MASTER OF SCIENCE (BIOTECHNOLOGY) [AKANKSHA KHOSLA] 2021

IN SILICO ANALYSIS OF POTENTIAL DRUGS AS NEUROPROTECTANTS TARGETING MITOCHONDRIAL PROTEIN *PINK1* OF PARKINSON'S DISEASE

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE
OF

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

Submitted by:

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MAY, 2021

CANDIDATE'S DECLARATION

I, **Akanksha Khosla**, (Roll No.: 2K19/MSCBIO/10) hereby certify that the work which is presented in the Major Project entitled **"In silico analysis of potential drugs and molecules regulating mitochondrial dysfunction against Parkinson's disease"** "in fulfillment of the requirement for the award of Degree of Masters in Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own work, carried during a period from 7-Jan-2021 to 28-May-2021, under the supervision of **Prof. Pravir Kumar.**

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other university. This work has been communicated in Scopus indexed journal with the following details:

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Name of the journal: Journal of Pharmacy and Pharmacology

Conference date with Venue (if applicable): NA

Have you registered for the conference (yes/no): NA

Status of the paper (Accepted/published/communicated): Accepted

Date of paper communication: 25th Oct 2021

Date of paper acceptance: 27th March 2021

Date of Paper Publication: 29th May 2021

2. Title of paper: "Unboxing the molecular modalities of mutagens in cancer."

Authors name: Smita Kumari, Sudhanshu Sharma, Dia Advani, **Akanksha Khosla**, Pravir Kumar, Rashmi Ambasta

Name of Journal: Environmental Science and Pollution Research.

Conference date with Venue (if applicable): NA

Have you registered for the conference (yes/no): NA

Status of paper: Communicated (Under Review)

Date of paper communicated: 22nd April 2021

Date of paper acceptance: -

Date of Paper Publication: -

3.Title of paper: "Unboxing the Mitochondrial dynamics and dysfunction in Parkinson's Disease: From cause to potential cure."

Authors name: **Akanksha Khosla**, Pravir Kumar

Name of Journal: Suranaree Journal of Science and Technology

Conference date with Venue (if applicable): NA

Have you registered for the conference (yes/no): NA

Status of paper: Communicated (Assigned to the editor)

Date of paper communicated: 15TH May 2021

Date of paper acceptance: -

Date of Paper Publication: -

Place: New Delhi

Date: 30.05.2021

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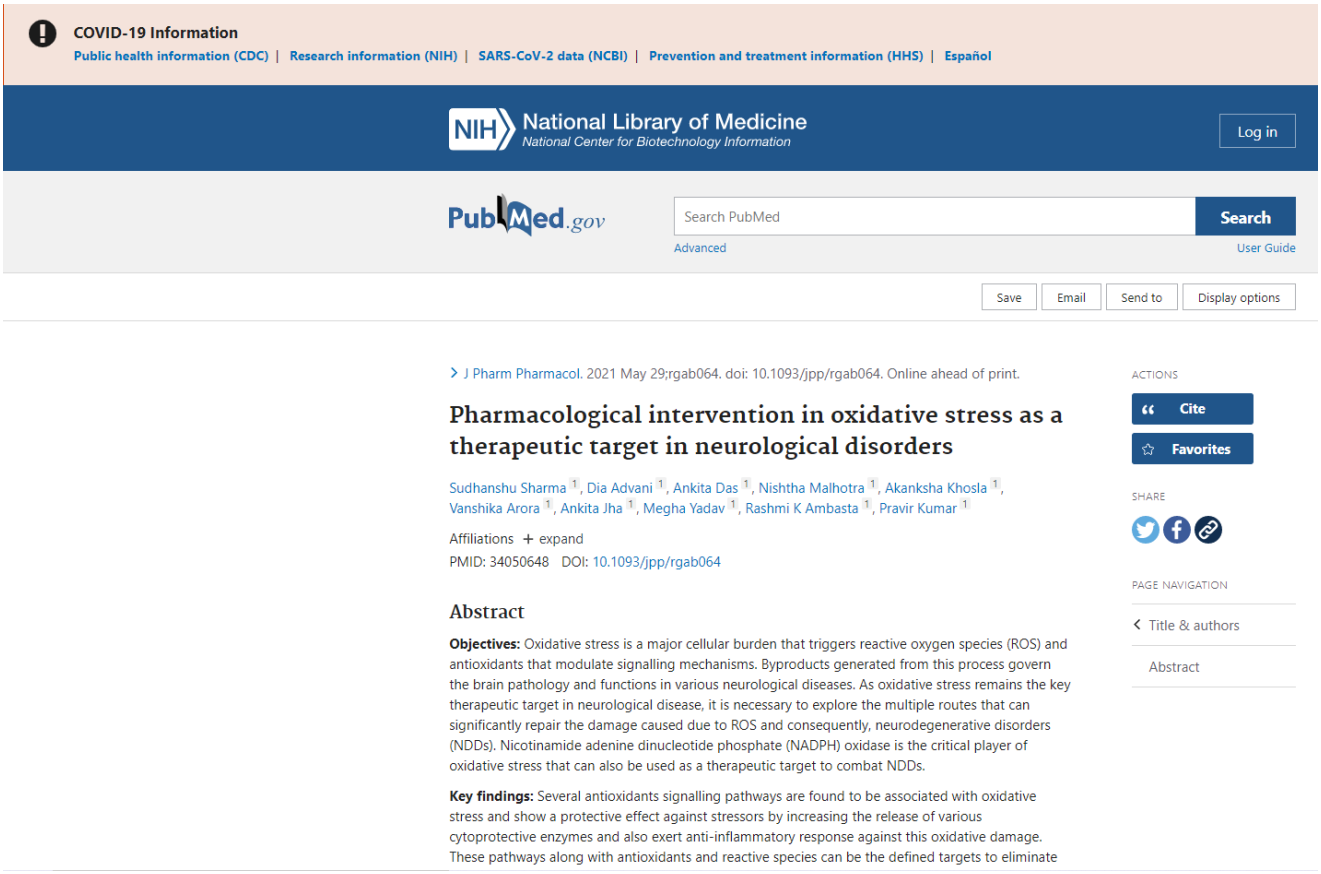

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Pharmacological intervention in oxidative stress as a therapeutic target in neurological disorders

Sudhanshu Sharma [1], Dia Advani [1], Ankita Das [1], Nishtha Malhotra [1], Akanksha Khosla [1], Vanshika Arora [1], Ankita Jha [1], Megha Yadav [1], Rashmi K Ambasta [1], Pravir Kumar [1]

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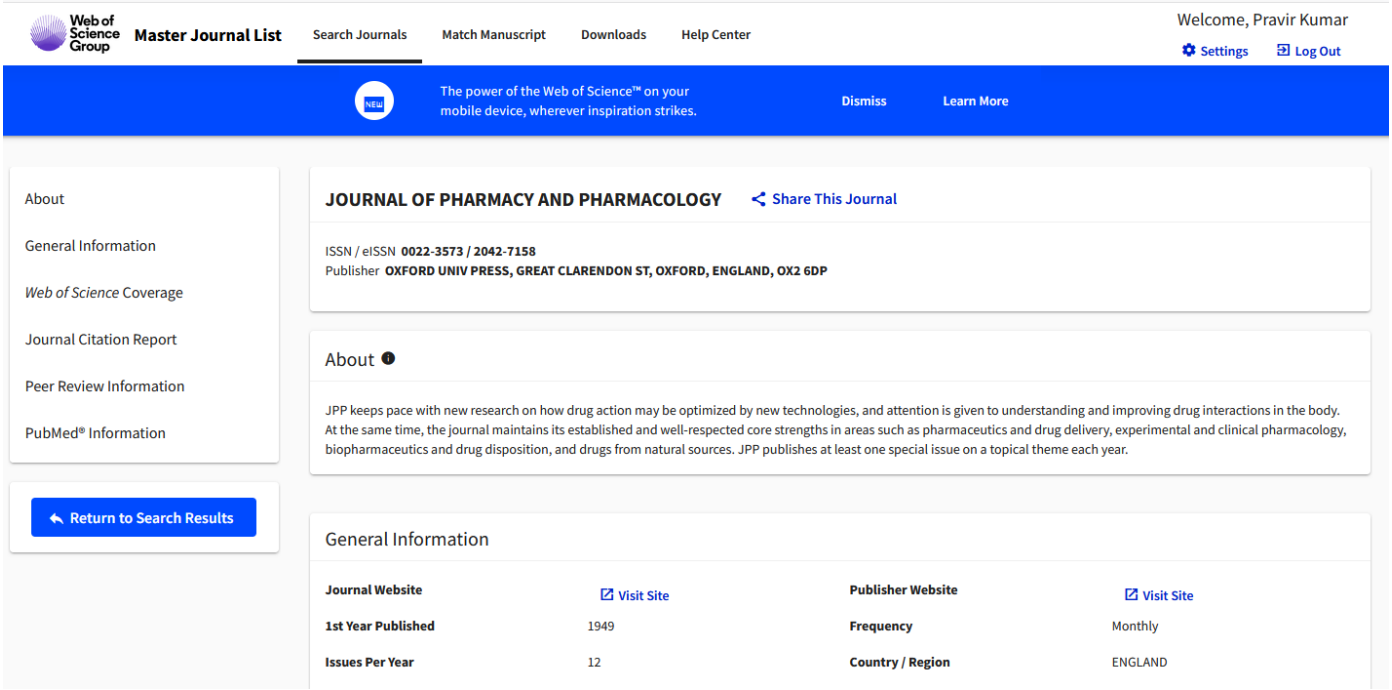
Abstract

Abstract

Objectives: Oxidative stress is a major cellular burden that triggers reactive oxygen species (ROS) and antioxidants that modulate signalling mechanisms. Byproducts generated from this process govern the brain pathology and functions in various neurological diseases. As oxidative stress remains the key therapeutic target in neurological disease, it is necessary to explore the multiple routes that can significantly repair the damage caused due to ROS and consequently, neurodegenerative disorders (NDDs). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is the critical player of oxidative stress that can also be used as a therapeutic target to combat NDDs.

Key findings: Several antioxidants signalling pathways are found to be associated with oxidative stress and show a protective effect against stressors by increasing the release of various cytoprotective enzymes and also exert anti-inflammatory response against this oxidative damage. These pathways along with antioxidants and reactive species can be the defined targets to eliminate



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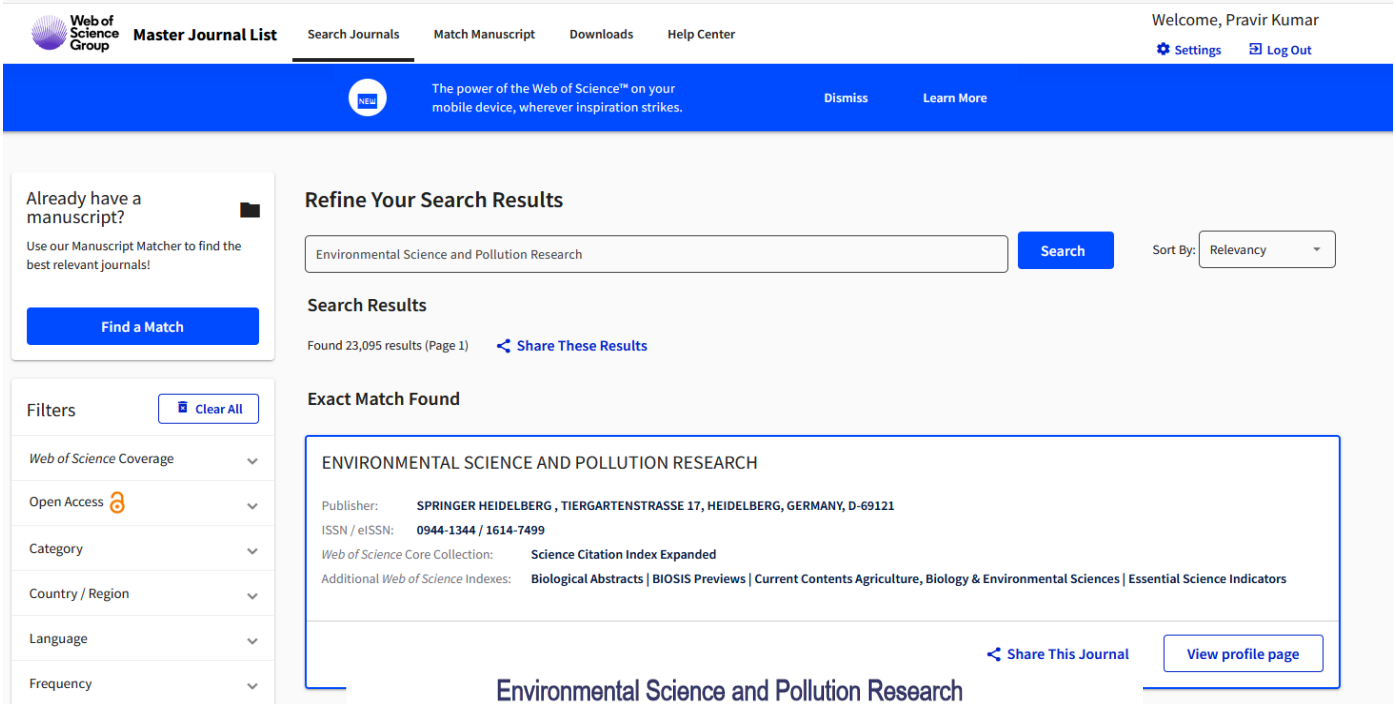

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Unboxing the molecular modalities of mutagens in cancer

--Manuscript Draft--

Manuscript Number:	ESPR-D-21-05202
Full Title:	Unboxing the molecular modalities of mutagens in cancer
Article Type:	Review Article
Keywords:	Mutation; DNA damage; DNA repair mechanism; Inflammation; Immunotherapy
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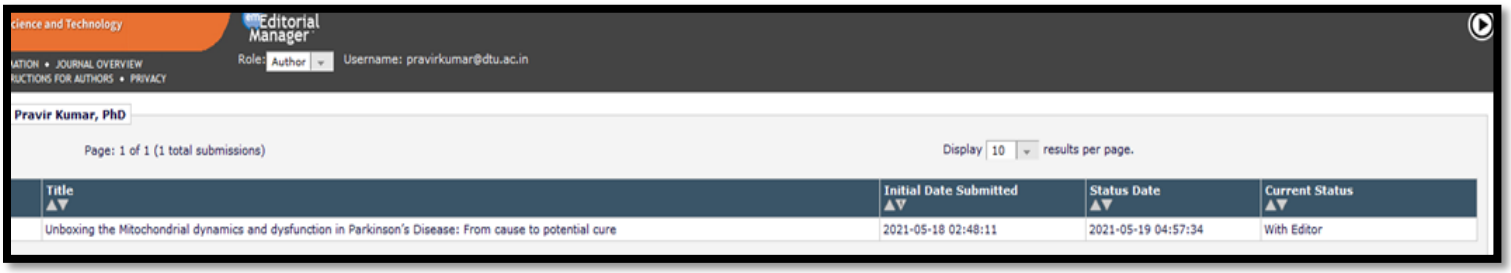
Abstract:	The etiology of the majority of human cancers are associated with a myriad of environmental causes, including physical, chemical, and biological factors. DNA damage induced by such mutagens is the initial step in the process of carcinogenesis resulting in the accumulation of mutations. Mutational events are considered the major triggers for introducing genetic and epigenetic insults such as DNA crosslinks, single and double-strand DNA breaks, formation of DNA adducts, mismatched bases, modification in histones, DNA methylation, and microRNA alterations. However, DNA repair mechanisms are devoted to protect the DNA to ensure genetic stability, any aberrations in these calibrated mechanisms provoke cancer occurrence. Comprehensive knowledge of the type of mutagens and carcinogens and the influence of these agents in DNA damage and cancer induction is crucial to develop rational anticancer strategies. This review delineated the molecular mechanism of DNA

3.Title of paper: "Unboxing the Mitochondrial dynamics and dysfunction in Parkinson's Disease: From cause to potential cure." (Submitted - Assigned to the editor)



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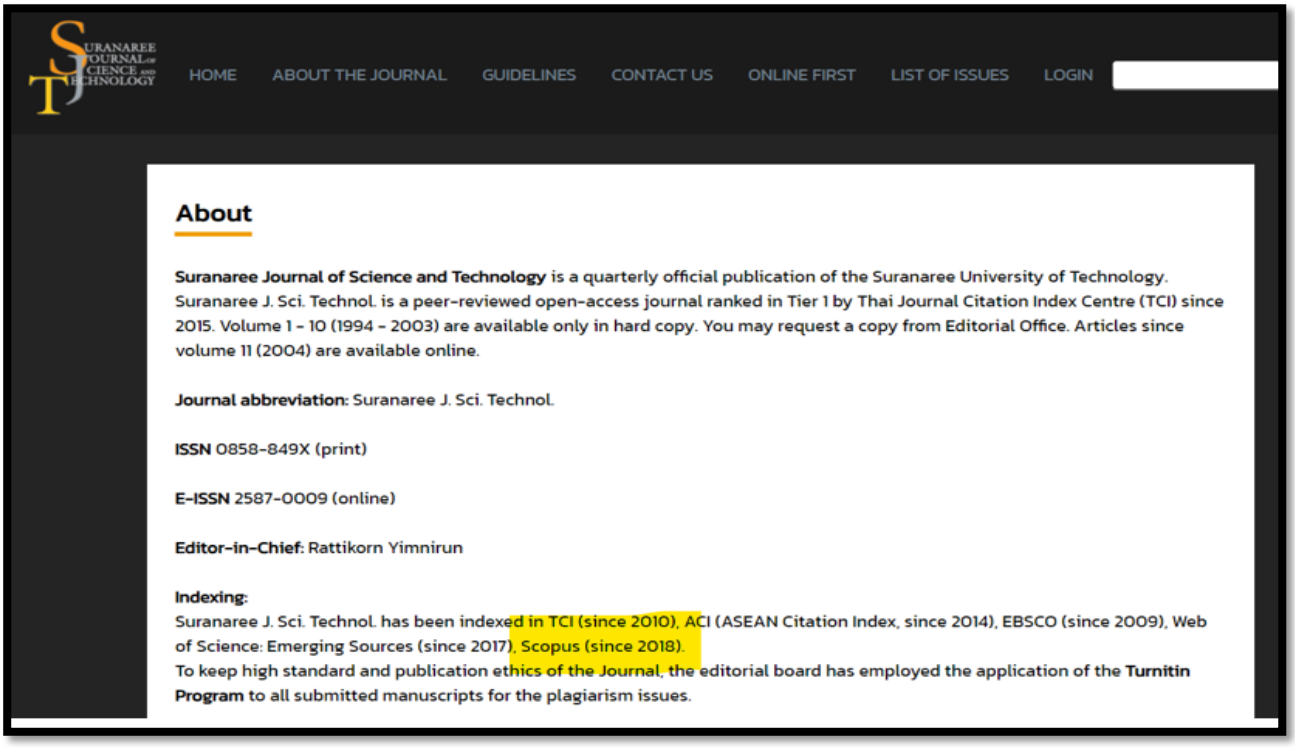

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my research guide **Prof. Pravir Kumar, Department of Biotechnology, Delhi Technological University,** for his unstinted inspiration, invaluable guidance, encouragement, keen interest, good wishes, and valuable suggestions throughout my entire research tenure.

I express my kind regards and gratitude to all the faculty members of the Department of Biotechnology, Delhi Technological University, and for helping in my project. I am equally grateful and wish to express my wholehearted thanks to respected lab seniors, Mr. Rohan Gupta, Ms. Smita Modi, Ms. Dia Advani, Mr. Rahul Tripathi, and Mr. Sudhanshu Sharma for their kind support and help in the course of my research work.

On a personal note, I wish to express my gratitude and affection to my family for their constant love and support. I also am thankful to Prasoon Prakash for giving me a morale boost and making my hopes alive with energy and enthusiasm to carry out the present work and helping hand I received during the tenure of study.

ABSTRACT

Parkinson's disease (PD) is regarded as one of the most chronic as well as a persistent neurodegenerative disorder, generally affecting elderly groups, with an obscure and complicated etiology. PD has a genetic association as it is caused due to mutations in different genes and thereby alters proteins they encode like DJ-1, Parkin, etc. The hallmarks of these diseases are the aggregation of altered proteins. Herein this review, we try to incorporate upon the details underlying mitochondrial dysfunction in PD and how it can be used as a curative agent providing vital acumens into its indispensable role in PD pathogenesis and serving as a purpose for future drug development. The mitochondria assist in various cellular processes, like bioenergetics, metabolism, cell signaling, redox homeostasis, and apoptosis. Even the well-being and integrity of mitochondria, depends on how precisely the protein is imported, folded, and regulated is defined as mitochondrial protein quality control; its impairment results in mitochondrial dysfunction, which can lead to different pathophysiological outcomes and also causes commencement of diseases like many irresistible proofs indicating that disturbance in mitochondrial dynamics is one of the prime factors involved in causing PD.

PINK1 is one of the central point associating mitochondrial dysfunction to PD and which may be targeted for PD therapy. Similar compounds to an approved drug-like Amphetamine were screen to target neuroprotective role against mitochondrial dysfunction in PD model, but due to its structural complication, it has black boxed warning and can cause cardiotoxicity, thus hunt for better nontoxic potential candidates continues.

Keywords: Parkinson's disease; Mitochondria; mitophagy; mitochondrial protein quality control; Molecular docking.

CONTENTS

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LIST OF SYMBOLS, ABBREVIATIONS AND NOMENCLATURE

S. No	Abbreviations	Full form
1	PD	Parkinson's disease
2	EOPD	Early onset of Parkinson's disease
3	LOPD	Late onset of Parkinson's disease
4	DA	Dopaminergic neurons
5	LB	Lewy body
6	SN	Substantia nigra
7	MAO	Monoamide oxidase
8	SNCA	Alpha Synuclein
9	AR	Autosomal recessive
10	AD	Autosomal dominant
11	OMM	Outer mitochondrial membrane
12	IMM	Inner mitochondrial membrane
13	IMS	Intermembrane space
14	mtDNA	Mitochondrial DNA
15	AD	Alzheimer's disease
16	Mfn	Mitofusion
17	OPA1	Optic atrophy 1
18	DRP1	Dynamin-related protein 1
19	FIS1	Fission protein1
20	PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
21	Tfam	Mitochondrial transcription factor A; also known as mtTFA
22	PRC	peroxisome proliferator-activated receptor gamma -1-related coactivator
23	AMPK	5'Adenosine monophosphate -activated protein kinase
24	ULK1	Unc-51-like autophagy activating kinase
25	SOD2	Superoxide dismutase-2
26	MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine

27	NMDA	*N*-methyl-d-aspartic acid
28	CR	Caloric restriction
29	MPQC	Mitochondrial protein quality control
30	TOM	Translocase of the outer membrane
31	SAM	Sorting and assembly machinery
32	TIM22	Translocase of the inner mitochondrial membrane 22
33	TIM23	Translocase of the inner mitochondrial membrane 23
34	mtUPR	Mitochondrial unfolded protein response
35	ROS	Reactive oxygen species
36	ETC	Electron transport chain
37	GSH	Glutathione
38	H_2O_2	Hydrogen peroxide
39	GSSG	Generating glutathione
40	mTOR	Mammalian target of rapamycin
41	PI3P	Phosphatidylinositol 3-phosphate
42	PAS	Pre-autophagosome structure
43	LC3	Light chain 3 protein
44	PINK1	PTEN-induced putative kinase 1
45	MAO-B	Monoamine oxidase-B
46	CoQ_{10}	Coenzyme Q10
47	MitoQ	Mitoquinone
48	TPP	Triphenylphosphonium
49	DBS	Drug based screening

CHAPTER - 1

INTRODUCTION

1.1 BACKGROUND

Parkinson's disease (PD), the second most chronic as well as a persistent neurodegenerative disorder, generally affecting elderly groups with an obscure and complicated etiology. This disease is characterized on the basis of the onset amongst people present in different age groups, .i.e. early onset of Parkinson's disease (EOPD) or late-onset of Parkinson's disease (LOPD)[1], [2]. Mostly 2 people out of 100 belonging to the above 60 years of age group is likely to be affected by PD. The degeneration of dopaminergic neurons (DA) along with the presence of Lewy bodies (LB), proteinaceous inclusion comprising mainly of ubiquitinated protein fibrillar α-synuclein, is regarded as a hallmark of PD. The bradykinesia, stiffness and rigid movements, frequent tremor, and imbalance in posture are four prime incapacitating signs. All these are significant evidence suggestive of PD pathogenesis, but the cause for the same is not known[3], [4].

The recent studies suggest that free radical formation is also identified, a consequence of oxidative imbalance research in PD and a massive decrease in the number of dopaminergic neurons present in substantia nigra (SN) pars compacta[5], [6]. This reduction in the number of SN dopaminergic neurons ranging between 50%-90% causes severe motor and non-motor dysfunction[7]. Some of the non-motor symptoms include memory fading, fatigue, abnormal sleep patterns, a decline in cognition, sensory abilities and behavioral changes[8]–[10].

Evidence supports that mutational changes in genes like PINK1, DJ-1, SNCA, EIF4G1, UCHL1, ATP13A2, GBA, PRKN and others are linked with PD development[11]. The genes PINK1, DJ-1 as well as *Parkin* responsible for autosomal recessive PD. On the other hand, mutations in LRRK2, SNCA and VPS35 lead to autosomal dominant PD[12]. At the same time, the predisposition in rest genes is also suspectable for the progression of the disease. PINK1 is one of the primary causative genes of autosomal early-onset PD. In general, the mutations in these genes initiate the loss of DA neurons and promote Lewy body formation[13].

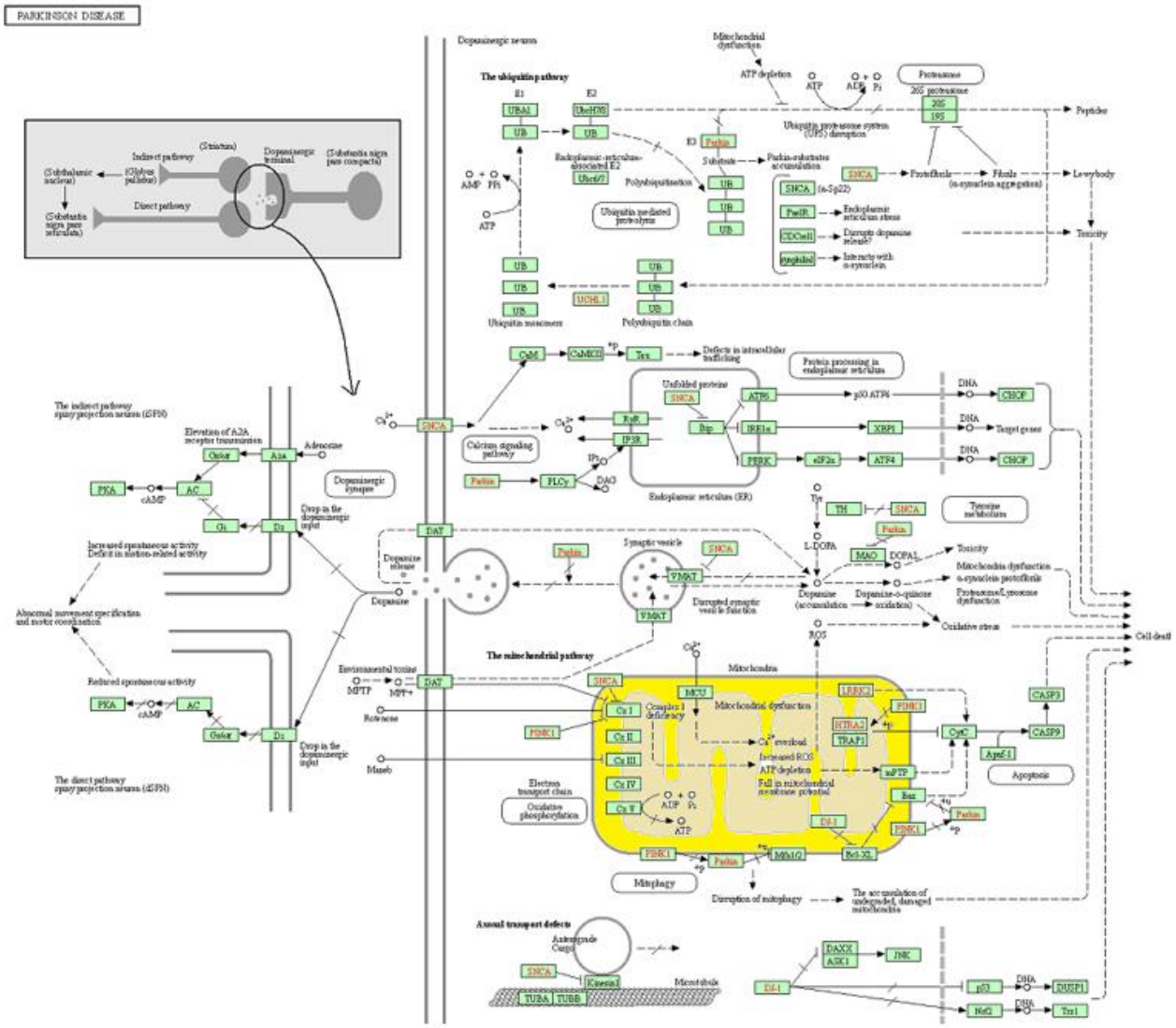

Figure1: Pathway for Parkinson's disease was retrieved from KEGG database

The molecular pathway was retrieved from KEGG database to study proteins that could be targeted to develop a potential therapeutic. The mutations in the proteins could also trigger mitochondrial dysfunction, proteosome system homeostasis, and finally leading to cell death.

1.2 PROBLEM STATEMENT

The report by Parkinson's disease Foundation suggests that nearly 1 million Americans are suffering from this. And by 2030, this will increase up to 1.2 million suffers worldwide. Also, the incidence rate depends on the geographical location too, like in India; due to the high rate of life expectancy, more older people are at risk of developing this disease[14]. But still, there is no prospective study to confirm the same. However, dementia is often seen in patients suffering

from PD but is not a characteristic symptom of this disease. Nearly 65% of elderly individuals above 85 years of age are at risk are experiencing dementia[15].

Currently, there is no cure for this disease, but the use of medications like levodopa, carbidopa has been effective in reducing symptoms; still neuroprotective roles are not confirmed[16]. Also, the treatment depends on the symptoms of the patients. Dopamine deficiency is the confirmed cause of PD, but the reason behind this degeneration of dopamine is still not understood. The need of the hour is to focus upon effective approaches to treat symptoms and to develop improved insight into molecular pathways involved in Parkinson's disease in order to develop potential drug candidates for its management.

Thus, future therapies should focus upon some novel drug targets through insights gained from the pathophysiology to increase efficacy against PD than the existing symptomatic treatments.

1.3 OBJECTIVE OF THE STUDY

- To find drugs targets which are inhibitor of monoamide oxidase (MAO)
- To screen approved as well as non- approved, potential inhibitors candidates with PINK1 proteins.
- To compare the screening result and identify if repurposing approved drugs can be used instead of current medication.

CHAPTER - 2

LITERATURE REVIEW

2.1 PARKINSON'S DISEASE AND ITS UNDERLYING MECHANISM

2.1.1 Epidemiology

James Parkinson, in 1817 wrote an essay on the Shaking Palsy, in which, for the first time, he described symptoms of the PD. PD is a neurodegenerative disorder being second most common amongst the other which affects the population of nearly 6 million at a global level. The prevalence of this disease is high aged people as it progresses with the aging and known to progress with progressive aging. With a maturing population and the anticipation of expanding life expectancy at a seemingly endless amount of time, the pervasiveness of PD is required to increment by over half by 2030.

The higher chances of incidence of PD are recorded in countries like North America, Europe as compared to Asian and African countries. The pathological is defined as a movement disorder with the triad of symptoms associating with rigidity, bradykinesia, and tremor[17]. While the risk factors include not only aging but gender also, having male: female ratio as being 3:2. The pathological reason behind the cause of PD is associated with degeneration of the dopaminergic nigrostriatal neurons and its associative pathway[18]. Although, dopamine substitution treatment has given the promise of suggestive treatment for the greater part of a century. Nonetheless, the advantages of indicative treatments are restricted by the rising side effects and the lack of efficacy after some time and the way that they try not to treat all manifestations of PD similarly well[19]. These limits arise partially in light of plastic changes in cerebrum circuits with respect to treatment, partially on account of the slippery movement) and also as other symptoms are not due to dysfunction of nigrostriatal[20]. PD also has some significant non-motor symptoms like mood-related disorders, cognitive problems, apathy, lack of smell, sleep pattern disturbances, chronic pain, which are not satisfactorily treated, adding to the nigrostriatal pathway. PD cases are generally erratic and appear to emerge from a complicated connection among hereditary and natural variables like the environment. The critical, hereditary examinations during the 90s

uncovered the presence of monogenic types of PD. From that point forward, PD has been distinguished as a hereditarily heterogeneous infection where a few monogenic types of the sickness and various hereditary menace have been recognized.

2.1.2 Pathophysiology

The presence of motor and non-motor symptoms suggests the manifestation of PD. The manifestations of motor symptoms are indicative that nearly 60% of the dopaminergic neurons in the SN are lost[21].

While the syndrome of parkinsonism is characterized by warning signs like resting tremor, bradykinesia, muscular rigidity, postural and gait impairment[22]. Also, the onset of a variety of non-motor symptoms associated with the disease is constipation, loss of smell, behavior and sleep disturbances. One of the key signs of PD is Dementia which occurs in the late course of the disease in patients who are suffering for more than 20 years [23].

Most PD patients have shown alpha-synuclein (SNCA) related pathology, regardless of etiology or genetic basis noticeable by the occurrence of LB and Lewy neurites. These LB are proteinaceous aggregates containing misfolded proteins which are seen inside the neurons of PD patients. [24].

Moreover, the classical pathology suggests that there is a significant loss of neurotransmitters, especially dopamine, in the SN. In addition, PD is not only limited to neurons in SN but also affects cholinergic neurons, serotonergic neurons as well as noradrenergic neurons[25]. The symptoms keep on worsening with progression as a greater number of cells are being affected are ultimately lost[26].

2.1.3 Pathogenesis

PD is a multifactorial disease which can be caused due aggregation of misfolded proteins, mitochondrial damage, disturbance in protein clearance pathways, oxidative stress, genetic mutations etc.

(i) Aggregation of SCNA: One of the hallmarks of PD is the accretion of LB in neurons containing misfolded SNCA aggregates SCNA. Also, several other studies have suggested that p-tau, Aβ, have been found in the brains of PD patients in misfolded conformation, suggesting the occurrence of dementia. Moreover, the assessment of the cerebrums of a few PD patients and found a combination of amyloid statements in their mind, which was

connected to intellectual decays without dementia, recommending amyloid adds to the psychological condition[27]. Correspondingly, SNCA gene mutation (like *A53T, A30P, E46K* and *H50Q*) is known to cause familial PD, which progresses rapidly in association with dementia. Even the SNCA accumulation in animal and cell culture models advocates abnormal mitochondria with deficit motility and reduced membrane potential[28]. The mitochondrial impairment along with SNCA inclusions in human DA neurons. Similar results were seen in knockout mice signifying the impairment in mitochondrial lipid and electron transport chain, making mice less subtle to mitochondrial toxins[29].

(ii) Hyper-phosphorylation of tau: It is known to be responsible for the buildup of neurofibrillary tangles connected to the accumulation of p-tau aggregation occurring in cortex and SN regions[30]. The p-tau can likewise be co-confined with LB, which is regularly connected with the advancement of irregular PD episodes[31].

(iii) Genetic mutation in PD: Many investigations have lately suggested that 5-10% of late-onset PD have been caused due to mutation of genes like SNCA, Parkin, DJ-1, PINK1 etc.[5] The table below summarizes genetic mutations related to PD.

(iv) Besides these, protein impairment and environmental toxins are also held responsible for the onset and progression of PD.

At present, no treatment can stop the disease progression; only indicative therapies are accessible. Medications that can fix intracerebral dopamine or trigger its receptors are best in class to manage motor symptoms. Perhaps the known far mediation is L-Dopa or Levodopa, which is the norm and starting treatment for patients. However, in later stages of the disease, patients experience a wearing-off impact and some other complications[32]. With respect to the catering of non-motor symptoms, various pharmacotherapies are accessible for utilizing previously approved drugs previously to treat these indications[33].

Table I: Genes related to Parkinson's Disease

Genes	Loci	Chromo-some	Inherit-ance	Type of PD (Onset)	Type of mutation	Risk Factor	Possible function	Lewy body patho logy	Changes in mitochondrial pathology	Distinct Clinical Features	Ref
Parkin	PARK 2	6q25.2–q27	AR	Early-Juvenile PD	Frameshift, nonsense and missense	High	Act as an E3 ubiquitin ligase, degrades mis-folded proteins, regulate release of DA from SNpc.	-	Downregulates PGC-1α leading to defective mitochondrial biogenesis	Often dystonia is observed with slow progression	[35]
DJ-1	PARK 7	1p36	AR	Early PD	Missense	High	Has neuroprotective role in dopaminergic neurons	-	High levels of ROS, impaired respiratory chain, defects in morphology and mitophagy	Focal dyskinesia with slow progression	[36]
SNCA	PARK 1/ PARK 4	4q21	AD	Late PD	Duplication, Missense & triplication	Very High	Involved in regulation of synapse vesicles	+	Interacts with mitochondria directly, inhibits complex I, mutations have shown increased mtDNA damage, mitophagy	Rapid progression, slight tremor	[37]
Glucoc erebro-sidase-1	GBA-1	1q21	Recessive	Familial PD	Lysosomal storage disorder	Interme diate	Plays crucial role in autophagic pathways in PD	-	- (under research)	Rapid eye movement, dementia, sleep pattern changes	[38]
PINK1	PARK 6	1p35–p36	AR	Early PD	Frameshift, nonsense & missense	High	protect cells against oxidative stress induced apoptosis	-	Complex I activity is reduced along with low level of ATP generation, impaired respiration and mitophagy defects.	Slow progression	[31]
LRRK2	PARK 8	12q12	AD	Idiopathic PD	Missense	High	Overexpression of *LRRK2* in leads to age-dependent DA-responsive reductions in locomotor activity and loss of DA neurons	-/+	Mitochondrial impairment has been observed in fibroblasts LRRK2 mutations.	-	[39]
ATP13 A2	PARK 9	1p36	AR	Juvenile PD	Deletion, insertion, frameshift & missense	Very High	Disrupts transport of polyamine which can lead to DNA damage and buildup of oxidative stress	-	High ROS levels observed	Rigidity and restriction in muscle movement; Dementia	[40]

*AR- autosomal recessive, AD- autosomal dominant

2.2 GENERAL ASPECTS AND UNDERLYING BIOLOGY OF MITOCHONDRIA

2.2.1 **Overview**

The mitochondria, a crucial organelle of all eukaryotic cells, being highly dynamic, have been defined as thread-like granules found in the cytosol of the cell. Mitochondria is a double membrane-bound structure possessing its own circular double-stranded DNA (mtDNA), which is quite distinct from the nuclear genome[34]. The origin of mitochondria as an organelle is supported by the endosymbiotic theory, which proposed that mitochondria have originated through the symbiosis of bacteria inside a eukaryotic host cell. This organelle is compartmentalized into four different sections:

- Outer mitochondrial membrane (OMM) – rich in phosphatidylcholine and phosphatidylethanolamine lipid and possess abundant porins and voltage-gated channels.
- Inner mitochondrial membrane (IMM) - acts as a center for the transportation of molecules between the cytosol and the mitochondrial matrix.
- Intermembrane space (IMS) and
- Matrix- possess many copies of mitochondrial DNA (mtDNA) as well as different enzymes.

The IMM bears numerous invaginations and thus is relatively larger than OMM; it is also sub-divided into (i) the inner boundary membrane (opposed to the OMM) and (ii) the folded cristae membranes. This organelle is commonly regarded as the powerhouse of the cell, thus generates ATP via the pathway of oxidative phosphorylation in order to meet the energy requirements of the cells. The oxidative phosphorylation is performed in a network comprising five protein complexes (complexes I–V). The electrochemical gradient between the inner membrane and the matrix of the mitochondria acts as the driving force for oxidative phosphorylation to occur, which results in ATP generation.

In addition to energy generation, mitochondria also serve a significant role in apoptosis (programmed cell death), amino acid metabolism, Fe-S cluster formation, calcium buffering as well as steroid hormone synthesis. It has been observed several times that the quality and functionality of the mitochondria decrease over time and are associated with aging, as mitochondrial dysfunction is considered to be a peculiar feature of age-related neurodegenerative disorders, especially in PD and Alzheimer's disease (AD)[35].

2.2.2 Mitochondrial Biogeny

With the discovery of fluorescent microscopy, mitochondrial dynamics became easier to be understood in both pathological and physiological circumstances[36]. It has been observed that mitochondria being a dynamic organelle, has a varied structure that undergoes changes, like fission and fusion, in order to adapt better to changing energy demands, as well as to enable the clearance of impaired organelles.

The mitochondrial network is controlled by the equilibrium because of fusion, fission; *de novo* mitochondrial biogenesis finally leads to the elimination of unwanted mitochondria by mitophagy.

Moreover, mitochondrial fusion is indulged into mitochondrial biogenesis by the trafficking of proteins and mtDNA. On the other hand, mitochondrial fission is involved in detaching the dysfunctional mitochondria; thereby, it is cleared by mitophagy. Furthermore, mitochondrial dynamics is considered as a process to control the status of the cell in terms of metabolic and bioenergetic[37].

2.2.3 Mitochondrial fusion

The mitochondria are double-membraned structures having OMM and IMM. The mitochondrial fusion is a process in which two mitochondria fuse with the help of mitofusin (MFN)1 located in OMM, MFN2 and optic atrophy 1 (OPA1), located on the IMM. These mitofusins are GTPases possessing conserved GTP-binding domains[38]. The two neighboring mitochondria interact through the heptad repeats, and the hydrolysis of GTP is the driving force for the fusion of the OMMs, which causes conformational change between the opposite membranes[39].

On the other hand, the ER has MFN2, which aids in the tethering of the ER to the mitochondria. It is also involved in the mitochondrial constriction of the fission process.

Whereas the IMM has OPA1 anchored protein which has a vital role in the fusion of IMMs[40]. Firstly, the OPA1 undergoes alternative splicing, which results in the formation of proteolytically cleaved short forms (S-OPA1) with the help of two IM peptidases: OMA1 and YME1L[41].

And cardiolipin, a mitochondria-specific lipid (another type of OPA1), is also significant to IMM fusion[42] as it the contact between cardiolipin and L-OPA1 present on either side of

the membrane which connects the two IMM, which undergoes OPA1-dependent GTP hydrolysis[43].

The S-OPA1 has been projected to increase the interaction of OPA1-CL as well as the fusion process[44]. Both the OPA1 and the mitofusin is controlled transcriptionally as well as by post-transcriptional mechanisms, though the destruction of mitofusin is regulated by ubiquitination, and phosphorylation regulates their destruction. Also, it has been stated that the fusion protein deficit leads to mitochondrial fragmentation.

2.2.4 Mitochondrial fission

Mitochondrial fission is employed to facilitate either isolation of damaged mitochondria from a healthy one or in order to transport the mitochondria through neuronal processes. The process of fission starts with the help of dynamin-related protein 1 (DRP1) and mitochondrial fission protein1 (FIS1). The DRP1 being a cytosolic GTPase protein is found in the OMM, where it forms a ring-like structure by undergoing oligomerization. The recruitment of DRP1 needs adaptors known as FIS1 in yeast but not in metazoans. FIS1 counterparts to OPA1, and their knockdown prevents programmed cell death, too[45]. FIS1 is the mitochondrial adaptor in yeast, but in metazoans, it is not required for fission[46]. The ER-mitochondrial contact sites are where fission occurs as tubules of ER help in constriction before recruitment of DRP1.[47].

After the phosphorylation of DRP1, it is translocated to mitochondria which tempt to bring about OMM fission and also leads to phosphorylation at Ser656[48].

However, whether IMM scission is influenced by DRP1-mediated OMM constriction is still not known. But the role of accumulation of S-OPA1 does support the fission process, which may give a new perspective to the research field in mitochondrial fission.

2.2.5 Biogenesis and its regulators

The process involving growth, development and multiplication of pre-existing mitochondria can be defined as mitochondrial biogenesis. It is influenced by a complex network of nuclear as well as mitochondrial expression events as majorly proteins are encoded in the nucleus, so machinery to ensure correct targeting and assembling of mitochondrial function and shape exists in nature. The peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α) is the crucial regulator which interacts with different transcription factors like

NFRs (nuclear respiratory factors) 1 and 2, and Tfam (mitochondrial transcription factor A; also known as mtTFA) to coordinate the biogenesis of mitochondria[49].

The activators and proteins involved in gene expression during mitochondrial biogenesis are as follows:

(i) Peroxisome proliferator-activated receptor-gamma -1 (PGC-) family: The PGC-1 family consists of PGC-1α, PGC-1β, and PGC-1-related coactivator (PRC), which resort to different signals to stimulate mitochondrial gene expression. The PGC-1α interacts with NRF1 to enhance transcription of genes, and it has been evident that impairment of this protein is often seen in many neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, and Huntington's disease[50]. Also, PRC is seen to enhance the gene transcription of NRF1 and is involved in the expression of genes in dividing cells. The lack of PRC suggests severe respiratory chain dysfunction as well as accumulation of mitochondria-bearing abnormalities[51]. While PGC-1β simply regulates the basal functions of mitochondria[52].

(ii) 5'Adenosine monophosphate-activated protein kinase (AMPK): It is regarded as the primary regulator of metabolism of eukaryotic cells as it raises the ratio of AMP/ATP, resulting in increased transportation of glucose and others[53]. It has a role in regulating both mitophagy as well as biogenesis as AMPK triggers destruction via Unc-51-like autophagy activating kinase (ULK1) and increasing transcription of genes regulated by PGC-1α respectively.

(iii) Sirtuins: They utilize one molecule of NAD^+ in every deacetylation cycle and are thus regarded as class III protein deacetylases. The mammalian cells have SIRT1-7. It has a prominent role in regulating aging[54]. It deacetylates PGC-1α and enhances the transcription of genes regulated PGC-1α[55]. The SIRT3 confines mitochondria and is crucial for mediating counter to oxidative stress by triggering superoxide dismutase-2 (SOD2). SIRT3 deacetylates SOD2 to augment its ROS scavenging activity[56]. The lack of SIRT3 in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson Disease mice aggravates deterioration of nigrostriatal dopaminergic neurons[57]. In comparison, the enhanced release of SIRT3 lowers the excitotoxicity of *N*-methyl-d-aspartic acid (NMDA) in neurons of the cortical region of cultured mice [58].

Also, other factors contributing to mitochondrial biogenesis involve environmental stress like low temperature, lack of physical activity, excessive sugar intake, oxidative stress, etc. The number and activity of mitochondria are greatly affected by physical activity, and this relationship is evident by comparing the mitochondrial content in diverse muscle groups, like the breast muscle of chickens bears a smaller number of mitochondria than that of pigeons, which also supports the fact that muscles of pigeons are involved in flying. Also, similar studies in rats and humans hold true[59]. In spite of these links, the connection between mitochondrial biogenesis and endurance-related workout remains subtle.

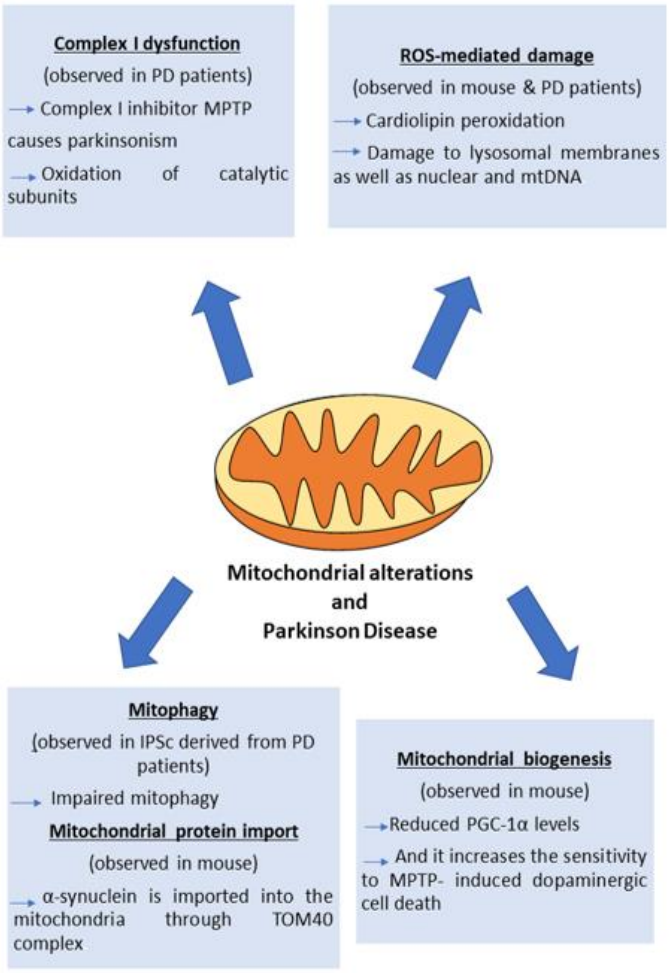

Figure 2.1: **Representation of pathways involved in mitochondrial dysfunction in Parkinson's disease pathophysiology**: Mitochondrial dysfunction associated with PD pathogenesis can be a consequence of compromised mitochondrial biogenesis, increased reactive oxygen species production, faulty mitophagy, impairment in mitochondrial protein trafficking.

(Khosla and Kumar)

The term caloric restriction (CR) signifies a reduction in the intake of calories intake, though still preserve all the vital nutrients (without being starved off or causing malnutrition). This CR input is known to reduce ROS levels and thereby decrease DNA as well as oxidative damage too. The sirtuins molecules also have been facilitating a significant role in CR-mediated longevity[60]. The overexpression of SIRT1, just as CR, is beneficial for reducing neurodegenerative diseases and their related symptoms. Also, the CR improves the number of mitochondria as well as cristae present per cell[61]. The other roles of CR suggest inhibition of PI3K/AKT pathway, calcium retention, enhanced mitophagy, thereby helping to maintain mitochondria homeostasis.

2.3 HOMEOSTASIS AND QUALITY CONTROL SCHEMES IN MITOCHONDRIA

The homeostasis in mitochondria is crucial for the proper import, targeting as well as folding of the protein. And collectively, these molecular events are defined as mitochondrial protein quality control (MPQC) system, whose dysregulation results in the development of various neurodegenerative diseases or cancer. Thus, mechanisms are it at molecular, or protein levels are being employed to maintain mitochondrial health.

2.3.1 Protein import system

The majority of the mitochondrial proteome (99%) is encoded by the nuclear DNA, which signifies the importance of mitochondrial protein import machinery. These proteins encoded by the nucleus are precursors having an unfolded conformation which makes them competent to translocation.

But these precursor proteins need to complex with the cytosolic chaperones (like TOM34, HSP70, HSP90) to prevent their aggregation and degradation[62]. In fact, these precursors carry information that directs them into an accurate compartment of mitochondria.

The main sites for MPQC are:

(i) at the OMM in association with the mitochondrial protein import machinery: The precursor proteins are placed into the OMM by the action of the translocase of the outer membrane (TOM) complex as well as by sorting and assembly machinery (SAM)

complex present in OMM itself. While for placing the β-barrel membrane proteins, cooperation between the two complexes is needed.

(ii) in the IMS, for targeting of the membrane proteins: The IMM bears many different proteins like transport chain complexes and ATP synthase. Mainly in three different ways, protein can be translocated by the TOM complex. Firstly, by the action of the translocase of the inner mitochondrial membrane 22 (TIM22) complex. Secondly, by a lateral insertion by the translocase of the inner mitochondrial membrane 23 (TIM23) complex and lastly by exporting from the matrix to the inner membrane. This TIM22 complex is formed by Tim22, a channel-forming protein Tim22; a subunit of respiratory chain complex II, i.e., SDH3 in humans, as well as some small TIM chaperones such as TIMM9, TIMM10A and TIMM10B in humans. Whereas in yeast, Tim54 and Tim18 are also present in addition to the above mention protein molecules.

(iii) in the matrix, which is responsible for the replacement of misfolded matrix and proteins located in IMM: Mainly, most proteins are present in the matrix of mitochondria which needs to be imported under the influence of the two mitochondrial translocase – TOM and TIM23 through OMM and IMM respectively[63]. Unlike TOM complex, the preprotein translocation through TIM23 is favored energetically by the membrane potential of mitochondria following the hydrolysis of ATP[64].

2.3.2 Protein homeostasis mechanism

Most proteins inside the mitochondria are encoded by the nuclear genes and transported back as precursor molecules. These precursors need to undergo proper assembly, cleavage and processing. And if this process is hindered, then degradation of proteins is necessary to prevent abnormality in mitochondria. Thus, to ensure proper protein functioning, mitochondria have chaperons and proteases as protein quality regulators. When there is an imbalance in protein homeostasis, nuclear signals elucidate mitochondrial unfolded protein response (mtUPR) in order to an equilibrium state.

(i) Chaperons: The molecular chaperons are constantly scrutinizing the mitochondrial protein import. The chaperons help other proteins to attain active conformation without themselves being a part of it and prevent misfolding of the precursors. While some are involved in protein trafficking like Mortalin, which and binds to unfolded protein forming

a central part of import motor amongst the matrix and TIM23 complex[65]. Other chaperons like TRAP1, HSP60, HSP90 also assist.

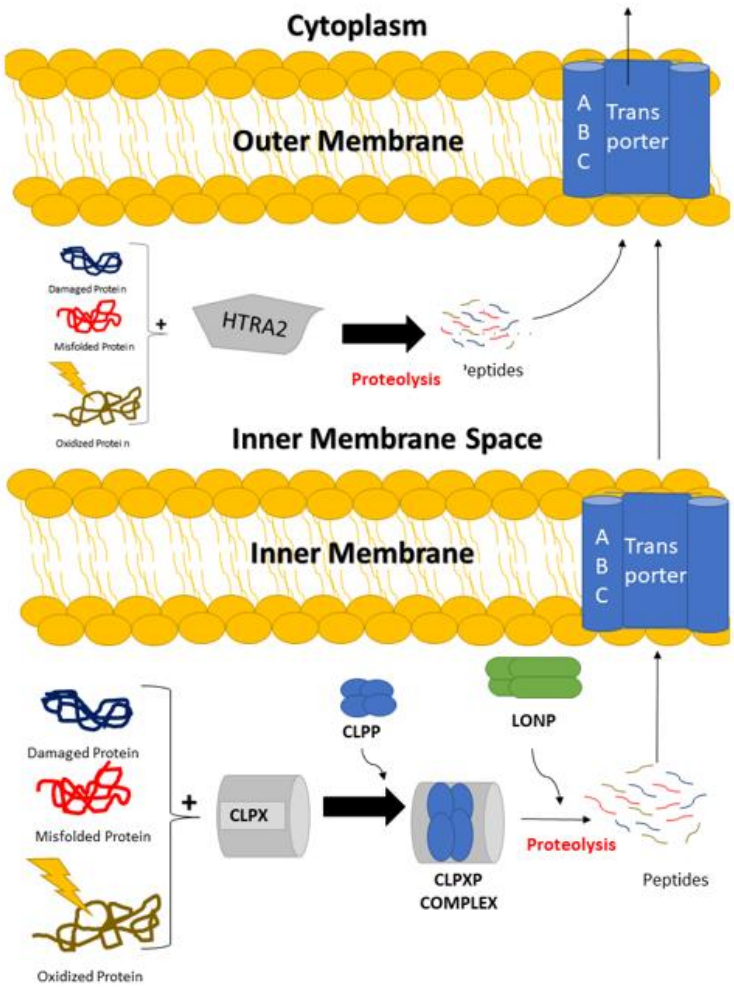

Figure 2.2: Mechanism of Mitochondrial Protein Quality Control System (MPQC): This system takes care of all the damaged/oxidized/misfolded proteins. Thereby protecting the cells against proteotoxic stress. Under diseased conditions, proteins can be harmed or denatured. These damaged proteins can be taken away by different proteases CLPX/CLPP is present in the matrix while HTRA2 is present in IMS. And finally transported by ABC transporter. HTRA2, High-temperature requirement protein A2; LON, Lon protease; CLPX, ATP-dependent Clp protease proteolytic subunit X; CLPP, ATP-dependent Clp protease proteolytic subunit P.

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(ii) Proteases: Proteases, as the name suggests, would cleave the damaged, misfolded proteins degrade misfolded and damaged proteins, which have exported into mitochondria. Additionally, they also provide protease-mediated quality control response, thereby

providing protection against damaged and non-assembled proteins produced as a result of ROS and mitonuclear imbalance, respectively[66]. Majorly there are three types of proteases: ATP-dependent, ATP-independent and oligopeptides.

(iii) mtUPR: The mitochondria resort to photostatic stress by the mitochondrial unfolded protein response (mtUPR). This response helps to build a communication network between the nucleus and mitochondria to support the activation of genes for maintaining homeostasis in mitochondria[67]. This mechanism of action of mtUPR is well understood in *C.elegans* but still not evident in how it works in humans. The mtUPR can serve as a promising pathway for stress response and maintain mitochondrial health.

Table II: Molecules involved in maintaining protein homeostasis and their function

Molecules	Type	Localization	Catalytic Class/Family	Function	Ref
LONP	Mitochondrial Protease	Inner Membrane Space	Cysteine Catalytic Class	Involved in PQC and biogenies of mitochondria	[75]
PITRM1	Mitochondrial Protease (ATP-dependent protease)	Inner Mitochondrial Membrane	Metallo Catalytic Class	Degrades peptides into amino acids	[73]
TRAP1	Chaperon	Matrix	HSP90 chaperon family	Negative regulator of mitochondrial respiration, possess an ATPase activity	[76]
MEP	Mitochondrial Protease	Inner Membrane Space	Metallo Catalytic Class	Hydrolysis of peptides.	[77]
HSP60	Chaperon	nearly 60% in matrix, 20% in inner membrane	Chaperonin family	Inhibits protein accumulation	[78]
PARL	Mitochondrial Protease (Cleaving)	Inner Mitochondrial Membrane	Serine Catalytic Class	Essential for proteolytic processing of OPA1	[79]
ATP23	Mitochondrial Protease (ATP-independent protease)	Inner Membrane Space	Metallo Catalytic Class	Maintenance of ETC complex as well as metabolism of mitochondrial phospholipid	[80]
CLPP	Mitochondrial Protease (ATP-dependent protease)	Matrix	Serine Catalytic Class	Removal of damaged matrix proteins	[81]
HSP27	Chaperon	Cytosol	sHsp (small heat shock protein) family	Helps in protein degradation, acts	[82]

2.3.3 Redox homeostasis

The mitochondria are the prime source of reactive oxygen species (ROS) as well as superoxide[68]. The complex I and III of the electron transport chain (ETC) are the chief producer of these superoxides. In addition to this, other sources like flavoprotein-ubiquitin oxidoreductase are also contributing to the generation of these highly reactive species[69].

These superoxide radicals then augmented ROS production leading to oxidative damage of lipid, proteins as well as DNA. Thus, in order to maintain a low threshold level of ROS, the mitochondria have several antioxidant defenses like catalases, thioredoxin, glutaredoxin. The glutathione (GSH) contributes to the detoxification of hydrogen peroxide (H_2O_2), generating glutathione (GSSG)[70]. GSSG also plays a crucial role in antioxidant defense by stimulating Mfn1- and Mfn2-dependent hyper-fusion of mitochondria[71]. The catalase enzyme converts H_2O_2 to water and oxygen. The recent studies suggested that the overexpression of mitochondrially-targeted catalase caused the deficiency of peroxiredoxin 3 in altered mesothelioma cells thereby, resulted in a hyper-fused mitochondrial phenotype[72].

2.3.4 Mitophagy

The degradation of mitochondria selectively occurs by a process called mitophagy. Thus, it is referred to as autophagy only, in which intracytosolic components like organelles are transported to the lysosome for the degradation process[73]. And this transportation is carried by the cargo sequestered autophagosome with whom lysosome fuses lately[74].

Based on the substrate to be eliminated, various autophagy paths have been described, like mitophagy for mitochondria, ribophagy for ribosomes; lipophagy for liposomes; aggrephagy for protein aggregates. The regulation of autophagy is under the action of the mammalian target of rapamycin (mTOR). The mTOR associates with regulatory proteins TOR (Raptor) and mLST8 to form mTOR Complex 1 (mTORC1). This mTORC1 binds as well as hinders the activity of ULK1, for initiation of autophagy dissociation of mTORC1-ULK1 is needed[75]. The ULK1 also causes translocation of Vps34 complex comprising of beclin 1, AMBRA, Vps34, Vps15 and Atg14L; Vps34 then produces phosphatidylinositol 3-phosphate (PI3P), which forms pre-autophagosome structure (PAS) by initiating the nucleation process.

The elongation of autophagy is done by two different ubiquitination-like cascades – first controlled by Atg12-Atg5-Atg16L and second under the control of protein-lipid conjugation. After this, both mechanisms unite for elongation through the lipidation of light chain 3 (LC3-

I) protein with phosphatidylethanolamine phospholipid, forming LC3-II[76]. There is an autophagy receptor responsible for recognition of specific destruction signals on cargo proteins as well as for forming autophagosome by binding of LC3/GABARAP proteins via LC3 interacting region[77].

The mitophagy pathway has three basic steps:

(i) The damaged mitochondria need to be discovered.
(ii) The organelle then needs to be enclosed by an autophagic membrane.
(iii) After that, the mitoautophagosomes fuse with the lysosomes.

Parkin and PTEN-induced putative kinase 1 (PINK1) play a crucial role in mitophagy. The Parkin and PINK-dependent pathway is the most well-understood pathway to identify mitochondria to undergo the degradation process. This PINK1 cytosol but keeps on accumulating in the OMM of abnormal mitochondria. Being a s the most common genetic cause of PD. Serine/threonine kinase is transported and destroyed inside mitochondria, functionating properly. Thus, its accrual is prevented on OMM. While it keeps assembling in dysfunctional mitochondria and leads to the formation of the TOM complex[78].

This accumulation of PINK1 induces recruitment of Parkin, a segment of E3 ubiquitin ligase for initiation of mitophagy[79]. But recent studies have also suggested that Parkin-independent mitophagy can also occur, which involves proteins like cardiolipin-LC3 interaction, etc. [80].

Also, endosomes are found to be essential in the elimination of impaired mitochondria as ubiquitin-tagged mitochondria are sequestered to Rab-5 expressing endosomes which ultimately fuses with lysosome leading to degradation. Moreover, studies suggest that disturbance in the endosomal pathway due to lack of Rab5 activity intensifies the chances of apoptosis cell as a consequence of mitochondrial stress[81].

2.4 MITOCHONDRIAL DYSFUNCTION AND NEURODEGENERATION

Profoundly, neurons rely on mitochondria for maintaining energy-driven processes like maintenance of membrane potential, neurotransmission, etc. Thus, impairment in the functioning of mitochondria will adversely affect neuronal survival[82]. The recent studies recommend the inclusion of mitochondrial anomalies that have been seen in age-related neurodegenerative sicknesses.

The neuronal mitochondria have a longer half-life than other cell types, so the quality control of mitochondria is particularly significant. Additionally, neuronal morphology is intricate and possesses long axons and dendrites, which also justify their high energy needs. Thus, management of mitochondrial elements, biogenesis as well as mitophagy is of prime importance in various neurodegenerative disorders[83].

2.4.1 Crossroads linking between mitochondria dysfunction & PD

In the 1980s, the underlying link between PD and mitochondria dysfunction was established, and the oxidative pressure hypothesis also supports the same by stating that the mitochondria act as a 'hotspot' for neurodegeneration. In PD, impaired activity related to mitochondrial complex-I has been noticed, which straightforwardly intrudes with the ATP generation process of the cell, prompting cell demise[84]. Likewise, the cerebrum monoamines neurotransmitters, like DA, Serotonin are known to act as antioxidants. Nonetheless, the degradation of DA by the action of monoamine oxidase-B (MAO-B) in the presence of oxygen stimulates ROS production[85]. Also, the relation between mitochondrial dysfunction and PD was evident with the use of a psychotropic drug that exposes 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine MPP+, which metabolizes to a complex I inhibitor which leads to nigral neuron loss and a Parkinson's phenotype[86]. Several scientists have discovered expanded oxidative pressure markers and related changes in PD patients, thereby suggesting mitochondrial dysfunction has been observed in both sporadic and genetic forms of PD. The expression of 'Bax'- an apoptotic marker protein, is seen to be stimulated in DA neurons of the SNpc in MPTP-treated mice[87]. Recent experiments have suggested that when mtDNA from PD patients have been placed into neuroblastoma cells, then LB was seen. These LB were similar to those observed in dopaminergic neurons in PD patients. These results strongly suggest that mitochondrial abnormalities play a critical role in PD.

a. In Familial PD

Nearly familial PD accounts for $1/10^{th}$ of all the known cases. Many models like a knockout mouse, Drosophila have been investigated to study familial PD. Familial PD are less diverse than sporadic PD models as they are controlled by genes, making them appropriate for reviewing mitochondrial dysfunction as well as potential therapeutics. Although there are many known causes for familial PD but the two most common genes linking PD with mitochondrial dysfunction are LRRK2, α-Synuclein and parkin/PINK1.

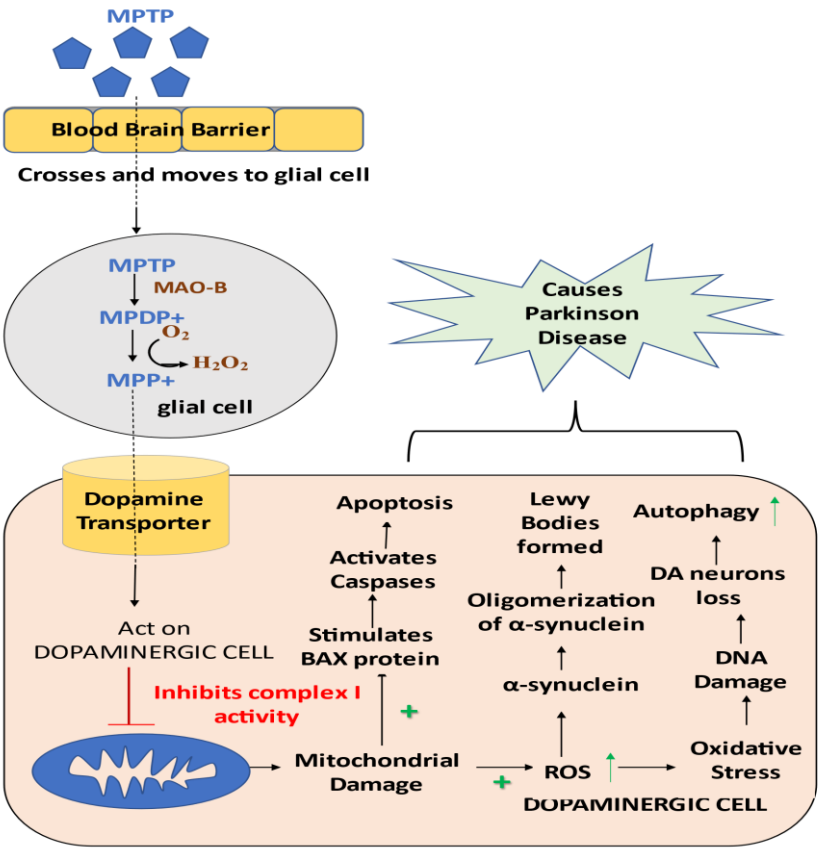

Figure 2.3: Mechanism of DA-neuronal loss in PD under the influence of MPTP: And pictorial representation to display crosslinks between mitochondrial damage and PD. MPTP firstly passes through the blood-brain barrier and reaches the glial cell (astrocytes), where it undergoes the catabolism process. Firstly, MPTP is converted to MPP+ in a glial cell by monoamine oxidase B in a two-step reaction having $MPDP^+$ as an intermediate. This MPP+ released from the glial cell is taken by the dopaminergic cell through dopamine transporter present across plasma membrane dopamine. Once MPP+ enters the cytosolic compartment of dopaminergic neurons, it inhibits the activity of complex I of mitochondrial by bind to it. This binding results in various changes resulting in damage to the mitochondrial physiology, leading to a surge in ROS levels and the building up of oxidative stress. Further, all these reactions lead to a cascade of events, ultimately causing apoptosis, autophagy of neuronal cells, Lewy body formation. All these have been reported to cause PD.

(Khosla and Kumar)

b. In Sporadic PD

Various studies have shown a decline in the activity of complex I in PD patients[88]. The complex I being the largest mitochondrial complex, is involved in electron transfer as well as proton translocation across the IMM. This abnormality in complex I suggest reduced ATP

production, increase ROS accumulation. Also, the samples were collected from PD patients undergoing one month of carbidopa/levodopa treatment. After undergoing one month of treatment, no significant improvements were detected in the activity of complex[89]. This suggests although mitochondrial deficiencies are present still, no medication has been turned out to be effective for the same. Hence, it is probable to infer that aiming at altering the mitochondrial function would be advantageous therapeutically. In addition, the activity of other complexes has also altered, and the plausible reason for it could have been the different methodologies adapted to study them.

Furthermore, a study has shown that even though the activity of complex I has decreased, but the protein level remains constant on native gel[90]. This needs pondering upon the fact how complex I inadequacy is related to, which is quite significant while focusing on it for novel therapeutics.

The mtDNA also plays a crucial role in the prognosis of PD as research has shown that many somatic mutations keep on accumulating over the PD patient's lifetime as well as neurons present in substantia nigra were exhibiting mtDNA damage in PD patients, in comparison to cortical neurons. In cytoplasmic hybrid (cybrid) cells, variations in the morphology of mitochondria were seen depicting the enlarged, swollen, less densely populated cristae mitochondria along with low MMP levels. Besides, these evidence still the studies involving the identification of the mitochondrial phenotype transmission did not confirm any deleterious mtDNA variations, suggesting more research needs to be done.

2.4.2 Role of genes involved in PD

a. PINK1

PINK1 (PTEN-induced putative kinase 1) is a mitochondrial protein kinase that is constitutively produced in mitochondria. This protein is mainly responsible mainly for maintaining mitochondrial homeostasis and morphology. The exact pathway is not well understood, but this is known to lead to apoptosis and hinder the accumulation of dysfunctional mitochondria, as stated by in vivo studies[91]. It has been hypothesized that mutations in this gene are caused due to the build-up of oxidative stress inside the cell which enhances the development of PD diseased condition in a later stage.

b. α-synuclein (SNCA)

α-synuclein is a protein that contains 140 amino acids which are expressed in terminals of presynaptic nerves. The mutation in this gene is known to cause autosomal dominant PD. The SNCA plays a vital role in modulating synaptic function as the misfolding of this protein leads to neurotoxicity and ultimately loss of DA neurons[92].

c. LRRK2

The mutation of LRRK2 (Leucine-rich repeat kinase 2) does also contributes to 1-5% of sporadic PD, but the mitochondrial functioning and morphology are pretty distinct. ROS acts as a critical mediator in both types of PD, thereby resulting in detrimental effects on mitochondrial morphology. In familial PD, G*2019S* substitution mutations of glycine to serine in LRRK2 increases kinase activity and is one of the common causes of PD on a genetic basis. It has been evident from various studies that LRRK2 has a modulatory role in autophagy, immune response and cytoskeleton maintenance[93]. The LRRK2 is known to interact with Rab GTPases, and impairment in the phosphorylation process causes neurotoxicity. Many Rab GTPases act as biological substrates of LRRK2, especially Rab35. This has led to speculations that LRRK2 and α-synuclein transmission can be interconnected to mediate neurodegeneration induced by LRRK2. Also, neuropathological studies propose that LRRK2 is involved in the accumulation of α-Synuclein and dementia in PD patients[94].

To date, the role of LRRK2 pathology in PD is being studied by research interests which mainly focused on the neurotoxic effects caused by pathogenic LRRK2 in the brain. Additionally, the mutant LRRK2 is known to suppress autophagy and thereby cause the accumulation of abnormal mitochondria. The exact mechanistic role of LRRK2 is not clear, but the mitochondrial abnormalities observed in mutant LRRK2 cell lines and models strongly suggest to be a potential therapeutic option.

2.5 THERAPEUTIC APPROACHES

The contribution of mitochondrial dysfunction in PD arguments to the likelihood that approaches intended at improving mitochondrial function can potentially slow down the advances in the dopaminergic neurodegeneration in PD. Thus, the strategies are aimed to address the mitochondria as a therapeutic target. Some of the approaches needed to be experimented with, while others are under clinical trials.

2.5.1. Antioxidant therapy

The most plausible therapeutics is antioxidant therapy, as these molecules will inhibit the oxidation process. The co-enzyme Q10 (CoQ10) is an antioxidant that shuttles between complexes I and III; some preliminary studies stated that CoQ10 was able to delay progression[95].

On the other hand, mitoquinone (MitoQ), a mitochondrially targeted antioxidant compound when coupled with triphenylphosphonium (TPP), is able to pass the lipid membrane and play an essential role in foraging superoxide, peroxyl and other ROS. This compound also has neuroprotective properties in PD associated with MPP+ induction. Furthermore, MitoQ is known to diminish fragmentation of mitochondria and translocate Bax when used to pre-treat SH-SY5Y neuroblastoma cells[96]. The various studies pertaining to deal with MitoQ are under the clinical trial stage. Some antioxidants can be exogenous in nature; these are taken as dietary supplements because the body cannot synthesis them. For example, Creatine, an exogenous antioxidant, is able to augment ATP synthesis, thus can act as a potential neuroprotectant in PD[95]. But still, more conclusive studies need to be done. Melatonin, being localized mitochondrially, is known to be a great ROS scavenger which promises it to be an excellent molecule for therapy against neurodegenerative diseases.

2.5.2. Peptide approach

To avoid the adverse effect of TPP^{+}, SS tetrapeptides have been used to actively targeting mitochondria. These are localized to the IMM and were found to have a neuroprotective role when studied in the MPTP-induced mice model. The pre-treatment of mice with SS-31 AND SS20 resulted in dopamine loss. Also, when mitochondria were isolated from mice, they showed depletion in ATP generation, oxygen consumption to inhibits lipid peroxidation[97]. But more research needs to be done in this regard.

2.5.3 Manipulating quality control and mitochondrial dynamics

The idea of using molecules that can manipulate the control process of mitochondria opens new therapeutic avenues. Here, using molecules that can mimic the action of genes whose mutations can lead to PD plays a very crucial role. For instance, Kinetin is found to have the same action as that of PINK1. It reduces apoptosis by signaling Parkin to recruit damaged mitochondria. But the complete detailed mechanism of action is yet to be known.

Mdivi-1 acts as a Drp1 inhibitor that prevents GTPase activity improves Neuronal cell life as well as dopamine release[98]. This therapy is quite promising but has some caveats, like it is not known how long-term exposure to Drp1 modulating species may affect other off-target molecules.

2.5.4 Deep brain stimulations (DBS) approaches

DBS approaches are quite beneficial for treating neurodegenerative diseases such as PD as it directly alters the mitochondrial function and has been proved to give positive outcomes in patients suffering from mitochondria-related disorders[99]. Thus, these results suggest that DBS can open new therapeutic avenues.

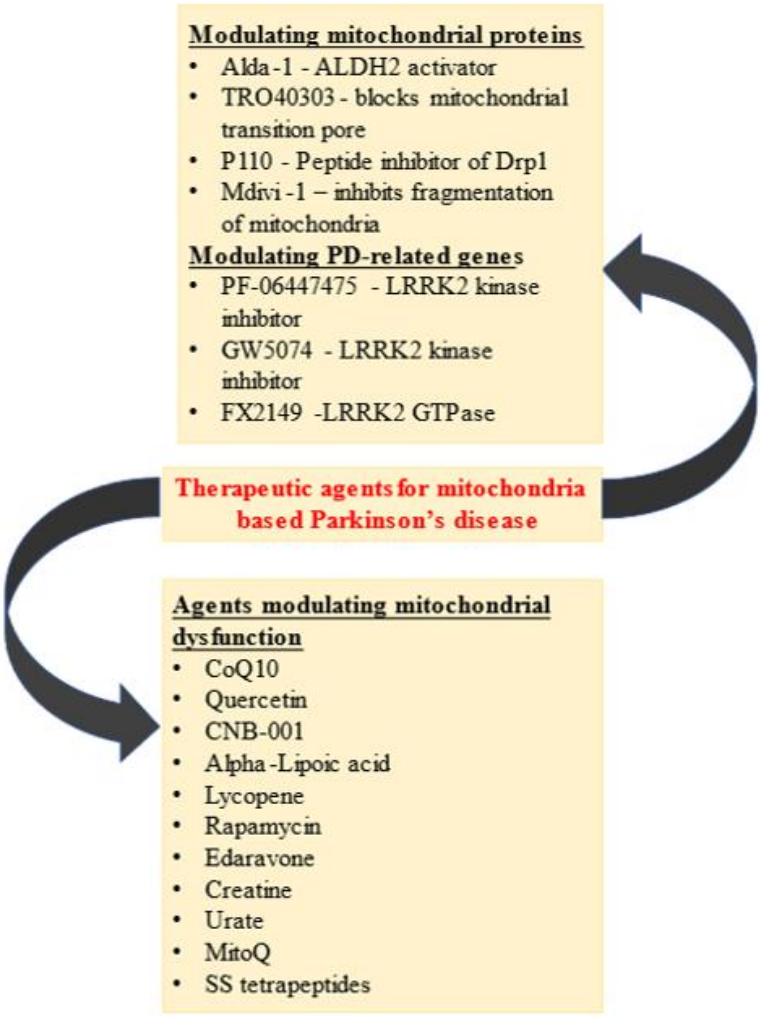

Figure 2.4: Therapeutic agents that target mitochondria for treatment of Parkinson's disease

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2.5.5 Drug screening

The curative studies are not perfectly known for PD. Thus, much research is ongoing to discover the same. In silico analysis and screening, computer-aided- drugs seem to be the most promising therapeutics as many novel targets can be expeditated, having a strong binding affinity for PD target. One can target signaling compounds involved in pathways responsible for PD pathogenies. This will help the researcher to understand and expand the new healing intervention to examine ligand-protein interaction. A ligand-primarily based drug layout totally may be completed in affiliation with molecular docking that's a structure-primarily based totally technique with underlying scoring capabilities to breed crystallographic ligand-binding modes. A variety of latest hit compounds with the remarkable inhibitory role can be diagnosed, which will allow us to apprehend the principal interplay with the binding sites. Rognan et al. in 2006 studied and computed conformational scaffolds like curcumin and glycosylated nornicotin with the help of the Artificial Neural Network (ANN) technique to study interaction with SNCA aggregation. These newly determined lead compounds reduce the formation of SNCA and PINK1 via impeding of the enzyme functioning[100].

Thus, all the above-stated neuroprotective strategies hold promises for treating PD on the grounds of targeting mitochondrial dysfunction. In spite of these, the mitochondrial impairment may be part of a more complicated multi-facet pathogenic process. And subsequently, ideal neuroprotective approaches for PD may need synchronized targeting of various pathways, be it within and outside the dopaminergic system.

CHAPTER - 3

MATERIAL AND METHODS

3.1 MATERIAL USED

The database used: Drugbank, ChEMBL, Pubchem

Software used: Autodock vina, LigPlot, Pymol, SwissADME, Toxicity Checker, Carcino-Pred EL

3.2 WORKFLOW

- **DRUG DISCOVERY STRATEGY**

PINK1 was selected as the target molecule from the literature survey and pathway analysis. The aim was to stop the conversion of MPTP to MPP^+ which is catalyzed by MAO-B enzyme and to see how it can modulate mitochondrial dysfunction against PD. The Drugbank database was searched to identify drugs that can target MAO enzymes. Further, the approved drug was selected as a ligand for the study.

3.3 METHODS FOR PREDICTION OF POTENTIAL COMPOUNDS

3.3.1 Protein Retrieval and Prediction

PTEN induced putative kinase 1(PINK1) protein (PDB ID: 5OAT) was retrieved using Pubchem. This target protein doesn't contain any heteroatoms.

3.3.2. Retrieval of Ligands

The Amphetamine was drug mined from Drugbank and similar compounds were searched using ChEMBL database against the PD targets. ChEMBL database provides compounds with details like molecular weight, a clinical phase of drug, rotatable bonds, number of hydrogen bond donor/acceptor. A total of 19 molecules were identified as similar molecules and their structures were retrieved from Pubchem. And out of these 7 approved and 2 non-approved were selected for further studies.

3.3.3 Molecular Docking was performed

The molecular docking was done to know about the protein-ligand interaction. The steps mentioned below were performed further:

a. Preparation of the target molecule

The downloaded proteins are prepared by converting the .sdf file to .pdb by using PyMol. Then this .pdb file was opened in Autodock vina. The Protein molecule was selected by clicking on the file option and then opened. The structure was modified and redefined by deleting unwanted molecules of water. Further, to compensate for water loss, polar hydrogen was added. The extra charges were compensated by adding Gasteiger charges. Finally, the file was saved in .pdbqt format

b. Preparation of ligand molecules

The desired ligand was opened in the Autodock vina tool by clicking on "Ligand" and then "Open." Then root was detected in the molecule, and then the number of rotatable, unrottable and non-rotatable bonds were calculated by clicking on the "Detect Root" option. At last, the ligand file was saved in .pdbqt format for further use.

c. Making of Grid

Firstly, the macromolecule was selected (in .pdbqt format). After "set map types" was selected under the grid, and ligand file (in .pdbqt format) was opened. This step is important to study energy calculations related to protein-ligand interaction. The grid box values were set to maximum. Then Autodock vina was run to get the desired output(in .pdbqt format) and docking score.

d. Analysis using Ligplot

Finally, ligPlot analysis was done to know about the interaction between the target protein and ligand as 2D structures. And the Pymol was used to known about 3D structure.

These steps were repeated for all the desired ligands.

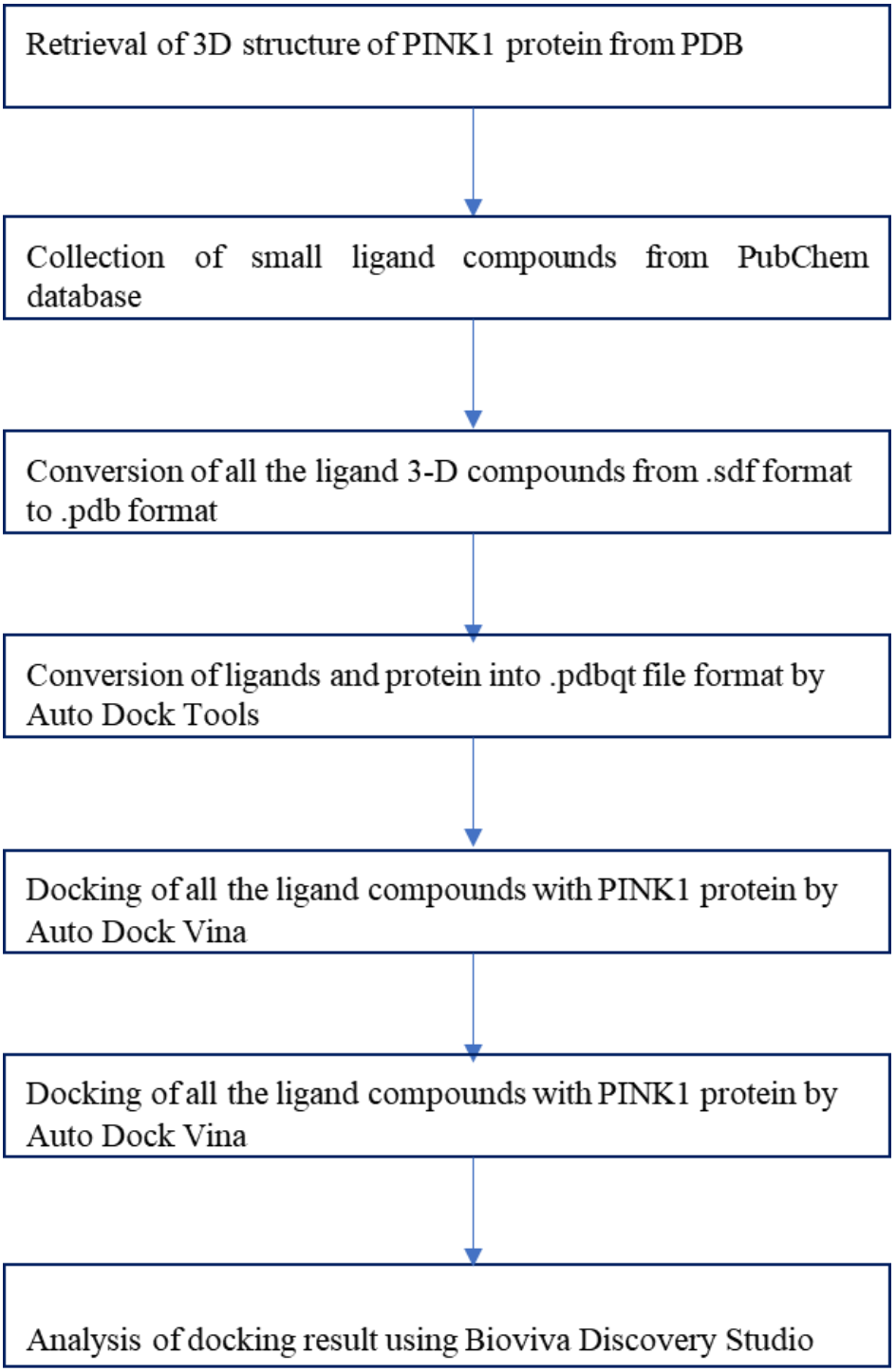
3.3.4. Prediction of ADMET properties and BBB permeation

Further, for these ligands, an ADMET prediction was made. It is a crucial step while identifying the lead molecules as it tells about the drug-likeness of the compounds on the basis of Absorption, Distribution, Metabolism, Excretion, and Toxicity. The ADME and BBB

permeation was predicted using SwissADME. While the toxicity was determined by using Toxicity Checker software.

3.3.5. Prediction of Carcinogenicity

The nature (carcinogenic or non-carcinogenic) of all these ligands were detected using Carcino-Pred EL.



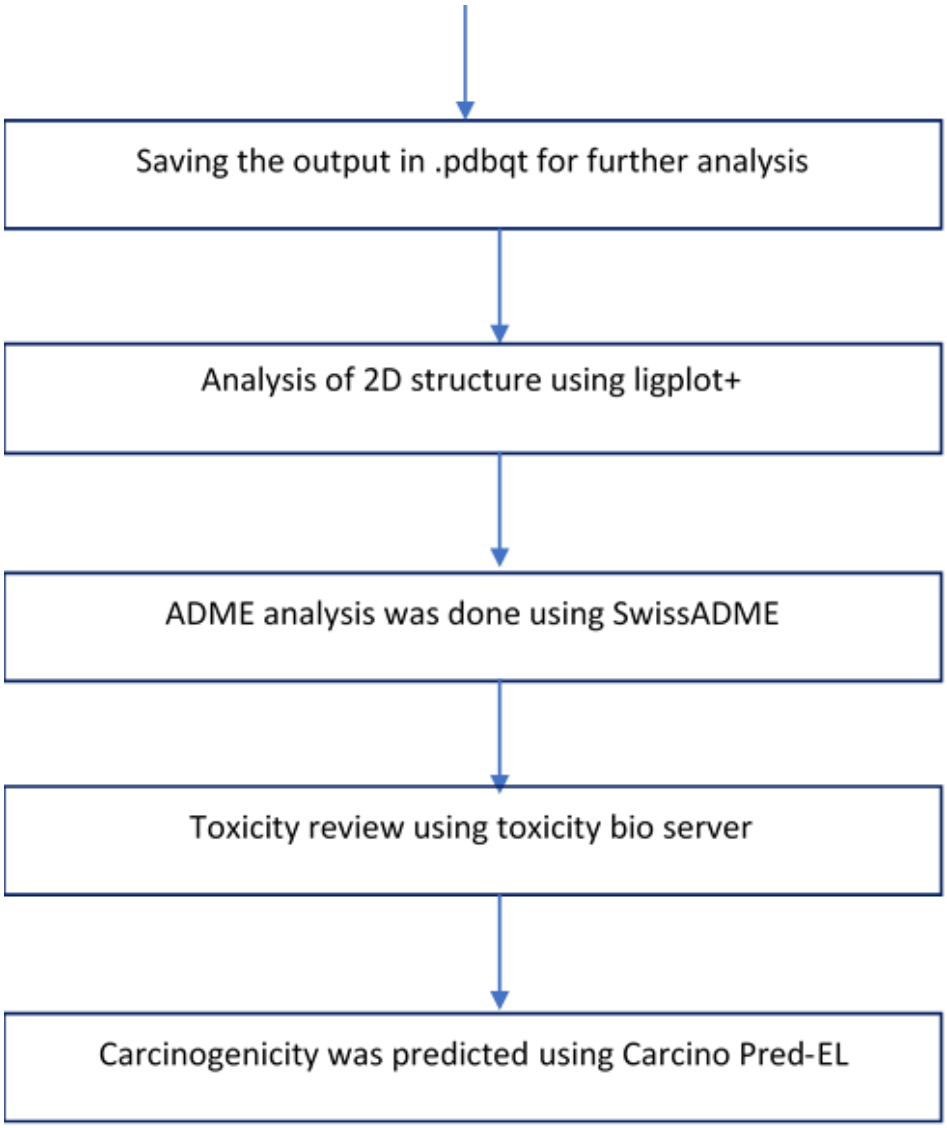


Figure 3.1: Flowchart depicting protocol followed

CHAPTER - 4

RESULT AND DISCUSSION

4.1 MAO-B inhibitors

From the Drugbank compounds targeting MAO was obtained and only Amphetamine out of these compounds was selected as parent drug as it was an approved drug known to inhibits the MAO- B
enzyme activity.

DRUGBANK ID	NAME	DRUG STATUS	PHARMACOLOGICAL ACTION	ACTIONS
DB09363	Amphetamine	Approved (illicit)	known	Inhibitor
DB00668	Epinephrine	Approved	unknown	substrate
DB00721	Procaine	Approved	unknown	inhibitor
DB06698	Betahistine	Approved	unknown	substrate
DB01171	Moclobemide	Approved	unknown	antagonist
DB01175	Escitalopram	Approved	unknown	substrate
DB01168	Procarbazine	Approved	known	Inhibitor

Table III: The approved drugs that target MAO enzyme

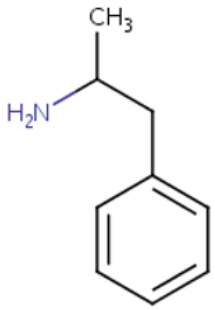
ChEMBL ID	STRUCTURE
CHEMBL405 (AMPHETAMINE)	

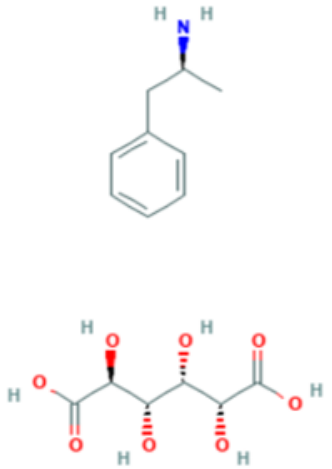
Table IV: The table depicts ChEMBL ID and 2D chemical structure of parent drug (Amphetamine)

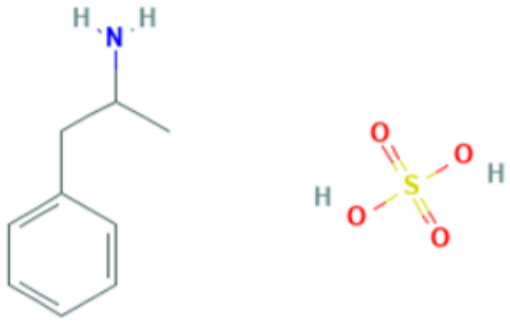
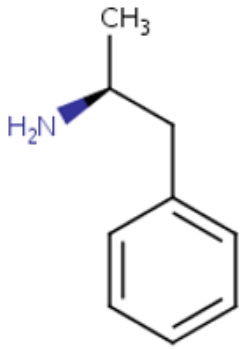
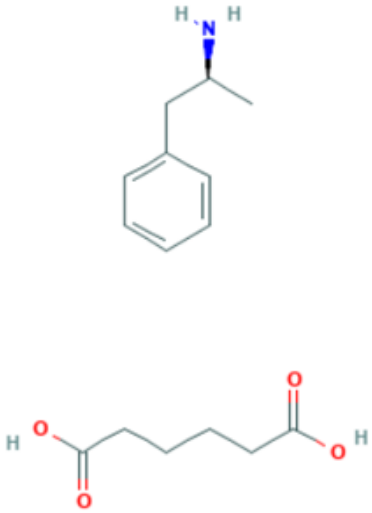
Properties	Amphetamine
Formula	C9H13N
Molecular weight	135.21 g/mol
Num. rotatable bonds	2
Num. H-bond donors	1
Num. H-bond acceptors	1
Bioavailability	0.55
Log P	2.08
GI absorption	High

Table V: Physiochemical properties parent drug (Amphetamine)

4.2 SIMILAR COMPOUNDS TO AMPHETAMINE

The 18 similar compounds were mined using ChEMBL database and their structures were mined Pubchem, and only 7 were approved out of them.

ChEMBL ID	STRUCTURE
CHEMBL3989844 (DEXTROAMPHETAMINE SACCHARATE) Compound I	

CHEMBL501 (AMPHETAMINE SULFATE) Compound II	
CHEMBL612 (DEXTROAMPHETAMINE) Compound III	
CHEMBL1200782 (DEXTROAMPHETAMINE ADIPATE) Compound IV	

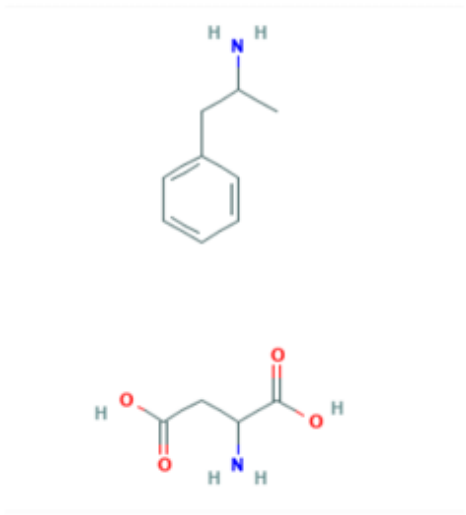
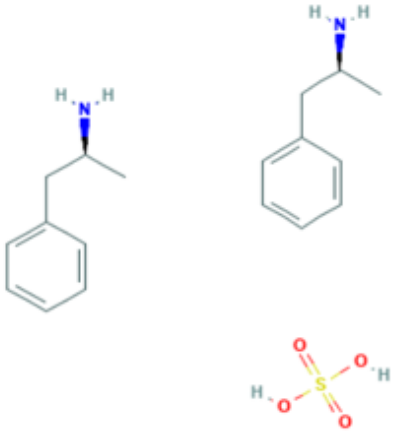
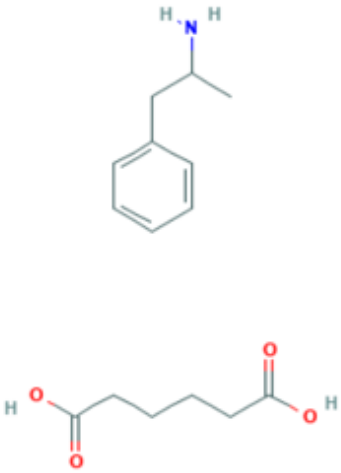
CHEMBL1200377 (AMPHETAMINE ASPARTATE) Compound V	
CHEMBL3544971 (DEXTROAMPHETAMINE SULFATE) Compound VI	
CHEMBL1200387 (AMPHETAMINE ADIPATE) Compound VII	

Table VI: ChEMBL ID and structure of approved drugs having similar structure to parent drug.

4.3 TARGET PROTEIN 'S STRUCTURE

The 3-D structure PINK1 protein showed in Fig 4.1. It is a hexametric protein. Having a molecular weight of 279.08 kDa

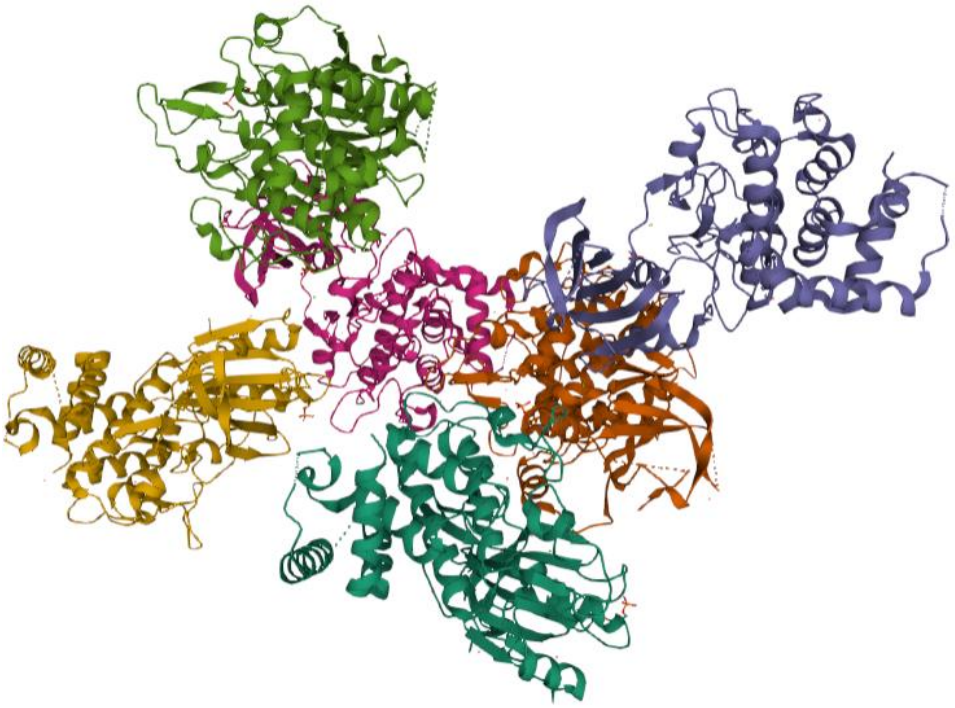


Figure 4.1: The 3- D structure of PINK1 obtained from PDB.

- **FOR APPROVED DRUGS**

4.4 ANALYSIS OF LIGAND-PROTEIN INTERACTION

The docking is done with various small ligand molecules which are similar in structure to the already available drugs, and the results were noted. The screened compounds were subjected to docking against PINK1, and their best confirmations were obtained. And the docking score for each ligand was obtained.

Mode	Affinity (kcal/mol)	Distance from Best mode	
		rmsd l.b.	rmsd u.b.
1	-6.8	0.000	0.000
2	-6.4	24.519	25.365
3	-6.3	48.832	49.914
4	-6.3	24.165	24.909
5	-6.3	24.674	25.363
6	-6.3	52.881	53.451
7	-6.3	48.095	49.092
8	-6.3	48.338	49.026
9	-6.3	24.291	24.921

Table VII: Docking Result for Parent drug

Mode	Affinity (kcal/mol)	Distance from Best mode	
		rmsd l.b.	rmsd u.b.
1	-3.9	0.000	0.000
2	-3.9	44.299	44.365
3	-3.9	31.684	32.339
4	-3.8	30.704	31.400
5	-3.7	41.888	41.940
6	-3.5	45.904	46.066
7	-3.3	51.496	52.174
8	-3.3	29.777	30.095
9	-3.3	20.306	20.888

Table VIII: Docking Result for Compound I

Mode	Affinity (kcal/mol)	Distance from Best mode	
		rmsd l.b.	rmsd u.b.
1	-6.4	0.000	0.000
2	-6.3	38.521	39.148
3	-6.3	14.178	15.585
4	-6.3	14.262	15.595
5	-6.3	37.708	38.627
6	-6.2	68.506	69.432
7	-6.2	13.180	13.975
8	-6.2	68.841	69.777
9	-6.2	38.403	39.122

Table IX: Docking Result for Compound II

Mode	Affinity (kcal/mol)	Distance from Best mode	
		rmsd l.b.	rmsd u.b.
1	-6.5	0.000	0.000
2	-6.4	65.314	66.407
3	-6.3	72.919	73.782
4	-6.3	66.771	68.017
5	-6.1	65.804	66.450
6	-6.1	72.847	73.620
7	-6.1	1.612	2.230
8	-6.0	1.711	3.064
9	-6.0	72.898	73.934

Table X: Docking Result for Compound III

Mode	Affinity (kcal/mol)	Distance from Best mode	
		rmsd l.b.	rmsd u.b.
1	-6.2	0.000	0.000
2	-6.2	56.113	56.839
3	-6.1	65.832	67.005
4	-6.1	76.984	78.367
5	-6.0	1.267	3.107
6	-6.0	56.043	56.762
7	-6.0	56.206	56.923
8	-6.0	77.175	78.265
9	-6.0	65.333	66.510

Table XI: Docking Result for Compound IV

Mode	Affinity (kcal/mol)	Distance from Best mode	
		rmsd l.b.	rmsd u.b.
1	-3.9	0.000	0.000
2	-3.9	31.705	32.358
3	-3.8	44.521	45.162
4	-3.8	45.339	45.440
5	-3.8	44.896	45.225
6	-3.6	1.979	2.737
7	-3.5	51.924	52.362
8	-3.5	69.638	69.750
9	-3.5	45.902	46.050

Table XII: Docking Result for Compound V

Mode	Affinity (kcal/mol)	Distance from Best mode	
		rmsd l.b.	rmsd u.b.
1	-3.9	0.000	0.000
2	-3.9	31.688	32.354
3	-3.8	71.750	71.917
4	-3.6	70.543	71.212
5	-3.5	45.901	46.055
6	-3.3	51.494	52.180
7	-3.3	29.784	30.106
8	-3.3	25.432	26.032
9	-3.2	36.535	36.726

Table XIII: Docking Result for Compound VI

Mode	Affinity (kcal/mol)	Distance from Best mode	
		rmsd l.b.	rmsd u.b.
1	-6.5	0.000	0.000
2	-6.5	56.184	56.836
3	-6.4	1.738	2.415
4	-6.4	29.826	30.530
5	-6.4	77.682	78.959
6	-6.4	114.163	115.663
7	-6.2	29.697	30.874
8	-6.2	1.690	2.954
9	-6.2	29.206	30.201

Table XIV: Docking Result for Compound VII

4.5 DRUG-LIKENESS OF THE SELECTED LIGANDS

The ADMET analysis is needed to study a) the cost for the drug discovery b) to identify which drug leads and candidates need to be focused on how safe they are for use. Thus, it is an essential step of the study. The result of ADMET analysis and carcinogenicity prediction for the approved compounds are as follows:

Characteristics	Compounds						
	I	II	III	IV	V	VI	VII
Molecular weight (g/mol)	345.35	281.35	135.21	281.35	268.15	368.49	281.35
Hydrogen bond donor	7	3	1	3	4	4	3
Hydrogen bond acceptor	9	5	1	5	6	6	5
Log P	1.64	2.28	1.90	1.89	0.79	2.25	2.25
Lipinski's Rule of five	No (1 violation)	Yes	Yes	Yes	Yes	Yes	Yes
GI absorption	Low	High	High	High	High	Low	Low
Toxicity	Toxic	Non-Toxic	Non-Toxic	Non-Toxic	Non-Toxic	Toxic	Non-Toxic
BBB Permeation	No	No	Yes	No	No	No	No
Carcinogenicity	All are non-carcinogenic in nature						

Table XV: Physicochemical properties of ligands

- **FOR NON-APPROVED DRUGS**

4.6 BBB ANALYSIS

Firstly, the BBB permeation for all (11) non-approved drugs was determined. And only those drugs which were permeable were selected for further studies.

S No.	ChEMBL ID	BBB Permeation	Compound No (As per our experimentation)
1	CHEMBL287386	Yes	VII
2	CHEMBL19393	Yes	IX
3	CHEMBL2106371	No	X
4	CHEMBL2106669	No	XI
5	CHEMBL554211	No	XII
6	CHEMBL3250774	No	XIII
7	CHEMBL3250772	No	XIV
8	CHEMBL3250773	No	XV
9	CHEMBL3250775	No	XV
10	CHEMBL3250771	No	XVI
11	CHEMBL3250770	No	XVII

Table XVI: BBB analysis for non-approved drugs

4.7 ADMET AND CARCINOGENICITY ANALYSIS

Only two non-approved drugs (compound VII, compound IX, compound XIII) which were able to cross BBB was selected for ADMET and carcinogenicity analysis.

Characteristics	Compounds	
	VIII	IX
Molecular weight (g/mol)	171.67	135.21
Hydrogen bond donor	1	1
Hydrogen bond acceptor	1	1
Log P	1.87	2.80
Lipinski's Rule of five	Yes	Yes
GI absorption	High	High
Toxicity	Non-Toxic	Non-Toxic
Carcinogenicity	All are non-carcinogenic in nature	

Table XVII: Drug-likeness of non-approved drugs

4.8 MOLECULAR DOCKING OF NON-APPROVED DRUG

After the ADMET Analysis, molecular docking of non – approved drugs was also performed.

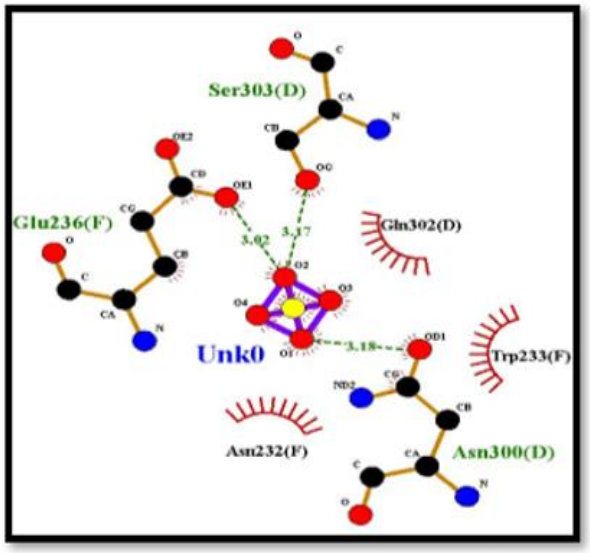
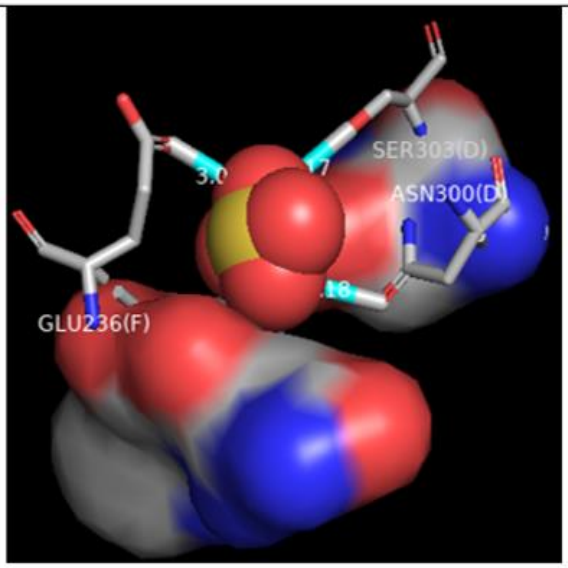
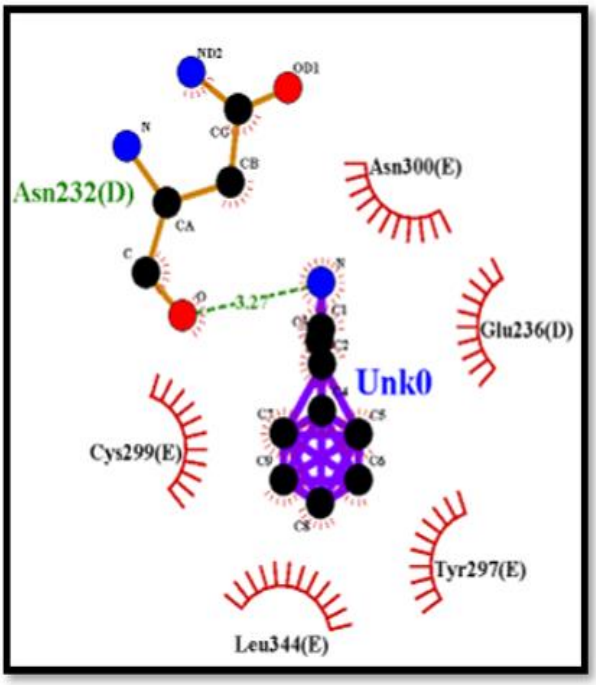
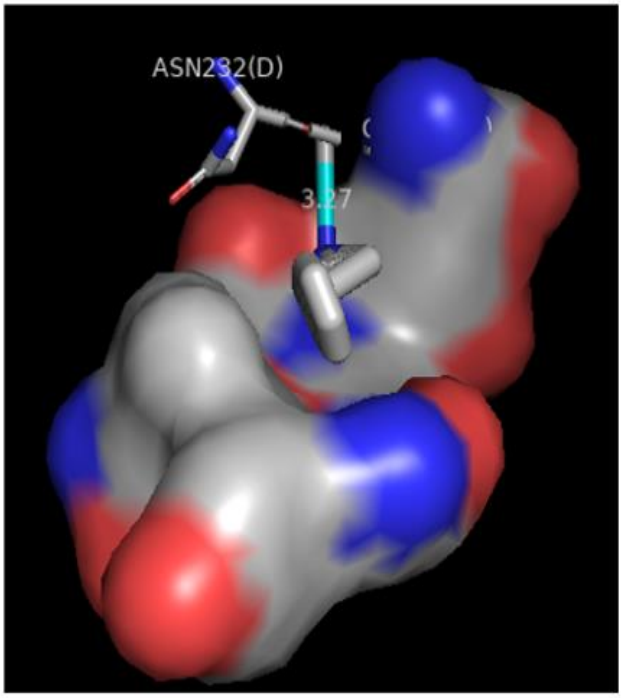
Mode	Affinity (kcal/mol)	Distance from Best mode	
		rmsd l.b.	rmsd u.b.
1	-6.5	0.000	0.000
2	-6.5	56.197	56.832
3	-6.4	91.643	92.058
4	-6.4	77.683	78.960
5	-6.4	114.164	115.663
6	-6.4	29.811	30.546
7	-6.3	1.246	1.822
8	-6.2	78.329	77.715
9	-6.2	1.678	2.944

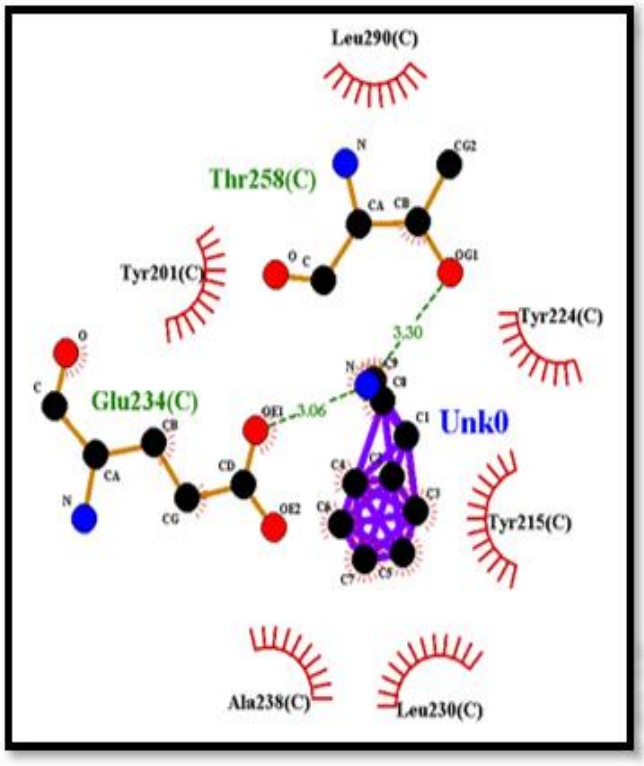
Table XVII: Docking Result for Compound VIII

Mode	Affinity (kcal/mol)	Distance from Best mode	
		rmsd l.b.	rmsd u.b.
1	-6.8	0.000	0.000
2	-6.4	53.375	54.581
3	-6.3	24.447	25.178
4	-6.3	69.046	69.871
5	-6.3	24.861	25.593
6	-6.3	25.104	25.749
7	-6.3	25.217	25.747
8	-6.3	53.400	54.063
9	-6.3	69.225	70.020

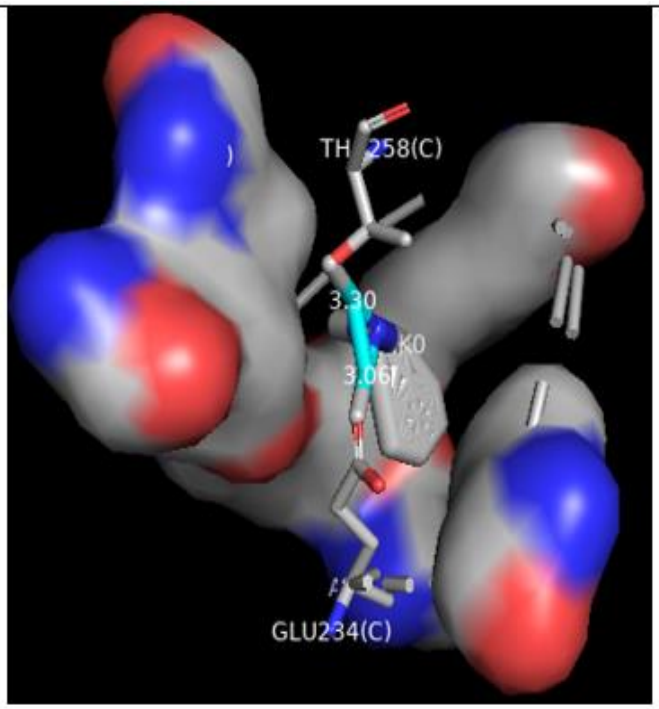
Table XVIII: Docking Result for Compound IX

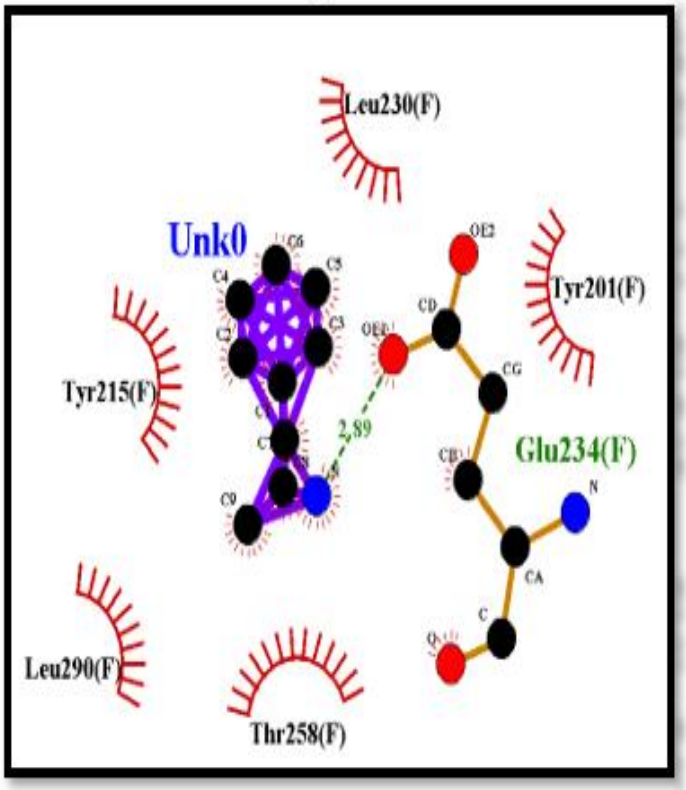
4.9 ANALYSIS USING LIGPLOT AND PYMOL

LigPlot (2-D structure)	Pymol (3-D structures)
 Compound I	
 Compound II	

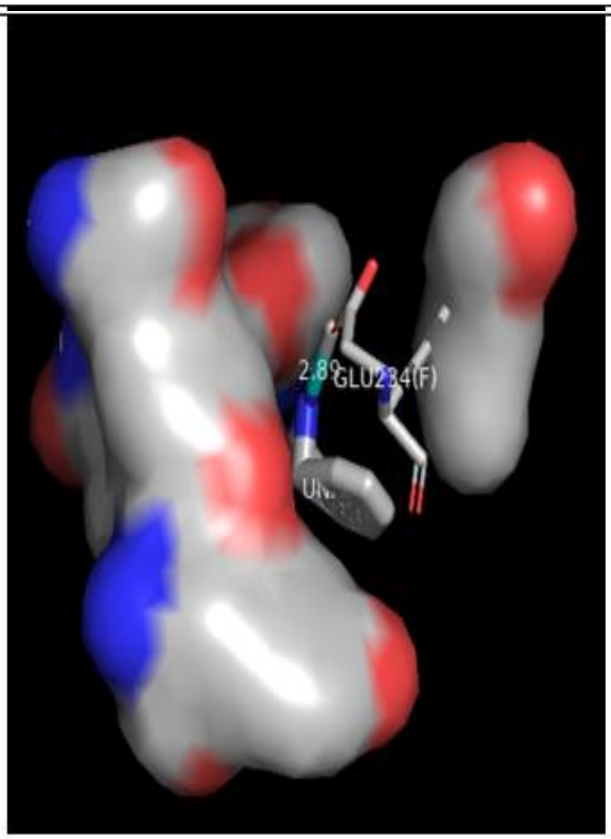


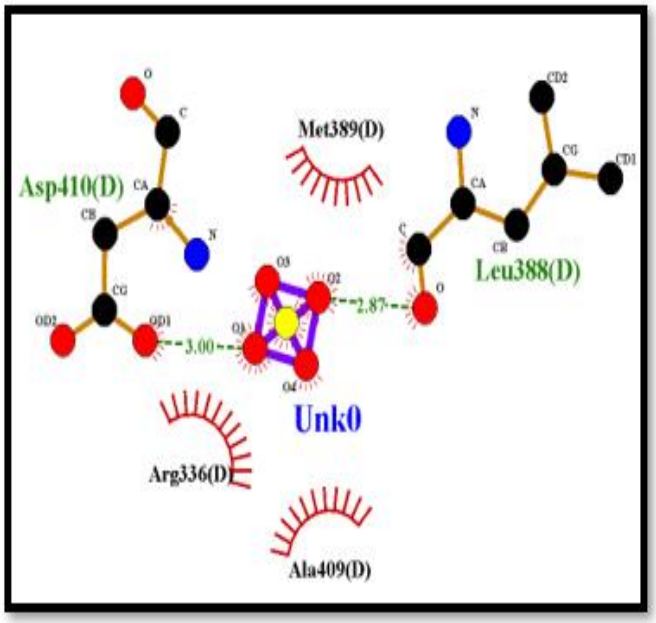
Compound III



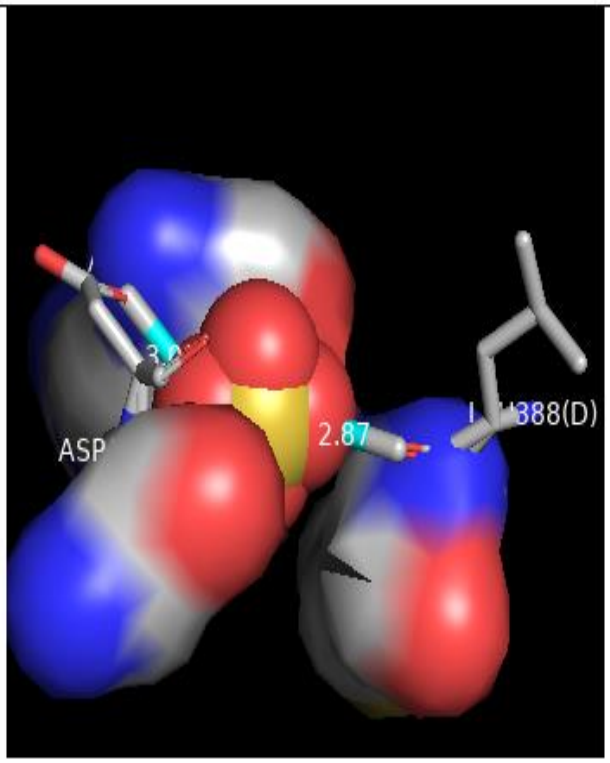


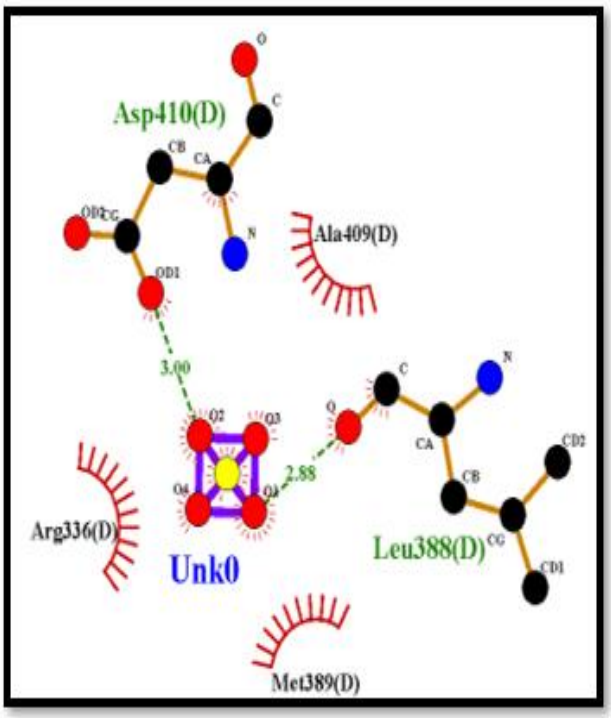
Compound IV



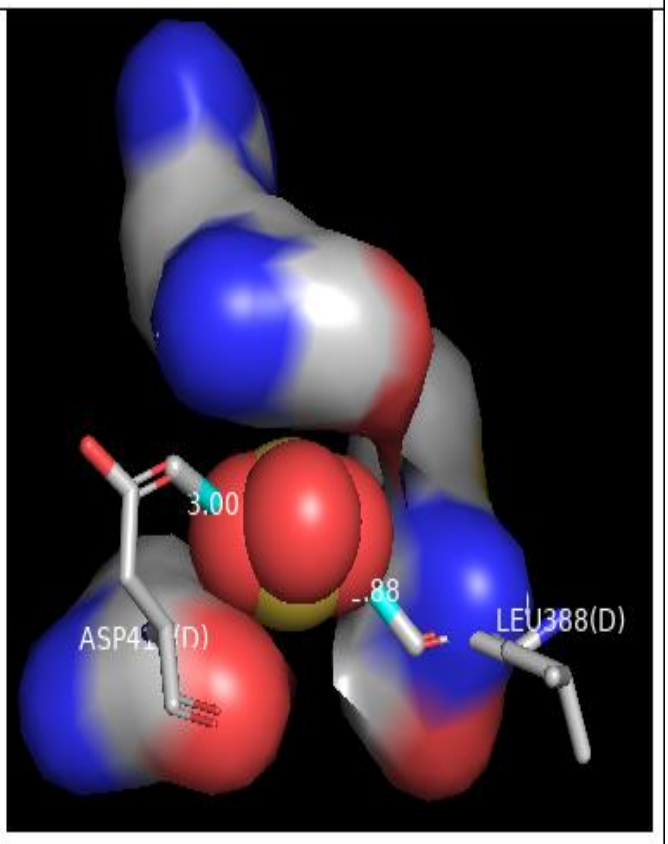


Compound V





Compound VI



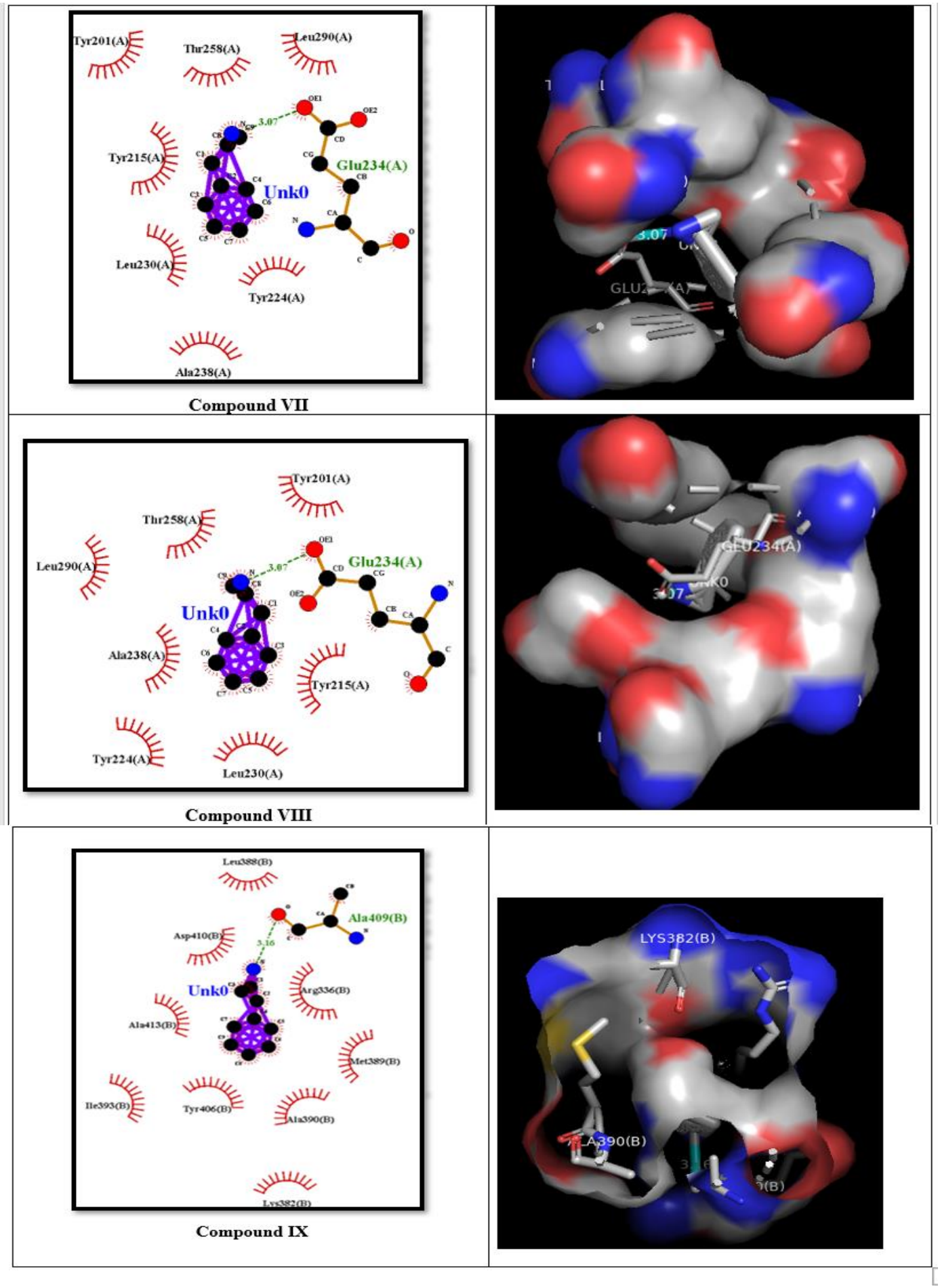


Figure 4.2: The docking results were analyzed using ligPlot for 2-D structures and Pymol for 3-D structures.

Amphetamine is an approved drug used in the treatment of attention deficit hyperactivity disorder. It is known to have a crucial role in the neuroactive metabolism of amines in the central nervous system and peripheral nervous system. Thus, this drug was chosen to study the interaction with *PINK1* protein. All the drugs having similar structures were mined.

Also, the selected compound having molecular weights less than 500KDa was preferred for permeability under Lipinski. As compounds having higher molecular weight are less likely to cross BBB. Thus, these screened compounds, which fall under this criterion, can be used as drugs against PD even along with current medication to improve the efficacy. Hence *In silico* analysis will help to provide a better and effective agent against PD.

CHAPTER - 5

CONCLUSION AND FUTURE PERSPECTIVE

Although, the current understanding of PD and factors contributing towards the disease progression are known. But still, there is no elusive curative for the same. The mutations in PINK1 genes are known to cause recessive autosomal PD. And it is a mitochondrial protein that is involved in maintaining mitochondrial health and providing protection against apoptosis induced as a result of oxidative stress. The docking of the ligands was carefully performed, and their interactions and orientations were also monitored. From this study, it can be considered that Amphetamine and molecules having similar structures have a high binding affinity for PINK1 protein. All the ligands were non-carcinogenetic. At last, three potential candidates, which are non-toxic, non-carcinogenic, ADMET investigated, capable of crossing BB barrier was found to have a neuroprotectant role against the *PINK1* gene.

Thus, the knowledge gained on the basis of docking score provides a piece of preliminary evidence for its potential as an anti-parkinsonian medication and may be implemented in designing effective therapeutics for PD in the future.

Furthermore, these selected compounds can be confirmed by analysis in cell culture assay. Also, the effect of compounds can be analyzed by cell viability and LDH assay. These tests will supplement the above evidence pertaining to the therapeutic capacity of these preferred compounds to be used for PD.

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