effects of medications used to treat HD symptoms include drowsiness, lethargy, decreased focus, restlessness, or hyperexcitability. Only when an individual's symptoms are bothering them should these medications be taken. Our review tried to look for natural alternatives that can be used as inhibitor for Huntington's disease with the help of molecular docking by taki ng three natural compounds to bind with the target protein namely *Withania somnifera*, *Curcuma longa and flavanoids*. The results for our molecular docking were positive for the natural compound *Withania somnifera* making it one of the prominent potential inhibitor for Huntington's disease.

FUTURE PROSPECTS.

After literature review and working on docking analysis we got to know about neuro-protective capabilities of certain natural compounds against HD, we plan to work on more natural substances for finding potent inhibitors for HD.

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Conflict of interest.
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All authors contributed to the study conceptionand design. The idea for the article was provided by Dr. Ashwani Kumar. Material preparation, data collection and analysis were performed by ritagya Gogoi. The first draft of the manuscript was written by Kritagya Gogoi and all authors Kritagya Gogoi. The first draft of the manuscript commented on previous versions of the manuscript. All authors read and approved the final manuscript.