Therapeutics Application of Anti-cancerous drugs in Neurodegenerative disorders

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Submitted by

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CERTIFICATE

This is to certify that the dissertation entitled "**Therapeutics Application of Anti-cancerous drugs in Neurodegenerative disorders"** submitted by **Abhishek Shrivastava (DTU/14/M.Tech/084)** in the partial fulfilment of the requirements for the award the degree of Master of Technology (Industrial Biotechnology), Delhi Technological University (Formerly Delhi College of Engineering), is a *bona fide* record of the candidate's own work carried out by him under my guidance. The information and data enclosed in this thesis is original and has not been submitted elsewhere for honoring of any other degree.

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DECLARATION

I, **Abhishek Shrivastava** hereby declare that the dissertation entitled **Therapeutics Application of Anti-cancerous drugs in Neurodegenerative disorders** has been undertaken by me for the award of Master of Technology in Industrial Biotechnology. I have completed this study under the guidance of **Dr. Pravir Kumar**, Associate professor at "Molecular Neuroscience and Functional Genomics Laboratory", Department of Biotechnology**,** Delhi Technological University, Delhi. I also declare that this dissertation has not been submitted for the award of any Degree, Diploma or any other title in this university or any other university.

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I Abhishek Shrivastava, student of M.Tech-Industrial Biotechnology, registration number-DTU/14/M.Tech/084 is presenting a project report on **"Therapeutics Application of Anti-cancerous drugs in Neurodegenerative disorders"**. I would like to acknowledge my mentor, Dr. Pravir Kumar for providing this wonderful opportunity to further my education and training. It has been a road filled with many trials and tribulations and Prof. D. Kumar has been committed to my success and development as a researcher and scholar. For this, I offer my deepest gratitude.

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LIST OF ABBREVIATIONS

NDDs	Neurodegenerative Disorders
PD	Parkinson's disease
AD	Alzheimer's disease
TDP-43	Tar DNA binding protein-43
Aβ	Amyloid beta
APP-CF	Amyloid precursor protein cytoplasmic fragment
BACE-1	Beta site APP cleaving enzyme-1
DLB	Dementia with Lewy bodies
MSA	Multiple system atrophy
HD	Huntington's disease
ALS	Amyotrophic Lateral Sclerosis
kDa	Kilo Daltons
SNCA	Alpha synuclein
NCBI	National Centre for Biotechnology Information
MPTP	Neurofibrillary tangles
SNCB	Beta synuclein
SNCG	Gamma synuclein
PDB	Protein Databank
LB	Lewy bodies
PTM	Posttranslational modifications
ATP	Adenosine triphosphate

Therapeutics Application of Anti-cancerous drugs in Neurodegenerative disorders

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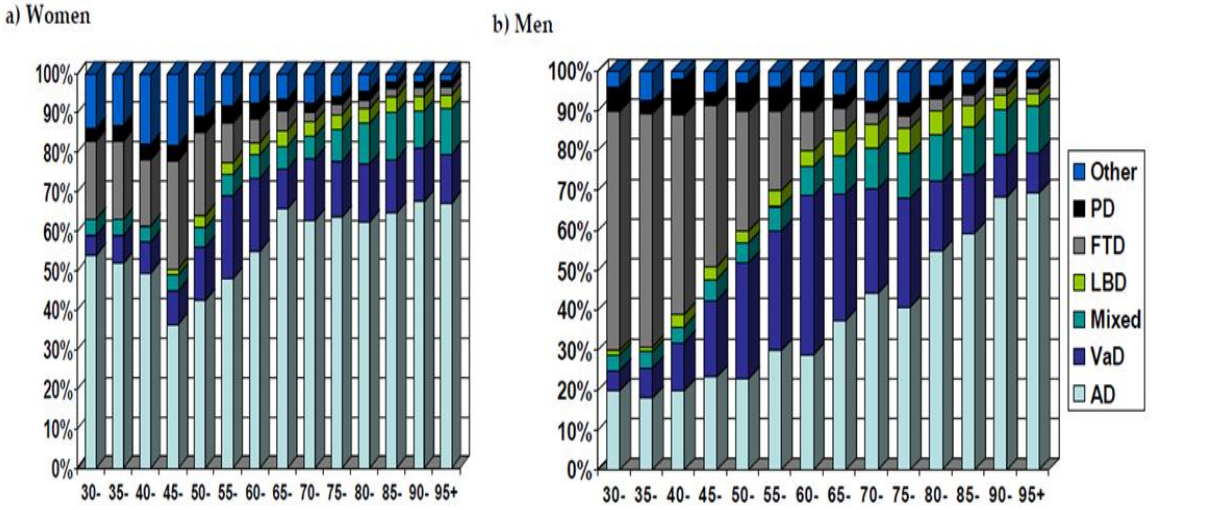
1. ABSTRACT

Neurodegenerative diseases (NDDs) are chronic devastating ailment manifested by selective loss of neurons associated with accumulation of altered proteins in the brain. Despite a plethora of modifications identified for different neurotoxic proteins, such as tau, amyloid-β, α-synuclein and prion protein, NDDs are classified on the basis of comprehensive and extensive evaluation of the morphological and structural features of aggregated protein deposits, and their association with clinical symptoms in the pathophysiology of disease. Compelling evidence suggests that accumulated protein deposits show a hierarchical involvement of distinct regions of brain and triggers the progression of a number of neurological disorders that have been categorized as tauopathies, TDP-43 proteinopathies and α-synucleinopathies. α-synuclein is among the center of focus to comprehend a number of NDDs involving Parkinson's disease (PD), Dementia with Lewy bodies (DLB) and Multiple System atrophy (MSA), known as α-synucleinopathies. α-synuclein has also been found secondarily in a number of other diseases such as Alzheimer's disease (AD), Huntington's disease (HD) and Amyotrophic Lateral Sclerosis (ALS). Understanding how a normal α-synuclein protein forms aggregates and deposits is an important aspect in the pathophysiology of these diseases. In this work, we have focused on the toxic form of α-synuclein protein. The objective of present study is to identify potent inhibitors (ligands)

which interact with the active site of toxic α-synuclein protein and inhibit its accumulation. In this study, insilico molecular docking studies was performed against α-synuclein using three plant derived compounds having anticancerous property: (a) Genistein (b) Hesperidine, and (c) Epigallocatechin-3-gallate. These compounds were analyzed using Lipinski filter, Autodock and LIGPLOT analysis tools. Further, our study revealed that Genistein was the best fit ligand against the active site of α-synuclein with minimum binding energy and inhibition constant. Finally, these natural compounds with the ability to interact with α-synuclein *in silico* can further be analyzed using *in vitro* and *in vivo* studies.

2. INTRODUCTION

Neurodegenerative disorders (NDDs) are one of the most dreadful adult-onset diseases these days and share a common phenomenon of degradation of neurons which progresses as the disease develops (Takalo et al., 2013). At some circumstances, partial relief is provided to patients by treating some of their symptoms but these diseases are still incurable (Beiske et al., 2009). Since, the underling mechanisms underlying the death of neurons in the specific areas of brain remains mysterious, degeneration of neurons continue inexorably and the symptoms often become unresponsive (Gorman, 2008). Further, it has been reported that accumulation of neurotoxic proteins such as α-synuclein, Amyloid beta (Aβ) and tau are accountable in the development of NDDs (Lonati et al., 2014). α-synuclein has been found to be implicated in numerous NDDs such as Parkinson's disease (PD) and Alzheimer's disease (AD) (Xie et al., 2014). Disorders characterized by the aggregation of α-synuclein are known as α-synucleinopathies (Brück et al., 2016). Together, these diseases have an emotional impact on approximately 5 million people worldwide and stand among one of the most devastating disorders in the United States and Europe (Cuny, 2012). Economical and psychological burden is posed on the caretakers, thus these diseases are troublesome not only for the patients but also for their caretakers (Habermann et al., 2013). Estimate of 2012 stated the progression of a number of NDDs worldwide which has been shown in **Figure-1**.



Source: *Dementia: a public health priority*. WHO, 2012.

PD. Parkinson's disease, FTD Frontotemporal dementia, LBD Lewy bodies dementia, VaD Vascular disease, AD Alzheimer disease.

(Image Source: www.who.int/medicines/areas/priority_medicines/BP6_11Alzheimer/)

Figure 1: Statistical analysis and data estimate of 2012 for the percentage distribution of NDDs in the differently aged people. This statistical data represents that AD is prevalent in 65% of aged people whereas the prevalence rate of PD, LBD and other NDDs are 95-99%.

Implication of α-synuclein is the most compelling evidence for the crucial role that it plays in the pathophysiology of PD and other synucleinopathies (Peelaerts et al., 2015). α-synuclein is the principal component of Lewy bodies, insoluble aggregates of protein, found in PD, AD and other Lewy body diseases (Schulz-Schaeffer, 2010). Lewy bodies are spherical and eosinophilic neuronal intracytoplasmic inclusions, composed of abnormal filamentous assemblies of α-synuclein (Spillantini et al., 1998). Moreover, presence of Lewy bodies in the cerebral cortex of dementia patients and in the substantia nigra of PD cases were reported. After the depiction of cortical Lewy bodies, similar cases were also reported and these diseases were considered as continuum disorders, and a frequent term Lewy body disease (LBD) was recommended (Hartmann, 2004).

Normal function of α-synuclein is to control vesicular neurotransmission at synaptic junctions, its pathogenic effects are associated with a wide range of cellular functions including mitochondrial activity, autophagic and proteasomal degradation of toxic proteins (Bobela et al., 2015). These functions are lost due to a number of modifications in the structure of α-synuclein, including phosphorylation, nitration, ubiquitination, and truncation (Schmid et al., 2013). Truncation of α-synuclein at the C-terminal leads to the aggregation of protein quicker than normal full-length protein and plays a critical role in the formation of Lewy bodies and progression of disease (Li et al., 2005).

Additionally, it has also been identified that α-synuclein acquires toxic or abnormal functions when neuronal cells are exposed to various other stressors such as genetic and environmental factors (Cook et al., 2012). Employment of various environmental toxins or heavy metals affects mitochondrial integrity leading to neuronal death (Ischiropoulos and Beckman, 2003). Thus, the pattern of α-synuclein pathology plays a key role in the central nervous system (CNS) but only specific neurons undergo degeneration (Osterberg et al., 2015). Similarly, Generation of free radicals and oxidative stress also play a major role in the pathogenesis of most of the neurological disorders (Guo et al., 2013). Reactive oxygen species (ROS) are active and dynamic in brain and neuronal tissue as neurotransmitters, excitatory amino acids and neuromodulators, and their metabolism serves as a source of oxidative stress (Alexander, 2004). ROS attack neurons and glial cells and are sensitive to free radicals, ultimately leading to neuronal damage (Uttara et al., 2009). It has been reported that antioxidant compounds possessing pharmacological activities such as anti-inflammatory, antitumor, anticarcinogenic, antiatherosclerotic, antibacterial, antimutagenic and antiviral activities upto some extent might also have ameliorative role in the treatment of most of the neurological disorders (Li et al., 2013). In numerous cases, increase in the level of oxidative stress is associated with

progression and development of several diseases and their complications are accompanied by the failure of antioxidative defense mechanism or increase in the production of free radicals (Pandey and Rizvi, 2009). Though, the consumption of natural antioxidants has been found to reduce the risk of diabetes, cardiovascular diseases, cancer and age related disorders (Rahman, 2007). Among a wide variety of natural compounds, terpenoids and flavonoids are the most diverse class of naturally occurring antioxidants and are identified as potential candidates for neuroprotection (Kumar and Khanum, 2012). Herein, we have focused our work on the aggregated form of α-synuclein. We have found that certain anti-cancerous drugs (Genistein, Hesperidine and Epigallocatechin-3-gallate) that might prevent the accumulation of toxic α-synuclein protein. Further, using *in silico* docking studies we have identified that Genistein was among the best fit ligand to inhibit aggregated form of α-synuclein. Finally, these natural compounds can be analyzed further to devise a potent therapeutic strategy to ameliorate α-synucleinopathies.

3. LITERATURE REVIEW

3.1 Structural properties of α-synuclein

α-synuclein (α-syn) is a small neuronal protein of 14.5kDa and is having 140 amino acids residues, is highly conserved in vertebrates and is localized in the presynaptic terminals of neurons, particularly in the hippocampus, neocortex, striatum, cerebellum and thalamus (Jiang et al., 2015). The protein is a member of synuclein family, α-, β- and γ-synuclein, from highly expressed human genes (SNCA, SNCB and SNCG) (Gallegos et al., 2015). The α-, β- and γ-synuclein genes (SNCA, SNCB and SNCG) have been mapped to human chromosomes 4q21, 5q35, and 10q23, respectively (Lavedan, 1998). All synucleins possess a similar sequence but only α-synuclein is associated with disease progression (Nishioka et al., 2010). The β-isoform of synuclein family also have a presynaptic location and co-localizes with α-syn at various, but not all presynaptic terminals, whereas glial cells and specific neuronal populations, mainly dopamine neurons shows the expression of γ-synuclein (Benskey et al., 2016). α-synuclein is a natively unfolded soluble protein having an extended structure composed primarily of random coils and lacks a well-defined secondary conformation and consequently belongs to intrinsically unstructured protein family (Uversky, 2003). Nevertheless, the notable conformational plasticity of α-synuclein enables it to undergo a wide range of conformational changes and to adopt various dynamic structures on the basis of environmental changes and binding partners present (Ferreon et al., 2009). For instance, interaction with a number of proteins and ligands that are likely to alter its native state conformation and α-synuclein gains flexibility to adopt several secondary structures (Uversky, 2007).

α-synuclein protein has two distinct alternatively spliced variants viz; the 112 amino acid and 126 amino acid variants which does not contain exon 5 and exon 3, respectively (McLean et al., 2012). The structure of α-synuclein protein is composed of three different regions (Stefanis, 2012). The amphipathic N-terminal region (residues 1 to 60) consists of 11 amino acid repeats including KTKEGV consensus sequence similar to the sequence found in the amphipathic helices of apolipoproteins (Uversky and Eliezer, 2009). The amphipathic α-helices are suggestive of the lipid binding domains of class A2 apolipoproteins which indicates that α-synuclein binds to the negatively charged phospholipids and this binding causes a change in structural conformation and it forms α-helical secondary structure (Pfefferkorn et al., 2012). Recent studies shown that lipidic environments accelerate the aggregation of α-synuclein along with its folding, suggesting that this conformation causes misfolding of α-synuclein which is prevalent in various neurodegenerative diseases (Melki, 2015). The α-helix forming region is composed of three independent missense mutations including A30P, E46K and A53T found in familial form of PD cases (Bekris et al., 2010).

The central hydrophobic region (residues 66-95) termed as non-amyloid-β component (NAC) is composed of two additional motifs and is one of the major components of brain amyloid plaques deposition in Alzheimer's disease (Olivares et al., 2009). It comprises of the amyloidogenic part which undergoes a conformational change in the structure of α-synuclein and the formation of cylindrical β-sheets and amyloid-β like fibrils and protofibrils occurs from a random coil of α-synuclein (Qin et al., 2007). These features contribute to distinguish α-synuclein from β- and γ-synuclein, which do not form copolymers with α-synuclein (Mor et al., 2016).

Finally, the C-terminal region (residues 96 to 140) is proline rich and highly acidic (Bisaglia et al., 2009). This region also has a regulatory role in fibril formation and aggregation of

protein (Moriarty et al., 2013). This region is highly variable in sequence and size among species (Kalia et al., 2013). It consists of an acidic domain (residues 125-140) that is composed of several posttranslational modification sites as phosphorylation, Ubiquitination and glycosylation sites that have a role in regulation of chaperone-like activity of α–synuclein (Nonaka et al., 2005). Recent studies have reported that truncation of C-terminal region of α-synuclein by proteolysis have a crucial role in fibrillogenesis of α-synuclein in various neurodegenerative diseases (Al-Mansoori et al., 2013). Partially truncated and full length insoluble aggregates of α-synuclein have been found in the aggregates of LBs (Baba et al., 1998). Different regions of α-synuclein have been depicted in **Figure-2**.

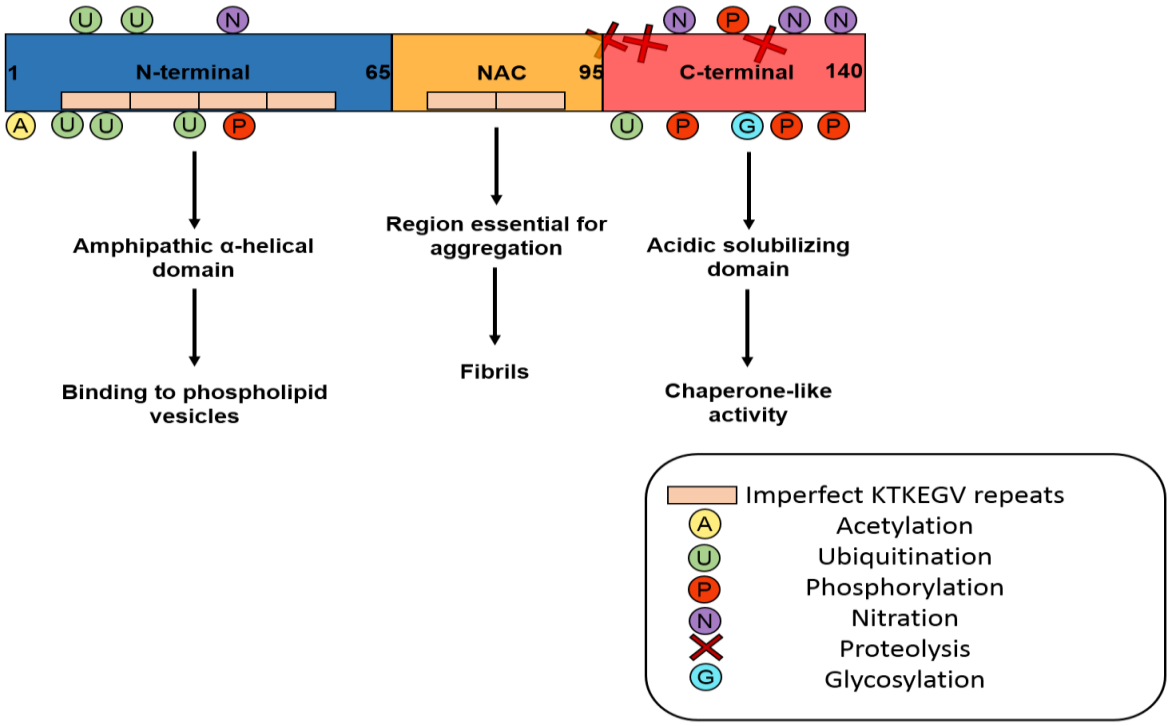


Figure 2: Schematic representation of α-synuclein regions. N-terminal region binds to phospholipid vesicles, C-terminal region is responsible for the chaperone like activity and the central hydrophobic region is known for the formation of fibrils.

3.2 Protein modification: A major contributor in α-synuclein protein folding and aggregation

α-synuclein exists as a monomer within the cytoplasm but under certain pathological conditions, it undergoes several conformational changes that cause the monomers of protein to aggregate and become insoluble (Bandopadhyay, 2016). It has been reported that changes in the structural and functional properties of α-synuclein are initiated when the protein undergoes a wide range of modifications (Popova et al., 2015). When α-synuclein interacts with various lipid surfaces such as phospholipid bilayers, lipid droplets or lipid membranes, it induces a dramatic change in the structure of α-synuclein from its natively unfolded form to an α-helical secondary structure (Rhoades et al., 2006). When the isolated monomers of α-synuclein, exposed to synthetic lipid membranes, readily bound and interact with the surface of membrane and formed various types of dimers and oligomers (Kim et al., 2014). The imperfect 11 amino acids repeats present in α-synuclein, comparable to the amphipathic α-helical motif of apolipoproteins play a key role in binding with lipid membrane (Bartels et al., 2010). The lipid composition of membrane, characterized by high concentrations of phospholipids, sphingolipids and cholesterol and altered surface charge that favors α-synuclein binding (Snead and Eliezer, 2014). This region is known as lipid rafts which serve as a platform to promote the binding and oligomerization of α-synuclein (Follmer et al., 2015).

Several other modifications such as posttranslational modifications of α-synuclein are prevalent and are reported as possible mediators of a number of pathological processes, including α-synuclein aggregation, Lewy body formation and neurotoxicity (Beye et al., 2009). Most common posttranslational modification of α-synuclein is phosphorylation, occurs at serine residues S129, S87 and at tyrosine residues Y125, Y133 and Y135 and has a

role on the function of numerous target proteins (Hejjaoui et al., 2012). Ser129 is phosphorylated approximately 90% in Lewy Bodies but no more than 4% of α-synuclein is phosphorylated at this residue in the C-terminal region under normal conditions in the human brain (Xu et al., 2015). This phosphorylation is regulated by several kinases and phosphatases such as casein kinases (CK1 and CK2), Polo-like kinases (PLK2 and PLK3) and G protein-coupled receptor kinases (GRK2, GRK3, GRK5 and GRK6) that are responsible for phosphorylation (Tenreiro et al., 2014). Protein Phosphoprotein phosphatase 2A (PPA2) is responsible for the dephosphorylation and reduced level of aggregated form of α-synuclein in human brains whereas the phosphorylation of Tyr-125 residue is regulated by two Src family protein tyrosine kinases (c-Src and Fyn) and Syk family protein tyrosine kinase (p72syk) (Peng et al., 2005). Src family kinases does not play their role to suppress or enhance the polymerization activity of α-synuclein but the putative anti-neurodegenerative activity of Syk family tyrosine kinases causes it lose its ability to form oligomers in human brains (Negro et al., 2002).

The second most common posttranslational modification is ubiquitination i.e. the attachment of ubiquitin molecules at the lysine residues of α-synuclein (Bisaglia et al., 2006). Though, α-synuclein consists of 15 lysine residues, isolated form of α-synuclein showed that the protein is ubiquitinated predominantly at K6, K10 and K12 residues (Meier et al., 2012). Ubiquitination at the lysine residues of α-synuclein causes changes in the functional properties of α-synuclein, affecting the localization and degradation processes of α-synuclein (Hasegawa et al., 2002). Another common posttranslational modification is nitration i.e. the attachment of a nitro molecule at the tyrosine residues (Y39, Y125, Y133 and Y136) of α-synuclein (Radi, 2013). Nitration of α-synuclein is considered to be an important factor in Lewy body diseases and is enhanced under conditions of elevated oxidative stress (Burai et

al., 2015). Recent studies have shown that mitochondrial impairment and nitration induced α-synuclein oligomer formation induces apoptosis via integrin pathway (Liu et al., 2011). In a PD model, nitration of α-synuclein caused increase in the level of toxic α-synuclein protein that causes cell death (McCormack et al., 2012).

Other post-translational modifications include glycosylation (Lehri-Boufala et al., 2015). O-glycosylated α-synuclein is a potent substrate for ubiquitination and is used by Ubiquitin proteasome system (UPS) and enhances the degradation process of toxic α-synuclein protein (Shimura et al., 2001). In this way, truncation of C-terminal region of α-synuclein also has a key role in various neurodegenerative diseases (Vamvaca et al., 2009). Other modifications due to environmental factors, oxidative stress, mitochondrial dysfunction, calcium dyshomeostasis and proteolytic cleavage for instance due to calpain-1 also play a crucial role in the aggregation of α-synuclein (Games et al., 2014).

3.3 Functional properties of α-synuclein

Since, α-synuclein is involved in various cellular processes but the etiology and functional mechanism of α-synuclein remains obscure (Bendor et al., 2013). Protein sequence of α-synuclein is highly conserved across species at the N-terminus of the protein but is having variability towards the C-terminus (Gitler et al., 2009). The highly conserved N-terminal domain of α-synuclein associates with synthetic lipid vesicles and facilitates dimerization (Auluck et al., 2010). The polar C-terminus of α-synuclein contains multiple post-translational modification sites and regulates its activity (Hodara et al., 2004). Researchers have also identified a role of α-synuclein as ATP-independent molecular chaperone to prevent the accumulation of misfolded or denatured proteins (Rekas et al., 2012). An experiment using α-synuclein knock out mice was carried out to find out the functions of α-synuclein, and had mild defects in synaptic transmission and metabolism and transfer of fatty

acids (Emanuele and Chieregatti, 2015). This study also revealed that α-synuclein is a negative regulator of dopamine neurotransmission and the striatal dopamine levels were also less affected by the action of MPTP (an inhibitor of mitochondrial complex 1) in α-synuclein knockout mice (Venda et al., 2010). Studies have also reported that under normal physiological conditions, overexpressed α-synuclein protects against paraquat-induced neurodegeneration (Manning-Bog et al., 2003). Researchers also proposed that under physiological conditions, α-synuclein exhibits a non-classical chaperone activity and inhibits the assembly of soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) machinery mediated vesicle fusion via its indirect interaction with SNARE proteins to regulate neurotransmission (Bonini and Giasson, 2005). N-terminus of α-synuclein binds to phospholipids while the C-terminus is necessary for synaptobrevin-2 binding and an indirect interaction is formed (Burré et al., 2010). A lipid regulator, arachidonic acid, encourages SNARE complex formation but α-synuclein segregates arachidonic acid and blocks the activation of SNAREs (Darios et al., 2010). Thus, α-synuclein have a role in synaptic vesicle trafficking and neurotransmission and prevents toxic insults-induced neurodegeneration (Vekrellis et al., 2011). Studies on α-synuclein knockout mice did not show short-term impairments in learning or probe trails but showed impairments in long term spatial and working memory, suggesting a role of α-synuclein in cognition and long-term memory (Leung and Jia, 2016). α-synuclein, confined to presynaptic terminals and axons of neurons, area responsible for memory, cognition and emotions (Kokhan et al., 2012).

A wide range of defects such as aggregation, mutations, misfolding and fibrillation factor and structural plasticity in α-synuclein protein are substantial to its association with various neurodegenerative diseases (Pacheco et al., 2012). When the unstable monomeric form of α-

synuclein becomes tangled or disordered, aggregates rich in cross- β-structures are formed (Esteban-Martín et al., 2013). These structures consist of several modifications such as binding to lipid vesicles and multiple post-translational modifications (PTMs) sites (Tokmakov et al., 2012). The PTMs and lipid binding of α-synuclein alter its size, shape, conformation and charge which contribute to the onset of various neurodegenerative diseases (Alderson and Markley, 2013).

3.4 Prion like similarities of α-synuclein

Prions are contagious proteins and are composed solely of aberrantly folded proteins (Kraus et al., 2013). They are deficient in nucleic acid composition and are responsible for the onset of disease progression. Since, prions undergo misfolding and a wide range of conformations with the formation of toxic and insoluble oligomers and amyloid like aggregates, rich in β-sheet conformation and causes neurodegeneration (Kupfer et al., 2009). Further, the abnormal or misfolded protein is transmitted from affected to healthy nerve cells and a chain reaction is formed in which the sequence is repeated continuously causing various neurodegenerative processes (Brettschneider et al., 2015). The well-studied prion protein in mammals is PrP^{Sc} which is formed from the precursor protein PrP^{C} by a complex mechanism involving the folding of α-helix–rich native protein into the PrP^{Sc} protein having high level of β-sheet conformation leading to the formation of toxic oligomers and amyloid plaques, and ultimately neurodegeneration with neuronal dysfunction (Cobb and Surewicz, 2009). Evidences suggest that mechanism of toxic PrP^{Sc} formation and neurodegeneration is comparable with the aggregation mechanism of α-synuclein and PD (Zhou et al., 2012). Both PrP^{C} and α-synuclein are native monomers but undergoes mutations and misfolding and form β-sheet conformation and accumulate into inclusions, fibrils and amyloid plaques accompanying neurodegeneration (Singh and Udgaonkar, 2016). Lewy bodies i.e. inclusions

of α-synuclein were identified in 2% to 8% of grafted neurons and SNc neurons of PD patients (Olanow and Brundin, 2013). Non-aggregated, soluble α-synuclein levels were also altered in grafted neurons (Luk et al., 2012). Autopsy studies of PD patients proposed a mechanism for the transmission of α-synuclein based on observations that the onset of disease initiates in the nose and/or gut and progresses to attack the brain in a number of stages (Mulak and Bonaz, 2015). Several subsequent studies reported that exogenous α-synuclein accumulates along the neuroanatomical pathways and triggers Lewy body pathology in the brain (Volpicelli-Daley et al., 2011). The conformation of the exogenous protein causes it to transmit to the endogenous protein inside the neurons and plays its role in the disease progression (Recasens and Dehay, 2014).

3.5 Intervening role of α-synuclein in Neurodegeneration

Although, α-synuclein has numerous roles in brain, it plays a significant role in the progression of several NDDs due to a wide range of modifications in the structure of protein (Danielson and Andersen, 2008). α-synuclein also act as chaperone due to its interaction with a wide variety of cellular proteins and ligands (Souza et al., 2000). Recently, it has been reported that alpha-syn exhibits 40% sequence conservation with molecular chaperone 14-3-3, signifying that the two proteins may serve the same function (Ostrerova et al., 1999). 14-3-3 is known for its ability to interact with various proteins which contain phosphorylated serine residues (Muslin et al., 1996). 14-3-3 plays a role in the development of neurons and control of cell growth due to its interaction with α-synuclein in the accumulated LBs (Ubl et al., 2002). α-synuclein binds to various other similar proteins including extracellular regulated kinase (ERK), protein kinase C (PKC) and BAD, a member of the Bcl-2 family which induces apoptosis (Shimada et al., 2013). BAD is phosphorylated at serine residues S112, S136, and S155 to stabilize its maintenance in the cytoplasm (Witt, 2013). 14-3-3

interacts with phosphorylated Bad and prevents apoptosis (Masters et al., 2001). Dephosphorylated Bad is localized along with Bcl-XL and Bcl-2 to mitochondria and triggers apoptosis (Gross et al., 1999). Moreover, Mutation in any of the phosphorylation sites in BAD enhances its cell-killing ability (Watabe and Nakaki, 2004).

Environmental toxins such as rotenone, inhibits mitochondrial complex I, promotes the release of α-synuclein and Bad dephosphorylation by enteric neurons (Dong et al., 2009). An elevated level of α-synuclein contributes to caspase-9 activation and ultimately apoptosis in human brain (Yamada et al., 2004). Elevated α-synuclein levels also triggers the secretion of IL-6, which is blocked by specific inhibitors of extracellular-regulated kinase (ERK1/2), p38 and c-Jun N terminal kinase (JNK) mitogen-activated protein (MAP) kinase pathways (Klegeris et al., 2006). Moreover, other toxins such as MPTP and paraquat causes microglia activation which secrete ROS, cytokines and prostanoids leading to cell death and neurodegeneration (Litteljohn et al., 2010). Further, modifications due to overexpression and mutations, certain environmental factors and enzymatic cleavage in the structure of α-synuclein also suggests that it has a major contribution in the development of neurodegenerative disorders (Robinson, 2008). There are numerous other factors so far, that have been reported to be associated with α-synuclein and NDDs and are shown in **Figure-3**.

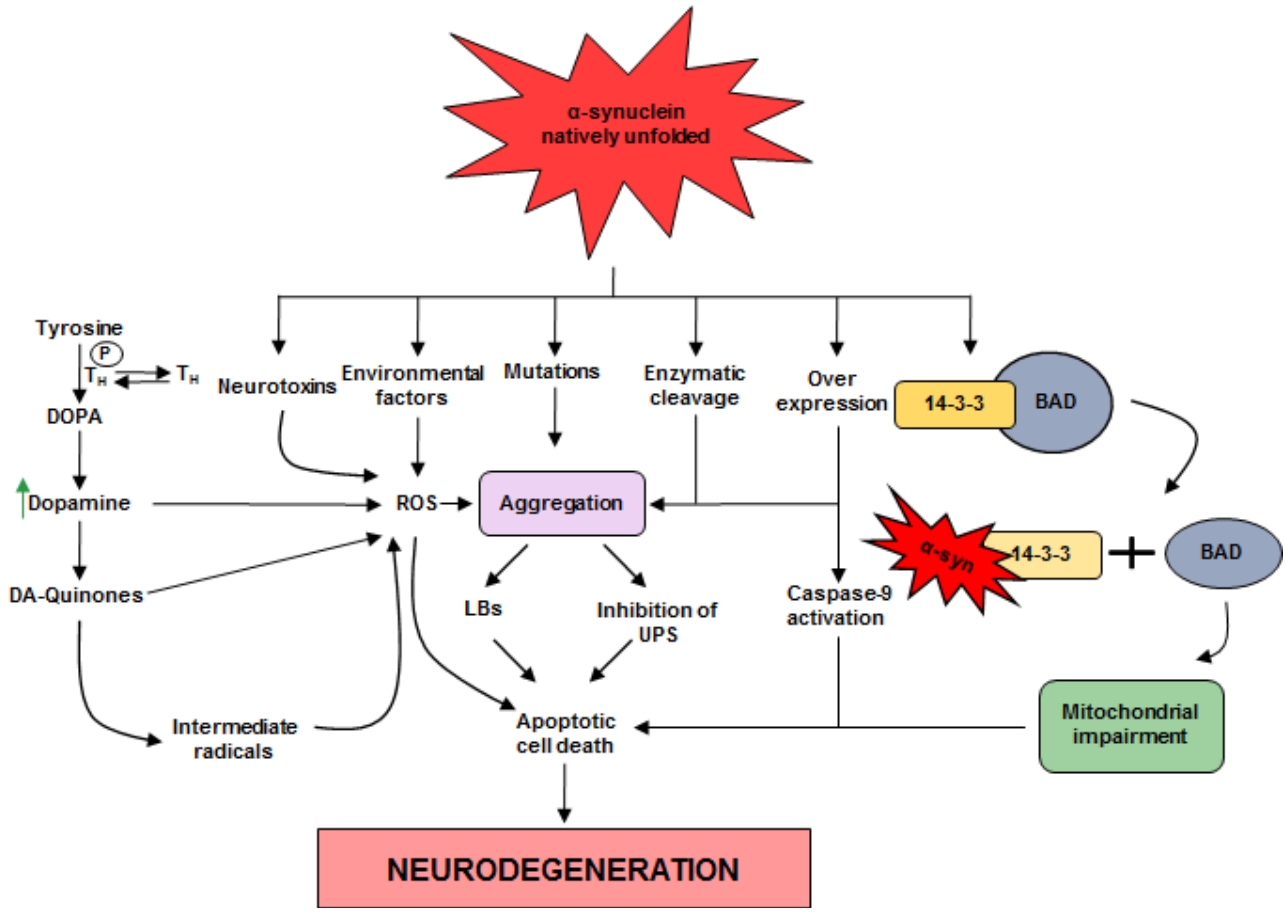


Figure 3: α-synuclein aggregation mechanism and its role in Neurodegeneration. Various genetic and environmental factors causes depletion in chaperone activity and aggregation of α-synuclein that leads to mitochondrial impairment and neurodegeneration.

3.5.1 Role in Parkinson's disease

Parkinson's disease (PD) is the most common movement neurodegenerative disorder accompanying the neuronal degradation in the nigrostriatal system (Fahn and Sulzer, 2004). It is typically abundant in the presence of Lewy Bodies i.e. intraneuronal protein inclusions which are responsible for dopaminergic neuronal loss thus leading to motor symptoms including muscle rigidity, resting tremor, postural instability and bradykinesia (Massano and Bhatia, 2012). LBs are spherical eosinophilic in shape with a diameter of 5-25 µm and is composed of aggregates of cytoplasmic protein α-syn, localized in the substantia nigra, cerebral cortex and in several structures of central nervous system (Narkiewicz et al., 2014).

LBs have affinity to specific dyes for instance Thioflavin S which identifies post-translational modifications such as phosphorylation and ubiquitination and β-sheet conformations to recognize misfolded α-synuclein (Zebrocki et al., 2005). Variations in solubility and phosphorylation pattern of α-synuclein occur prior to Lewy body formation in PD (Zhou et al., 2011). The amount of soluble α-synuclein decreases but the phosphorylation level of α-synuclein increases greatly over the course of PD (Breydo et al., 2012).

During the presymptomatic phase of disease progression, non-motor symptoms such as vagal dysfunction, olfaction impairment and sleep disorders are developed but there is no definitive diagnostic test (Pellicano et al., 2007). Clinical diagnosis relies mainly on the detection of motor symptoms of PD which appear when there is a loss of 50–60% of dopaminergic neurons (Cheng et al., 2010). Appearances of cognitive, psychiatric and autonomic problems are often accompanied with the PD symptoms (Varanese et al., 2011). Due to the appearance of these clinical symptoms, PD is identified as a complex clinicopathological entity (Jankovic, 2008). Majority of PD cases are sporadic and various environmental factors such as proteasome inhibition, oxidative stress and genetic susceptibility seem to be the pathological cause of PD (Klein and Westenberger, 2012).

3.5.2 Role in Alzheimer's disease

Several theories have also shown that α-synuclein and tau share some similarities and the co-existence of pure synucleinopathies and tauopathies and an overlap between neurodegenerative diseases, particularly among Parkinsonism and dementia has been observed (Moussaud et al., 2014). In this theory, two proteins tau and α-synuclein are central and are able to form intracellular inclusions and cause neurodegeneration (Nonaka et al., 2010). Current theories suggest that α-synuclein and tau interact with each other and this interaction is responsible for the development of wide range of neurodegenerative disorders

(Jellinger, 2011). AD is the most common cognitive neurodegenerative disorder characterized by progressive neuronal damage, loss of memory and other cognitive skills leading to severe dementia (Jahn, 2013). The cognitive decline in AD is accompanied by the accumulation of insoluble aggregates of amyloid beta 42 peptide (Aβ42) in the form of senile plaques and Neurofibrillary tangles (NFTs), formed by the hyperphosphorylation of microtubule associated protein, tau (τ) (Mokhtar et al., 2013). However, some additional signs of proteinopathies such as intracellular cytoplasmic inclusions of phosphorylated or ubiquitinated α-synuclein, well-known as Lewy bodies and Lewy neurites (LB/LN) have been found in 30–40% of AD cases (Zaccai et al., 2008). These additional lesions display a more rapid rate of memory loss and decline of cognitive functions comparable to AD alone (Karantzoulis and Galvin, 2011). Recent studies have reported that α-syn, tau and Aβ overexpression or enhanced level of mutant forms of these proteins promote the acceleration of accumulation of each other and cognitive dysfunction in humans (Clinton et al., 2010). Several reports have also revealed that overexpression or enhanced level of wild-type α-synuclein cause alterations in the neuronal physiology that leads the inhibition of synaptic vesicle recycling and ultimately reduces release of neurotransmitter (Lashuel et al., 2013). Another study have shown that molecular chaperone 14-3-3 promotes phosphorylation and aggregation of tau in the form of neurofibrillary tangles (NFTs) with the help of protein kinase A (PKA) and ultimately leads to neurodegeneration (Kumar et al., 2015).

3.5.3 Role in Dementia with Lewy Bodies

Lewy body diseases such as Parkinson's disease (PD), Lewy body variant of Alzheimer's disease (LBV) and diffuse Lewy body disease (DLBD) possess similar physiological and pathological characteristics (Mrak and Graffin, 2007). Lewy body diseases are progressive prior to cognitive decline and fatality (Olichney et al., 1998). Recent studies reported that

pathology of Lewy body propagates via transmission of α-synuclein aggregates from one neuron to another (Guo and Lee, 2014). Aging is known to be the most predominant cause of Lewy Body disease (McKeith, 2004). Environmental toxins, Oxidative stress and mitochondrial dysfunction also contribute to the disease progression (Spano et al., 2015). Aging has various effects on cellular protein degradation machinery for instance, proteolysis of presumptive cytotoxic protomer, changes in pathways of protein degradation such as UPS and autophagy and also the trypsin- and chymotrypsin-like activities of the proteasome is affected with age (Fecto et al., 2014). These physiological alterations are able to modify proteins over time, enhance aggregation and initiates disease progression (Golde et al., 2013).

Dementia with Lewy bodies (DLB) is a common neurodegenerative dementia in the aging population (Ubhi et al., 2010). It is a cognitive disorder and is characterized by visual hallucination, unstable cognitive functioning and Parkinsonism (Fernandez et al., 2003). Diagnosis of patients with such clinical features would be easy through careful neurological examinations but the accurate diagnosis of DLB might become laborious when patients also suffer with AD pathology, affecting the clinical symptoms of Parkinsonism and visual hallucinations (Zupancic et al., 2011). Accurate clinical diagnosis of DLB is significant and the use of cholinesterase inhibitors improves neuropsychiatric symptoms and cognitive function of DLB (Trinh et al., 2003).

3.5.4 Involvement in Huntington's disease

Huntington's disease (HD) is the most common inherited neurological disorder characterized by expansion of polyglutamine (polyQ) sequence having propensity to aggregate and induce neurotoxicity (Arrasate and Finkbeiner, 2012). It has been reported that polyQ expansion might affect other proteins prone to misfolding (Finkbeiner, 2011). α-synuclein, with tendency to aggregate and form Lewy bodies ultimately causes familial PD (Kahle et al.,

2002). It has also been proposed that overexpression of wild-type α-synuclein affects macroautophagy, a mechanism for the clearance of cytoplasmic inclusions like hyperphosphorylated tau and huntingtin (Yasuda et al., 2013). Recent studies have shown that α-synuclein is a mediator in polyQ induced neurotoxicity since, HD and other polyQ diseases are immunopositive for α-synuclein (Chánez-Cárdenas and Vázquez-Contreras, 2012). Wild-type α-synuclein overexpression in HD patients triggers the progression of tremors and causes weight loss while its deletion increases the number of autophagosomes which accompanying a reduction in the rate of weight loss and tremors in HD patients (Corrochano et al., 2012). Accordingly, depletion of α-synuclein results in reduced amount of inclusions of N-mutHtt in transfected neurons (Tomás-Zapico et al., 2012). A functional link is therefore established between these two proteins which show that wild-type α-synuclein has a role in the regulation of autophagy even at physiological levels (Waelter et al., 2001). This study validates that α-synuclein modifies polyQ induced neurotoxicity and there is a great probability of reducing the rate of HD using potential PD related therapies to counteract α-syn toxicity (Pocas et al., 2015).

3.5.5 Involvement in Amyotrophic Lateral Sclerosis (ALS)

ALS is another complex neurodegenerative disease characterized pathologically by degeneration of upper and lower motor neurons in the motor cortex, spinal cord, brainstem and astrogliosis confined to neurodegeneration (Wijesekera and Leigh, 2009). The etiology of ALS is a complex mechanism of numerous pathogenic processes involving oxidative stress, mitochondrial dysfunction, misfolded protein aggregates, TDP-43 abnormalities, dysfunction of the UPS pathway and impaired axonal transport (Jiang et al., 2015). The absolute diagnosis of ALS is essential and is diagnosed pathologically and clinically by the presence of cytoplasmic inclusions, immunoreactive to neurofilament and/or peripherin (Chen et al.,

2013). Likewise α-synuclein, mutations in SOD-1 triggers the formation of soluble oligomers, inclusion bodies and insoluble fibrils (Furukawa, 2013). Recently, extrapyramidal symptoms due to nigrostriatal dysfunction and phosphorylated α-synuclein inclusions have been reported in ALS patients suggesting that PD related pathologies might have a role in ALS (Helferich et al., 2015). Further, increased expression of α-synuclein was observed in spheroids of spinal cord and glial cells of ALS patients and co-occurrence of α-synuclein and SOD1 have also been identified in the some protein aggregates (Miller et al., 2004). This study indicates the involvement of α-synuclein in ALS but more research is needed to identify the molecular interaction of α-synuclein in ALS cases (Yang and Choi, 2013). Furthermore, insoluble aggregates of Bunina Bodies, Hyaline conglomerate inclusions (HCIs) and Axonal spheroids are also found in neurofilaments and peripherins of approximately 85% of ALS cases (Xiao et al., 2006).

3.5.6 Involvement in Multiple System Atrophy

Multiple system atrophy (MSA) is considered as one of major α-synucleinopathies and is a sporadic neurodegenerative disorder involving the clinicopathological entities like Shy–Drager syndrome (SDS), striatonigral degeneration (SND) and olivopontocerebellar atrophy (OPCA) affecting blood pressure, muscle control, movement, bladder function and heart rate (Yoshida, 2007). The pathological events in MSA are the clinical triads' similar cerebellar ataxia, autonomic failure and Parkinsonism along with myelin dysregulation and demyelination and neurodegeneration (Bleasel et al., 2014). Since, MSA is a rare neurological disorder (Wenning and Stefanova, 2009), recent studies have reported that genetic variants, posttranslational modifications such as phosphorylation and ubiquitination and various other modifications such as formation of protofibillary α-synuclein are the causal factors of MSA (Dickson et al., 1999). Various mutation sites in the coding sequence of α-

synuclein have not been identified so far and more research needs to be done to identify the causal mutations and etiology of MSA (Stemberger et al., 2011).

Histopathology of MSA includes the presence of misfolded, modified and fibrillar structure of α-synuclein in the cytoplasm like PD and DLB but the principal site for the deposition of α-synuclein is oligodendrocytes (Jellinger and Wenning, 2016). The accumulation/aggregation of abnormal α-synuclein in MSA has been reported in various cellular sites, as Glial cytoplasmic inclusions (GCIs) in oligodendrocytes, neuronal cytoplasmic and neuronal nuclear inclusions (NCIs and NNIs), glial nuclear inclusions (GNIs) in oligodendroglial nuclei and in neurites (Burn and Jaros, 2001). These findings suggest that modified α-synuclein is widespread and primary fibrillar and nonfibrillar aggregation of α-synuclein also occur in neurons in MSA cases (Stefanova and Wenning, 2016). Immunostaining studies of neuronal cytoplasm or nucleus indicates that accumulation of non-fibrillar α-synuclein occur prior to the formation of fibrillar inclusion (Bleasel et al., 2016). On the basis of these studies, several researchers identified the neuroprotective role of nonfibrillar α-synuclein to seize its toxic protofibrils (Uversky, 2008).

3.6 Oxidative stress: A prevalent cause of neurodegeneration

Oxidative stress is a condition of imbalance between ROS production and the level of antioxidants leading to tissue damage that ultimately becomes a cause of a number of diseases (Shukla et al., 2011). Initially, the production of ROS was considered as an impact of imbalance between the metabolisms of ROS/RNS but recent reports have suggested that ROS play a crucial role in the alteration of cellular functions and in the development of neurodegeneration (Hsieh and Yang, 2013). ROS targets different critical processes of cell such as lipid peroxidation, damage to DNA, RNA and protein (Gandhi and Abramov, 2012). High levels of oxidative stress can cause ATP depletion, necrosis and apoptotic cell death

(Jha et al., 2014). For instance, Haber Weiss and Fenton reaction reported that ROS generation and free radical formation stimulates mitogen activated protein (MAP) kinase signaling cascade and have their great contribution towards neuronal loss due to excitotoxic calcium mobilization, mitochondrial (Mt) dysfunctions and ultimately apoptotic cell death which causes aging and neurodegenerative diseases (Kehrer, 2000). The basic mechanism underlying oxidative stress mediated neurodegeneration has been represented in **Figure-4**.

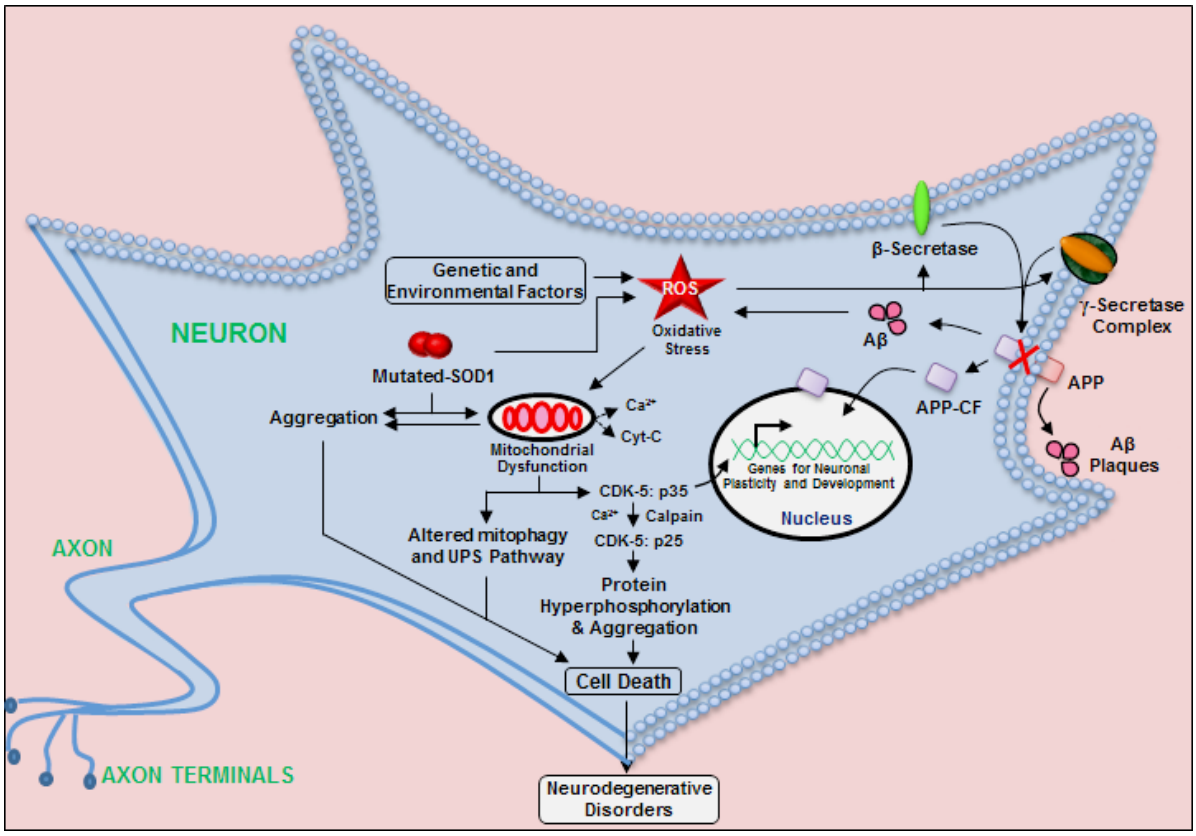


Figure 4: Oxidative stress induced neurodegeneration. Several factors causes increase in ROS level that leads to mitochondrial impairment and increases the level of β and γ secretase that causes neuronal death.

In Alzheimer's disease (AD), oxidative stress is found to elevate the levels of *β*-secretase (BACE-1) (Mouton-Liger et al., 2012) and *γ*-secretase complex proteins via activation of PKR-eIF2α and other signaling pathways that caused A*β* aggregation into insoluble senile plaques and triggered apoptotic cell death (Jo et al., 2010). Further oxidative stress causes an

increase in cellular ROS level and mitochondrial dysfunctions resulting in partial reduction of oxygen during oxidative phosphorylation (Praticò, 2008). Moreover, role of mutations in Superoxide dismutase-1 (SOD-1) have been observed to cause motor neuronal damage but the underlying mechanism(s) behind oxidative stress mediated mutations in SOD-1 and disease progression is still unclear (Pickles et al., 2016). Furthermore, Oxidative Stress and Mitochondrial dysfunction, particularly in complex-I (NADH dehydrogenase) of the mitochondrial electron-transport chain in the substantia nigra are found to trigger a sequence of events that caused cell death (Hwang, 2013). However in case of PD, DLB and other α-synucleinopathies, Oxidative stress obstructed the cellular homeostatic processes involving mitophagy and ubiquitin-proteasome system (UPS) (Dias et al., 2013). Recent studies on animal models have revealed that oxidative stress contributes towards neuronal damage via PI3K/AKT and p38 MAPK signaling pathways (Jha et al., 2015). Moreover, dopamine metabolism also contributes to oxidative stress, causing modification of macromolecules that leads to a gain of toxic functions in PD and LBD (Sayre et al., 2008).

Further, numerous evidences indicate the crucial role of CdK-5, a member of cyclin dependent kinase family has been detected in various NDDs (Wei and Tomizawa, 2007). Under normal condition, Cdk-5 plays a significant role in the development of neurons by the phosphorylation of specific serine/threonine residues of various cytoskeletal proteins accompanying neuronal migration, synaptic transmission, synaptogenesis as well as synaptic plasticity but under the conditions of oxidative stress, excitotoxicity, Aβ exposure, calcium dyshomeostasis, mitochondrial dysfunctions and inflammation, level of intracellular Ca^{2+} arises (Mushtaq et al., 2016). Thus elevated Ca^{2+} concentration triggers calpain to cleave p35 to p25 and form a stable but hyperactive complex of CdK-5/p25 which in turn causes hyperphosphorylation of a large number of cytoskeletal proteins that leads to a wide variety

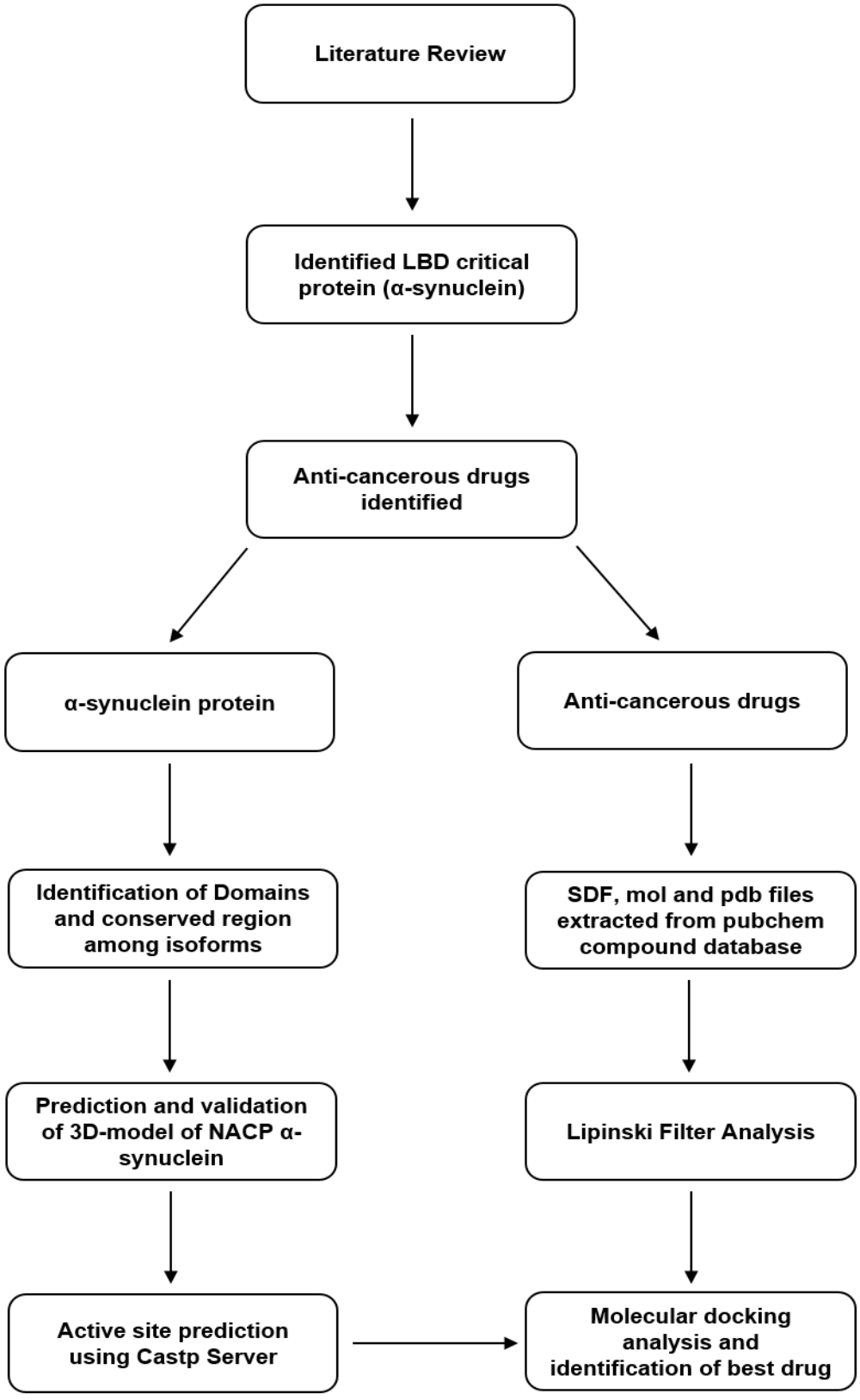
of neurodegenerative disorders like AD, PD and other Lewy body diseases (Yousuf et al., 2016).

3.7 Role of Natural compounds

Natural plant products possess some remarkable properties and find their use for medicinal purposes for a long time as they are having a wide range of pharmacological properties like antioxidant, anti-carcinogenic, hypercholesterolemic and anti-inflammatory (Zhao, 2005). The recent advancement in the separation, extraction, sequestration and isolation techniques of plant derived products and the attempts to develop natural plant products as ameliorative have made it possible to produce 63% of the plant-derived drugs since 1981 (Yoo and Park, 2012). Among various natural products, poly phenols (terpenoids and flavonoids) are the vast and most diverse class of phytochemicals that play an important role in the therapeutics of different disorders and syndromes (Macedo et al., 2015). Some of these synthetic and semi-synthetic compounds are identified and studied for the therapeutics of a large number of neurodegenerative disorders in the last few years (Trosset and Carbonell, 2015). Recent studies revealed that a range of pure compounds with anti-cancerous activity derived from herbal materials & herbal formulations are effective against in vitro/ in vivo PD models (Fridlender et al., 2015). Genistein obtained from soybeans, Hesperidine obtained from citrus fruits and Epigallocatechin-3-gallate obtained from green tea have been used as health supplements and anticancerous drugs, play an important role in the therapeutics of various neurological disorders (Shay et al., 2015). Genistein is one of the naturally occurring flavanones that exists in soybeans either in the form of glucosides or in free form (aglucons) (Kamaraj et al., 2009). Genistein containing soybean pastes exert an antioxidant effect by scavenging oxygen derived free radicals and inhibiting lipid peroxidation and exhibits improved cognitive learning skills (Sonee et al., 2004). On the other hand, Hesperidin is a

major constituent of citrus fruits and can be isolated in large amounts from *Citrus unshiu* (satsuma mandarin), *Citrus sinensis* (sweet orange) and the peels of *Citrus aurantium* (bitter orange) (Cho, 2016). Hesperidin possesses a wide range of pharmacological properties such as antioxidant, antihypercholesterolemic, anticarcinogenic and anti-inflammatory properties (Tamilsevam et al., 2013). Epigallocatechin-3-gallate is an aromatic compound with phenolic hydroxyl groups on its aromatic rings and is present in green tea and it enhances the cell resistance to oxidative stress (OS) beyond the iron chelating and simple scavenging activities and have an impact in those pathologies, where iron and OS are involved (Weinreb et al., 2009). Recent studies have shown that green tea catechins have a crucial role in lowering the adverse effects of free radicals in *in vitro* and *in vivo* activities, associated with several chronic disorders such as cancer, atherosclerosis, stroke and neurodegenerative disorders (Mandel et al., 2006).

4. MATERIALS AND METHODS



Steps 1: A large pool of data have been investigated for the appropriate information needed to include in the research findings of this project through the NCBI archives as well as other reputed journals.

Step 2: In the work, it was found that α-synuclein protein is the center of focus in Lewy body diseases For instance, Parkinson's disease, Alzheimer's disease and Dementia with Lewy bodies which are often known as α-synucleinopathy.

Step 3: In the literature survey, it was also found that there are many bio-molecules and drug compounds which act on toxic α-synuclein protein to inhibit its accumulation.

Step 4: Three biomolecules with anti-cancerous activity were identified as having neuroprotective role and were selected through the journals on which undergoing trials are being performed towards finding a better cure for α-synucleinopathy.

Step 5: From here, there goes two ways for the protein taken as receptor molecules and drugs chosen as Ligands and their further study.

4.1 Retrieval of α-synuclein protein and its function recognition

For primary structure analysis, the amino acid sequence of SNCA with accession number P37840.1 was retrieved from NCBI database and was used for homology search using Basic Local Alignment Search Tool. Protein functional elucidation was done using Interproscan server (http://www.ebi.ac.uk/interpro/search/sequence-search/).

4.2 Phylogenetic relationship and Physico-chemical properties

For multiple sequence analysis, ClustalW2 tool (http://www.ebi.ac.uk/Tools/msa/clustalw2/) was used and phylogenetic tree was constructed based on NJ (Neighbor joining) plot without distance correction. ProtParam (http://web.expasy.org/protparam/) was used to predict

physico-chemical properties. The parameters computed by ProtParam included the molecular weight, theoretical PI, aliphatic index and grand average of hydropathicity (GRAVY).

4.3 Homology modelling, visualization and quality assessment of 3D structure of α-synuclein protein.

Homology modelling was used to determine the 3D structure of α-synuclein isoforms. A BLASTP search with default parameters was performed against the Brookhaven Protein Data Bank (PDB) to find suitable templates for homology modelling. Template with PDB ID: 1XQ8 was retrieved for SNCA protein from Protein Data Bank (PDB). The Protein Structure Prediction Server Swiss model (http://swissmodel.expasy.org/) was used for homology model construction. Once the 3D structure of protein was generated, structural evaluation and stereo chemical analysis was performed using RAMPAGE (http://www.mordred.bioc.cam.ac.uk/~rapper/rampage.php). Errat server was used to find the accuracy of the structure and visualization of determined structures was performed using UCSF Chimera.

4.4 Active site prediction

Castp Server (http://www.sts.bioe.uic.edu/castp/) was used to predict the active sites of protein. Castp could also be used to measure area, circumference of mouth openings of each binding site in solvent and molecular accessible surface. PDB file of protein was uploaded in the server and it showed the ligand binding sites present in protein and the most conserved site was selected and all the amino acid residues involved in binding with ligands were retrieved.

4.5 Ligand optimization

Reported ligand molecules along with their physical and chemical properties were retrieved from PubChem Compound Database (http://www.pubchem.ncbi.nlm.nih.gov/). Pubchem is a composite database backed up by three primary databases, i.e. PCsubstance, PCcompund, and PCBioAssay. Pubchem provides biological activity, chemical information of small molecules. PCsubstance contains information about the substances; PCcompound contains information about chemical compounds, and PCBioAssay provides information about Bioassays. Three compounds (Genistein, Hesperidine and Epigallocatechin-3-Gallate) were selected, SDF files of Ligands were converted in PDB file with the help of Open Babel tool that could be used for docking study. Visualization of Molecular Structure of compounds was done using Pymol.

4.6 Lipinski filter analysis of screened drugs

An online tool Lipinski Filter (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) was used to retrieve the information about drug likeness of drugs with the help of Lipinski Rule of five. Lipinski rule (or Lipinski rule of five) helps to differentiate drug and nondrug like molecules. It is used to identify the possibility of success or failure due to drug likeness for molecules fulfilling with two or more of the following rules: (a) Molecular Mass should be less than 500 Dalton, (b) High Lipophilicity (expressed as logP less than 5), (c) Less than 5 hydrogen bond donors, (d) Less than 10 hydrogen bond acceptors, and (e) Molar refractivity should be between 40 -130.

4.7 Preparation of Protein and ligand molecules and docking study

Preparation of protein involves the addition of polar hydrogen atoms, neutralization of charge and removal of any miscellaneous structures from the protein molecule by Autodock 4.2.1

whereas ligand preparation involves the neutralization of charge. Prepared and optimized structures of ligands and protein were ultimately used for molecular docking studies to predict the possible protein–ligand interactions and the results that include the understanding of the association that involves H-bonding and hydrophobic interactions were analyzed using LIGPLOT1.4.5, a program to generate schematic diagrams of protein-ligand interactions.

5. RESULTS AND DISCUSSIONS

5.1 Retrieval of α-synuclein Protein and its function elucidation

Based on functional domain sequence of well characterized gene/protein, homology search was done using Basic Local Alignment Search Tool (BLAST). We have successfully hunted 5 isoforms of SNCA protein on the basis of families and domains identified from Interproscan results. Interproscan study revealed that all homologues proteins for SNCA were belonging to Synuclein family (IPR001058) and α-synuclein family (IPR002460). These isoforms belong to Synuclein, α-synuclein and NACP/ α-synuclein family **(Figure-5)**.

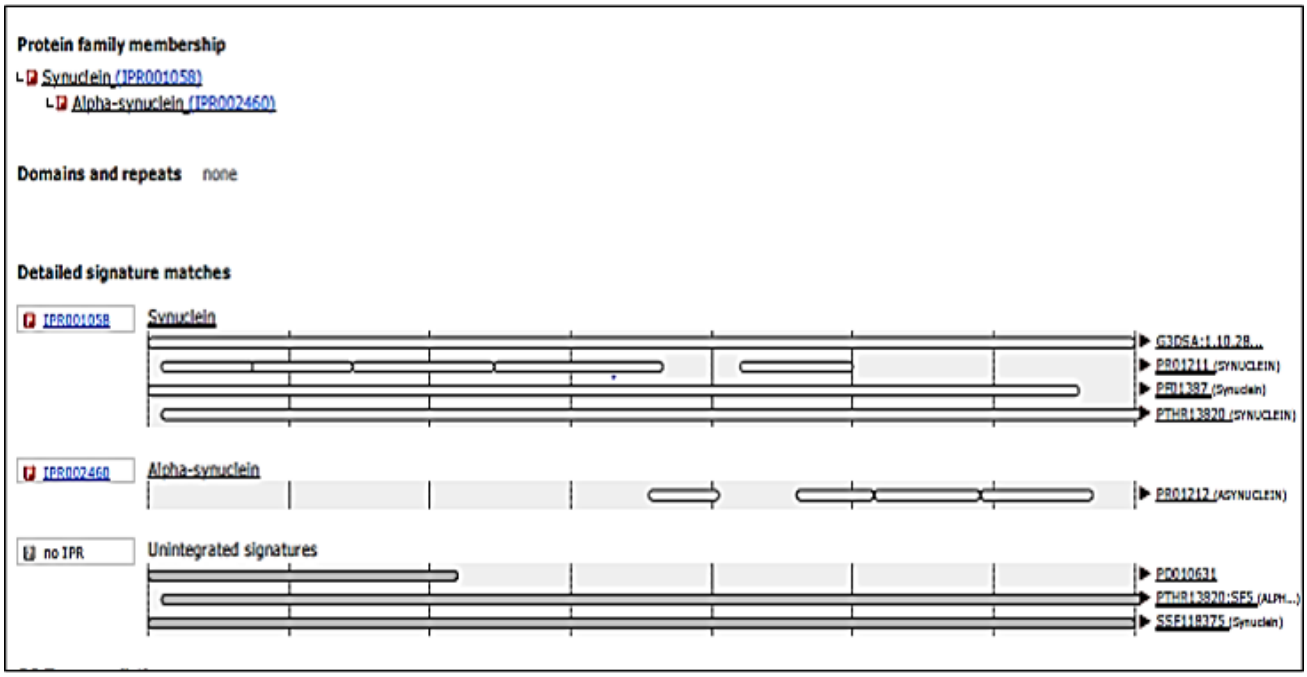


Figure 5: Interproscan result for SNCA domain identification. Interproscan analysis shows that SNCA does not have domains and repeats.

S.No.	Accession	Protein	Score	Identity	E Value
1	AAP36433.1	synuclein, α (non A4 component of amyloid precursor)	270	100%	4.00E-90
2	NP_000336.1	α-synuclein isoform NACP140	270	100%	4.00E-90
3	AKI70670.1	SNCA	268	99%	1.00E-89
4	AAC02114.1	NACP/α-synuclein	265	99%	2.00E-88
5	AAA98493.1	synuclein	234	99%	3.00E-76

Table 1: Hunted α-synuclein and synuclein proteins

5.2 Phylogenetic relationship and Physico-chemical properties

For multiple sequence analysis, *ClustalW2* tool was used and found that amino acid residues were conserved in most of the isoforms of the protein SNCA. Phylogenetic study of SNCA revealed that α-synuclein isoform NACP140 and NACP/α-synuclein were differing from others and SNCA and synuclein, α (non A4 component of amyloid precursor) were in same cluster as share more homology **(Figure-6)**. ProtParam showed that Mol. wt. of SNCA was 14460.1 Daltons. An isoelectric point for SNCA was found to be 4.67 which indicate that protein was negatively charged. The GRAVY index of -0.403 indicates that SNCA protein is hydrophilic and soluble.

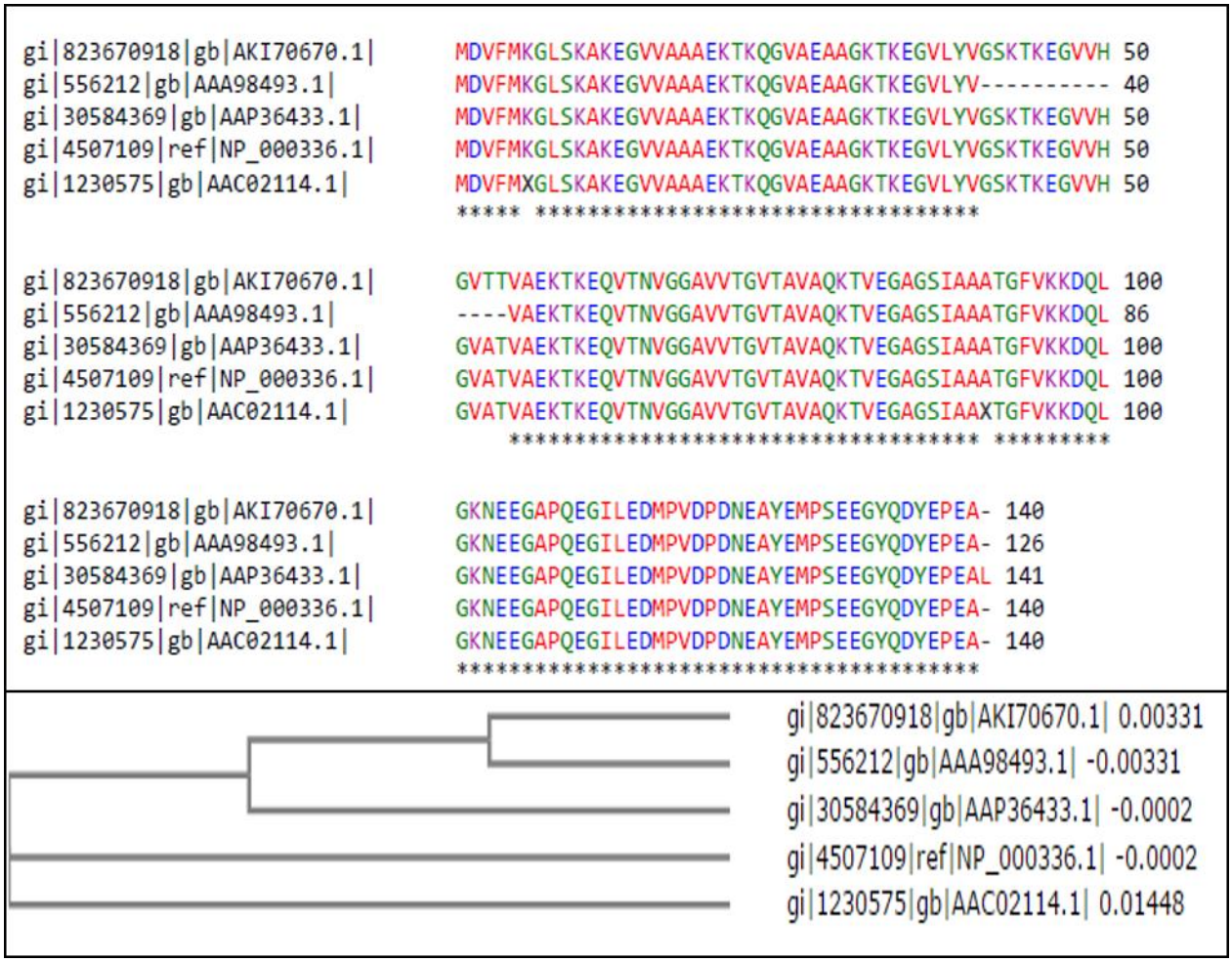


Figure 6: Multiple Sequence Alignment of all SNCA isoforms and Tree generation for SNCA using NJ Plot without distance correction. MSA analysis shows that the hunted sequences are conserved with respect to one another and phylogeny predicts that NACP140 and NACP/α-synuclein were differing from others and SNCA and synuclein, α (non A4 component of amyloid precursor) were in same cluster.

Properties	SNCA
Molecular Formula	$C_{627}H_{1012}N_{166}O_{216}S_4$
Molecular Weight (Daltons)	14460.1
Theoretical PI	4.67
Aliphatic Index	69.64
Grand Average of Hydropathicity (GRAVY)	-0.403

Table 2: Physico-chemical properties of SNCA

5.3 Homology modelling

Prediction of 3D structure of proteins provides us precise functional information of how proteins interact and localize in their stable conformation. Homology or comparative modelling is one of the most common structure prediction methods in structural genomics and proteomics. Numerous online servers and tools have become available for homology or comparative modelling of proteins. The best matching template with PDB ID: 1XQ8 was selected for the target protein on the basis of sequence homology using PDB Advance Blast. Template is experimentally determined 3D structure of protein that share sequence similarity with target sequence. A well-defined alignment is very important for the reliable prediction of a 3D structure. The template sequence and the target protein sequence were aligned using BLASTP alignment tool. Template showed sequence identity of 100% for α-synuclein isoforms. 3D structure of α-synuclein was generated using Swiss Model Server. The Z-score is indicative of overall model quality and is used to check whether the input structure is within the range of scores typically found for native proteins of similar size. Z score of the template and query model was obtained by SWISS MODEL. Z score for α-synuclein was -0.559 suggesting a good structure **(Table 3)**.

Gene Name	Modelled residue range	Based on template	Sequence Identity	QMEAN Z-Score
SNCA	1-140	1XQ8	100%	-0.559

Table 3: Swiss Model server result showing template structure used in homology modelling, Sequence Identity and quality score of the model generated.

5.4 3D structure Visualization and quality assessment

3D structure of SNCA was generated. Even though there were no steric clashes in the structure generated, these were assessed for geometric and energy aspects **(Figure-7 a)**. Ramachandran plot was used to check the reliability of predicted 3D structure of SNCA

protein. RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) checks the stereo chemical quality of a protein structure by analyzing residue-by-residue geometry and overall structure geometry **(Figure-7 b)**. Ramachandran plot was obtained for SNCA for quality assessment. RAMPAGE displayed 92.6% of residues in the most favored regions, 7.2% residues in additionally allowed and 0.2% disallowed regions. Errat (http://nihserver.mbi.ucla.edu/ERRATv2/) server was used to find the accuracy of the model. Result of Errat showed that the structure of SNCA protein was 93.016% accurate.

5.5 Active site prediction

CastP server was used to predict the ligand binding site. This server calculates the possible active sites from the 3D atomic coordinates of the protein. Active site prediction is useful to determine potential sites for ligand binding in molecular docking. Residues involved in ligand binding site, site volume and protein volume for ten active sites for SNCA were predicted. Among the ten binding sites obtained from CastP for SNCA, site 10 was highly conserved within the active site of the protein **(Figure-7 c)**. The Predicted site 10 consisted 783.61 Cubic angstroms site volume out of the 1095.56 Cubic Angstroms of protein volume. The residues in site 10 are LYS^{10}, GLU^{13}, GLY^{14}, Val^{15}, ALA^{17}, ALA^{18}, Glu^{20}, LYS^{21}, Gln^{24}, Ala^{28}, LYS^{58}, GLU^{61}, GLN^{62}, ASN^{65}, VAL^{66}, ALA^{69}, VAL^{70}, GLY^{73}, VAL^{74}, ALA^{76}, VAL^{77}, Gln^{79}, LYS^{80} and Glu^{83}.

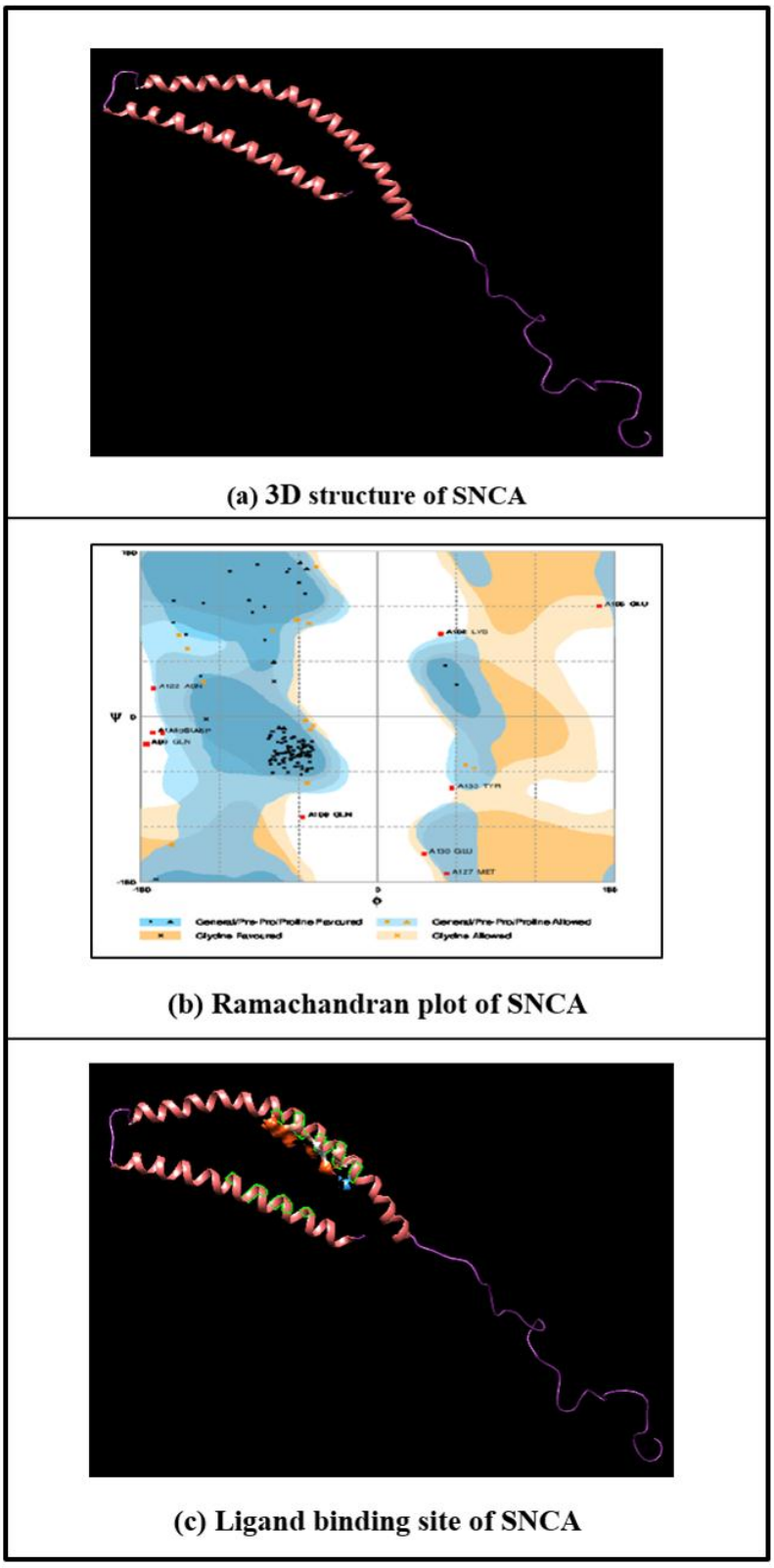


Figure 7: 3D structures, Ramachandran Plot and Active site of generated SNCA model. Generated model had no steric clashes and its most conserved active site is located in the hydrophobic region of α-synuclein protein.

5.6 Physico-chemical Properties of ligands

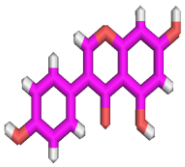
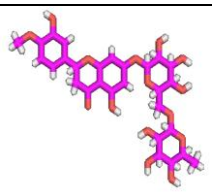
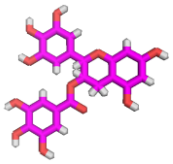
Characteristics	Genistein	Hesperidine	Epigallocatechin-3-Gallate
Molecular weight	270.237 g/mol	610.56056 g/mol	458.37172g/mol
Molecular Formula	$C_{15}H_{10}O_5$	$C_{28}H_{34}O_{15}$	$C_{22}H_{18}O_{11}$
Molecular Structure			
IUPAC Name	5,7-dihydroxy-3-(4-hydroxyphenyl)chromen-4-one	(2S)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxy-2,3-dihydrochromen-4-one	[(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl] 3,4,5-trihydroxybenzoate
Rotatable Bond Count	1	7	4
Topological Polar Surface Area	$87A^2$	$234A^2$	$197A^2$
Heavy Atom Count	20	43	33
Complexity	411	940	667
Covalently Bonded Unit Count	1	1	1

Table: 4 Characteristics of Anticancerous drugs used for docking study

Biomolecules	Molecular Mass (Daltons)	Lipophilicity (xlogP)	Hydrogen Bond Donors	Hydrogen Bond Acceptors	Molar Refractivity
Genistein	268	1.23237	0	5	59.309498
Hesperidine	660	8.671936	7	15	193.869644
Epigallocatechin-3-Gallate	468	3.537589	0	11	108.926483

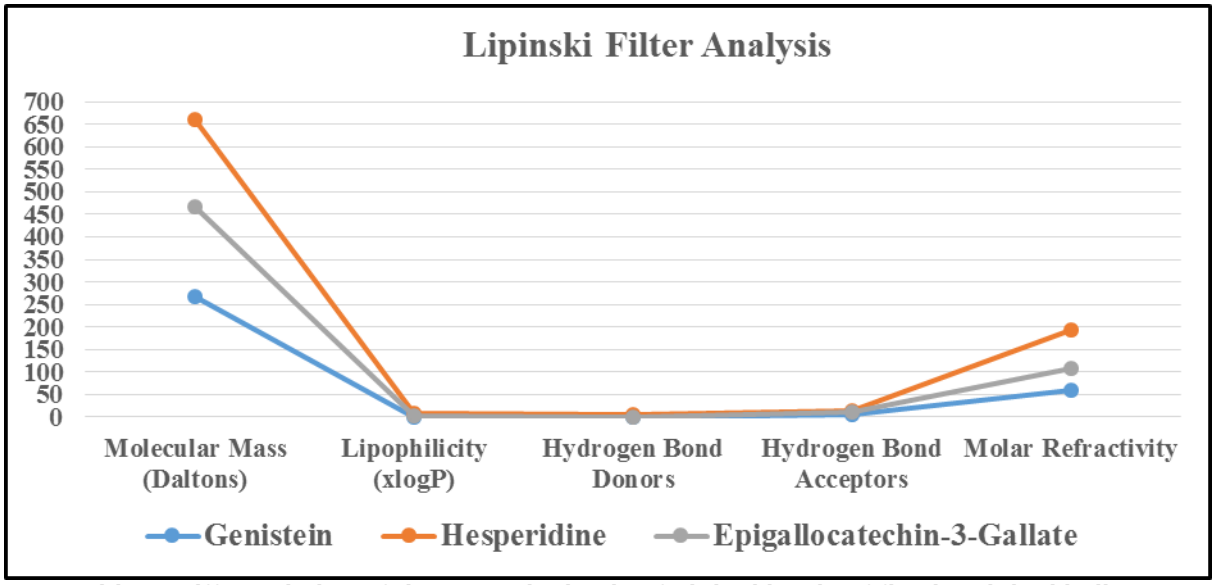


Table 5: Differentiation of drugs on the basis of Lipinski Rule of five by Lipinski Filter

This initial screening of the ligand molecules was done on the basis of Lipinski's rule of five. Lipinski filter analysis revealed that Genistein had more drug likeness which was followed by Epigallocatechin-3-Gallate and Hesperidine possessed least drug likeness **(Table 5)**.

5.7 Docking calculation of compounds with SNCA

SNCA interaction with Genistein

Free energy of binding with Genistein was -4.28 kcal/mol and Est. Inhibition Constant, Ki was found to be 733.64 µM **(Figure-8 a)**. Intermolecular Energy was found to be -5.47 kcal/mol. VdW + Hbond + desolv Energy and Electrostatic Energy was -5.32 kcal/mol and

-0.15 kcal/mol. Total Internal Energy and Torsional Free Energy was found to be 16.68kcal/mol and 1.19 kcal/mol.

SNCA interaction with Hesperidine

Free energy of binding with Hesperidine was -3.04 kcal/mol and Est. Inhibition Constant, Ki was found to be 5.88 mM **(Figure-8 b)**. Intermolecular Energy was found to be -7.52 kcal/mol. VdW + Hbond + desolv Energy and Electrostatic Energy was -7.34 kcal/mol and -0.18 kcal/mol. Total Internal Energy and Torsional Free Energy was found to be 27.93 kcal/mol and 4.47 kcal/mol.

SNCA interaction with Epigallocatechin-3-Gallate

Free energy of binding with Epigallocatechin-3-Gallate was -3.04 kcal/mol and Est. Inhibition Constant, Ki was found to be 5.88 mM **(Figure-8 c)**. Intermolecular Energy was found to be -7.52 kcal/mol. VdW + Hbond + desolv Energy and Electrostatic Energy was -7.34 kcal/mol and -0.18 kcal/mol. Total Internal Energy and Torsional Free Energy was found to be 27.93 kcal/mol and 4.47 kcal/mol.

Compound Name	Est. Free Energy of Binding	Est. Binding Constant	Est. Intermolecular Energy	vdW+Hbond+desolv Energy	Electrostatic Energy	Est. Internal Energy	Torsional Free Energy
Genistein	-4.28 (kcal/mol)	733.64 µM	-5.47 (kcal/mol)	-5.32 (kcal/mol)	-0.15 (kcal/mol)	+16.68 (kcal/mol)	+1.19 (kcal/mol)
Hesperidine	-3.04 (kcal/mol)	5.88 mM	-7.52 (kcal/mol)	-7.34 (kcal/mol)	-0.18 (kcal/mol)	+27.93 (kcal/mol)	+4.47 (kcal/mol)
Epigallocatechin-3-Gallate	-2.78 (kcal/mol)	9.16 mM	-6.36 (kcal/mol)	-5.60 (kcal/mol)	-0.76 (kcal/mol)	-5.89 (kcal/mol)	+3.58 (kcal/mol)

Table 6: Docking calculation of compounds with SNCA

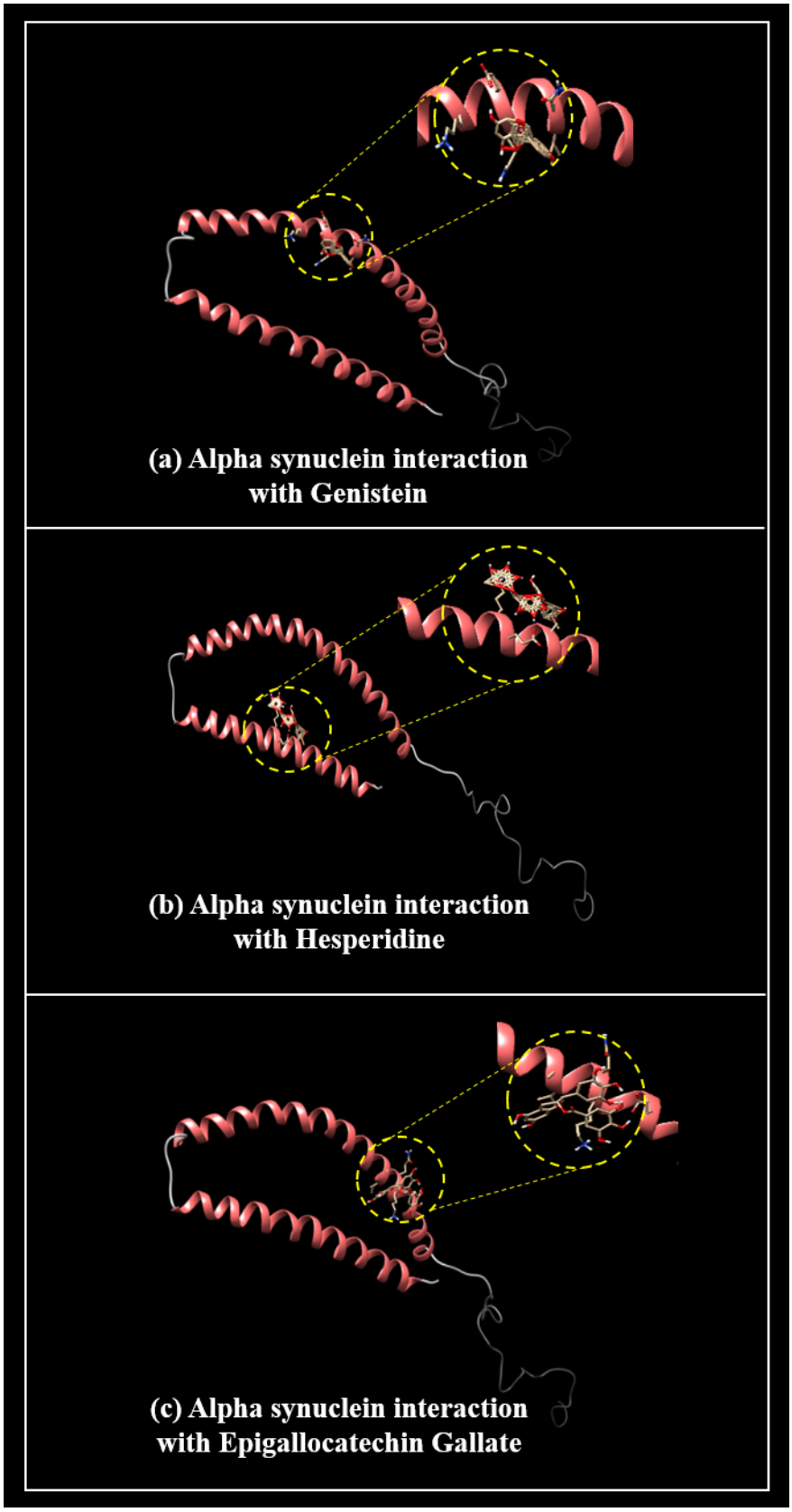


Figure 8: Docking study of SNCA with selected anti-cancerous drugs. Docking study shows that Genistein and Epigallocatechin-3-gallate binds to the hydrophobic region while Hesperidine does not bind to this region.

5.8 Binding site of SNCA with selected compounds along with its reported Inhibitory active site

Binding site residues of SNCA interacting with Genistein, Hesperidine and Epigallocatechin-3-Gallate was found to be the same as the residues involved in binding with earlier used inhibitor. Interacting residues of SNCA with its inhibitor were LYS^{10}, GLU^{13}, GLY^{14}, VAL^{15}, ALA^{17}, ALA^{18}, GLU^{20}, LYS^{21}, GLN^{24}, ALA^{28}, LYS^{58}, GLU^{61}, GLN^{62}, ASN^{65}, VAL^{66}, ALA^{69}, VAL^{70}, GLY^{73}, VAL^{74}, ALA^{76}, VAL^{77}, GLN^{79}, LYS^{80} and GLU^{83} (**Table 7)**. Analysis of ligand and protein interaction revealed that Genistein forms H bonding pattern with GLU^{61} residue and Hydrophobic bonding pattern with LYS^{58}, GLU^{61}, GLN^{62}, ASN^{65} and VAL^{66} residues **(Figure-9 a & d)** of SNCA protein. Hesperidine forms H bonding pattern with ALA^{17}, GLU^{20} and LYS^{21} residues and Hydrophobic bonding pattern with GLY^{14}, VAL^{15}, ALA^{17}, GLU^{20}, LYS^{21}, GLN^{24} and ALA^{28} residues **(Figure-9 b & e)** of SNCA protein. Epigallocatechin-3-Gallate forms H bonding pattern with GLU^{83} residue and Hydrophobic bonding pattern with ALA^{76}, VAL^{77}, GLN^{79}, LYS^{80} and GLU^{83} residues **(Figure-9 c & f)** of SNCA protein.

Compound	Interacting residues
Reported Inhibitory Active Site	LYS^{10}, GLU^{13}, GLY^{14}, VAL^{15}, ALA^{17}, ALA^{18}, GLU^{20}, LYS^{21}, GLN^{24}, ALA^{28}, LYS^{58}, GLU^{61}, GLN^{62}, ASN^{65}, VAL^{66}, ALA^{69}, VAL^{70}, GLY^{73}, VAL^{74}, ALA^{76}, VAL^{77}, GLN^{79}, LYS^{80}, GLU^{83}.
Genistein	LYS^{58}, GLU^{61}, GLN^{62}, ASN^{65}, VAL^{66}
Hesperidine	GLY^{14}, VAL^{15}, ALA^{17}, GLU^{20}, LYS^{21}, GLN^{24}, ALA^{28}
Epigallocatechin-3-Gallate	ALA^{76}, VAL^{77}, GLN^{79}, LYS^{80}, GLU^{83}

Table 7: SNCA reported inhibitory site and selected compounds interacting residues

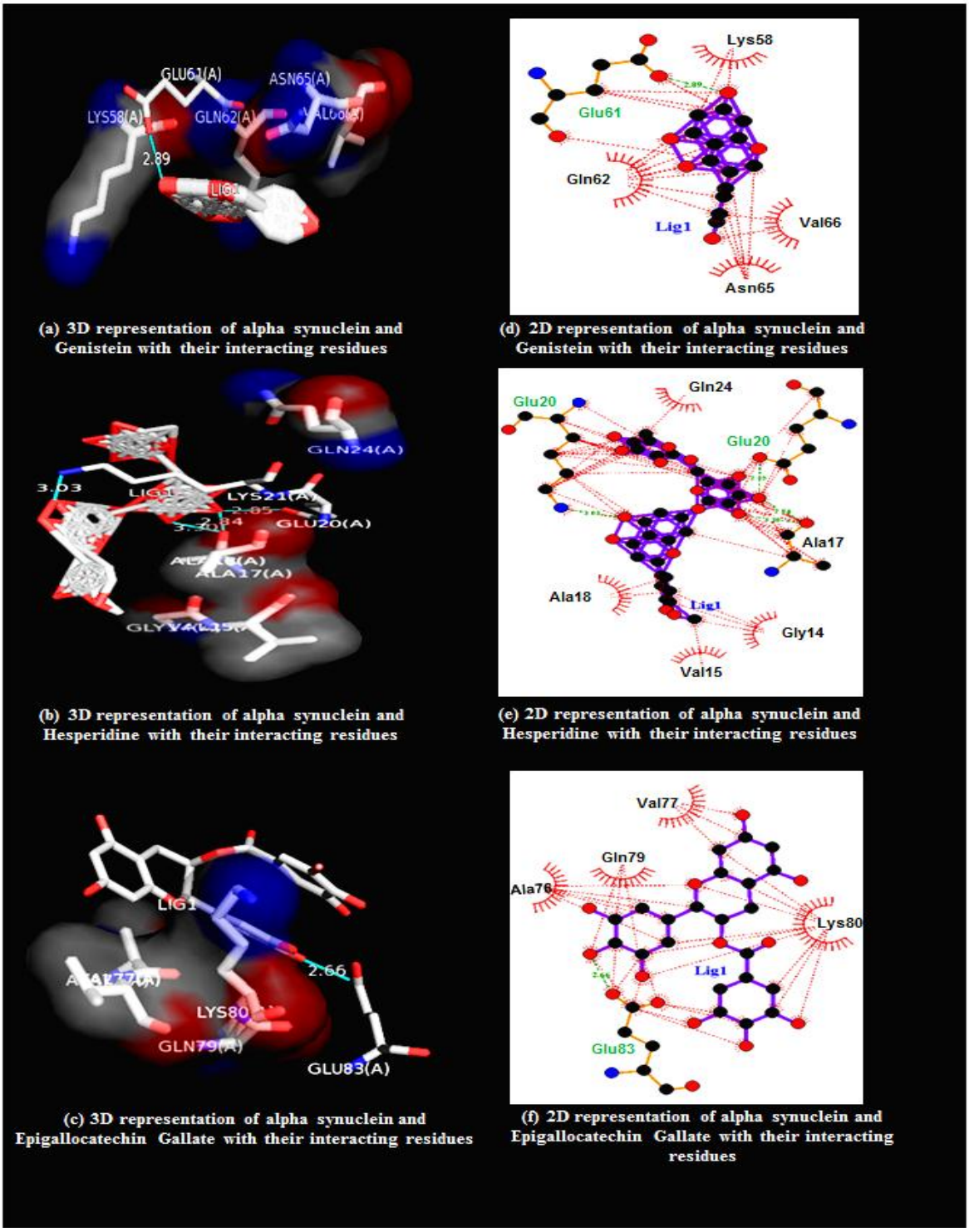


Figure 9: Binding site of SNCA with selected compounds along with its reported inhibitory active site. 3D and 2D pattern of protein-ligand interaction shows the interacting residues of SNCA ligand binding.

Recent therapeutics advancement in α-synucleinopathies reveal the promising role of anti-cancerous drugs as potent neuroprotective agents for the treatment of Lewy body diseases. Here in *Insilico* investigation, we have successfully hunted 5 unique hits based on functional domain sequence using BLAST **(Table 1)** (Altschul et al., 1990) and optimized the full length genes of SNCA on the basis of families identified from Interproscan results. These isoforms belong to Synuclein family (IPR001058) and α-synuclein family (IPR002460) and catalyze functions based on its interaction with tubulin and have potential microtubule associated protein, tau like activity **(Figure-5)** (Kawakami and Ichikawa, 2015). Phylogenetic study using ClustalW2 revealed that α-synuclein isoform NACP140 and NACP/α-synuclein were differing from others and SNCA and synuclein, α (non A4 component of amyloid precursor) were in same cluster as share more homology **(Figure-6)** (Thompson et al., 2002). ProtParam results showed that isoelectric point was 4.67 which indicate that protein was negatively charged. The GRAVY index of -0.403 indicates that SNCA protein is hydrophilic and soluble **(Table 2)**. Template showed 100% sequence identity for SNCA. 3D structure of SNCA was generated by using Swiss Model Server (Arnold et al., 2006) and visualized using UCSF chimera **(Figure-7 a)** (Yang et al., 2012). Z score for SNCA was 0.559 suggesting that input structure is within the range of scores typically found for native proteins of similar size **(Table 3)** (Schwede et al., 2003). RAMPAGE displayed 92.6% of residues in the most favored regions, 7.2% residues in additionally allowed and 0.2% disallowed regions, showing that stereo chemical quality of protein structure is good **(Figure-7 b)**. Result of Errat showed that the structure of SNCA protein was 93.016% accurate. Among the ten binding sites obtained from CastP Server for SNCA, site 10 was highly conserved within all the binding sites of SNCA protein **(Figure-7 c)** (Dundas et al., 2006). Active site prediction is useful to determine potential sites for ligand binding in molecular docking. Three compounds

(Genistein, Hesperidine and Epigallocatechin-3-Gallate) which are extracted from different plants were selected for molecular docking study at *In-silico* level.

Lipinski Filter Analysis of all the compounds revealed that Genistein had more drug likeness which was followed by Epigallocatechin-3-Gallate and Hesperidine possessed least drug likeness respectively **(Table 5)** (Lipinski, 2004). Docking study revealed that all the three compounds are interacting at the reported active binding site (Pradeepkiran et al., 2015). Inhibition Constant, Ki of Genistein, Hesperidine and Epigallocatechin-3-Gallate for SNCA was found to be 733.64 µM, 5.88 mM and 9.16 mM respectively, suggesting that all the selected compounds are effective as SNCA (**Table 6)**. Investigation of binding sites along with the reported active binding site within SNCA protein gives a better idea for a valuable drug target site and drug interaction with highest affinity. In this result, the most effective compound was found to be Genistein as showing minimum Inhibition Constant, Ki and lowest free energy of binding with maximum interacting surface area (Park et al., 2006). Furthermore, Genistein binds to the hydrophobic region present in the active site of SNCA protein, Epigallocatechin-3-gallate also binds to the hydrophobic region of active site but with lesser efficiency but Hesperidine does not bind to the hydrophobic region in the active site of SNCA protein **(Figure-8 & 9)** and hence it may not have a role in the inhibition of aggregated form of α-synuclein in dementia with Lewy bodies (DLB) and Alzheimer's disease. Hesperidine can play its role in some other signaling mechanism at the molecular level of neurodegenerative disorders.

6. CONCLUSION

In conclusion, *in silico* studies revealed that anti-cancerous drugs might have a role in the inhibition of α-synuclein in dominantly inherited. Neuronal damage is caused due to oxidative stress that results from the imbalance between antioxidants and reactive oxygen species. The human diseases such as aging, arthritis, shock, ischemic injury, and neurodegenerative diseases (Parkinson's and Alzheimer's disease) all are influenced by this phenomenon. Furthermore, anti-cancerous drugs such as Genistein, Hesperidine and Epigallocatechin-3-Gallate might provide protection against these diseases such as LBD. However, the role of anti-cancerous drugs Genistein with α-syn provides a novel therapeutic approach among all three drugs on the basis of docking studies. We conclude that anti-cancerous drug Genistein plays a crucial role in the therapeutics of α-synucleinopathies and other related neurological disorders.

7. FUTURE PERSPECTIVES

The results can be validated through laboratory trials and clinical trials of the drugs as one of future perspective options. One can also find the aberrant unknown side effects associated with these drugs that could arise upon combination with other potentiating drug molecules. Moreover, differential expression of α-synuclein can be analyzed that would be knocked down or perturbed due to ingestion of these drug combination or changes brought in any signaling pathway leading to faulty mechanisms inside body. Further, their association studies could be done in order to explore the metabolic pathways that might get affected with the implication of these drugs. Furthermore, mutational analysis could be done to find the potential threat to the crucial proteins upon drug treatment. Drug processing and development should to be addressed so as to minimize the drug diffusion in order to deliver it at the target site. Finally, linking all the results through various cross-checks and thus proclaiming about finding a new possibility to treat α-synucleinopathies or Lewy body diseases can be made.

8. REFERENCES

Alderson TR, Markley JL. Biophysical characterization of α-synuclein and its controversial structure. *Intrinsically Disord Proteins*. 2013; 1:18-39.

Alexander GE. Biology of Parkinson's disease: pathogenesis and pathophysiology of a multisystem neurodegenerative disorder. *Dialogues Clin Neurosci*. 2004; 6:259-80.

Al-Mansoori KM, Hasan MY, Al-Hayani A, El-Agnaf OM. The role of α-synuclein in neurodegenerative diseases: from molecular pathways in disease to therapeutic approaches. *Curr Alzheimer Res*. 2013; 10:559-68.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol*. 1990; 215:403-10.

Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics*. 2006; 22:195-201.

Arrasate M, Finkbeiner S. Protein aggregates in Huntington's disease. *Exp Neurol*. 2012; 238:1-11.

Auluck PK, Caraveo G, Lindquist S. α-Synuclein: membrane interactions and toxicity in Parkinson's disease. *Annu Rev Cell Dev Biol*. 2010; 26:211-33.

Baba M, Nakajo S, Tu PH, Tomita T, Nakaya K, Lee VM, Trojanowski JQ, Iwatsubo T. Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. *Am J Pathol*. 1998; 152:879-84.

Bandopadhyay R. Sequential Extraction of Soluble and Insoluble Alpha-Synuclein from Parkinsonian Brains. *J Vis Exp*. 2016; 107.

Bartels T, Ahlstrom LS, Leftin A, Kamp F, Haass C, Brown MF, Beyer K. The N-terminus of the intrinsically disordered protein α-synuclein triggers membrane binding and helix folding. *J Biophys*. 2010; 99:2116-24.

Beiske AG, Loge JH, Rønningen A, Svensson E. Pain in Parkinson's disease: Prevalence and characteristics. *Pain*. 2009; 141:173-7.

Bekris LM, Mata IF, Zabetian CP. The genetics of Parkinson disease. *J Geriatr Psychiatry Neurol*. 2010; 23:228-42.

Bendor JT, Logan TP, Edwards RH. The function of α-synuclein. *Neuron*. 2013; 79:1044-66.

Benskey MJ, Perez RG, Manfredsson FP. The contribution of alpha synuclein to neuronal survival and function - Implications for Parkinson's disease. *J Neurochem*. 2016; 137:331-59.

Beyer K, Domingo-Sàbat M, Ariza A. Molecular pathology of Lewy body diseases. *Int J Mol Sci*. 2009; 10:724-45.

Bisaglia M, Mammi S, Bubacco L. Structural insights on physiological functions and pathological effects of alpha-synuclein. *FASEB J*. 2009; 23:329-40.

Bisaglia M, Trolio A, Bellanda M, Bergantino E, Bubacco L, Mammi S. Structure and topology of the non-amyloid-beta component fragment of human alpha-synuclein bound to micelles: implications for the aggregation process. *Protein Sci*. 2006; 15:1408-16.

Bleasel JM, Halliday GM, Kim WS. Animal modeling an oligodendrogliopathy--multiple system atrophy. *Acta Neuropathol Commun*. 2016; 4:12.

Bleasel JM, Wong JH, Halliday GM, Kim WS. Lipid dysfunction and pathogenesis of multiple system atrophy. *Acta Neuropathol Commun*. 2014; 2:15.

Bobela W, Aebischer P, Schneider BL. Alpha-Synuclein as a Mediator in the Interplay between Aging and Parkinson's Disease. *Biomolecules*. 2015; 5:2675-700.

Bonini NM, Giasson BI. Snaring the function of alpha-synuclein. *Cell*. 2005; 123:359-61.

Brettschneider J, Del Tredici K, Lee VM, Trojanowski JQ. Spreading of pathology in neurodegenerative diseases: a focus on human studies. *Nat Rev Neurosci*. 2015; 16:109-20.

Breydo L, Wu JW, Uversky VN. A-synuclein misfolding and Parkinson's disease. *Biochim Biophys Acta*. 2012; 1822:261-85.

Brück D, Wenning GK, Stefanova N, Fellner L. Glia and alpha-synuclein in neurodegeneration: A complex interaction. *Neurobiol Dis*. 2016; 85:262-74.

Burai R, Ait-Bouziad N, Chiki A, Lashuel HA. Elucidating the Role of Site-Specific Nitration of α-Synuclein in the Pathogenesis of Parkinson's Disease via Protein Semisynthesis and Mutagenesis. *J Am Chem Soc*. 2015; 137:5041-52.

Burn DJ, Jaros E. Multiple system atrophy: cellular and molecular pathology. *Mol Pathol*. 2001; 54:419-26.

Burré J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Südhof TC. Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. *Science*. 2010; 329:1663-7.

Chánez-Cárdenas ME, Vázquez-Contreras E. The Aggregation of Huntingtin and α-Synuclein. *J Biophys*. 2012; 2012:606172.

Chen S, Sayana P, Zhang X, Le W. Genetics of amyotrophic lateral sclerosis: an update. *Mol Neurodegener*. 2013; 8:28.

Cheng HC, Ulane CM, Burke RE. Clinical progression in Parkinson disease and the neurobiology of axons. *Ann Neurol*. 2010; 67:715-25.

Cho J. Antioxidant and neuroprotective effects of hesperidin and its aglycone hesperetin. *Arch Pharm Res*. 2006; 29:699-706.

Clinton LK, Blurton-Jones M, Myczek K, Trojanowski JQ, LaFerla FM. Synergistic Interactions between Abeta, tau, and alpha-synuclein: acceleration of neuropathology and cognitive decline. *J Neurosci*. 2010; 30:7281-9.

Cobb NJ, Surewicz WK. Prion diseases and their biochemical mechanisms. *Biochemistry*. 2009; 48:2574-85.

Cook C, Stetler C, Petrucelli L. Disruption of protein quality control in Parkinson's disease. *Cold Spring Harb Perspect Med*. 2012; 2:a009423.

Corrochano S, Renna M, Carter S, Chrobot N, Kent R, Stewart M, Cooper J, Brown SD, Rubinsztein DC, Acevedo-Arozena A. α-Synuclein levels modulate Huntington's disease in mice. *Hum Mol Genet*. 2012; 21:485-94.

Cuny GD. Foreword: neurodegenerative diseases: challenges and opportunities. *Future Med Chem*. 2012; 4:1647-9.

Danielson SR, Andersen JK. Oxidative and nitrative protein modifications in Parkinson's disease. *Free Radic Biol Med*. 2008; 44:1787-94.

Darios F, Ruipérez V, López I, Villanueva J, Gutierrez LM, Davletov B. Alpha-synuclein sequesters arachidonic acid to modulate SNARE-mediated exocytosis. *EMBO Rep*. 2010; 11:528-33.

Dias V, Junn E, Mouradian MM. The role of oxidative stress in Parkinson's disease. *J Parkinson's Dis*. 2013; 3:461-91.

Dickson DW, Liu W, Hardy J, Farrer M, Mehta N, Uitti R, Mark M, Zimmerman T, Golbe L, Sage J, Sima A, D'Amato C, Albin R, Gilman S, Yen SH. Widespread alterations of alpha-synuclein in multiple system atrophy. *Am J Pathol*. 1999; 155:1241-51.

Dong XX, Wang Y, Qin ZH. Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacol Sin*. 2009; 30:379-87.

Dundas J, Ouyang Z, Tseng J, Binkowski A, Turpaz Y, Liang J. CASTp: computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues. *Nucleic Acids Res*. 2006; 34:W116-118.

Emanuele M, Chieregatti E. Mechanisms of alpha-synuclein action on neurotransmission: cell-autonomous and non-cell autonomous role. *Biomolecules*. 2015; 5:865-92.

Esteban-Martín S, Silvestre-Ryan J, Bertoncini CW, Salvatella X. Identification of fibril-like tertiary contacts in soluble monomeric α-synuclein. *J Biophys*. 2013; 105:1192-8.

Fahn S, Sulzer D. Neurodegeneration and neuroprotection in Parkinson disease. *NeuroRx*. 2004; 1:139-54.

Fecto F, Esengul YT, Siddique T. Protein recycling pathways in neurodegenerative diseases. *Alzheimers Res Ther*. 2014; 6:13.

Fernandez HH, Wu CK, Ott BR. Pharmacotherapy of dementia with Lewy bodies. *Expert Opin Pharmacother*. 2003; 4:2027-37.

Ferreon AC, Gambin Y, Lemke EA, Deniz AA. Interplay of alpha-synuclein binding and conformational switching probed by single-molecule fluorescence. *Proc Natl Acad Sci U S A*. 2009; 106:5645-50.

Finkbeiner S. Huntington's Disease. *Cold Spring Harb Perspect Biol*. 2011; 3.

Follmer C, Coelho-Cerqueira E, Yatabe-Franco DY, Araujo GD, Pinheiro AS, Domont GB, Eliezer D. Oligomerization and Membrane-binding Properties of Covalent Adducts Formed by the Interaction of α-Synuclein with the Toxic Dopamine Metabolite 3,4-Dihydroxyphenylacetaldehyde (DOPAL). *J Biol Chem*. 2015; 290:27660-79.

Fridlender M, Kapulnik Y, Koltai H. Plant derived substances with anti-cancer activity: from folklore to practice. *Front Plant Sci*. 2015; 6:799.

Furukawa Y. Redox environment is an intracellular factor to operate distinct pathways for aggregation of Cu,Zn-superoxide dismutase in amyotrophic lateral sclerosis. *Front Cell Neurosci*. 2013; 7:240.

Gallegos S, Pacheco C, Peters C, Opazo CM, Aguayo LG. Features of alpha-synuclein that could explain the progression and irreversibility of Parkinson's disease. *Front Neurosci*. 2015; 9:59.

Games D, Valera E, Spencer B, Rockenstein E, Mante M, Adame A, Patrick C, Ubhi K, Nuber S, Sacayon P, Zago W, Seubert P, Barbour R, Schenk D, Masliah E. Reducing C-terminal-truncated alpha-synuclein by immunotherapy attenuates neurodegeneration and propagation in Parkinson's disease-like models. *J Neurosci*. 2014; 34:9441-54.

Gandhi S, Abramov AY. Mechanism of Oxidative Stress in Neurodegeneration. *Oxid Med Cell Longev*. 2012; 428010-11.

Gitler AD, Chesi A, Geddie ML, Strathearn KE, Hamamichi S, Hill KJ, Caldwell KA, Caldwell GA, Cooper AA, Rochet JC, Lindquist S. Alpha-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. *Nat Genet*. 2009; 41:308-15.

Golde TE, Borchelt DR, Giasson BI, Lewis J. Thinking laterally about neurodegenerative proteinopathies. *J Clin Invest*. 2013; 123:1847-55.

Gorman AM. Neuronal cell death in neurodegenerative diseases: recurring themes around protein handling. *J Cell Mol Med*. 2008; 12:2263-80.

Gross A, McDonnell JM, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. *Genes Dev*. 1999; 13:1899-911.

Guo C, Sun L, Chen X, Zhang D. Oxidative stress, mitochondrial damage and neurodegenerative diseases. *Neural Regen Res*. 2013; 8:2003-14.

Guo JL, Lee VM. Cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases. *Nat Med*. 2014; 20:130-8.

Habermann B, Hines D, Davis L. Caring for parents with neurodegenerative disease: a qualitative description. *Clin Nurse Spec*. 2013; 27:182-7.

Hartmann A. Postmortem studies in Parkinson's disease. *Dialogues Clin Neurosci*. 2004; 6:281-93.

Hasegawa M, Fujiwara H, Nonaka T, Wakabayashi K, Takahashi H, Lee VM, Trojanowski JQ, Mann D, Iwatsubo T. Phosphorylated alpha-synuclein is ubiquitinated in alpha-synucleinopathy lesions. *J Biol Chem*. 2002; 277:49071-6.

Hejjaoui M, Butterfield S, Fauvet B, Vercruysse F, Cui J, Dikiy I, Prudent M, Olschewski D, Zhang Y, Eliezer D, iuel HA. Elucidating the role of C-terminal post-translational modifications using protein semisynthesis strategies: α-synuclein phosphorylation at tyrosine 125. *J Am Chem Soc*. 2012; 134:5196-210.

Helferich AM, Ruf WP, Grozdanov V, Freischmidt A, Feiler MS, Zondler L, Ludolph AC, McLean PJ, Weishaupt JH, Danzer KM. α-synuclein interacts with SOD1 and promotes its oligomerization. *Mol Neurodegener*. 2015; 10:66.

Hodara R, Norris EH, Giasson BI, Mishizen-Eberz AJ, Lynch DR, Lee VM, Ischiropoulos H. Functional consequences of alpha-synuclein tyrosine nitration: diminished binding to lipid vesicles and increased fibril formation. *J Biol Chem*. 2004; 279:47746-53.

Hsieh HL, Yang CM. Role of Redox Signaling in Neuroinflammation and Neurodegenerative Diseases. *BioMed Res Int*. 2013; 484613-18.

Hwang O. Role of oxidative stress in Parkinson's disease. *Exp Neurobiol*. 2013; 22(1):11-7.

Ischiropoulos H, Beckman JS. Oxidative stress and nitration in neurodegeneration: cause, effect, or association? *J Clin Invest*. 2003; 111:163-9.

Jahn H. Memory loss in Alzheimer's disease. *Dialogues Clin Neurosci*. 2013; 15:445-54.

Jankovic J. Parkinson's disease: clinical features and diagnosis. *J Neurol Neurosurg Psychiatry*. 2008; 79:368-76.

Jellinger KA. Interaction between α-synuclein and other proteins in neurodegenerative disorders. *ScientWorld J*. 2011; 11:1893-907.

Jellinger KA, Wenning GK. Multiple system atrophy: pathogenic mechanisms and biomarkers. *J Neural Transm*. 2016; 123:555-72.

Jha NK, Jha SK, Kar R, Ambasta RK, Kumar P. Role of Oxidative Stress, ER Stress and Ubiquitin Proteasome System in Neurodegeneration. *MOJ Cell Sci Rep*. 2014; 1: 00010.

Jha SK, Jha NK, Kar R, Ambasta RK, Kumar P. p38 MAPK and PI3K/AKT Signalling Cascades in Parkinson's Disease. *Int J Mol Cell Med*. 2015 spring; 4:67-86.

Jiang Z, Hess SK, Heinrich F, Lee JC. Molecular details of α-synuclein membrane association revealed by neutrons and photons. *J Phys Chem B*. 2015; 119:4812-23.

Jiang Z, Wang W, Perry G, Zhu X, Wang X. Mitochondrial dynamic abnormalities in amyotrophic lateral sclerosis. *Transl Neurodegener*. 2015; 4:14.

Jo DG, Arumugam TV, Woo HN, Park JS, Tang SC, Mughal M, Hyun DH, Park JH, Choi YH, Gwon AR, Camandola S, Cheng A, Cai H, Song W, Markesbery WR, Mattson MP. Evidence that gamma-secretase mediates oxidative stress-induced beta-secretase expression in Alzheimer's disease. *Neurobiol Aging*. 2010; 31:917-25.

Kahle PJ, Haass C, Kretzschmar HA, Neumann M. Structure/function of alpha-synuclein in health and disease: rational development of animal models for Parkinson's and related diseases. *J Neurochem*. 2002; 82:449-57.

Kalia LV, Kalia SK, McLean PJ, Lozano AM, Lang AE. α-Synuclein oligomers and clinical implications for Parkinson disease. *Ann Neurol*. 2013; 73:155-69.

Kamaraj S, Ramakrishnan G, Anandakumar P, Jagan S, Devaki T. Antioxidant and anticancer efficacy of hesperidin in benzo (a) pyrene induced lung carcinogenesis in mice. *Invest New Drugs*. 2009; 27:214-222.

Karantzoulis S, Galvin JE. Distinguishing Alzheimer's disease from other major forms of dementia. *Expert Rev Neurother*. 2011; 11:1579-91.

Kawakami F, Ichikawa T. The Role of α-Synuclein and LRRK2 in Tau Phosphorylation. *Parkinsons Dis*. 2015; 2015:734746.

Kehrer JP. The Haber-Weiss reaction and mechanisms of toxicity. *Toxicology*. 2000; 149:43-50.

Kim WS, Kågedal K, Halliday GM. Alpha-synuclein biology in Lewy body diseases. *Alzheimers Res Ther*. 2014; 6:73.

Klegeris A, Giasson BI, Zhang H, Maguire J, Pelech S, McGeer PL. Alpha-synuclein and its disease-causing mutants induce ICAM-1 and IL-6 in human astrocytes and astrocytoma cells. *FASEB J*. 2006; 20:2000-8.

Klein C, Westenberger A. Genetics of Parkinson's disease. *Cold Spring Harb Perspect Med*. 2012; 2:a008888.

Kokhan VS, Afanasyeva MA, Van'kin GI. α-Synuclein knockout mice have cognitive impairments. *Behav Brain Res*. 2012; 231:226-30.

Kraus A, Groveman BR, Caughey B. Prions and the potential transmissibility of protein misfolding diseases. *Annu Rev Microbiol*. 2013; 67:543-64.

Kumar GP, Khanum F. Neuroprotective potential of phytochemicals. *Pharmacogn Rev*. 2012; 6:81-90.

Kumar P, Jha NK, Jha SK, Ramani K, Ambasta RK. Tau phosphorylation, molecular chaperones, and ubiquitin E3 ligase: clinical relevance in Alzheimer's disease. *J Alzheimers Dis*. 2015; 43:341-61.

Kupfer L, Hinrichs W, Groschup MH. Prion protein misfolding. *Curr Mol Med*. 2009; 9:826-35.

Lashuel HA, Overk CR, Oueslati A, Masliah E. The many faces of α-synuclein: from structure and toxicity to therapeutic target. *Nat Rev Neurosci*. 2013; 14:38-48.

Lavedan C. The synuclein family. *Genome Res*. 1998; 8:871-80.

Lehri-Boufala S, Ouidja MO, Barbier-Chassefière V, Hénault E, Raisman-Vozari R, Garrigue-Antar L, Papy-Garcia D, Morin C. New roles of glycosaminoglycans in α-synuclein aggregation in a cellular model of Parkinson disease. *PLoS One*. 2015; 10:e0116641.

Leung C, Jia Z. Mouse Genetic Models of Human Brain Disorders. *Front Genet*. 2016; 7:40.

Li J, O W, Li W, Jiang ZG, Ghanbari HA. Oxidative stress and neurodegenerative disorders. *Int J Mol Sci*. 2013; 14:24438-75.

Li W, West N, Colla E, Pletnikova O, Troncoso JC, Marsh L, Dawson TM, Jäkälä P, Hartmann T, Price DL, Lee MK. Aggregation promoting C-terminal truncation of alpha-synuclein is a normal cellular process and is enhanced by the familial Parkinson's disease-linked mutations. *Proc Natl Acad Sci U S A*. 2005; 102:2162-7.

Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discov Today Technol*. 2004; 1:337-341.

Litteljohn D, Mangano E, Clarke M, Bobyn J, Moloney K, Hayley S. Inflammatory mechanisms of neurodegeneration in toxin-based models of Parkinson's disease. *Parkinsons Dis*. 2010; 2011:713517.

Liu Y, Qiang M, Wei Y, He R. A novel molecular mechanism for nitrated {alpha}-synuclein-induced cell death. *J Mol Cell Biol*. 2011; 3:239-49.

Lonati E, Sala G, Bulbarelli A. Protein Misfolding and Accumulation as Root Cause in Neurodegeneration. *Austin Alzheimers J Parkinsons Dis*. 2014; 1:10.

Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, Trojanowski JQ, Lee VM. Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in non-transgenic mice. *Science*. 2012; 338:949-53.

Macedo D, Tavares L, McDougall GJ, Vicente Miranda H, Stewart D, Ferreira RB, Tenreiro S, Outeiro TF, Santos CN. (Poly) phenols protect from α-synuclein toxicity by reducing oxidative stress and promoting autophagy. *Hum Mol Genet*. 2015; 24:1717-32.

Mandel S, Weinreb O, Reznichenko L, Kalfon L, Amit T. Green tea catechins as brain-permeable, nontoxic iron chelators to "iron out iron" from the brain. *J Neural Transm Suppl*. 2006; 71:249-57.

Manning-Bog AB, McCormack AL, Purisai MG, Bolin LM, Di Monte DA. Alpha-synuclein overexpression protects against paraquat-induced neurodegeneration. *J Neurosci*. 2003; 23:3095-9.

Massano J, Bhatia KP. Clinical approach to Parkinson's disease: features, diagnosis, and principles of management. *Cold Spring Harb Perspect Med*. 2012; 2:a008870.

Masters SC, Yang H, Datta SR, Greenberg ME, Fu H. 14-3-3 inhibits Bad-induced cell death through interaction with serine-136. *Mol Pharmacol*. 2001; 60:1325-31.

McCormack AL, Mak SK, Di Monte DA. Increased α-synuclein phosphorylation and nitration in the aging primate substantia nigra. *Cell Death Dis*. 2012; 3:e315.

McKeith I. Dementia with Lewy bodies and other difficult diagnoses. *Int Psychogeriatr*. 2004; 16:123-7.

McLean JR, Hallett PJ, Cooper O, Stanley M, Isacson O. Transcript expression levels of full-length alpha-synuclein and its three alternatively spliced variants in Parkinson's disease brain regions and in a transgenic mouse model of alpha-synuclein overexpression. *Mol Cell Neurosci*. 2012; 49:230-9.

Meier F, Abeywardana T, Dhall A, Marotta NP, Varkey J, Langen R, Chatterjee C, Pratt MR. Semisynthetic, site-specific ubiquitin modification of α-synuclein reveals differential effects on aggregation. *J Am Chem Soc*. 2012; 134:5468-71.

Melki R. Role of Different Alpha-Synuclein Strains in Synucleinopathies, Similarities with other Neurodegenerative Diseases. *J Parkinsons Dis*. 2015; 5:217-27.

Miller DW, Cookson MR, Dickson DW. Glial cell inclusions and the pathogenesis of neurodegenerative diseases. *Neuron Glia Biol*. 2004; 1:13-21.

Mokhtar SH, Bakhuraysah MM, Cram DS, Petratos S. The Beta-amyloid protein of Alzheimer's disease: communication breakdown by modifying the neuronal cytoskeleton. *Int J Alzheimers Dis*. 2013; 2013:910502.

Mor DE, Ugras SE, Daniels MJ, Ischiropoulos H. Dynamic structural flexibility of α-synuclein. *Neurobiol Dis*. 2016; 88:66-74.

Moriarty GM, Janowska MK, Kang L, Baum J. Exploring the accessible conformations of N-terminal acetylated α-synuclein. *FEBS Lett*. 2013; 587:1128-38.

Moussaud S, Jones DR, Moussaud-Lamodière EL, Delenclos M, Ross OA, McLean PJ. Alpha-synuclein and tau: teammates in neurodegeneration? *Mol Neurodegener*. 2014; 9:43.

Mouton-Liger F, Paquet C, Dumurgier J, Bouras C, Pradier L, Gray F, Hugon J. Oxidative stress increases BACE1 protein levels through activation of the PKR-eIF2α pathway. *Biochim Biophys Acta*. 2012; 1822:885-96.

Mrak RE, Griffin WS. Dementia with Lewy bodies: Definition, diagnosis, and pathogenic relationship to Alzheimer's disease. *Neuropsychiatr Dis Treat*. 2007; 3:619-25.

Mulak A, Bonaz B. Brain-gut-microbiota axis in Parkinson's disease. *World J Gastroenterol*. 2015; 21:10609-20.

Mushtaq G, Greig NH, Anwar F, Al-Abbasi FA, Zamzami MA, Al-Talhi HA, Kamal MA. Neuroprotective Mechanisms Mediated by CDK5 Inhibition. *Curr Pharm Des*. 2016; 22:527-34.

Muslin AJ, Tanner JW, Allen PM, Shaw AS. Interaction of 14-3-3 with signaling proteins is mediated by the recognition of phosphoserine. *Cell*. 1996; 84:889-97.

Narkiewicz J, Giachin G, Legname G. In vitro aggregation assays for the characterization of α-synuclein prion-like properties. *Prion*. 2014; 8:19-32.

Negro A, Brunati AM, Donella-Deana A, Massimino ML, Pinna LA. Multiple phosphorylation of alpha-synuclein by protein tyrosine kinase Syk prevents eosin-induced aggregation. *FASEB J*. 2002; 16:210-2.

Nishioka K, Wider C, Vilariño-Güell C, Soto-Ortolaza AI, Lincoln SJ, Kachergus JM, Jasinska-Myga B, Ross OA, Rajput A, Robinson CA, Ferman TJ, Wszolek ZK, Dickson DW, Farrer MJ. Association of alpha-, beta-, and gamma-Synuclein with diffuse lewy body disease. *Arch Neurol*. 2010; 67:970-5.

Nonaka T, Iwatsubo T, Hasegawa M. Ubiquitination of alpha-synuclein. *Biochemistry*. 2005; 44:361-8.

Nonaka T, Watanabe ST, Iwatsubo T, Hasegawa M. Seeded aggregation and toxicity of {alpha}-synuclein and tau: cellular models of neurodegenerative diseases. *J Biol Chem*. 2010; 285:34885-98.

Olanow CW, Brundin P. Parkinson's disease and alpha synuclein: is Parkinson's disease a prion-like disorder? *Mov Disord*. 2013; 28:31-40.

Olichney JM, Galasko D, Salmon DP, Hofstetter CR, Hansen LA, Katzman R, Thal LJ. Cognitive decline is faster in Lewy body variant than in Alzheimer's disease. *Neurology*. 1998; 51:351-7.

Olivares D, Huang X, Branden L, Greig NH, Rogers JT. Physiological and pathological role of alpha-synuclein in Parkinson's disease through iron mediated oxidative stress; the role of a putative iron-responsive element. *Int J Mol Sci*. 2009; 10:1226-60.

Osterberg VR, Spinelli KJ, Weston LJ, Luk KC, Woltjer RL, Unni VK. Progressive aggregation of alpha-synuclein and selective degeneration of lewy inclusion-bearing neurons in a mouse model of parkinsonism. *Cell Rep*. 2015; 10:1252-60.

Ostrerova N, Petrucelli L, Farrer M, Mehta N, Choi P, Hardy J, Wolozin B. alpha-Synuclein shares physical and functional homology with 14-3-3 proteins. *J Neurosci*. 1999; 19:5782-91.

Pacheco C, Aguayo LG, Opazo C. An extracellular mechanism that can explain the neurotoxic effects of α-synuclein aggregates in the brain. *Front Physiol*. 2012; 3:297.

Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev*. 2009; 2:270-8.

Park H, Lee J, Lee S. Critical assessment of the automated AutoDock as a new docking tool for virtual screening. *Proteins*. 2006; 65:549-54.

Peelaerts W, Bousset L, Van der Perren A, Moskalyuk A, Pulizzi R, Giugliano M, Van den Haute C, Melki R, Baekelandt V. α-Synuclein strains cause distinct synucleinopathies after local and systemic administration. *Nature*. 2015; 522:340-4.

Pellicano C, Benincasa D, Pisani V, Buttarelli FR, Giovannelli M, Pontieri FE. Prodromal non-motor symptoms of Parkinson's disease. *Neuropsychiatr Dis Treat*. 2007; 3:145-52.

Peng X, Tehranian R, Dietrich P, Stefanis L, Perez RG. Alpha-synuclein activation of protein phosphatase 2A reduces tyrosine hydroxylase phosphorylation in dopaminergic cells. *J Cell Sci*. 2005; 118:3523-30.

Pfefferkorn CM, Jiang Z, Lee JC. Biophysics of α-synuclein membrane interactions. *Biochim Biophys Acta*. 2012; 1818:162-71.

Pickles S, Semmler S, Broom HR, Destroismaisons L, Legroux L, Arbour N, Meiering E, Cashman NR, Vande Velde C. ALS-linked misfolded SOD1 species have divergent impacts on mitochondria. *Acta Neuropathol Commun*. 2016; 4:43.

Poças GM, Branco-Santos J, Herrera F, Outeiro TF, Domingos PM. α-Synuclein modifies mutant huntingtin aggregation and neurotoxicity in Drosophila. *Hum Mol Genet*. 2015; 24:1898-907.

Popova B, Kleinknecht A, Braus GH. Posttranslational Modifications and Clearing of α-Synuclein Aggregates in Yeast. *Biomolecules*. 2015; 5:617-34.

Pradeepkiran JA, Kumar KK, Kumar YN, Bhaskar M. Modeling, molecular dynamics and docking assessment of transcription factor rho: a potential drug target in Brucella melitensis 16M. *Drug Des Devel Ther*. 2015; 9:1897-1912.

Praticò D. Oxidative stress hypothesis in Alzheimer's disease: a reappraisal. *Trends Pharmacol Sci*. 2008; 29:609-15.

Qin Z, Hu D, Han S, Hong DP, Fink AL. Role of different regions of alpha-synuclein in the assembly of fibrils. *Biochemistry*. 2007; 46:13322-30.

Radi R. Protein tyrosine nitration: biochemical mechanisms and structural basis of functional effects. *Acc Chem Res*. 2013; 46:550-9.

Rahman K. Studies on free radicals, antioxidants, and co-factors. *Clin Interv Aging*. 2007; 2:219-36.

Recasens A, Dehay B. Alpha-synuclein spreading in Parkinson's disease. *Front Neuroanat*. 2014; 8:159.

Rekas A, Ahn KJ, Kim J, Carver JA. The chaperone activity of α-synuclein: Utilizing deletion mutants to map its interaction with target proteins. *Proteins*. 2012; 80:1316-25.

Rhoades E, Ramlall TF, Webb WW, Eliezer D. Quantification of alpha-synuclein binding to lipid vesicles using fluorescence correlation spectroscopy. *Biophys J*. 2006; 90:4692-700.

Robinson PA. Protein stability and aggregation in Parkinson's disease. *Biochem J*. 2008; 413:1-13.

Sayre LM, Perry G, Smith MA. Oxidative stress and neurotoxicity. *Chem Res Toxicol*. 2008; 21:172-88.

Schmid AW, Fauvet B, Moniatte M, Lashuel HA. Alpha-synuclein post-translational modifications as potential biomarkers for Parkinson disease and other synucleinopathies. *Mol Cell Proteomics*. 2013; 12:3543-58.

Schulz-Schaeffer WJ. The synaptic pathology of alpha-synuclein aggregation in dementia with Lewy bodies, Parkinson's disease and Parkinson's disease dementia. *Acta Neuropathol*. 2010; 120:131-43.

Schwede T, Kopp J, Guex N, Peitsch MC. SWISS-MODEL: An automated protein homology-modeling server. *Nucleic Acids Res*. 2003; 31:3381-3385.

Shay J, Elbaz HA, Lee I, Zielske SP, Malek MH, Hüttemann M. Molecular Mechanisms and Therapeutic Effects of (-)-Epicatechin and Other Polyphenols in Cancer, Inflammation, Diabetes, and Neurodegeneration. *Oxid Med Cell Longev*. 2015; 2015:181260.

Shimada T, Fournier AE, Yamagata K. Neuroprotective function of 14-3-3 proteins in neurodegeneration. *Biomed Res Int*. 2013; 2013:564534.

Shimura H, Schlossmacher MG, Hattori N, Frosch MP, Trockenbacher A, Schneider R, Mizuno Y, Kosik KS, Selkoe DJ. Ubiquitination of a new form of alpha-synuclein by

parkin from human brain: implications for Parkinson's disease. *Science*. 2001; 293:263-9.

Shukla V, Mishra SK, Pant HC. Oxidative Stress in Neurodegeneration. *Advances in Pharmacological Sciences*. 2011; 572634-13.

Singh J, Udgaonkar JB. The Pathogenic Mutation T182A Converts the Prion Protein into a Molten Globule-like Conformation Whose Misfolding to Oligomers but Not to Fibrils Is Drastically Accelerated. *Biochemistry*. 2016; 55:459-69.

Snead D, Eliezer D. Alpha-synuclein function and dysfunction on cellular membranes. *Exp Neurobiol*. 2014; 23:292-313.

Sonee M, Sum T, Wang C, Mukherjee SK. The soy isoflavone, genistein, protects human cortical neuronal cells from oxidative stress. *Neurotoxicology*. 2004; 25:885-891.

Souza JM, Giasson BI, Lee VM, Ischiropoulos H. Chaperone-like activity of synucleins. *FEBS Lett*. 2000; 474:116-9.

Spano M, Signorelli M, Vitaliani R, Aguglia E, Giometto B. The possible involvement of mitochondrial dysfunctions in Lewy body dementia: a systematic review. *Funct Neurol*. 2015; 30:151-8.

Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M. alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proc Natl Acad Sci U S A*. 1998; 95:6469-73.

Stefanis L. α-Synuclein in Parkinson's disease. *Cold Spring Harb Perspect Med*. 2012; 2:a009399.

Stefanova N, Wenning GK. Review: Multiple system atrophy: emerging targets for interventional therapies. *Neuropathol Appl Neurobiol*. 2016; 42:20-32.

Stemberger S, Scholz SW, Singleton AB, Wenning GK. Genetic players in multiple system atrophy: unfolding the nature of the beast. *Neurobiol Aging*. 2011; 32:1924.e5-14.

Takalo M, Salminen A, Soininen H, Hiltunen M, Haapasalo A. Protein aggregation and degradation mechanisms in neurodegenerative diseases. *Am J Neurodegener Dis*. 2013; 2:1-14.

Tamilselvam K, Braidy N, Manivasagam T, Essa MM, Prasad NR, Karthikeyan S, Thenmozhi AJ, Selvaraju S, Guillemin GJ. Neuroprotective effects of hesperidin, a plant flavanone, on rotenone-induced oxidative stress and apoptosis in a cellular model for Parkinson's disease. *Oxid Med Cell Longev*. 2013; 2013:102741.

Tenreiro S, Eckermann K, Outeiro TF. Protein phosphorylation in neurodegeneration: friend or foe? *Front Mol Neurosci*. 2014; 7:42.

Thompson JD, Gibson TJ, Higgins DG. Multiple sequence alignment using ClustalW and ClustalX. *Curr Protoc Bioinformatics*. 2002; Chapter 2, 2.3.

Tokmakov AA, Kurotani A, Takagi T, Toyama M, Shirouzu M, Fukami Y, Yokoyama S. Multiple post-translational modifications affect heterologous protein synthesis. *J Biol Chem*. 2012; 287:27106-16.

Tomás-Zapico C, Díez-Zaera M, Ferrer I, Gómez-Ramos P, Morán MA, Miras-Portugal MT, Díaz-Hernández M, Lucas JJ. α-Synuclein accumulates in huntingtin inclusions but forms independent filaments and its deficiency attenuates early phenotype in a mouse model of Huntington's disease. *Hum Mol Genet*. 2012; 21:495-510.

Trinh NH, Hoblyn J, Mohanty S, Yaffe K. Efficacy of cholinesterase inhibitors in the treatment of neuropsychiatric symptoms and functional impairment in Alzheimer disease: a meta-analysis. *JAMA*. 2003; 289:210-6.

Trosset JY, Carbonell P. Synthetic biology for pharmaceutical drug discovery. *Drug Des Devel Ther*. 2015; 9:6285-302.

Ubhi K, Peng K, Lessig S, Estrella J, Adame A, Galasko D, Salmon DP, Hansen LA, Kawas CH, Masliah E. Neuropathology of dementia with Lewy bodies in advanced age: a comparison with Alzheimer disease. *Neurosci Lett*. 2010; 485:222-7.

Ubl A, Berg D, Holzmann C, Krüger R, Berger K, Arzberger T, Bornemann A, Riess O. 14-3-3 protein is a component of Lewy bodies in Parkinson's disease-mutation analysis and association studies of 14-3-3 eta. *Brain Res Mol Brain Res*. 2002; 108:33-9.

Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr Neuropharmacol*. 2009; 7:65-74.

Uversky VN. α-Synuclein Misfolding and Neurodegenerative Diseases. *Curr Protein Pept Sci*. 2008; 9:507-40.

Uversky VN. A protein-chameleon: conformational plasticity of alpha-synuclein, a disordered protein involved in neurodegenerative disorders. *J Biomol Struct Dyn*. 2003; 21:211-34.

Uversky VN, Eliezer D. Biophysics of Parkinson's disease: structure and aggregation of alpha-synuclein. *Curr Protein Pept Sci*. 2009; 10:483-99.

Uversky VN. Neuropathology, biochemistry, and biophysics of alpha-synuclein aggregation. *J Neurochem*. 2007; 103:17-37.

Vamvaca K, Volles MJ, Lansbury PT Jr. The first N-terminal amino acids of alpha-synuclein are essential for alpha-helical structure formation in vitro and membrane binding in yeast. *J Mol Biol*. 2009; 389:413-24.

Varanese S, Birnbaum Z, Rossi R, Di Rocco A. Treatment of advanced Parkinson's disease. *Parkinsons Dis*. 2011; 2010:480260.

Vekrellis K, Xilouri M, Emmanouilidou E, Rideout HJ, Stefanis L. Pathological roles of α-synuclein in neurological disorders. *Lancet Neurol*. 2011; 10:1015-25.

Venda LL, Cragg SJ, Buchman VL, Wade-Martins R. α-Synuclein and dopamine at the crossroads of Parkinson's disease. *Trends Neurosci*. 2010; 33:559-68.

Volpicelli-Daley LA, Luk KC, Patel TP, Tanik SA, Riddle DM, Stieber A, Meaney DF, Trojanowski JQ, Lee VM. Exogenous α-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. *Neuron*. 2011; 72:57-71.

Waelter S, Boeddrich A, Lurz R, Scherzinger E, Lueder G, Lehrach H, Wanker EE. Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. *Mol Biol Cell*. 2001; 12:1393-407.

Watabe M, Nakaki T. Rotenone induces apoptosis via activation of bad in human dopaminergic SH-SY5Y cells. *J Pharmacol Exp Ther*. 2004; 311:948-53.

Wei FY, Tomizawa K. Cyclin-dependent kinase 5 (Cdk5): a potential therapeutic target for the treatment of neurodegenerative diseases and diabetes mellitus. *Mini Rev Med Chem*. 2007; 7:1070-4.

Weinreb O, Amit T, Mandel S, Youdim M. Neuroprotective molecular mechanisms of (2)-epigallocatechin-3-gallate: a reflective outcome of its antioxidant, iron chelating and neuritogenic properties. *Genes Nutr*. 2009; 4:283–96.

Wenning GK, Stefanova N. Recent developments in multiple system atrophy. *J Neurol*. 2009; 256:1791-808.

Wijesekera LC, Leigh PN. Amyotrophic lateral sclerosis. *Orphanet J Rare Dis*. 2009; 4:3.

Witt SN. Molecular chaperones, α-synuclein, and neurodegeneration. *Mol Neurobiol*. 2013; 47:552-60.

Xiao S, McLean J, Robertson J. Neuronal intermediate filaments and ALS: a new look at an old question. *Biochim Biophys Acta*. 2006; 1762:1001-12.

Xie A, Gao J, Xu L, Meng D. Shared mechanisms of neurodegeneration in Alzheimer's disease and Parkinson's disease. *Biomed Res Int*. 2014; 2014:648740.

Xu Y, Deng Y, Qing H. The phosphorylation of α-synuclein: development and implication for the mechanism and therapy of the Parkinson's disease. *J Neurochem*. 2015; 135:4-18.

Yamada M, Iwatsubo T, Mizuno Y, Mochizuki H. Overexpression of alpha-synuclein in rat substantia nigra results in loss of dopaminergic neurons, phosphorylation of alpha-synuclein and activation of caspase-9: resemblance to pathogenetic changes in Parkinson's disease. *J Neurochem*. 2004; 91:451-61.

Yang EJ, Choi SM. α -Synuclein Modification in an ALS Animal Model. *Evid Based Complement Alternat Med*. 2013; 2013:259381.

Yang Z, Lasker K, Schneidman-Duhovny D, Webb B, Huang CC, Pettersen EF, Goddard TD, Meng EC, Sali A, Ferrin TE. UCSF Chimera, MODELLER, and IMP: an integrated modeling system. *J Struct Biol*. 2012; 179:269-78.

Yasuda T, Nakata Y, Mochizuki H. α-Synuclein and neuronal cell death. *Mol Neurobiol*. 2013; 47:466-83.

Yoo KY, Park SY. Terpenoids as potential anti-Alzheimer's disease therapeutics. *Molecules*. 2012; 17:3524-38.

Yoshida M. Multiple system atrophy: alpha-synuclein and neuronal degeneration. *Neuropathology*. 2007; 27:484-93.

Yousuf MA, Tan C, Torres-Altoro MI, Lu FM, Plautz E, Zhang S, Takahashi M, Hernandez A, Kernie SG, Plattner F, Bibb JA. Involvement of aberrant Cdk5/p25 activity in experimental traumatic brain injury. *J Neurochem*. 2016.

Zabrocki P, Pellens K, Vanhelmont T, Vandebroek T, Griffioen G, Wera S, Van Leuven F, Winderickx J. Characterization of alpha-synuclein aggregation and synergistic toxicity with protein tau in yeast. *FEBS J*. 2005; 272:1386-400.

Zaccai J, Brayne C, McKeith I, Matthews F, Ince PG; MRC Cognitive Function, Ageing Neuropathology Study. Patterns and stages of alpha-synucleinopathy: Relevance in a population-based cohort. *Neurology*. 2008; 70:1042-8.

Zhao B. Natural antioxidants for neurodegenerative diseases. *Mol Neurobiol*. 2005; 31:283-93.

Zhou J, Broe M, Huang Y, Anderson JP, Gai WP, Milward EA, Porritt M, Howells D, Hughes AJ, Wang X, Halliday GM. Changes in the solubility and phosphorylation of α-synuclein over the course of Parkinson's disease. *Acta Neuropathol*. 2011; 121:695-704

Zhou M, Ottenberg G, Sferrazza GF, Lasmézas CI. Highly neurotoxic monomeric α-helical prion protein. *Proc Natl Acad Sci* U S A. 2012; 109:3113-8.

Zupancic M, Mahajan A, Handa K. Dementia with lewy bodies: diagnosis and management for primary care providers. *Prim Care Companion CNS Disord*. 2011; 13.

9. APPENDIX

10. CONFERENCE PROCEEDINGS

P-18

Relevance of Terpenoids and Alkaloids in Neuroprotection

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Abstract:

Cognitive dysfunctions are the major medical challenges faced by the ageing population in21st century. Many neuropsychiatric disorders and neurodegenerative disorders, such as seizures, schizophrenia, Alzheimer's disease, dementia, cerebrovascular impairment, and Parkinsonism severely affects the ageing population. Recent studies highlighted various neurotransmitters, signaling molecules and a wide variety of natural compounds that have been identified as the potential therapeutic targets of cognitive decline. Additionally, phytochemicals from medicinal plants play a vital role in maintaining the brain homeostasis by influencing the function of several proteins and their receptors for the major inhibitory neurotransmitters. It has been observed that a number of herbal compounds and phytochemicals used in Ayurveda may have a neuroprotective role which may be beneficial in different neuropsychiatric and neurodegenerative disorders. However, the presence of receptors or transporters for the phytochemicals of the medicinal plants in brain tissues has to be determined, the molecules with numerous proteins and receptorsas potent targets appear as a promising class of compounds for the therapeutics of cognitive diseases with a multifactorial etiology.In this study, we focused on phytochemical compoundseliciting their neuroprotectiveproperties. Further, using different *in silico* techniques, we have validated the potential protective role of terpenoidsand alkaloids in cognitive disorders.

Relevance of Terpenoids and Alkaloids in Neuroprotection

Abhishek Shrivastava[1*], Puspendra Mishra[*], Dhiraj kumar[1], Saurabh Kumar Jha[1], Niraj Kumar Jha[1], Rashmi K Ambasta[1] and Pravir Kumar[1,2]

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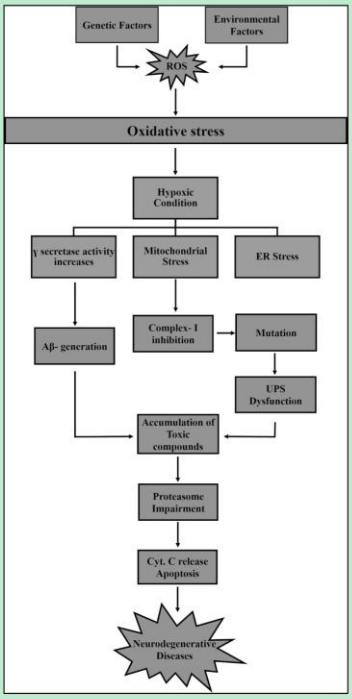
ABSTRACT

Cognitive dysfunctions are the major medical challenges faced by the ageing population in 21st century. Many neuropsychiatric disorders and neurodegenerative disorders, such as seizures, schizophrenia, Alzheimer's disease, dementia, cerebrovascular impairment, and Parkinsonism severely affects the ageing population. Recent studies highlighted various neurotransmitters, signaling molecules and a wide variety of natural compounds that have been identified as the potential therapeutic targets of cognitive decline. Additionally, phytochemicals from medicinal plants play a vital role in maintaining the brain homeostasis by influencing the function of several proteins and their receptors for the major inhibitory neurotransmitters. It has been observed that a number of herbal compounds and phytochemicals used in Ayurveda may have a neuroprotective role which may be beneficial in different neuropsychiatric and neurodegenerative disorders. However, the presence of receptors or transporters for the phytochemicals of the medicinal plants in brain tissues has to be determined, the molecules with numerous proteins and receptors as potent targets appear as a promising class of compounds for the therapeutics of cognitive diseases with a multifactorial etiology. In this study, we focused on phytochemical compounds eliciting their neuroprotective properties. Further, using different *in silico* techniques, we have validated the potential protective role of terpenoids and alkaloids in cognitive disorders. **Key words**: Neurodegenerative diseases (NDD), Phytochemicals, Receptors, Terpenoids, Alkaloids

INTRODUCTION

The Major health concern in today's world is Neurodegenerative diseases, it is estimated that there is key influence of Neurodegenerative disease on mortality rate by 2050 [1]. Neurodegenerative diseases display a common pathophysiological process in which aggregates of misfolded protein start the degeneration and its progression in different parts of brain [2]. These brain disease may treated in two way either by preventing the formation of misfolded aggregates of protein or removing the formed aggregates of protein. Plant products has been used in treatment of brain disorders since long ago. Differentiated cells of plant contains secondary metabolites, which is not utilize by cell themselves but it may be useful in metabolism of whole plant [3]. Previous research demonstrate the role of plant secondary metabolites in treatment of Neurodegenerative disease for instance anti-cholinergic and Monoamine oxidase Inhibitory effect of Alkaloids on the central nervous system [4, 5]. Terpenoids also proven as anti-Alzheimer's therapeutic agent [6]. In current research we are assessing the drug likeness of plant secondary metabolites and effect of these molecules on protein associated with neurodegeneration by molecular docking.

MECHANISM



MATERIALS AND METHODS

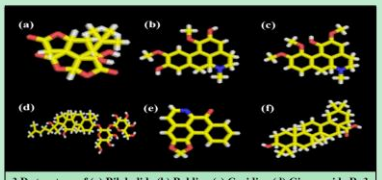
[1] Chemical Structure of Alkaloidal and Terpenoidal ligands were retrieved and analyzed using Pubchem database and Pymol.

[2] PDB files of proteins AβPP (PDB ID:1AAP), DJ-1 (PDB ID:4ZGG) and SOD-1 (PDB ID:2JLP) were retrieved from protein data bank and analyzed using Pymol.

[3] Lipinski filter was used to analyze the drug likeness of ligands.

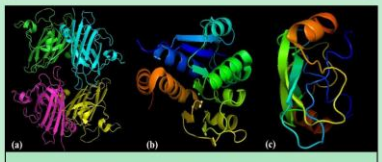
[4] Interaction of ligands with receptors was performed with the help of Hex tool and result was analyzed by comparing their energy values.

MOLECULAR STRUCTURE OF LIGANDS



3 D structure of (a) Bilobalide (b) Boldine (c) Coridine (d) Ginsenoside Rg3 (e) Liriodinine (f) Oleanolic acid

MOLECULAR STRUCTURE OF PROTEINS

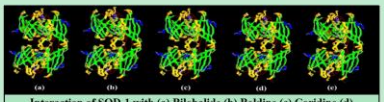


3 D structure of proteins (a) SOD-1 (b) DJ-1 (c) AβPP

LIPINSKI FILTER ANALYSIS OF LIGANDS

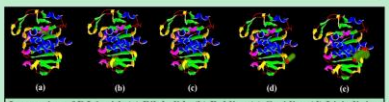
Ligand Name	Bilobalide	Boldine	Corydine	Ginsenoside Rg3	Liriodenine	Oleanolic acid
Type of Metabolite	Terpenoid	Alkaloid	Alkaloid	Terpenoid	Alkaloid	Terpenoid
Molar Mass (Da)	351	325	341	832	277	460
H-bond donor	3	1	0	5	0	2
H-bond acceptor	8	4	4	13	4	3
Lipophilicity	1.364	2.353	2.63	8.565	1.87	7.3
Molar refractivity	128.53	88.7	94.42	163.24	69.54	154.44

DOCKING RESULT OF LIGANDS WITH SOD-1



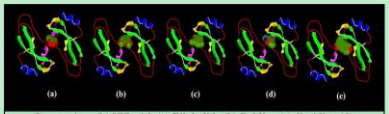
Interaction of SOD-1 with (a) Bilobalide (b) Boldine (c) Coridine (d) Liriodinine (e) Oleanolic acid

DOCKING RESULT OF LIGANDS WITH DJ-1



Interaction of DJ-1 with (a) Bilobalide (b) Boldine (c) Coridine (d) Liriodinine (e) Oleanolic acid

DOCKING RESULT OF LIGANDS WITH AβPP



Interaction of AβPP with (a) Bilobalide (b) Boldine (c) Coridine (d) Liriodinine (e) Oleanolic acid

FREE ENERGY CALCULATION

Receptors	Bilobalide	Boldine	Corydine	Liriodinine	Oleanolic Acid
SOD-1	-181.98	-208.43	-207.46	-158.55	-243.97
DJ-1	-196.63	-215.22	-219.01	-190.75	-247.39
APP	-184.47	-212.01	-227.57	-201.8	-245.36

FREE ENERGY PLOT

CONCLUSION

[1] Lipinski filter analysis revealed that all the selected molecules except Ginsenoside Rg3 have drug likeness and can be used for docking purposes.

[2] Energy value analysis of ligand and receptor interaction revealed that the above used molecules bind more effectively to DJ-1 and AβPP as compared to SOD-1 and may have a role in the therapeutics of oxidative stress induced neurodegeneration.

REFERENCES

1. Buendia I, Michalska P, Navarro E, Gameiro I, Egea J, León R. Nrf2-AREPathway: An emerging target against oxidative stress and neuroinflammation in neurodegenerative diseases. *Pharmacol Ther*. **2015**.
2. André Nieoullon.Neurodegenerative diseases and neuroprotection: current views and prospects. *J Appl Biomed*, **2011**, Vol. 9, pp.173-183.
3. Scotti L, Scotti MT. Computer Aided Drug Design Studies in the Discovery of Secondary Metabolites Targeted Against Age-Related Neurodegenerative Diseases. *Curr Top Med Chem*. **2015**, Vol. 15(21), pp.2239-2252.
4. Huma Naaz, Swati Singh, Veda P Pandey, Priyank Singh, Upendra N Dwivedi. Anti-cholinergic alkaloids as potential therapeutic agents for Alzheimer's disease: An Insilico approach, *Indian Journal of Biochemistry and Biophysics*. **2013**,Vol. 50, pp.120-125.
5. Carolina Dos Santos Passos, Claudia Simoes-Pires, Amelia Henriques, Muriel Cuendet, Pierre-Alain Carrupt, Philippe Christen. Alkaloids as Inhibitors of Monoamine Oxidases and Their Role in the Central Nervous System. *Studies in Natural Products Chemistry*. **2014**,Vol. 43, [Book chapter].
6. Yoo KY, Park SY. Terpenoids as potential anti-Alzheimer's disease therapeutics. *Molecules*. **2012**, Vol. 17(3), pp.3524-3538.

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The Authors would like to thank senior management of Delhi Technological University for constant encouragement and support.

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In silico study of cannabinoids in neurodegenerative disorders

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[1]Delhi Technological University, India
[2]Tufts University School of Medicine, USA

Oxygen is requisite in biological systems to converge their energy demands and supply of nutrients in our body. The generation of free radicals is the most common consequence of oxygen consumption which may lead to the damaging of cells. Importantly, antioxidant system of the body has a pertinent role in prevention of any loss that arises due to the generation of free radicals. However, dysfunction of antioxidant system or due to overproduction of free radicals may lead to some serious consequences which affect normal brain functionality. The brain tissue is greatly prone to the effects of reactive oxygen species due to its high demand for oxygen. Apart from several other environmental factors, oxidative stress (OS) plays a critical role in free-radical attack on neural cells leads to the loss of function that eventually contributes to neurodegeneration. However, antioxidants have defensive role against such kind of oxidative stress to prevent neuronal damage. Antioxidants may also be used as a therapeutic agent against intense neuronal loss, as they have the ability to neutralize free-radicals. Diet is a chief source of antioxidants, as well as medicinal herbs are also found to be a commercial source of antioxidants at present. Currently, we have focused on the natural compounds with good antioxidant and anti-inflammatory property that elicit neuroprotection. Herein, using different *in silico* techniques, we have validated the potential protective role of Cannabinoids in neurodegeneration. Furthermore, *in silico* techniques encompass string database for protein-protein interaction and docking for finding proper interaction between drugs and protein molecules have been identified.

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IN SILICO ANALYSIS OF CANNABINOIDS IN NEURODEGENERATION

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Presenting author: Abhishek Shrivastava

ABSTRACT

Oxygen is requisite in biological systems to converge their energy demands and supply of nutrients in our body. The generation of free radicals is the most common consequence of oxygen consumption, which may lead to the damaging of cells. Importantly, antioxidant system of the body has a pertinent role in prevention of any loss that arises due to the generation of free radicals. However, dysfunction of antioxidant system or due to overproduction of free radicals may lead to some serious consequences which affect normal brain functionality. The brain tissue is greatly prone to the attack of reactive oxygen species. Apart from several other environmental factors, oxidative stress (OS) plays a critical role in free-radical attack on neural cells leads to the loss of function that eventually contributes to neurodegeneration. However, antioxidants have a defensive role against such kind of oxidative stress to prevent neuronal damage. Antioxidants may also be used as a therapeutic agent against intense neuronal loss, as they have the ability to neutralize free-radicals. Diet is a chief source of antioxidants, as well as medicinal herbs are also found to be a commercial source of antioxidants at present. Currently, we have focused on the natural compounds with good antioxidant and anti-inflammatory property that elicit neuroprotection. Herein, using different *in silico* techniques, we have validated the potential protective role of Cannabinoids in neurodegeneration. Furthermore, *in silico* techniques encompass multiple sequence alignment, phylogenetic analysis and docking for finding proper interaction between drugs and protein molecules have been identified.

Keywords: Neurodegenerative diseases (NDD), Anti-oxidant, Free radicals

INTRODUCTION

Neurodegenerative disorders are the progressive and chronic disorders illustrated by functional loss of cognitive neurons that leads to deficient nervous system functioning. It can result due to various factors such as oxidative stress, free radical's accumulation, mitochondrial dysfunction, impaired ubiquitin proteasomal system and several other determinants can regulate prognosis in NDDs (Jha et.al, 2014). Oxidative stress plays an important role in the development of several ages related brain disorders. Reactive oxygen species (ROS) are involved in various biochemical activities of cells such as gene transcription, signal transduction, and regulation of soluble guanylate cyclase activity. The most common cellular free radicals are nitric monoxide (NO-), superoxide (O2-), and hydroxyl (OH-) (Mahajan et.al. 2009). However, several differences in the redox state lead to toxicity via production of peroxides and free radicals damaging lipids, proteins and DNA of the cell (Pant et.al, 2011). Human body produces oxygen-free radicals and other reactive oxygen species as by products through various physiological, environmental and biochemical processes. Moreover, disturbed equilibrium between antioxidant homeostasis can make ROS and free radicals who are detrimental for neurons. The antioxidants prevent free radical mediated damage of cells that's led to various neurodegenerative diseases (Gandhi and Abramov, 2012). Antioxidants, such as selenium, zinc, vitamin E, vitamin C, vitamin A, glutathione, arginine, citrulline, taurine, creatine, and tea polyphenols facilitate controlling the ROS thus generated. However, antioxidant is further maintained with antioxidant enzymes, e.g. glutathione peroxidase, catalase, glutathione reductase and superoxide dismutase, those apply synergistic actions in removing free radicals (Mahajan et.al, 2009). Currently, we have focused on the natural compounds' cannabinoids using different in silico techniques, for validation of potential therapeutics role in NDDs.

MOLECULAR STRUCTURE OF CANNABINOIDS

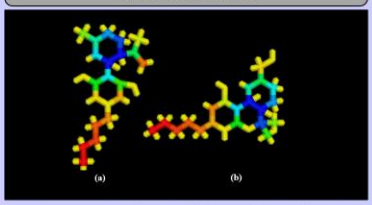
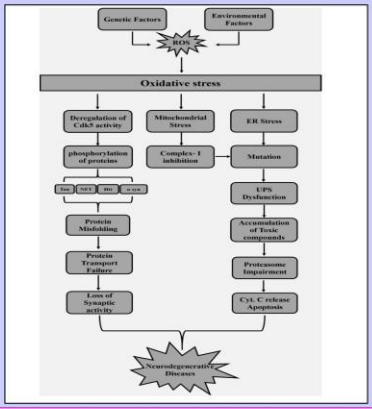


Figure: Structure of (a) Cannabidiol (b) Tetrahydrocannabinol

MECHANISM OF OXIDATIVE STRESS



MATERIALS AND METHODS

[1] Chemical Structure of Cannabidiol and Tetrahydrocannabinol were retrieved from PubChem database.

[2] Cannabidiol and Tetrahydrocannabinol drugs were analyzed with the help of Pymol.

[3] Multiple Sequence Alignment (MSA) and Phylogenetic Tree for the proteins CdK5(Accession no. Q00535.3), DJ-1(Accession no. Q99497.2), PINK-1(Accession no. O60260.2) and SOD-1(Accession no. P24706.1) were obtained with the help of Muscle Software.

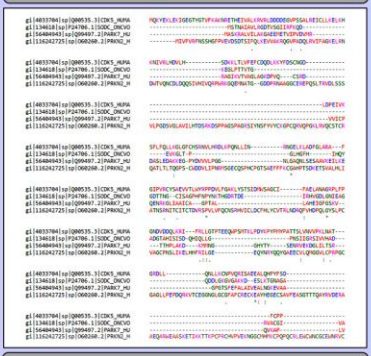
[4] PDB files of Proteins CdK5(PDB ID: 3O0G), DJ-1(PDB ID: 4ZGG), PINK-1(PDB ID: 4WZP) and SOD-1(PDB ID: 2JLP) were retrieved from protein data bank.

[5] 3 Dimensional structure of proteins were analyzed with the help of Pymol and verified with the help of Errat Server.

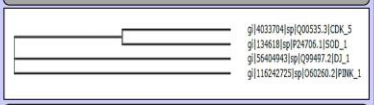
[6] Analysis of Screened drugs was performed with the help of Lipinski filter.

[7] Interaction of ligands with the receptors was performed with the help of Hex tool and result was analyzed by comparing their energy values.

MULTIPLE SEQUENCE ALIGNMENT



PHYLOGENETIC TREE



3 D STRUCTURE OF PROTEINS

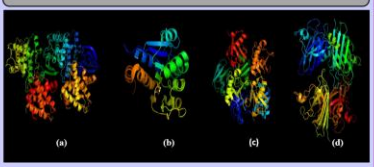


Figure: 3 D structure of (a) CdK-5 (b) DJ-1 (c) PINK-1 (d) SOD-1

LIPINSKI FILTER ANALYSIS

Biomolecules	Molecular Mass	Lipophilicity (xlogP)	Hydrogen Bond Donors	Hydrogen Bond Acceptors	Molar Refractivity
Cannabidiol	308	4.086	0	2	90.841
Tetrahydrocannabinol	326	4.407	0	3	94.211

DOCKING RESULT OF RECEPTORS WITH CANNABIDIOL

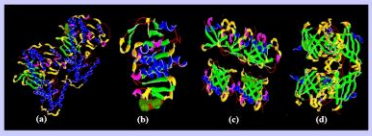


Figure: Interaction of Cannabidiol with (a) CdK-5 (b) DJ-1 (c) PINK-1(d) SOD-1

DOCKING RESULT OF RECEPTORS WITH TETRAHYDROCANNABINOL

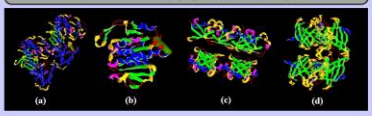


Figure: Interaction of Tetracannabinol with (a) CdK-5 (b) DJ-1 (c) PINK-1(d) SOD-1

DOCKING CALCULATION

Receptors	E Value of Cannabidiol	E Value of Tetrahydrocannabinol
CdK-5	-21.36	-20.06
DJ-1	-234.87	-240.2
PINK-1	-242.83	-231.63
SOD-1	-222.53	-243.37

CONCLUSION

[1] It has been observed that proteins CdK-5, DJ-1, PINK-1 and SOD-1 have 45% sequence similarity which might be the cause of their response to oxidative stress.

[2] It has also been find out that proteins CdK-5 and SOD-1 are in same cluster as they share more homology while proteins DJ-1 and PINK-1 are different from others.

[3] Lipinski filter analysis revealed that both the molecules selected may have drug likeness and can be used for docking purposes.

[4] Energy value analysis of ligand and receptor interaction revealed that Cannabidiol and Tetrahydrocannabinol can bind more effectively to PINK-1 and SOD-1 respectively as compared to the other receptors and may have a role in the therapeutics of oxidative stress induced neurodegeneration.

REFERENCES

[1] Gandhi S, Abramov AY (2012) Mechanism of Oxidative Stress in Neurodegeneration. Oxidative Medicine and Cellular Longevity 1: 1-11.

[2] Jha NK, Jha SK, Kar R, Ambasta RK, Kumar P (2014) Role of Oxidative Stress, ER Stress and Ubiquitin Proteasome System in Neurodegeneration. MOJ Cell Sci Report 1(2): 00010.

[3] Pant HC, Mishra SK, Shukla V (2011) Oxidative Stress in Neurodegeneration. Advances in Pharmacological Sciences 1:1-13.

[4] Mahajan RT, Zamboni P, Singh AV, Uttara B (2009) Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options. Current Neuropharmacology 7(1): 65-74.

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Role of DNA Damage And Repair Defects In Neurodegenerative Disorders

PP-35

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Progressive neuronal DNA damage has been reported in aging brains that are associated with the onset of various neurological disorders such as Amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). Increasing scientific reports suggests that neurodegeneration takes place as a result of DNA damage and functional deficit in DNA repairing abilities of cell. Although evidences advocates that DNA damage is a consequence of neurodegeneration but there might be a direct role of DNA damage with the cause of neurodegeneration. However neurons are prone to DNA damage under oxidative stress but identification of mutations in DNA repairing genes clearly links DNA damage and DNA repair anomalies with the progression of neurodegenerative disorders. Moreover, DNA alterations have also contributed to the post translational modifications (PTMs) of proteins that act as a pathogenic mechanism of neurodegenerative disorders. Therefore identification of DNA aberrations and their impact on PTMs of pathogenic proteins could provide a valuable insight into the mechanism of these disorders. Herein, (i) we have identified the factors associated with DNA damage in Alzheimer's and Amyotrophic lateral sclerosis, (ii) demonstrated the point mutations in their pathogenic proteins and functional consequences, (iii) illustrated the mechanism of DNA induced neurodegeneration in Alzheimer's disease and Amyotrophic lateral sclerosis and (iv) depicted the DNA damage induced PTMs in these proteins.

DNA DAMAGE AND ITS ROLE IN NEURODEGENERATION

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ABSTRACT

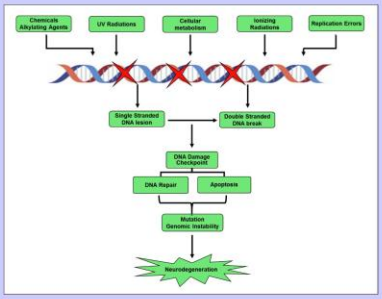
Progressive neuronal DNA damage has been reported in aging brains that are associated with the onset of various neurological disorders such as Amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). Increasing scientific reports suggests that neurodegeneration takes place as a result of DNA damage and functional deficit in DNA repairing abilities of cell. Although evidences advocates that DNA damage is a consequence of neurodegeneration but there might be a direct role of DNA damage with the cause of neurodegeneration. However neurons are prone to DNA damage under oxidative stress but identification of mutations in DNA repairing genes clearly links DNA damage and DNA repair anomalies with the progression of neurodegenerative disorders. Moreover, DNA alterations have also contributed to the post translational modifications (PTMs) of proteins that act as a pathogenic mechanism of neurodegenerative disorders. Therefore identification of DNA aberrations and their impact on PTMs of pathogenic proteins could provide a valuable insight into the mechanism of these disorders. Herein, (i) we have identified the factors associated with DNA damage in Alzheimer's and Amyotrophic lateral sclerosis, (ii) demonstrated the point mutations in their pathogenic proteins and functional consequences, (iii) illustrated the mechanism of DNA induced neurodegeneration in Alzheimer's disease and Amyotrophic lateral sclerosis and (iv) depicted the DNA damage induced PTMs in these proteins.

Keywords: Neurodegenerative disorders (NDD), Alzheimer's disease (AD), Amyotrophic Lateral Sclerosis (ALS), DNA Damage, Post translational Modifications (PTMs)

INTRODUCTION

Neurodegenerative disorders are a heterogeneous group of degenerative conditions, leading to a gradual and progressive loss of neuronal cells. Neurodegenerative diseases include complex pathologies such as Huntington's disease (HD), Parkinson's disease (PD), Alzheimer's disease (AD) and Amyotrophic lateral sclerosis (ALS) that affect many activities such as cognitive or movement impairments and memory loss. In recent years, it has been detected that interaction between genetic polymorphisms of metabolic enzymes and environmental agents have a major role in the onset of neurodegenerative disorder (Coppede et al. 2006). Due to the substantial requirement of oxygen for maintenance of CNS tissue, neurons cope with oxidative and metabolic stress that can result in DNA strand breaks and effective DNA strand-break surveillance and repair mechanisms are essential to deal with these types of lesions. DNA repair mechanism play a significant role in maintaining the homeostasis in brain and the genetic mutations inactivate the repair mechanism and show enhanced level of neuronal death (Katyal and McKinnon. 2008).

DNA DAMAGE IN NEURODEGENERATION



FACTORS CAUSING DNA DAMAGE

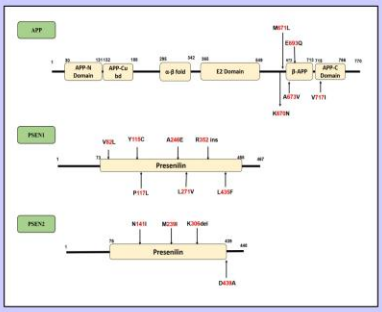
DNA DAMAGING AGENTS

DNA Damaging Agents	DNA Damage	Repair Mechanism	References
UV Radiations Polycyclic aromatic hydrocarbons	6-4 photoproduct Cyclobutane pyrimidine primer Bulky Adduct	Nucleotide Excision Repair (NER)	Subba RK. 2007
X-rays Reactive Oxygen Species (ROS) Alkylating Agents	Abasic site, Oxidised, deaminated and alkylated bases DNA Single Stranded Break	Base Excision Repair (BER)	Jeppesen et al. 2011 Maynard et al. 2009
X-rays Hydroxy urea UV Radiations Anti-cancer Agents	DNA Double Stranded Break	Double Strand Break Repair: Homologous Recombination (HR) Non-homologous end-joining (NHEJ)	Bohgaki et al. 2010 Jeppesen et al. 2011
Replication Errors Recombination Errors	Base mismatches Insertion Deletion	Mismatch Repair (MMR)	Jeppesen et al. 2011

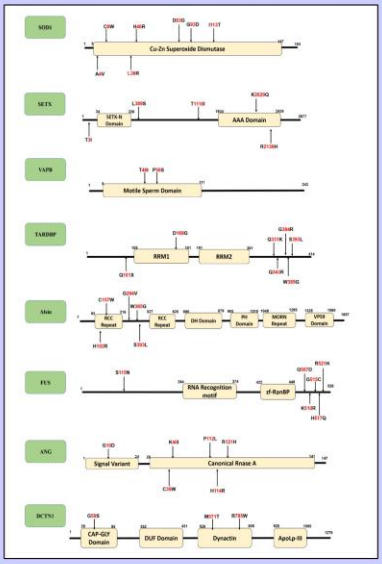
METHODOLOGY

1. Literature review has been done for the identification of mutations and the post translational activity in AD and ALS due to the breakage of DNA by various endogenous and exogenous factors.
2. Point mutations in AD and ALS have been obtained by using Alzforum network and ALSoD database.
3. Domain structure of mutated genes has been obtained from conserved domain database of NCBI.
4. Mutational mapping has been done on the structural domain of mutated proteins and its impact has been studied.

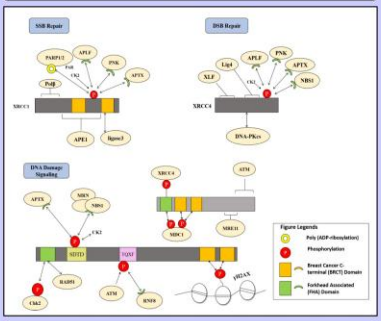
MUTATIONAL MAPPING OF AD PROTEINS



MUTATIONAL MAPPING OF ALS PROTEINS



PTM IN DNA REPAIR



CONCLUSION

1. It has been observed that DNA damage may lead to Apoptosis or the DNA repair mechanism gets activated, inefficient repair leads to mutation that may cause of neurodegenerative disorders.
2. In AD, APP, PSEN1 and PSEN2 are widely affected proteins but in ALS, most widely affected proteins are SOD1, TARDBP, Alsin, FUS and ANG.
3. Out of various mutations, it has been observed that in AD mutated APP and PSEN1 directly increase the ratio of $A\beta_{42}$ to $A\beta_{40}$ and in ALS mutated SOD1, FUS, Alsin and TARDBP directly lead to hexanucleotide repeat GGGGCC that is a major cause of ALS.
4. It has been observed that cellular metabolism, Ionizing Radiations, UV Radiations and anti-cancer agents are the most adverse factors for neurodegeneration.

REFERENCES

- Bohgaki et al. (2010) DNA double-strand break signaling and human disorders. Genome Integrity. 1(1):1-15.
- Coppede et al. (2006) Genes and the Environment in Neurodegeneration. Biosci Rep. 26(5):341–367.
- Jeppesen et al. (2011). DNA Repair Deficiency in Neurodegeneration. Prog Neurobiol. 94(2):166–200.
- Katyal and McKinnon. (2008) DNA strand breaks, neurodegeneration and aging in the brain. Mech Ageing Dev. 129(7-8):483–491.
- Maynard et al. (2009) Base excision repair of oxidative DNA damage and association with cancer and aging. Carcinogenesis. 30:2-10.
- Subba RK. (2007) Mechanisms of Disease: DNA repair defects and neurological disease. Nature Clinical Practice Neurology 3:162-172.

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Microbial involvement in cause and treatment of Alzheimer's disease

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A complex ecosystem formed by the microorganisms that reside within human organs is gastrointestinal tract, urogenital tract, skin and nasal and oral mucosa. Albeit, largest pool of microbiome in the human body is gastrointestinal tract that comprising 99% anaerobic bacteria and remaining are fungi, protozoan and archaebacteria. The ratio of prokaryotic microbial cells within the human body to eukaryotic human cells is 100:1 and gene ratio is 150:1. Recently, it has been identified that Microbiome resides in human predict four major division of bacterium resides in GI tract namely actinobacteria (3%), Bacteroidetes (23%), firmicutes (64%) and proteobacteria (8%). Remainder 2% consists of diverse minor taxonomic division. Further, such kind of microbial cells has been found to involve in the progression of neurodegenerative disorders. Alzheimer's disease is one of the most lethal disorders which befalls several damages in the brain due to accumulation of toxic Amyloid Beta (Aβ), directed by mainly dementia, cognitive disabilities and tauopathy. Interestingly, amyloid is secreted by various species of microbiome including bacteria and fungi. Blood examination of AD patients has also revealed through the presence of disperse Mycoses and amyloidogenic fungal protein which was found to link with increased risk of AD due to chronic fungal infection. Molecular mimicry is another factor underlying mechanism for neurodegeneration through bacterial amyloid. In spite of this, many plant and animal viruses are also associated with molecular mimicry and altered protein expression in AD. Based on this ground, we demonstrated the microbial source of amyloid causing AD, illustrated underlying mechanism of AD due to microbial amyloid, elucidated the amyloid protein interaction with other proteins and finally, analyzed the microbial product for AD treatment using *in silico* techniques.

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Microbial involvement in cause and treatment of Alzheimer's disease

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ABSTRACT

A complex ecosystem formed by the microorganisms that reside within human organs is gastrointestinal tract, urogenital tract, skin and nasal and oral mucosa. Albeit, largest pool of microbiome in the human body is the gastrointestinal tract that comprising 99% anaerobic bacteria and remaining are fungi, protozoan and archaebacteria. The ratio of prokaryotic microbial cells within the human body to eukaryotic human cells is 100:1 and gene ratio is 150:1. Recently, it has been identified that Microbiome resides in human predict four major division of a bacterium resides in GI tract namely actinobacteria (3%), bacteroidetes (23%), firmicutes (64%) and proteobacteria (8%). Remainder 2% consists of diverse minor taxonomic division. Further, such kind of microbial cells has been found to involve in the progression of neurodegenerative disorders. Alzheimer's disease is one of the most lethal disorders, which befalls several damages in the brain due to accumulation of toxic Amyloid Beta (Aβ), directed by mainly dementia, cognitive disabilities, and tauopathy. Interestingly, amyloid is secreted by various species of microbiome, including bacteria and fungi. Blood examination of AD patients has also revealed through the presence of disperse Mycoses and amyloidogenic fungal protein, which was found to link with increased risk of AD due to chronic fungal infection. Molecular Mimicry is another factor underlying mechanism for neurodegeneration through Bacterial amyloid. In spite of this, many plant and animal viruses are also associated with molecular mimicry and altered protein expression in AD. Based on this ground, (i) we demonstrated the microbial source of amyloid causing AD, (ii) illustrated underlying mechanism of AD due to microbial amyloid, and finally, analyzed the Microbial product for AD treatment using in silico techniques.

Key words: Alzheimer's disease (AD), Amyloid Beta (Aβ), Microbiome

INTRODUCTION

Alzheimer's disease is the leading cause of dementia, characterized by mainly the presence of Aβ and hyperphosphorylated tau in the brain tissues of the diseased people [1]. Epigenetic or environmental factors besides genetic factors may contribute to the pathology of AD. Moreover, nowadays recognition of pathogenic microbes has been identified as one of the environmental risk factor for AD. The microbes which are involved in AD have been classified into diverse groups. For instance, HCV, HIV-1, HSV-1, Cytomegalovirus, Viroids, Fungus, Toxoplasma species, Chlaymydophila pneumonia and other pathogenic bacteria. Although, microbial infection also involved in numerous activities, including, amyloidogenesis, inflammation, brain cell atrophy and altered gene expression, thereby leading to cognitive impairments and memory deficits [2]. Recent discoveries reveal the role of microbes derived LPS to neurodegenerative disease such as AD. The secretory product of microbes induces to complement proteins of host and inflammatory cytokine that in turn hasten the generation of ROS/RNS and free radical. The pathological feature such as, immunogenicity, vascular permeability and activation of innate immune system also caused to aggregation of Aβ in SP lesion which aggravates the symptoms of AD [3]. Researchers found that the intestinal microbes metabolize many dietary polyphenols, including GSPE to phenolic acids. Further, agglomeration of two phenolic acids namely 3-hydroxybenzoic acid and 3-(3'-hydroxyphenyl) propionic acid in a brain interferes with the assembly of β-amyloid peptides thus blocked the formation of toxic Aβ. Previous study has also reported that the Intestinal microbes may contribute in protection against the advancementof AD [4].

MATERIALS AND METHODS

[1] 3-hydroxybenzoic acid (3-HBA) and 3-(3'-hydroxyphenyl) propionic acid (3-(3'HP) PA) structural file were retrieved from PubChem and Human amyloid beta protein file was obtained from PDB protein data bank.

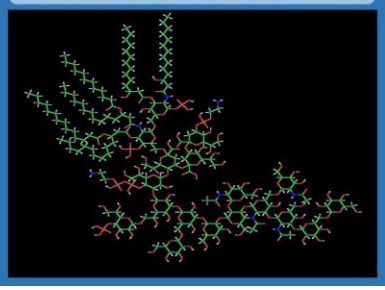
[2] Lipinski filter was used for screening drug likeness of 3-HBA and 3-(3'HP) PA molecules.

[3] Swiss dock tool was used for docking between Human amyloid beta protein and Ligands 3-HBA and 3-(3'HP) PA.

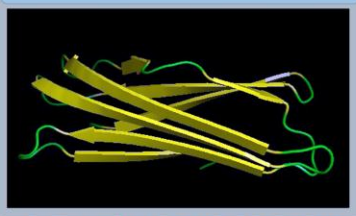
MICROBIAL SOURCE OF AMYLOID BETA

		Reference
Bacteria	*Escherichia coli*	Zhao et al, 2015
	Streptomyces species	
	Bacillus species	Hill et al, 2015
	Pseudomonas species	
	Staphylococcus species	
	Chlaymydophila pneumoniae	Hill et al, 2014
	Borrelia species	
	Helicobacter pylori	
	Treponema denticola	
	Tannerella forsythia	
Virus	*Pseudomonas aeuroisvalis*	
	Herpes simplex virus-1	
	Human immunodeficiency virus	
	Hepatitis C virus	
	Human cytomegalovirus	
Protozoa	*Toxoplasma gondii*	
Fungi	*Candida famata*	Alonso et al, 2014
	Candida albicans	
	Candida parapsilosis	
	Candida glabrata	
	Candida tropicalis	
	Candida krusei	

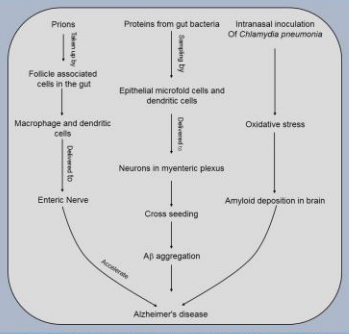
MOLECULAR STRUCTURE OF BACTERIAL LIPOPOLYSACCHARIDE



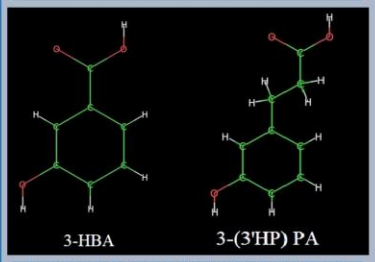
BACTERIAL AMYLOID CURLI (CSGC)



MECHANISM BEHIND AD ASSOCIATED WITH MICROBIAL FACTORS



2 D-STRUCTURE OF BIOMOLECULES OBTAINED FROM BACTERIAL SOURCE



PROPERTIES OF BIOMOLECULES (BY LIPINSKI FILTER ANALYSIS)

Properties	3-HBA	3-(3'-HP) PA
Molecular Mass (Da)	137	163
H-bond doner	0	0
H-bond Acceptor	3	3
LOG P	0.509	0.809
Molar refractivity	28.05	36.8

DOCKING RESULT OF HUMAN AMYLOID BETA WITH 3-HBA MOLECULE

DOCKING RESULT OF HUMAN AMYLOID BETA WITH 3-(3'HP) PA MOLECULE

CONCLUSION

[1] Microbes and Microbial products like LPS and Amyloid include in cause and progression of AD.

[2] Microbial metabolic products 3-HBA and 3-(3'-HP) PA can be helpful in reducing amyloid beta aggregates and lead to act as a potential therapeutics agent for the treatment of AD.

[3] Docking result and Lipinski filter analysis has also confirmed that both 3-HBA and 3, 3-(3'-HP) PA can be used for curing AD.

REFERENCES

[1] Reitz C, Brayne C, Mayeux R. Epidemiology of Alzheimer disease. *Nat Rev Neurol.* 2011, Vol.7 (3), pp.137-152.

[2] Hill JM, Clement C, Pogue AI, Bhattacharjee S, Zhao Y, Lukiw WJ. Pathogenic microbes, the microbiome, and Alzheimer's disease (AD). *Front Aging Neurosci.* 2014. Vol. 6, pp.127.

[3] Zhao Y, Lukiw WJ. Microbiome-generated amyloid and potential impact on amyloidogenesis in Alzheimer's disease (AD). *J Nat Sci.* 2015, Vol.1 (7), pp.e138.

[4] Wang D, Ho L, Faith J, Ono K, Janle EM, Lachcik PJ, Cooper BR, Jannasch AH, D'Arcy BR, Williams BA, Ferruzzi MG, Levine S, Zhao W, Dubner L, Pasinetti GM. Role of intestinal microbiota in the generation of polyphenol-derived phenolic acid mediated attenuation of Alzheimer's disease β-amyloid oligomerization. *Mol Nutr Food Res.* 2015, Vol.59 (6), pp.1025-1040.

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Genetic Aberrations In Neurodegenerative Disorders: A Molecular Link Between Parkinson's And Huntington's Disease PP-34

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Neurodegenerative disorders are a complex assembly of pathologies governs by the deposition of neurotoxic proteins in response to genetic alterations in DNA. Familial and sporadic forms of neurodegenerative disorders have been reported that possess a morphological link with DNA damage repair defects. The model diseases are Parkinson's and Huntington's disease that are helpful for understanding the pathophysiology behind the neurodegenerative disorders. However they appear to differ in their symptoms but several studies indicated that they might be related at molecular level. DNA damage in these disorders have been linked with the intricate interplay between genetic and environmental factors. Furthermore, these disorders have been attributed to defective DNA-repairing machinery in nucleus and mitochondria. Though the protein aggregates in these disorders differs but they possess similarity in their neurodegenerative mechanisms. Thus molecular analysis of the pathological proteins is the prime necessity for the elucidation of exact molecular mechanism behind their pathogenesis. Herein, (i) we demonstrated the DNA lesions involved in the onset of PD and HD, (ii) identified the susceptible genes with these disorders, (iii) elucidated the comparative interactomic protein-protein interaction network between PD and HD causative proteins, (iv) defined the factors involved in the pathogenesis of these diseases and (v) illustrated the plausible mechanism involved in the pathogenesis of Parkinson's and Huntington's disease.

GENETIC ABERRATIONS IN NEURODEGENERATIVE DISORDERS: A MOLECULAR LINK BETWEEN PARKINSON'S AND HUNTINGTON'S DISEASE

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ABSTRACT

Neurodegenerative disorders are a complex assembly of pathologies govern by the deposition of neurotoxic proteins in response to genetic alterations in DNA. Familial and sporadic forms of neurodegenerative disorders have been reported that possess a morphological link with DNA damage repair defects. The model diseases are Parkinson's and Huntington's disease that are helpful for understanding the pathophysiology behind the neurodegenerative disorders. However they appear to differ in their symptoms but several studies indicated that they might be related at molecular level. DNA damage in these disorders have been linked with the intricate interplay between genetic and environmental factors. Furthermore, these disorders have been attributed to defective DNA-repairing machinery in nucleus and mitochondria. Though the protein aggregates in these disorders differs but they possess similarity in their neurodegenerative mechanisms. Thus molecular analysis of the pathological proteins is the prime necessity for the elucidation of exact molecular mechanism behind their pathogenesis. Herein, (i) we demonstrated the DNA lesions involved in the onset of PD and HD, (ii) identified the susceptible genes with these disorders, (iii) elucidated the comparative interactomic protein-protein interaction network between PD and HD causative proteins, (iv) defined the factors involved in the pathogenesis of these diseases and (v) illustrated the plausible mechanism involved in the pathogenesis of Parkinson's and Huntington's disease.

Keyword: Neurodegenerative diseases (NDD), Parkinson's diseases (PD), Huntington's diseases (HD).

Introduction

Neurodegenerative diseases affecting millions of people worldwide, specific areas of brain affected by a diverse group of degenerative conditions, leading to movement or cognitive losses, depending on the type of neuronal cells undergoing selective degeneration with the disease. Neurodegenerative pathologies implicated with DNA damage induced neurotoxicity, provoked post transcriptional activity which may be an important contributor to the progressive deterioration of Neurons. Several factors have been identified implicated with DNA damage together with susceptibility genes and environmental factors in Neurodegenerative disease. Parkinson's disease ranked second among all the neurodegenerative disease, characterized by the production of bradykinesia, tremor, rigidity, and impairment of postural reflexes, Mutation in genes *SNCA, Parkin, PINK-1, DJ-1, ATP13A2, UCH-L1, GIGYF2, Omi/HTRA2, PLA2G6* and *FBXO7* leads to PD. Mitochondrial dysfunction, accumulation of misfolded protein, defective Ubiquitin Proteasome pathway, oxidative stress implicated with PD. Huntington's disease caused by mutant Huntingtin (mHtt) protein, produced as a result of CAG expansion beyond 40 in Huntingtin gene (*HTT*). a toxic gain of function of the mutant huntingtin and a loss of function of the normal protein cause the disruption of multiple intracellular pathway. vesicle transport, postsynaptic signaling, protein trafficking, transcriptional regulation, and apoptosis associated with normal functioning Huntingtin, progressive degeneration observed in HD associated with dopamine toxicity, metabolic impairment, mitochondrial dysfunction, oxidative stress, excitotoxicity, apoptosis and autophagy. A growing body of evidence elucidated the underlying mechanism in these two Neurodegenerative disorder.

DNA damage associated with Neurodegenerative disorder (PD, HD)

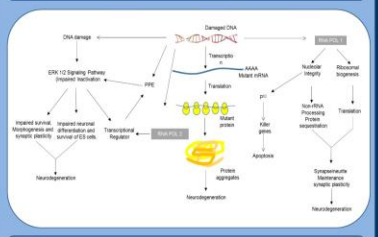
Neurodegenerative disorders	Types of DNA damage	References
PD	Duplication, Triplication, Insertion, Deletion, Substitution, Frameshift, Nonsense, Missense,Silent mutation, Polymorphism	1
HD	36 to more than 120 CAG repeats, 36 to 39 may or may not develop sign and Symptome, people with 40 or more repeats always develop the disorder	2

DNA damaging Agents associated with Neurodegenerative disorder (PD, HD)

S.No.	DNA Damaging Agent	Mechanism	Disease	References
1	ROS	Enzyme inactivation affect all component of DNA repair Machinary	PD	[3-5]
2	ROS/RNS	c-abl signalling implicated in several pathway.	PD	
3	NOX 1/RAC1	JNK-mediated Signaling pathways	PD	
4	MPTP	PARP activation	PD	
5	Rotenone/MPTP	Inhibit complex-I of Mitochondria	PD	
6	MSH2-MSH3	MSH2-MSH3 complex bind to TNR hairpin and promoting TNR expansion	HD	

Post-Transcriptional Activity

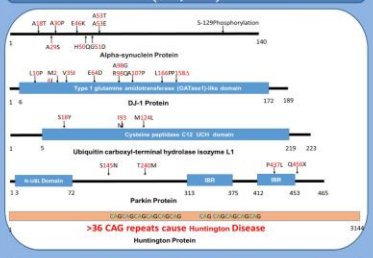
Mechanism of Neurodegeneration



Genes in Neurodegenerative disorder (PD, HD)

S.No.	Designation	Locus	Gene	Inheritance	Disease	References
1	PARK 1/ PARK 4	4q21	*SNCA*	AD	PD	[2-6]
2	PARK 8	12q12	*LRRK2*	AD	PD	
3	PARK 2	6q25.2–q27	*Parkin*	AR	PD	
4	PARK 6	1p35-36	*PINK1*	AR	PD	
5	PARK 7	1p36	*DJ-1*	AR	PD	
6	PARK 9	1p36	*ATP13A2*	AR	PD	
7	PARK 5	4p14	*UCH-L1*	AD	PD	
8	PARK 11	2q36-37	*GIGYF2*	AD	PD	
9	PARK 13	2p12	*Omi/HTRA2*	AD	PD	
10	PARK 14	22q13.1	*PLA2G6*	AR	PD	
11	PARK 15	22q11.2-qter	*FBXO7*	AR	PD	
12	D4S10	4p16.3	*IT15*	AD	HD	

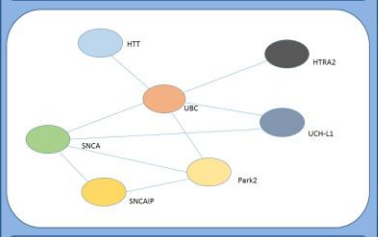
Gene mutation in Neurodegenerative disorder (PD, HD)



Susceptibility Genes in Neurodegenerative disorder(PD, HD)

S.No.	Susceptibility Gene	Increased Risk	References
1	APOE(ε2-variant)	Parkinson's disease	[7]
2	NAT2	Parkinson's disease	
3	MAO-B	Parkinson's disease	
4	CYP2D6	Parkinson's disease	
5	APOE	Huntington's disease (in males)	
6	GluR6	Huntington's disease	

Interactomic interaction of PD and HD



Conclusion

1-We have analysed the mechanism of neurodegeneration, Agents involve, associated gene, gene mutation and susceptibility gene in neurodegeneration.

2-We further investigated the Interactomic interaction of PD and HD, we found that the HTT Protein of Huntington's disease and PARK2, SNCA, HTRA2, UCH-L1 of Parkinson's disease interact through UBC protein

References

1. Abbas et al. (1999) A wide variety of mutations in the parkin gene are responsible for autosomal recessive parkinsonism in Europe. French Parkinson's Disease Genetics Study Group and the European Consortium on Genetic Susceptibility in Parkinson's Disease. Hum Mol Genet. 8(4): 567-74.

2. Bates GP. (2005) History of genetic disease: the molecular genetics of Huntington disease - a history. Nat Rev Genet. 6(10): 766-73.

3. Sarah et al. (2013) Parkinson's Disease: A Complex Interplay of Mitochondrial DNA Alterations and Oxidative Stress. Int. J. Mol. Sci. 14: 2388-09.

4. Dong-Hee et al. (2012) NADPH Oxidase 1-Mediated Oxidative Stress Leads to Dopamine Neuron Death in Parkinson's Disease. Antioxidants & Redox Signaling. 16(10): 1033-45.

5. Yuan et al. (2012) DNA base excision repair: A mechanism of Trinucleotide repeat Expansion. Trends Biochem Sci. 37(4): 162–72.

6. Coppede F. (2012) Genetics and Epigenetics of Parkinson's Disease. ScientificWorld Journal. 2012.

7. Coppede et al. (2006) Genes and the Environment in Neurodegeneration . Biosci Rep 26: 341–67.

8. Sonia Angeline et al. (2013) Sesamol and Naringenin reverse the effect of rotenone induced PD rat models. Neuroscience 254: 379-94.

9. Ravagnan et al. (2001) Heat-shock protein 70 antagonizes apoptosis inducing factor. Nat Cell Biol 3:839-843.

10. Rosen et al (2006) Parkin protects against mitochondrial toxins and beta amyloid accumulation in skeletal muscle cells. J Biol Chem 28: 12809-12816.

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