SIGNALLING MECHANISM AND THERAPEUTICS ACTION OF BIOMOLECULES IN NEURODEGENERATIVE DISORDER

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IN

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By

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Dedicated

To

My Parents

DECLARATION

I hereby declare that the thesis entitled **"Signalling Mechanism and Therapeutics Action of Biomolecules in Neurodegenerative Disorder"** submitted by me, for the award of the degree of ***Doctor of Philosophy*** to **Delhi Technological University (Formerly DCE)** is a record of *bona fide* work carried out by me under the guidance of Prof. Pravir Kumar.

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

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CERTIFICATE

This is to certify that the thesis entitled **"Signalling Mechanism and Therapeutics Action of Biomolecules in Neurodegenerative Disorder"** submitted by **Mr. Saurabh Kumar Jha** to **Delhi Technological University (Formerly DCE)**, for the award of the degree of "Doctor of Philosophy" in Biotechnology is a record of *bona fide* work carried out by him. Saurabh Kumar Jha has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to our knowledge has reached requisite standards.

The results contained in this thesis are original and have not been submitted to any other university or institute for the award of any degree or diploma.

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ABSTRACT

Neurodegenerative disorder (NDD) continues to traumatize an aging proportion of the human population, especially in the industrialized world. Aging has long been recognized as a compound process of damage accretion that ultimately leads to notice able disruption of multiple cellular and molecular proceedings, which ultimately are translated into various chronic ailments such as Parkinson's disease (PD), Alzheimer's disease (AD), Multiple Sclerosis (MS) and many more**.** Parkinson's disease (PD) is a chronic neurodegenerative condition which has the second largest incidence rate among all other neurodegenerative disorders after AD. Currently, there is no cure and researchers continue to probe the therapeutic prospect of PD. Out of several factors contributing to PD prognosis, the role of p38 MAPK and PI3K/AKT signalling module in PD brains is crucial because impaired balance between the pro-apoptotic and anti-apoptotic pathways trigger unwanted phenotypes such as microglia activation, neuroinflammation, oxidative stress, and apoptosis. These factors continue challenging the brain homeostasis in initial stages thereby essentially assisting the dopaminergic (DA) neurons towards progressive degeneration in PD. Owing to the limitations of sample availability of PD patients, the current research scenario is focused on cellular models of PD which are an excellent source of large drug screening and easy to maintain. Prior to *in vitro* study, we have done *in silico* analysis of two major proteins including, Parkin and DJ-1, which are reportedly involved in neuronal damage. Consequently, we have targeted these proteins with selected biomolecules in order to regulate their altered expression in the cells. Further, to validate our results we have performed *in vitro* experiment as well, where we used 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced PD model in SHSY5Y neuroblastoma cell lines to study of signalling mechanism and screening of biomolecules in an attempt to reverse disease symptoms in PD.

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(Saurabh Kumar Jha)

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LIST OF ABBREVATIONS

PD	**:**	Parkinson`s disease
AD	**:**	Alzheimer`s disease
ALS	**:**	Amyotrophic lateral sclerosis
HD	**:**	Huntington's disease
BBB	**:**	Blood brain barrier
CHIP	**:**	C-terminaus HSP 70 interacting protein
CNS	**:**	Central nervous system
DJ-1	**:**	PARK-7
DLB	**:**	Dementia with Lewy Bodies
HSP_S	**:**	Heat shock proteins
LB_S	**:**	Lewy bodies
LN_S	**:**	Lewy neuritis
MT	**:**	Microtubules
NDDs	**:**	Neurodegenerative disorders
NFTs	**:**	Neurofibrillary tangles
NOS	**:**	NO synthase
PINK1	**:**	PTEN-induced kinase 1
Parkin	**:**	PARK -2
ROS	**:**	Reactive oxygen species
SNCA	**:**	α-synuclein
SNP	**:**	Single nucleotide polymorphism
TH	**:**	Tyrosine hydroxylase
UBL	**:**	Ubiquitin like domain
UCH	**:**	Ubiquitin carboxy- terminal hydrolase
UPD	**:**	Unique parkin domain
UPS	**:**	Ubiquitin-proteasome system
MPTP	**:**	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

Chapter I

Introduction & Review of Literature

CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

1.1 INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder characterized by dopamine depletion in the striatum. PD is mainly characterized by bradykinesia, tremor, rigidity, and weakening of postural reflexes (Massano *et al.,* 2012). The dramatic loss of neuromelanin containing dopaminergic neurons is convoyed by the presence of Lewy bodies in the remaining neurons. The hallmark of PD includes fibrillar cytoplasmic inclusions consisting of aggregated and abnormally accumulated proteins, the most prominent being α-synuclein, neurofilaments, ubiquitin, and ubiquitinated proteins (Gundersen V. 2010). Additionally, at least 13 loci and nine genes have been proposed to be linked with PD, but only six genes are widely accepted to be associated with Mendelian forms of the disease (Exner *et al.,* 2012). Mutations in these genes potentially lead to autosomal dominant (α -synuclein and LRRK2), or autosomal recessive PD (Parkin, PINK1, DJ1, and ATP13A2) and shown in **(Table 1.1)** respectively. Recent epidemiological studies have shown that less than 10% of PD cases are of familial origin with the majority being sporadic (Pirkevi *et al.,* 2009). The sporadic form of PD is caused by mutated DJ1 and Parkin which shows reduced nuclear localization and translocation to mitochondria. These are ubiquitously expressed in a number of pathways associated with PD pathogenesis and have ubiquitin E3-ligase activity which also reduces α-synuclein aggregation (Cookson *et al.,* 2012). Although mutations associated with Parkin and DJ-1lead to the onset of familial PD, the exact mechanism behind the pathogenesis are still unknown (Klein *et al.,* 2012). Multiple lines of evidence from molecular and cellular to epidemiological studies suggest that innate and environmental factors such as aging, genetics, MPTP, 6-hydroxydopamine (6-OHDA) metals, mitochondrial dysfunction induced by environmental toxins, such as mitochondrial complex I inhibitors rotenone, traumatic brain injury, and shortage of trophic factors that can play a role in PD neuropathology (Gao *et al.,* 2010, Giuliani *et al.,* 2012 & Low and Aebischer, 2012). In addition, lifestyle factors viz. cigarette smoking and coffee consumption, with a gender bias, can also influence the onset of PD. Notably; the

neurotoxin 6-hydroxydopamine (6-OHDA) is extensively used to mimic the PD associated neurodegeneration in both *in vivo* and *in vitro* experimental models. On the treatment front, the use of levodopa, dopamine agonists, herbal medicines, health supplement foods, and acupuncture are on the rise all over the world among patients afflicted with PD, however, without much benefit (Tapia-González *et al.,* 2011, Giuliani *et al.,* 2012 & Kawamata *et al.,* 2011).

In the last 15 years, the procedure of deep brain stimulation (DBS) has emerged as a touchstone to mitigate the adverse neuropathological symptoms witnessed in advanced forms of the disease. The procedure is mainly designated for PD victims who are dopamine-responsive but with disabling motor complications such as motor fluctuation, dyskinesia, or unendurable side-effects of anti-PD suppositories. It is well known that motor fluctuations like wearing-off and peak-dose dyskinesia are motor anomalies observed few years after patients are started on medical treatment. When these complications breach the edge of severity, despite maximal fine-tuning of pharmacological agents, DBS has been shown to be effective and safe with benefits lasting for no less than 10 years. In fact, bilateral sub thalamic nucleus DBS has arisen as a treatment of choice and proven to have an unquestionable influence on motor symptoms, countenancing the minimization of drug treatment and its side effects (Mylène *et al.,* 2013). Numerous other reports have demonstrated the effectiveness of neurosurgery, specifically on motor symptoms and on health related quality of life. However, a vital concern has been that most of those studies have testified no significant perfection in social adaptation after sub thalamic nucleus DBS in some patients. Furthermore, the lack of post-operative improvement in the psychosocial dimension of health-related quality of life and its link to coping strategies is still unclear and there are contentious reports surrounding the use of DBS in the early stage of PD and most medication centers will offer this modality typically to infirm with disruptive motor complications obstinate to drug treatment (Chan *et al.,* 2014). Therefore, it is of urgency to examine novel mechanisms that can be used as a benchmark therapy of choice in patients bloated with Parkinson's in future.

It is well documented that oxidative stress (OS), impaired ROS/NO balance, microglia activation, and chronic inflammation are striking pathological features observed in PD brains. These factors altogether have detrimental effects on the integrity of dopaminergic (DA) neurons thus potentially leading to neuronal apoptosis and

subsequent neurodegeneration. Not surprisingly, these critical neuro determinants are under the control of an array of proteins and signalling networks, and accruing genetic studies in recent years have advocated prominently on the role of improper phosphorylation events, dysfunctional kinases, and aberrantly functioning kinases associated signalling events as few of many responsible determinants involved in a convoluted network defining PD progression and pathogenesis. One such mechanism contributing to microglia response and neuroinflammation in both inherited and sporadic forms involve the protein named LRRK2 (leucine-rich repeat kinase 2). LRRK2 can effectively moderate the neuroinflammatory ambiance in traumatized neurons in response to a panel of pathological stimuli. LRRK2 is a large multi-domain protein belonging to the cohort of mammalian ROCO (Ras of complex) proteins. It can be functionally characterized by the presence of an enzymatic core, comprising of ROC/GTPase, COR (C-terminus of ROC) and serine threonine kinase domains. In addition, there are multiple protein-protein interaction domains including ankyrin and leucine-rich repeat motifs at the N-terminus, and WD40 repeats at the C-terminus. LRRK2 mutations can induce microglias through hyperpolymerization and hyperphosphorylation of cytoskeleton and vesicle components thereby directing these cells towards a pro-inflammatory ambiance, which in turn can result in aggravated inflammation and subsequent neurodegeneration. The profound investigation into the innumerable functionalities of misregulated signalling cascades involving kinases such as p38 mitogen-activated protein (MAP) kinase, protein kinase B (AKT) kinase, and C-Jun N- terminal kinase (JNK), extracellular signal-regulated kinases (ERK), PI3K/AKT shall unravel novel mechanism for drug targeting in future.

In that regard, identification of microglia-specific kinase substrates, GTPase downstream effectors, and interactors shall reveal acute therapeutic hot spots and outline credible prototypes for attenuation of the cardinal symptoms and motor complications in this group of disorders (Wang *et al.,* 2012, Russo *et al.,* 2014). Numerous intracellular signalling cascades that congregate on MAPK exist in all eukaryotic cells and play critical roles in various cellular activities. The p38 MAPK as also can be designated as stress-activated protein kinases (SAPKs), are especially triggered by a range of cytotoxic stress stimuli and cytokines. In response, p38 potentially drive crucial cellular activities such as

proliferation, differentiation, survival, and stress-induced apoptosis. In the central nervous system (CNS), p38 MAPK is central towards the maintenance of synaptic plasticity, and as a result, anomalies' resulting from the deviant functioning of p38 MAPK pathway in neurons has observed in brain diseases like AD, PD, and ALS. Consistent activation of JNK or p38 MAPK is critical towards facilitating neuronal apoptosis in AD, PD, and ALS brains (Takeda *et al.,* 2002, Kim *et al.,* 2010 & Sônia *et al.,* 2012). On contrary, PI3K/AKT pathway modulates cellular activities like neuronal cell proliferation, migration, and plasticity. The cytoprotective phenotype of PI3K/AKT provides an important signalling for neuroprotection, however, it prerequisites that the pathway is optimally activated in PD brains; this could possibly antagonize the detrimental effects of the p38 MAPK activation in degenerating DA neurons and thus can assist in establishing a neuro-protective setting in insulted brains. In general, activation of ERK or p42/p44 MAP kinase and the PI3K/AKT pathway encourage cell survival (cytoprotective pathways), whereas SAPK's, c-Jun N-terminal kinases (JNK's) and the P38 MAPK, moderate cell mortality (Yasuko *et al.,* 2012, Morrison *et al.,* 2000). Nevertheless, several plausible cyto-dynamics involving P38 MAPK- PI3K/AKT and their subtle contribution towards progressive neurodegenerative remains an area of active curiosity for research in PD. Moreover, active biomolecules targeting the impaired P38-PI3K/AKT balance could significantly contribute to neuroprotection in PD challenged brains.

Table 1.1: Mode of inheritance and genes postion on different chromosome in the case of Parkinson's disease

S.No	Locus	Gene	Location	Mode of Inheritance	Reference
1.	PARK-1	α -Synuclein	4q21	Autosomal dominant	(Polymeropoulos *et al.,* 1996)
2.	PARK-2/8	Parkin	6q25	Autosomal recessive	(Matsuminen *et al*., 1997)
3.	PARK-3	Unknown	2p13	Autosomal dominant	(DeStefano *et al*., 2001)
4.	PARK-4	Unknown	4p15	Autosomal dominant	(Farrer *et al*., 1999)
5.	PARK-5	Ubiquitin	4p14	Autosomal dominant	(Leroy *et al*., 1998)
6.	PARK7	DJ-1	1p36	Autosomal recessive	(Van Duijn *et al*., 2001)

Under the ambit of this chapter we have made an attempt to explore the role of oxidative stress, UPS, signalling and mitochondrial dysfunction respectively in neurodegenerative conditions. This interaction shall than be crucial in embellishing the development of potential neurotherapeutics. Further, implementation of several biomolecules and chaperones for targeting NDDs induced by oxidative stress, UPS and mitochondrial dysfunction has also been elaborated.

1.2. ROLE OF OXIDATIVE STRESS IN NEURODEGENERATION

Oxidative stress (OS) condition in the brain results from imbalance between ROS and the body's detoxification mechanism, which results in accumulation of ROS and subsequent neuronal damage. Hence, the outcome of oxidative stress on neuronal cells depends upon the ability of the cell to maintain oxidative homeostasis. High stress levels can cause ATP depletion, necrosis and prevent apoptotic cell death (Beal M.F, 2005). Any disproportion in the usual redox state can result in toxicity via the activation of peroxides and free radicals which in turn damages lipids, proteins and cellular DNA. A mammalian cell as a consequence of mitochondrial aerobic respiration generates superoxide radical. Superoxide is sequentially reduced to hydroxyl radicals and hydrogen peroxide that cause severe traumatic injury to the DNA thus leading to mutations, which might be causative factors leading to severe neurodegeneration (Halliwell B, 1994). Reactive oxygen species (ROS) also plays a discrete role in cell signalling, by a mechanism known as redox signalling. In order to sustain proper cellular homeostasis, a balance must be reached between ROS production and consumption. Therefore, it is obvious that free radicals need to either be reduced and detoxified by converting them into metabolically non-destructive molecules or be neutralized right after their generation. Any aberration in the cellular antioxidant defence system, which protects the neurons from free radical assaults, therefore can lead to neurodegenerative conditions and aging (Yu B.P, 1994).

Brain is the most metabolically active organ of the body that including the spinal cord

comprises the central nervous system (CNS), which even in resting condition consumes an estimated 20-22% of the total oxygen uptake. In addition, during active state the brain oxygen demand considerably rises in order to establish normal physiological homeostasis. Blockage or oxygen deprivation can lead to severe and irreversible injuries to the neurons. Oxygen consumption in the brain of oxygen results in production of free radicals and higher oxygen levels in brain leads to even higher concentration of reactive oxygen/nitrogen species. However, in spite of the fact that brain has higher necessity for oxygen, it is relatively deficient in the enzymes capable of metabolizing a number of these toxic oxygen-based reactants to harmless residues. In contrast, CNS is highly enriched with polyunsaturated fatty acids and toxic oxygen derivatives oxidize these polyunsaturated fatty acids (Jaiswal *et al.,* 2006, Perry *et al.,* 2002).

This then makes the neurons more vulnerable to oxidation related damages and the role of the cellular detoxification machinery in these conditions is vital. Oxidative stress has major impact on several NDDs such as Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD).

1.2.1. Parkinson's disease

Oxidative process can modulate pathogenesis in PD. It is the most common neurodegenerative disorder and is clinically demarcated by bradykinesia, progressive rigidity and tremor. Like all other neurodegenerative disorders determinants such as environmental factors, mitochondrial dysfunction, oxidative damage, and genetic predisposition together play a crucial role in both sporadic as well as familial PD (Jenner *et al.,* 2003). Neurotoxic compounds, such as N-methyl-4-phenyl-1, 2, 3, 6-tetrahydroyridine or its active derivative, MPPT and 6-hydroxydopamine (6-OHDA) can provoke oxidative stress, impair mitochondrial respiration and energy metabolism which in turn leads to neurodegeneration. Postmortem tissues from PD patients have revealed a significant insight into the failure of complex I in the *substantia nigra*. Complex I is involved in the mitochondrial electron-transport chain, and 30–40% decrease in activity

may be the central prognosis of sporadic PD (Dawson *et al.,* 2003). The decreased activity of complex I could be the result of self-inflected oxidative damage, underproduction of certain complex 1 subunits, and may be due to complex 1 disassembly (Keeney *et al.,* 2006). Immunocytochemical confirmation of protein glycation and nitration in substantia nigra region of human PD brain revealed oxidative damage to DNA and protein resulting from persistent oxidative trauma (Floor *et al.,* 1998).

1.3. UPS AND E3 LIGASES IN NEURODEGENERATION

Dysfunction of the ubiquitin proteasome system is one of the major events that lead to the progression of neuronal loss. An in vivo report suggests that oxidative stress is caused directly by neuronal proteasome dysfunction in the mammalian brain (Jaiswal *et al.,* 2012, Dasuri *et al.,* 2012 & Elkharaz *et al.,* 2013). The UPS plays a vital role in regulated degradation of cellular proteins under diverse physiological conditions. Aggregation of misfolded proteins has been attributed in the progression of various neurodegenerative diseases, such as AD, PD and HD. Ubiquitin E3 ligases are key regulators involved in mediating the proteasomal degradation of misfolded proteinsin the endoplasmic reticulum (ER), as a result protecting neurons against oxidative stress, mitochondrial dysfunction and ER stress (Mei *et al.,* 2010). Furthermore, Ubiquitin proteasome system can critically modulate the level of proteins in cells, and robustly control cellular mechanisms. Aberration in UPS function in susceptible neurons results in protein aggregation, increased, oxidative stress, ER stress, and ultimately neuronal death. Conversely, neurons depend on the proper functioning of E3 ligases and UPS to maintain neuronal homeostasis and also highlights **(Table 1.2)** the prospective role E3 ligases in neurodegeneration (Jara *et al.,* 2013).

Table 1.2: E3 ligases in the brain and their functional prospect in neurodegeneration

E3 LIGASE	SUBSTRATES	FUCNTIONAL SIGNFICANCE	REFERENCES
Keap1	Nrf2	Involved in degradation of Nrf2. disruption of protein degradation systems and sustained activation of the Keap1-Nrf2 system occur in the AD brain.	(Tanji *et al.,* 2014)
PARK2	AIMP2	Parkin is an E3 ubiquitin ligase that has been shown to be a key regulator of the autophagy pathway. Although, mutations in Parkin results into Parkinson's Disease.	(Segura-Aguilar *et al.,* 2014, Imam *et al.,* 2014)
HECW1	DVL1, p53, and mutant SOD1	NEDL1 is another name of HECW1. It mainly interacts with p53 and the Wnt signaling protein DVL1, and may play a critical role in p53-mediated cell death in neurons.	(Li *et al.,* 2008)
HUWE1	TopBP1, N-Myc, C-Myc, p53, Mcl-1	HUWE1 regulates neuronal differentiation by destabilizing N-Myc, and also modulates p53-dependent and independent tumor suppression via ARF. It is also known as Mule. HUWE1 is a HECT domain E3 ubiquitin ligase which involves in degradation of Mcl-1 and thus regulates DNA damage-induced apoptosis.	(Zhong *et al.,* 2005)
MGRN1		Involved in melanocortin signaling. Losses of mahogunin function leads to neurodegeneration and loss of pigmentation, and also have mechanism of action in prion disease.	(Pérez-Oliva *et al.,* 2009)
MYCBP2	TSC2, Fbxo45	MYCBP2 associates with Fbxo45 to play a crucial role in neuronal development. MycBP2 is an E3 ubiquitin ligase also known as PAM. MycBP2 also modulates the mTOR pathway through ubiquitination of TSC2.	(Han *et al.,* 2008)
UHRF2	PCNP	UHRF2 ubiquitinates PCNP and has been shown to play a role in degradation of nuclear aggregates containing polyglutamine repeats mediated neureodegeneration.UHRF2 is also known as NIRF. UHRF2 is a nuclear protein that may regulate cell cycle progression through association with Chk2.	(Mori *et al.,* 2004)
ZNRF1		Highly expressed in neuronal cells. ZNRF1 is found in synaptic vesicle helpful in neuronal transmissions and plasticity. It also contains a RING finger motif, which expression is upregulated in the Schwann cells mediaated nerve injury.	(Araki *et al.,* 2003, Saitoh *et al.,* 2010)
NEDD4		Highly expressed in the early mouse embryonic central nervous system. It downregulates both neuronal voltage-gated Na+ channels and epithelial Na+ channels in response to increased intracellular Na+ concentrations.	(Goulet *et al.,* 1998)

E3 LIGASE	SUBSTRATES	FUCNTIONAL SIGNFICANCE	REFERENCES
NEDD4L	Smad2	It also highly expressed in the early mouse embryonic central nervous system. NEDD4L negatively regulates TGF-β signaling by targeting Smad2 for degradation.	(Gao *et al.,* 2009)
HECTD2		HECTD2 is a likely E3 ubiquitin ligase and may act as a vulnerable gene for neurodegeneration especially in prion disease.	(Lloyd *et al.,* 2009)
PJA2		Expressed in neuronal synapses. The exact role and substrates of PJA2 are unclear.	(Yu *et al.,* 2002)
RNF19	SOD1	RNF19 is also known as Dorfin. Accumulation of mutant SOD1 results into ALS disease. RNF19 ubiquitinates mutant SOD1 protein, causing less neurotoxicity in brain.	(Sone *et al.,* 2010)
HECTD1		HECTD1 is required for normal development of the mesenchyme and neural tube closure.	(Zohn *et al.,* 2007)
MULAN	mnd2	Involved in degradation of mnd2. mnd2 causes neuromuscular disorder due to loss of Omi/HtrA2's protease activity.	(Cilenti *et al.,* 2014)
HACE1	NRF2	HACE1 plays a crucial in the NRF2 mediated antioxidative stress response pathway and also involed in HD pathogenesis.	(Rotblat *et al.,* 2014)
CUL4	TSC2	It promotes proteasomal degradation of TSC2. As a result, Tnfaip8 l1/Oxi-β competes with TSC2 to bind FBXW5, increasing TSC2 stability through preventing its ubiquitination in PD progression.	(Ha *et al.,* 2014)
TRIM13		Involved in regulation of ER stress induced cell death. However, the expression of TRIM13 sensitizes cells to ER stress induced neuronal cell death.	(Tomar *et al.,* 2013)
MGRN1		Over expression of MGRN1 protects against cell death mediated by ER and oxidative stress and also interacts with Cytosolic Hsp70. Lack of MGRN1 functionalities are the hall mark of age dependent spongiform disease in the brain.	(Chhangani *et al.,* 2013)
NEDD4-1	FOXM1B	Upregulated in cultured neurons in response to various neurotoxins, including, hydrogen superoxide, and zinc via transcriptional activation likely mediated by the reactive oxygen species. A level of the insulin-like growth factor receptor (IGF-1Rβ) is also maintained due to upregulation of NEDD4-1.	(Kwak *et al.,* 2012)
APC/C		Involved in cell cycle progression in proliferating cells, plays a significant role in post-mitotic neurons. APC/C-activating cofactor, Cdh1, is also helpful for the function of APC/C in neuronal survival.	(Almeida A, 2012)

1.4. ROLE OF MITOCHONDRIAL DYSFUNCTION IN NEURODEGENERATIVE DISORDERS

Mitochondria is an important organelle which is also known as the power house of cells and predominantly required for determining many cellular functions ranging from metabolic to catabolic activities. Mitochondria perform numerous crucial functions within the cell, which include cellular ATP production, Ca^{2+} buffering, regulation of apoptotic process and involvement in the synthesis of key metabolites. Nevertheless, it also acts as a primary source of endogenous reactive oxygen species (ROS) under oxidative stress. Additionally, In order to maintain the normal brain function, the immense amount of energy (ATP) is required. Among all the essential tissues of the body, including, brain, heart and skeletal muscle, brain is the most energy-dependent tissue. The main source of energy which is predominantly required for the normal functioning of the brain is mainly produced by normal activity of mitochondrial cells. In mitochondria, ATP production is mainly associated with an ETS where, the passage of electrons through diverse electron carriers is coupled with the transport of protons from the mitochondrial matrix into the inner-membrane space, and thereafter these protons re-enter into the mitochondrial matrix by dint of ATP synthase for the generation of ATP (Su *et al.,* 2010, Onyango *et al.,* 2010).

However, mitochondrial metabolism is also accountable for the ROS production in cells when unpaired electrons leave the ETS and react with molecular oxygen and thus generate superoxide. This superoxide further interacts with lipids, DNA and proteins and plays a crucial role in numerous signaling processes, which is associated with the disease phenomenon of both aging and NDDs. Moreover, ROS can also react with nitric oxide (NO) to produce reactive nitrogen species (RNS) (Patel *et al.,* 1999). Furthermore, apart from altered energy and increased ROS production, mitochondrial dysfunction is also responsible for altered Ca^{2+} buffering, apoptosis and senescence that may lead to the progressive decline of post mitotic cells and consequently, results into aging and NDDs (Seo *et al.,* 2010). There are numerous NDDs identified so far, which

have been reported to be associated with stress and mitochondrial dysfunctioning, which include AD, PD, HD and ALS have also been depicted in (**Figure 1.1)**.

1.4.1. Mitochondrial dysfunction and Parkinson's disease

Further, we have discussed the role of mitochondrial dysfunction in pathogenesis in PD. There are various factors but its cause remained mysterious, although numerous evidences support that mitochondrial dysfunctions and oxidative stress to be associated with the disease pathophysiology. For instance, mitochondrial DNA damage in dopaminergic neurons has been reported as one of the causing factors of striatal dysfunctions in a PD mouse model (Pickrell *et al.,* 2011). However, Glucocerebrosidase 1 (GBA1) gene mutations are identified as another causative factor responsible for perturbing normal mitochondrial function by increasing ROS production and by decreasing oxygen consumption, adenosine triphosphate (ATP) production and membrane potential. GBA1 mutations are also accountable for agglomerating dysfunctional and fragmented mitochondria (Migdalska-Richards *et al.,* 2016). Furthermore, quinine oxidation products responsible for ROS production have also been evidenced to be associated with mitochondrial dysfunction and dopamine cytotoxicity (Jana *et al.,* 2011). Similarly, the selective loss of alpha-ketoglutarate dehydrogenase complex (KGDHC) and mitochondrial complex I in the nigral neurons is another reason of PD pathogenesis. Such selective loss causes mitochondrial respiratory system failure and lead to cause oxidative damage in neurons. These abnormalities can trigger apoptotic cell death as well (Gibson *et al.,* 2011). Further, the pesticide rotenone which is known to inhibit mitochondrial complex I in PD is also responsible for microtubule (MT) destabilization that contributes to the blockage of mitochondrial voltage-dependent anions channels (VDACs) by tubulin and thus leads to PD (Cabeza-Arvelaiz *et al.,* 2012). Upcoming research identified hepatic mitochondrial dysfunctions in a rodent model of PD that arise as a result of nigrostriatal degeneration and interference in CNS-liver interaction due to thyroid hormone (Vairetti *et al.,* 2012). Likewise, deficiency in Phosphatase and tensin homolog (PTEN) induced putative kinase 1 causes Ca^{2+}accumulation in mitochondria that lead to both ROS production and intrinsic cell death in PD.

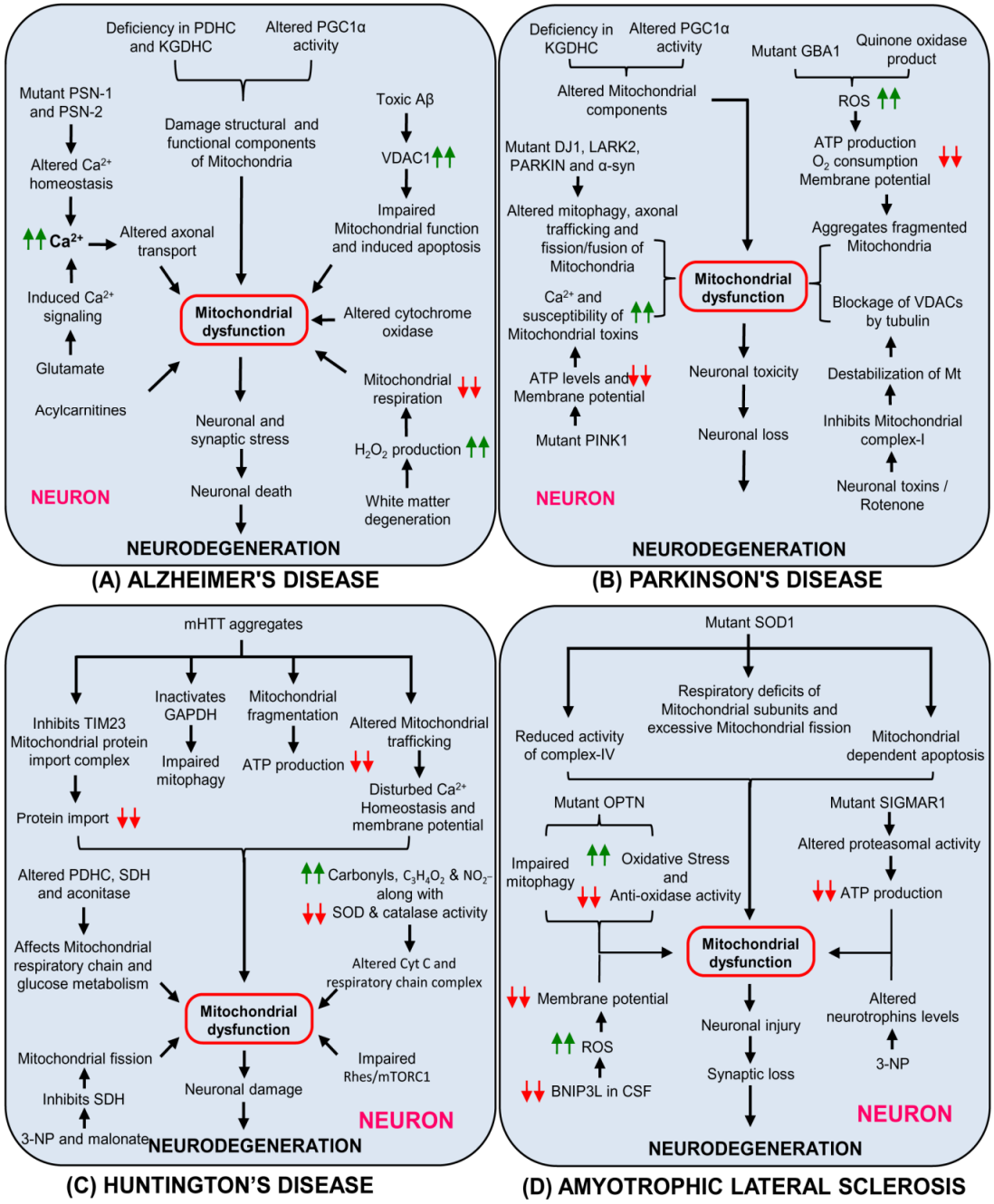


Figure 1.1: Molecular mechanism involved in mitochondrial dysfunction mediated pathophysiology of neurodegenerative disorders. PDHC, pyruvate dehydrogenase complex; KGDHC, ketoglutarate dehydrogenase complex; PGC1α, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PSN-1 & PSN-2, Presenilin-1 & Presenilin-2; VDAC1, voltage dependent anion channel 1; GBA1, glucocerebrosidase 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PINK1, PTEN-induced putative kinase 1; TIM23, translocase of the inner membrane 23; SDH, succinate dehydrogenase; 3-NP, 3-Nitropropionic Acid; SOD1, superoxide dismutase; OPTN, optineurin; SIGMAR1, sigma-1 receptor (Sig-1R); BNIP3L, pro-apoptotic mitochondrial proteins; CSF, cerebrospinal fluid.

It has revealed that mutations in PTEN-induced putative kinase 1 (PINK1) lead to a recessive form of PD. Since, PINK1 is responsible for mitochondrial quality control and its partial knock-down provokes numerous mitochondrial dysfunctions, including reduced membrane potential, decreased cellular ATP levels and enhanced susceptibility against mitochondrial toxins (Thomas *et al.,* 2009). Apart from PINK1, mutations in numerous other PD associated genes have also been reported to be involved in impaired mitochondrial dynamics. Such genes include DJ-1, α-Synuclein, parkin and LRRK2, which caused several defects in mitochondrial dynamics, such as altered fission/fusion, biogenesis, trafficking in retrograde and anterograde directions and impaired mitophagy process (Van Laar *et al.,* 2013). Impairment in the function and expression of PGC-1α, a key regulator of mitochondrial biogenesis is also responsible for PD (Pacelli *et al.,* 2011). Further, mitochondrial transcription termination factor 2 and 4 (MTERF2 and MTERF4) has been identified in 1-methyl-4-phenylpyridinium (MPP+)-induced PD brain that is responsible for mitochondrial dysfunction (Han *et al.,* 2016, Ye *et al.,* 2015). Recently, SUMOylation, an epigenetic modification has also been found to be involved in mitochondrial dysfunction associated with PD progression (Guerra de Souza *et al.,* 2016). Numerous studies have reported that the brain of PD patients manifests similar cellular and functional changes with signs of reduced mitochondrial function, increased oxidative stress, reduced glucose uptake and augmented peroxidation of cellular membranes (Dias *et al.,* 2013).

1.4.2. Biomolecules mediated therapy for altered mitochondrial dynamics

Since, there is not a single cause responsible for such alterations but diverse factors are conducing to such abnormalities. Therefore, it is decisive to implicate different biomolecules in multiple conditions in order to regain the normal function against altered mitochondrial dynamics. For this reason, these days, several biomolecules are being utilized for targeting defective mitochondria and its associated disease phenomenon in NDDs. For instance, vitamin Co-Q10 supplementation is used during the treatment of obesity, oxidative stress and the inflammatory process in NDDs. Co-Q10 served as an antioxidant by acting as a cofactor and activator of mitochondrial

uncoupling proteins, responsible for the reduction in ROS production, endothelial dysfunction and hypertension (Tangvarasittichai *et al.,* 2015, Alam *et al.,* 2014). Similarly, numerous other vitamins with antioxidant properties have also been developed to treat NDDs induced by oxidative stress and mitochondrial dysfunctions. These vitamins are α-lipoic acid, vitamin E, vitamin C, N-acetylcysteine (NAC) and inducers of the heme oxygenase, which are responsible for reduced ROS production (Sivitz *et al.,* 2010). Further, treatment with antiepileptic drug, Levetiracetam has been found to be associated with ameliorating numerous aspects of mitochondrial dysfunction such as alterations of fission-fusion balance in a cell model for aging and early/late-onset AD (Stockburger *et al.,* 2015). Likewise, another biomolecule Icariin has also benefitted against mitochondrial fragmentation associated with $A\beta_{1\text{-}42}$ accumulation in 3×Tg-AD neurons (Chen *et al.,* 2015). Similarly, Salicylic acid has currently reported to provide neuroprotection against mitochondrial dysfunction, oxidative stress and apoptosis in PD brain due to its antioxidant and anti-inflammatory effects on neuronal cell (Thrash-Williams *et al.,* 2016). Further, potential protective effect of Succinobucol against mitochondrial metabolic impairment and oxidative stress induced by 3-NP has been reported in HD brain (Colle *et al.,* 2016). Another biomolecule, N-acetyl-l-tryptophan (L-NAT) has been presently reported as an inhibitor of mitochondrial cytochrome c release and thereby acting as a possible neuroprotective agent for ALS (Sirianni *et al.,* 2015). Similarly, many other biomolecules have been identified and implicated so far to target altered mitochondrial dynamics in the PD which have been outlined in (**Table 1.3)**. These biomolecules act by binding to their specific targets and reduce the severity of neurological outcomes associated with altered mitochondrial dynamics.

Moreover, potential protective mechanisms of numerous chaperones against altered mitochondrial dynamics in PD have been summarized in (**Table 1.4)**

Table 1.3: list of biomolecules, mitigating the effect of mitochondrial dysfunctions in Parkinson's disease

BIOMOLECULES	FUNCTION RELATED TO MITOCHONDRIA	PD CAUSED DUE TO MITOCHONDRIAL DYSFUNCTION	REFERENCES
Asiatic acid (AA)	Protects against glutamate toxicity, rotenone-induced mitochondrial dysfunction and oxidative stress-mediated apoptosis in differentiated SH-SYS5Y cells	PD	(Nataraj et al., 2016)
Ghrelin	Protects dopaminergic neurons against rotenone via reinstating mitochondrial dysfunction and inhibiting mitochondrial dependent apoptosis	PD	(Bayliss et al., 2013)
Ursodeoxycholic acid (UDCA)	Exerts a beneficial effect on mitochondrial dysfunction and neuronal dysfunction *in vivo*	PD	(Mortiboys et al., 2015)
Curcumin	In order to protect against apoptosis induced by mutation of α-synuclein it stabilizes mitochondrial membrane potential and helps in opening of mito-KATP channel	PD	(Chen et al., 2015)
Crocin	Protects PC12 cells against MPP (+)-induced injury through inhibition of ER stress and mitochondrial dysfunction	PD	(Zhang et al., 2015)
Allicin	Inhibits ROS production, reduces lipid peroxidation linked to suppressed mitochondrial dysfunction and promotes the mitochondrial Ca^{2+} buffering capacity	PD	(Liu et al., 2015)
5-(methylamino)methyl)-8-hydroxyquinoline	Protects against mitochondrial-induced oxidative damage and neuronal death	PD	(Mena et al., 2015)
Nesfatin-1	Attenuates rotenone-induced mitochondrial membrane potential collapse, ROS production and the subsequent caspase-3 activation and restore the function of mitochondrial respiratory chain complex I	PD	(Tan et al., 2015)
Folic acid	Alleviates the behavioral defects, oxidative stress, and represses mitochondrial dysfunction as well	PD	(Srivastav et al., 2015)

BIOMOLECULES	FUNCTION RELATED TO MITOCHONDRIA	PD CAUSED DUE TO MITOCHONDRIAL DYSFUNCTION	REFERENCES
Resveratrol	Protects dopaminergic cells against MPTP-induced apoptotic death and motor dysfunction by improving mitochondrial dysfunction and oxidative stress. Further, it is also having a potential protective effect against mitochondrial metabolic impairment and oxidative stress induced by 3-NP. Lowered mRNA levels of COX-2 and TNF-α mRNA in the substntia nigra and also attenuated oxidative stress, lipid peroxidation and protein carbonyl in case of PD.	PD	(Khan et al., 2010)
Silibinin	Protects dopaminergic cells against MPTP-induced apoptotic death and motor dysfunction by improving mitochondrial membrane disruption and oxidative stress	PD	(Lee et al., 2015)
Myricitrin	Alleviates MPP^+-induced mitochondrial dysfunction and increases cell viability in a DJ-1-dependent manner in SN4741 cells	PD	(Cai et al., 2015)
Naringenin	Inhibited LPS induced NOS and COX2 expression and suppressed the production of NO in microglial cells. It also inhibited LPS/IFN-ϒ induced p38 and STAT-1 phosphorylation.	PD	(Chao et al., 2010)
Quercetin	Inhibited LPS induced TNF-α and IL-1α gene expression in glial cells, reduced level of inflammation induced neuronal death and play significant role in clearance of β amyloid induced apoptosis, iNOS expression, NO and peroxynitrite production by increasingthe level of glutathione. It played major role in free radicals scavanging, Ser/Thr and Tyr phosphate activity and clearance of lipid raft accumulation. Quercetin Inhibited LPS induced IKK, NF-κβ and AP-1 activation and also inhibited STAT-1	PD	(Mercer et al., 2005)
Melatonin	Shows Neuroprotective effect against mitochondrial dysfunction induced by oxidative stress	PD	(Srinivasan et al., 2013)

Table 1.4: List of chaperones, mitigating the effect of mitochondrial dysfunctions in Parkinson's disease

CHAPERONES	MOLECULAR MECHANISMS ASSOCIATED WITH MITOCHONDRIA	PRINCIPLE PHENOTYPES	REFERENCES
HSP70	Hsp70 overexpression protect neuronal cells against proteolytic and mitochondrial stress in a manner which is similar to that brought about by parkin overexpression	PD	(Zhang et al., 2016)
HSP70 and HSP90	Both HSP70 and mitochondrial HSP90 mitigate the toxic effect of α-synuclein associated with mitochondrial dysfunction. Further, mitochondrial HSP70 is also known to play a crucial role in the mitochondrial import complex and in mitochondrial biogenesis	PD	(Leak et al., 2014, Butler et al., 2012)
Mortalin	Mitochondrial chaperone mortalin maintains mitochondrial homeostasis through mitochondrial protein quality control	PD	(Burbull et al., 2010)

1.5. SIGNALLING CASCADES IN PARKINSON'S DISEASE

In this chapter we have focused solely on the viable contributions made by the p38 MAPK and PI3K pathways towards maintaining neuronal dynamics in PD brains. At a later stage we have enumerated a group of probable neurotherapeutics molecules, which can offer neuroprotection by mechanisms involving the likely targeting of the cell survival (PI3K/AKT) and death pathways (p38 MAPK), and actually can attenuate or prevent neurodegenerative symptoms associated with PD brains in future.

1.5.1. MAP KINASE pathway: At a glance

P38 MAPK (mitogen-activated protein kinase) signalling cascade provides a mechanism for cells to respond to a catalogue of external mitogens (signals) and respond accordingly by mediating a wide range of cellular effects. In fact, the diversity and specificity in cellular responses as depicted by the cascade is facilitated via a simple linear architecture, which comprises of sequentially operating core of

three evolutionarily conserved protein kinases namely; MAPK, MAPK kinase (MAPKK), and MAPKK kinase (MAPKKK). MAPKKKs are serine/threonine kinases, which are activated via phosphorylation and/or because of their interaction with a small GTP-binding protein of the Ras/Rho family in response to extracellular stimulus. MAPKKK activation results in phosphorylation and activation of MAPKKs, which consequently stimulate MAPK activity through dual phosphorylation of threonine and tyrosine residues positioned in the activation loop of kinase subdomain VIII (Cuadrado *et al.,* 2010). The activated MAPKs now phosphorylates target substrates specifically on serine or threonine residues followed by a proline. MAPKKs such as MEK3 and MEK6 are activated by a wide range of MAPKKKs (MEKK1 to 3, MLK2/3, ASK1, Tpl2, TAK1, and TAO1/2), which themselves become activated in response to oxidative stress, UV irradiation, hypoxia, ischemia, and cytokines, including interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) (Zarubin *et al.,* 2005).

At present, five different MAP kinases (MAPks) have been characterized and investigated namely; extracellular signal-regulated kinases (ERKs) 1 and 2 (ERK1/2), c-Jun amino-terminal kinases or stress-activated protein kinases (JNKs/SAPKs) 1, 2, and 3, p38 isoforms α, β, γ, and δ, ERKs 3 and 4, and ERK5. The kinase p38α (p38) was initially isolated as a 38-kDa protein which was observed to be rapidly phosphorylated at tyrosine motifs in response to LPS stimulation. Later, p38 was cloned and studied as a molecule capable of binding puridinyl imidazole derivatives; these derivatives inhibits the biosynthesis of inflammatory mediators like interleukin-1 (IL-1) and tumor-necrosis factor (TNF) in LPS activated monocytes. p38 (also known as CSBP, mHOG1, RK, and SAPK2) kinases are more responsive towards stress stimuli such as osmotic shock, ionizing radiation, and cytokine stimulation and four different variants of p38 arising out of alternative splicing are known viz. p38α, p38β, p38γ (ERK6, SAPK3), and p38δ (SAPK4). Among these, p38 and p38β are ubiquitously expressed in tissues whereas p38γ and p38δ shows variegated expression in a tissue specific manner (Roux *et al.,* 2004). Each of the p38 variants comprises of a Thr-Gly-Tyr (TGY) dual phosphorylation motif and sequence comparison performed earlier suggests that each p38 isoform shares approximately 60% identity with

other members of the p38 group but only 40-45% with other MAP kinase family members. The activity of p38 is controlled and coordinated in vitro by three different kinases: MKK3, MKK4, and MKK6. In vivo, MKK3 and MKK6 are necessary for tumor necrosis factor-stimulated p38 MAPK activation whereas, ultraviolet radiation-mediated p38 MAPK activation requires MKK3, MKK4, and MKK6 (Brancho *et al.,* 2003).

Emerging proofs advocate a role for the p38MAPK and MKP-1 in the maintenance and demise of dopaminergic neurons. Mitogen-activated protein kinase phosphatase-1 (MKP-1) is a negative regulator of p38 activity and other MAPKs such as ERK, and c-Jun NH (2) -terminal kinase (JNK). MKP-1 was found to be expressed in DA neurons cultured from E14 rat ventral mesencephalon (VM) and it was reported that DA neurons when transfected to overexpress MKP-1, triggered a substantial increase in neurite length and branching with maximum upsurge observed in primary branches (Collins *et al.,* 2013). In addition, DA neurons displaying over-expressed MKP-1 patterns are subjected to neuroprotection against the effects of PD inducing neurotoxin 6-OHDA. MKP-1 can also promote the growth and elaboration of dopaminergic neuronal processes suggesting that MKP-1 is actively involved in DA neuronal maintenance and therefore deviant MKP-1 expression is a hallmark of damaged DA neurons in PD (Korbecki *et al.,* 2013). Therefore formulating strategies aimed at augmenting MKP-1 expression in order to appropriate p38 activity may be advantageous in shielding dopaminergic neurons from PD induced damage (Comalada *et al.,* 2012).

1.5.2. PI3K/AKT/mTOR pathway

The PI3K-PKB/Akt pathway is highly conserved, tightly controlled and a multistep signalling cascade. Since its discovery in the 1980s, lipid kinase termed phosphoinositide 3-kinases (PI3Ks) has been proven time and time again to facilitate crucial cellular dynamics viz. survival, proliferation and differentiation. PI3Ks critically operate downstream of receptor tyrosine kinases (RTKs) and G protein coupled receptors (GPCRs) and are responsible for propagating a wide array of signals arising out from numerous growth factors and cytokines into intracellular communications by generating phospholipids, which in turn activate the

serine/threonine kinase AKT and several other effector pathways (Liu *et al.,* 2009). PI3Ks can be divided into three classes based on their structural physiognomies and substrate specificity; of these, the most commonly investigated are the class I enzymes that are activated directly by cell surface receptors. Class I PI3Ks can further be segmented into class IAs that are activated by RTKs, GPCRs and oncogenes like the small G protein Ras, and class IBs, that are entirely moderated by GPCRs. Activated receptors can directly trigger class 1A PI3Ks bound via their regulatory subunit or adapter molecules like the insulin receptor substrate (IRS) proteins. Once activated, class I PI3Ks generate the phospholipid PI(3, 4, 5)P3 that serves as a secondary messenger, driving multiple effector pathways influencing key cellular processes. PI3K cascade is negatively regulated by the tumour suppressor PTEN (phosphatase and tensin homolog deleted from chromosome 10) and the cellular levels of PI(3,4,5)P3 is closely regulated by the antagonizing PTEN levels. In fact, PTEN down regulates PI3K activity via its intrinsic lipid phosphatase activity that diminishes the cellular pool of PIP3 by converting PI (3,4,5)P3 back to PI(4,5)P2. Therefore, loss of function of PTEN will result in uncontrolled PI3K signalling thereby leading to baffling disorders like cancer and neurodegeneration (Hemmings *et al.,* 2012). AKT is a serine/threonine kinase which is expressed as three isoforms, AKT1, AKT2 and AKT3, which are encoded by three PKB genes namely α (PKBα), β (PKBβ), and γ (PKBγ). All the three isoforms can be characterized based upon a parallel architecture comprising of an N-terminal PH domain, a central serine/threonine catalytic domain, and a small C-terminal regulatory domain. Initial AKT activation is facilitated by translocation to the plasma membrane mediated by docking of the PH domain to the membranous phospholipid PI(3,4,5)P3, resulting a change in AKT conformational and subsequently divulging the two critical amino acid residues for phosphorylation. Phosphorylation at both the exposed sites, T308 by PDK1 and S473 by PDK2, are a prerequisite in order for the AKT to achieve full activation status. Once AKT is activated by phosphorylation at T308 and S473, it then facilitates the phosphorylation of targets viz. GSK3 (glycogen synthase kinase 3) and FOXOs (the forkhead family of transcription factors). A variety of PDKs are currently known to operate in the process, including ILK (integrin-linked kinase), PKCbII, DNA-PK (DNA-dependent protein kinase), and ATM (ataxia telangiectasia mutated),

while AKT itself has PDK functionality. However, it is strongly believed that mTORC2 (the mTOR/rictor complex) is the chief source of PDK2 activity under most circumstances (LoPiccolo *et al.,* 2008). mTOR belongs to a group of Ser/Thr protein kinases group of more distantly related enzymes (related to class I,II and III PI3Ks) and occasionally referred to class IV PI3Ks and includes members like ATM, ATR (ataxia telangiectasia and Rad3 related), DNA-PK and SMG-1 (SMG1 homolog, phosphatidylinositol 3-kinase-related kinase). PI3K/AKT pathway upstream of mTOR, moderates mTOR activity. mTOR is made up of two distinct complexes namely mTORC1 and mTORC2. The mTORC1 subunit is responsible for mTOR catalytic activity and harbours other components such as Raptor (regulatory associated protein of mTOR), PRAS40 (proline-rich AKT substrate 40 kDa) and the protein mLST8/GbL. Likewise, mTORC2 comprises of mTOR, Rictor (rapamycin insensitive companion of mTOR), mSIN1 (mammalian stress-activated protein kinase interacting protein 1) and mLST8/GbL. AKT is capable of activating mTOR by phosphorylating both PRAS40 and TSC2 (tuberous sclerosis complex) thereby offsetting the inhibitory effects on mTORC1. PKB/Akt binds to PIP3 at the plasma membrane in that way allowing PDK1 to access and phosphorylate the exposed T308 site in the "activation loop," thus leading to partial activation of the PKB/Akt component. This amendment is however adequate to stimulate mTORC1 by directly phosphorylation and inactivation of PRAS40 and TSC2 as mentioned above (Bartholomeusz *et al.,* 2012). Second phosphorylation of Akt at S473 in the carboxy-terminal hydrophobic motif, either by mTOR or by DNA-PK; result in full activation of PKB/Akt. mTOR can also operate as PDK2 and phosphorylate AKT when it is bound to Rictor in the mTORC2 subunit. mTOR is pivotal to cell growth and proliferation as it monitors variables like monitoring nutrient availability, cellular energy levels, oxygen levels, and mitogenic signals. Well characterized effector targets of mTORC1 includes 4E-BP1 (4E-binding protein), ribosomal protein S6 kinase and S6K1 (p70S6 kinase) which, in turn, phosphorylates the ribosomal protein S6 (S6/RPS6) (Wong *et al.,* 2010). mTOR also regulate autophagy, the failure to which leads to deficiency in elimination of abnormal and toxic protein aggregates which subsequently trigger catastrophic cellular stress, failure and ultimately death. Autophagy is also modulated by starvation, growth factors, and cellular stressors and

has long been proven to play a critical role in PD neuropathology. Yet, the cross-talk between PI3K/AKT/mTOR and autophagy is compound and comprehensive examination of tissue from patients suffering from PD and of animal and cellular models shall provide further valuable (Heras-Sandoval *et al.,* 2014).

1.5.3. p38 MAPK mediates microglial response and neuroinflammation

Microglial cells arise from mesodermal/mesenchymal progenitors and are the resident macrophages in the CNS. Once matured, these cells are disseminated into all regions of the CNS, spread through the brain parenchyma, and attain a specific ramified morphological appearance known as "resting microglia" In the normal brain, microglia however have highly motile procedures by which they patrol their territorial domains. Additionally, these cells can communicate with macroglial cells and neurons and with other cells of the immune system, the interaction mediated by a wide range of cell signalling mechanisms. Microglial cells display characteristic receptors as labelled for brain-specific communication such as neurotransmitter receptors and immune cell-specific such as for cytokines. Interestingly, microglial cells are the most vulnerable sensors of brain homeostasis and upon any detection of anomaly such as traces of brain lesions; nervous system dysfunction or external insults (trauma, toxicants). These cells surpass a multi-staged activation prototype to transform from “resting microglia” to “activated microglia”. The activated microglial cells have the enormous capacity to secrete a repertoire of molecules which can either act damaging or advantageous to the neighbouring population. Activated microglial cells migrate to the site of injury where it proliferates further and phagocytize damaged cells and cellular compartments (Kettenmann *et al.,* 2011, Cicchetti *et al.,* 2014).

1.5.4. Microglial activation and neuroinflammation in PD pathology

Human Brain and immune system are convolutedly involved in a crosstalk so as to maintain tissue homeostasis. A panel of evidences obtained from human and animal research have advocated the principle that neuroinflammation significantly contributes to the neuronal loss in PD. Available reports highlight the centrality of non-cell-autonomous pathological mechanisms in PD, which in most cases are

regulated by the activation of glial and peripheral immune population. Neuroinflammation in PD is a chronic mechanism that can be connected with alteration of glial cells, including astrocytes and microglia. Microglia activation in PD brains acutely involves a panel of microglial-derived neurotoxic factors such as reactive oxygen species (ROS), inducible nitric oxide synthase (iNOS), elevated pro-inflammatory cytokine levels, and upregulated inflammatory-associated factors such as cyclooxygenase-2, which altogether cooperate to stabilise microglial response in PD brains (**Figure 1.2**). Therefore, and it is not surprising that prolonged use of anti-inflammatory drugs can indeed reduce the risk for the disease. The neuronal response to microglia activation triggers unwanted trauma viz. oxidative stress (OS), neuroinflammation, cytokine-receptor-mediated apoptosis, which eventually contribute to DA neuronal mortality and subsequent disease progression. Interestingly, recent reports on transgenic mice related model mice were supportive of the idea that neuroinflammation in PD can be ambiguous, that is protective in the initial stages of degeneration but becomes severely damaging as the disease progress (Hunot *et al.,* 2003, Hirsch *et al.,* 2009 & Kazunari *et al.,* 2012).

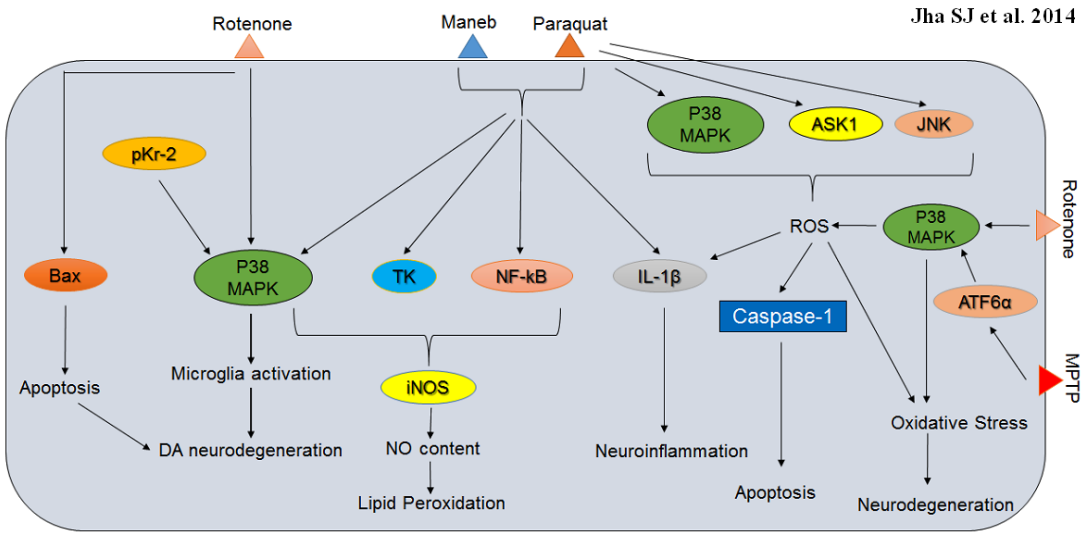


Figure 1.2: P38 MAPK interactions involved in Parkinson's disease neuropathology and associated neurodegeneration. Neurotoxins viz. Rotenone, Maneb, Paraquat and MPTP evokes numerous detrimental phenotypes in degenerating neurons and P38 MAPK is responsible for microglia activation, induction of oxidative stress, apoptosis, neuroinflammation and neurodegeneration as triggered by these toxins.

1.5.5. p38 MAPK mediates oxidative stress in diseased neurons

Neural cells suffer severe functional or sensory loss in neurodegenerative disorders and as discussed in previous sections, microglia-derived inflammatory neurotoxins play a major role in disease pathogenesis. Although, oxygen is the basis for life, defective metabolism and excess ROS production contributes to severe trauma and in contribution with several other environmental or genetic factors, oxidative stress ultimately leads to ROS accumulation in neural cells. Nevertheless, the human body's innate antioxidant defence plays a decisive role in prevention of any loss due to ROS but imbalance in defence mechanism of antioxidants, overproduction or incorporation of free radicals from environment to living system can lead to serious penalty and calamitous neurodegeneration. Moreover, a spectrum of environmental prompts, ER-stress, mitochondrial dysfunction, DNA injury, accretion of damaged misfolded proteins due to defective proteasome function, neuro-inflammation, tissue damage and subsequent neural cell apoptosis also subject the brain to severe insults. These factors affect glial function thereby aiding to hasten the cadence of the disease. Understanding the connections between oxidative stress, free radical formation, neuro-inflammation, and neurotoxicity is critical to deciphering novel disease mechanisms and the development of model neurotherapeutics to antagonize disease progression (Uttara *et al.,* 2009, Reynolds *et al.,* 2007 & Jha *et al.,* 2012). Oxidative stress in DA neurons can trigger the p38 MAPK pathway thus leading to activation of both mitochondrial and extra mitochondrial apoptotic pathways PD culture models. These results suggest that oxidative stress and p38 MAPK pathways operate to balance the pro and anti-apoptotic phenotypes of DA neurons (Choi *et al.,* 2004).

Paraquat (PQ) elicits a dose-dependent increase in ROS which results in death of SH-SY5Y neuroblastoma cells. This observation can be closely associated with the activation of ASK1 and the stress kinases p38 and JNK SH-SY5Y cells. It was recently reported that chemical inhibition of either p38 or JNK can confer resistance from L-DOPA-induced apoptosis. Moreover, direct knockdown of ASK1 protects from L-DOPA-induced neuronal cell death. Furthermore, suppression of the 6-OHDA-generated ROS by treating the cells with N-acetyl-L-cysteine effectively constrains the 6-OHDA-triggered activation of ASK1, p38 and JNK, and thereby protects the cells from apoptosis. It must

be noted here that ROS mediated caspase-1 activation and mature IL-1β release are strictly reliant on the p38 MAPK levels in 6-OHDA model systems. These studies clearly show the path from ROS generation to initiation of p38/JNK signalling via activation of ASK1 and subsequent apoptosis in investigated PD systems (Liedhegner *et al.,* 2011, Ouyang *et al.,* 2006). Rotenone can also meritoriously generate ROS, the concentration levels of which can be directly correlated with the activity of P38 MAPK in the microglia populace (Gao *et al.,* 2013, Oh *et al.,* 2011). These studies clearly show the path from ROS generation to initiation of p38/JNK signalling via activation of ASK1 and subsequent apoptosis in investigated PD systems.

ATF6α is an ER-membrane-bound transcription factor in mammalian cells that is activated as a consequence of protein misfolding in the ER. ATF6α functions as a critical regulator of ER quality control. MPTP, a dopaminergic neurotoxin well known to generate OS, activates ATF6α and increases the level of ER chaperones and ER-associated degradation (ERAD) component in DA neurons. This induced oxidative stress not only stimulates phosphorylation of p38 MAPK but also augments the interaction between phosphorylated p38MAPK and ATF6α, leading to an increment in the transcriptional activity of ATF6α. This mechanism provides a credible link between oxidative stress and ER stress by underscoring the reputation of ATF6α in the protection of the DA neurons from MPTP induced neurotoxicity that occurs via OS-induced activation of ATF6α and p38MAPK-mediated enrichment of ATF6α transcriptional activity (Egawa *et al.,* 2011).. Mutations in PINK1 (phosphatase and tensin homolog (PTEN)-induced putative kinase 1) gene is causative behind autosomal recessive PD. Recent studies investigated the impact of PINK1 on HO-1 (heme oxygenase-1) activation in SH-SY5Y cell lines following H2O2 or 1-methyl-4-phenylpyridinium [MPP (+)] treatment. It was suggested that the H2O2 induced HO-1 induction was dependent on Akt and ERK phosphorylation, moreover, in cells expressing PINK1 G309D mutant and knockdown of tumour necrosis factor receptor-associated protein-1(TRAP1), the phosphorylation of ERK and Akt was inhibited but not p38 MAPK phosphorylation. These results identified a novel mechanism involving p38 MAPK by which the defect in PINK1 inhibits the oxidative stress -induced HO-1 production. Above all, aberrant HO-1 production following oxidative stress has tens the DA neurodegeneration and directs the

brain to a traumatic state in PD patients with PINK1 defect (Chien *et al.,* 2011). Finally, uncharacteristic expression of matrix metalloproteinases (MMPs) play their part in PD prognosis and contributing factors such as ROS, PI3K, NF-κB, and AP-1 are commonly involved in 6-OHDA- and MPP (+)-induced MMP-9 gene expression during PD. SK-N-BE(2)C human neuroblastoma and Cath.a mouse DA cell lines when treated with 6-OHDA and MPP(+), resulted in an induction of MMP-9 expression, where the role p38 MAPK was found to be only differential (Kim *et al.,* 2004).

1.5.6. PI3K/AKT/mTOR pathway mediates neuroprotection in PD

Accumulating evidences strongly suggest on PI3K/Akt and mTOR to being neuroprotectiveand hence malfunctioned in PD brains; this is actually of relevance to longevity and may present strategic targets for therapeutic improvement (Elstner *et al.,* 2011). Recent research statistics strongly advise that the vulnerability of DA neuron could arise from elevated metabolic stress levels, resulting from numerous perturbed cascades designated for the control of energy metabolism and cell survival in response to growth factors, oxidative stress, and nutrient deprivation (PI3K/AKT, mTOR, eIF4/p70S6K and Hif-1α). Altogether, these factors operate in a convoluted network thereby adding to archetypal phenotypes observed in PD patients. One of the cardinal symptom observed in diseased brains is neuro-inflammation and PTEN induced putative kinase 1 (PINK1), an autosomal recessive familial PD gene, regulates the inflammatory ambience during traumatic states. Dearth in PINK1 levels expedites neuro-inflammation in PD brains through diminished AKT activation and enhanced IκB degradation in response to traumatic brain injury (Kim *et al.,* 2013). In-fact, mutations in PINK1 genes have provided a credible basis to a certain extent to meticulously monitor and comprehend the otherwise complex etiology of PD. PINK1 mutations were found to be severely damaging in C2-ceramide (neurotoxin) challenged brains thereby suggesting on the neuroprotective role of PINK1 in preventing mitochondrial dysfunction and reinforcing the anti-apoptotic and neuronal survival pathways such as Bcl-2 and PI3K/AKT (Sánchez-Mora *et al.,* 2012). PINK1 and PARKIN are responsible for mitochondrial damage limitation during active durations of stress and cooperate together in autophagy following mitochondrial injury. Examination of primary mouse cells acquired from PINK1-knockout mice directed that PARKIN induction and lysosomal translocation proceeded autonomous of PINK1. Moreover, suppression of

the PI3K/AKT-mTOR pathway by therapeutic proxies can vary PARKIN expression accordingly. These results altogether validates that PARKIN and PINK1 are co-regulated during starvation and suggest a likely role of PI3K/AKT-mTOR in response to trophic signals and starvation stress (Klinkenberg *et al.,* 2012).

PI3K/AKT pathway can also play a key role in IGF-mediated cell survival and prevention of apoptosis in MPP+ induced human neuroblastoma SH-EP1 cells. This defensive activity of AKT is principally reliant on the BIO mediated inactivation of GSK-3β, the result of which could imitate the protective influence of IGF-1 in SH-EP1 cells. Interestingly, the IGF-1 potentiated PI3K/AKT activity was found to further down regulate the JNK related apoptotic activity and this negative regulation was reported to be facilitated via AKT-dependent GSK-3β inactivation (**Figure 1.3**).

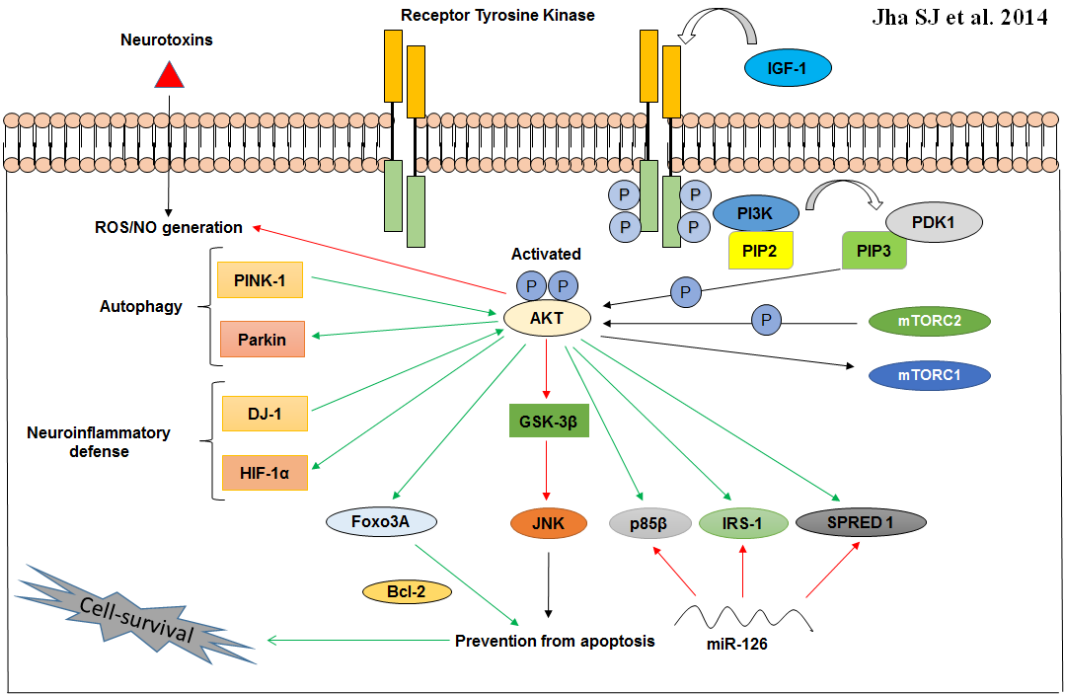


Figure 1.3: Neuroprotective cross-talk involving the cytoprotective PI3K/AKT pathway. AKT when by optimally activated by phosphorylation at serine and threonine residues, thereafter, it can interact with a spectrum of molecules to erect an anti-inflammatory (DJ-1 and HIF-1α) and anti-apoptotic (Bcl-2) ambience in vulnerable neurons. In addition, phosphorylated AKT can also promote autophagy via PINK-1 and Parkin. IRS-1 activation takes place via IGF-1/AKT cascade and other AKT targets including p85β and SPRED 1 are known to be down regulated by miR-126 in PD neurons. Activation (green arrows); prevention or suppression (red arrows).

Moreover, these results acknowledges that IGF-1 protects SH-EP1 cells from MPP+-induced apoptotic cell mortality via the cytoprotective PI3K/AKT/GSK-3β pathway involving GSK-3β inactivation (Chong *et al.,* 2014, Bao *et al.,* 2014 & Wang *et al.,* 2010). Most recently, it was studied in DA neuronal cell systems that upregulation of miR-126 impaired IGF-1 signalling and increased the susceptibility of such systems to 6-OHDA, possibly by stamping down factors involved in IGF-1/PI3K signalling, including its downstream targets p85β, IRS-1, and SPRED1. Micro RNAs (miRs/miRNAs) act as post transcriptional regulators of gene expression and therefore it is unsurprising that they could be critically modulating pathogenesis in PD. Notably, blocking miR-126 activity increased IGF-1 trophism and thereby combating the cataclysmic events of 6-OHDA. This result strongly ascertain the criticality IGF-1/ PI3K cascade in DA neuron maintenance and also suggests that higher expression patterns of miR-126 might be contribute towards DA neurodegeneration aided by downregulation of IGF-1/PI3K/AKT signalling (Kim *et al.,* 2014).

1.5.7. Neuroprotective molecules of relevance in p38 and PI3K/AKT mediated PD prognosis

Neurotherapeutics research in recent times have probed a spectrum of protective biomolecules which can either activate the PI3K/AKT cascade, while others assist in limiting the activation of p38 MAPK in neurons; **(Table 5)** below provides a list of neuro-protective therapeutic modalities that summon either the misfiring p38 MAPK and/or PI3K/AKT cascades in some form or the other, en route to providing a resilient neuroprotective shield against the hastened degeneration of neurons in PD.

Table 1.5: Neuroprotective Biomolecules offering neuroprotection in neurotoxin challenged Parkinson's model systems presumably via p38 MAPK and/or PI3K/AKT cascade.

Biomolecules	Nature	PD Model systems	neurotoxin	Proteins involved	Mode of action	References
Guanine based purines	anti-apoptotic	SH-SY5Y	6-OHDA	AKT, p38, JNK, and Bcl-2	Triggers an early upsurge in the phosphorylation of AKT and subsequent activation of the cytoprotective PI3K/AKT/PKB pathway; prevents the 6-OHDA intermediated activation of p38 and JNK and cause an upsurge in the expression level of the anti-apoptotic Bcl-2 protein.	(Giuliani *et al.,* 2012)
Human albumin	anti-oxidant and anti-apoptotic	PC12	6-OHDA	JNK, c-Jun, ERK, and p38 MAPK	Attenuates 6-OHDA-inflicted ROS generation and apoptosis; inhibits 6-OHDA-induced activation of JNK, c-Jun, ERK, and p38 MAPK signalling.	(Zhang *et al.,* 2012)
Peroxiredoxin (PRX-2)	anti-oxidant and anti-apoptotic	MN9D DA neurons	6-OHDA	ASK1, c-Jun , p38	Inhibits 6-OHDA-induced ASK1 activation by regulating the redox properties of the endogenous ASK1 inhibitor Trx; display significant anti-apoptotic properties via suppression of ASK1-dependent activation of the c-Jun N-terminal kinase/c-Jun and p38 pro-mortality cascades; lastly, PRX2 over expression preserves Trx in a reduced state by blocking the cysteine thiol-disulfide interchange, thus preventing the dissociation of thioredoxin from ASK1.	(Hu *et al.,* 2011, Schreiber *et al.,* 2011)
NOSH-ASA (NO- and H2S-releasing hybrid of aspirin)	anti-inflammatory	IFNγ-stimulated human astroglia and U373 cells, SH-SY5Y		TNFα, IL-6 , P38 MAPK and NFκB	Results in reduced TNFα and IL-6 levels along with a concomitant deactivation of P38 MAPK and NFκB proteins.	(Zhang *et al.,* 2012)

Biomolecules	Nature	PD Model systems	neurotoxin	Proteins involved	Mode of action	References
Bu-7	anti-apototic	PC12	Rotenone	JNK,p38,p53,caspase-3,Bax,Bcl-2	Protects the cells from rotenone triggered apoptosis and subsequent death; limits the rotenone induced potential reduction in mitochondria of the treated cells, prevents the rotenone induced activation of JNK, p38, p53, cleaved caspase-3 and decreases the Bax/Bcl-2 ratio.	(Lee *et al.,* 2013)
3,4-Dihydroxybenzalacetone (DBL)	anti-oxidant, anti-inflammatory, and anti-tumorigenic	SH-SY5Y	6-OHDA	Akt, ERK, p38 MAPK, PI3K	Induce stress-associated kinases such as AKT, ERK, and p38 MAPK, and PI3K or AKT inhibitors, but not ERK, p38, or JNK inhibitors; activates the Nrf2/glutathione cascade via PI3K/AKT, and facilitates survival of SH-SY5Y cells.	(Li *et al.,* 2011)
Tetrahydroxystilbene glucoside (TSG)	anti-apoptotic	PC12 and mice	MPTP	DAT, AKT, GSK3β, Bcl-2, BAD, caspase-3 and caspae-9	Protects DA neurodegeneration by averting MPTP-induced reduction of SN tyrosine hydroxylase (TH)-positive cells and striatal dopaminergic transporter (DAT) protein expression; increase in striatal AKT and GSK3β phosphorylation, up-regulation of the Bcl-2/BAD ratio, and inhibition of caspase-9 and caspase-3 activity; offers neuroprotective effects against MPP-prompted damage and apoptosis in PC12 cells, presumably through PI3K/AKT. Activation.	(Gunjima *et al.,* 2013)
Tyrosol [2-(4-hydroxyphenyl) ethanol]	anti-apoptotic	CATH.a	MPP(+)	PI3K, AKT, SOD-1, SOD-2 and DJ-1	Is neuroprotective against (MPP(+))-induced CATH.a neuronal death in a dose dependant manner by its ability to activate the PI3K/Akt signalling cascade; Tyrosol also upregulate SOD-1, SOD-2 and DJ-1.	(Zhang *et al.,* 2013)

Biomolecules	Nature	PD Model systems	neurotoxin	Proteins involved	Mode of action	References
Oxicam non-steroidal anti-inflammatory drugs (NSAIDs)	anti-inflammtory	SH-SY5Y and mice	MPTP	PI3K, AKT, and COX	Offers protection via the PI3K/AKT cascade independentally of cyclooxygenase (COX) inhibition.	(Dewapriya *et al.,* 2013)
Tocotrienols (T3s)	anti-oxidant	SH-SY5Y	MPP(+)	ER β and PI3K/AKT	γT3 and δT3 treatments triggers the PI3K/Akt signalling module and this could perhaps be under the control of estrogen receptor (ER) β; ER β being an upstream regulator of PI3K/AKT; T3s and, especially, γT3/δT3 in conjunction with the activation of ERβ/PI3K/AKT cascade, display not only antioxidant activity but also offers a receptor signal-mediated neuroprotection.	(Tasaki *et al.,* 2012)
Danshensu (beta-3,4-dihydroxyphenyl-lactic acid)	ROS scavenger and anti-oxidant	PC12 and Zebra fish DA neurons	6-OHDA	PI3K/AKT, Nrf-2, and HO-1	Induces AKT phosphorylation, and the induced cytoprotective effects are reversed by PI3K, AKT and HO-1 inhibitors; enhances HO-1 expression in order to suppress 6-OHDA-induced oxidative stress via PI3K/AKT/Nrf2 cascade.	(Nakaso *et al.,* 2014)
Puerarin	anti-oxidant	Mice	MPTP	PI3K/AKT, GSH, and GDNF	Puerarin administration enhances glutathione (GSH) activity, glial cell line-derived neurotrophic factor (GDNF) expression and activates the PI3K/AKT pathway; dampens MPTP-reduced lysosome-associated membrane protein type 2A (Lamp 2A) expression.	(Chong *et al.,* 2012)
Eucommia ulmoides Oliv. Bark. (EUE)	anti-oxidant	SH-SY5Y	6-OHDA	JNK, PI3K/AKT, GSK-3β, and NF-κB	EUE reduces 6-OHDA-induced ROS formation, mitochondrial dysfunction, cell death and cytotoxicity; mitigates oxidative stress through induction of JNK, PI3K/AKT, GSK-3β, and NF-κB cascades.	(Kwon *et al.,* 2014)

Biomolecules	Nature	PD Model systems	neurotoxin	Proteins involved	Mode of action	References
Rotigotine	anti-oxidant, anti-apototic	primary dopaminergic cultures	glutamate	D3 receptor, AKT, and GSK-3-β	The molecule most likely stimulates the dopamine D3 receptor; abates the production and accumulation of superoxide radicals; consistent exposure to Rotigotine promotes Akt phosporylation, and results in deactivation of the pro-apoptotic component GSK-3-β.	(Oster *et al.,* 2014)
Squamosamide derivative FLZ		Rats	6-OHDA	PI3K/AKT, α-Syn, and TH	FLZ protects TH activity and DA neurons by diminishing α-synuclein (α-Syn) expression and the cooperation between α-Syn and TH, FLZ neuroprotection involves the PI3K/AKT cascade and blocking the cascade attenuates α-Syn expression and subsequently the protection offered by FLZ is lost.	(Bao *et al.,* 2014)

Environmental exposures to toxic mediators lead to neurodegenerative sickness that has common pathophysiology and clinical findings with PD. It is conjectured that decisive factors like microglia activation, neuroinflammation, oxidative stress due to ROS accumulation, NO activity, and neuronal apoptosis resulting from all these modes are at the base of DA neuronal toxicity and subsequent damage in Parkinson's brains. However, the precise identity and functional prototypes of molecular intermediates leading to neuronal mortality still remains to be deciphered. Recent studies have highlighted the fundamental role of p38 MAPK in controlling all of the above detrimental consequences, and thereby in the process upset DA neuronal homeostasis, which ultimately progresses to an advanced diseased state and incurable neurodegeneration. Using cell systems like SH-SY5Y, several factors like inducible NO synthase, ATF6α (oxidative stress), IL-1β, and TNF-α (neuroinflammation), and Bax and ASK1 (apoptosis) were found to be relevant in DA neuronal death. Unprecedented observations that these factors operate in unison with the p38 MAPK cascade strongly advocates the cyto-destructive nature of the cascade in degenerating neurons. Conversely, normal functioning of the PI3K/AKT pathway ensures that the neuroprotective defence is active in order to negate the destructive aftermath of p38 MAPK activation in degenerating neurons. In the process, the activated AKT interacts with several mediators like JNK, FoxO, and GSK3β etc. thereby to render neuroprotection by limiting apoptosis, preventing microglia activation and neuroinflammation, preventing ROS accumulation and by keeping oxidative stress levels under check. However, the pathway is misregulated in PD brains and eventually it fails to render its protective veneer in traumatised brains. It is thereby necessary to identify modalities which can repair the misbalanced p38/PI3K interactome in order to limit poor prognosis in PD patients.

1.6. METHODS TO STUDY PARKINSON'S DISEASE

There are three methods through which the etiology of PD can be studied. Each of these can be employed based on their availability and purpose of study. These methods include:

(i) *In silico* study

(ii) Cell culture model

(iii) Animal models

Currently there is no cure and researchers continue to probe the therapeutic prospect in *In silico* study, cell cultures and animal models of PD. Owing to the limitations of sample availability of PD patients and scarcity of animals, the current research scenario has shifted focus on cellular models of PD which are an excellent source of large drug screening and are easy to maintain.

Firstly, we have done *In silico* studied, which will be discussed in Chapter *II* and *III* and uses cell culture model to study the toxicity effects of a neurotoxin; MPTP in SH-SY5Y neuroblastoma cell line. Moreover, the neuroprotective effect of biomolecules against MPTP induced toxicity has been studied which will be discussed in Chapter *V*.

1.7. AIMS AND OBJECTIVES OF PRESENT STUDY

1.7 1. Aim: To investigate the protective role of biomolecules in Parkinson's disease

1.7 2 Objectives

To fulfill the aim of the present work, following objectives were outlined:

1. To investigate the protective role of biomolecules, induced E3 ligase activity of DJ-1 and Parkin, which can have possible clinical relevance in PD pathogenesis.

2. To identify the mechanistic approach of biomolecules targeting the impaired p38-PI3K/AKT balance could significantly contribute to neuroprotection in PD.

3. To establish the therapeutic action of biomolecules prevents MPTP-Induced neurotoxicity in dopaminergic SH-SY5Y cell line in PD.

1.8 PLAN OF WORK

The present work is divided into different sections to achieve the following objectives

I. Molecular docking study of neuroprotective plant-derived biomolecules in Parkinson's disease

a. *Retrieval of Ubiquitin E3 Ligase (Parkin and DJ-1) their functional elucidation*

b. *Phylogenetic relationship and Physico-chemical properties.*

c. *Homology modelling, visualization and quality assessment of 3D structure of Ubiquitin E3 Ligase Parkin and DJ-1.*

d. *Active site prediction of Ubiquitin E3 Ligase Parkin and DJ-1.*

e. *Assessing the drug-likeliness of given compounds.*

f. *Ligand optimization and Molecular docking study of Ubiquitin E3 Ligase Parkin and DJ-1.*

II. Study the effect of biomolecules in ameliorating toxicity in SH-SY5Y cells

a. *Study the dose-dependent and time-dependent effect of toxin; MPTP on neuronal SH-SY5Y cells line*

b. *Study the effect of MPTP on cell viability*

c. *Check the dose-dependent and time-dependent efficacy of biomolecules against MPTP toxicity*

d. *Assess the effect of biomolecules on cell viability*

III. Check the expression of protective proteins in response to MPTP administration and after treatment with biomolecules

The experimental procedures and methods employed in studying the objectives mentioned above have been discussed in the succeeding chapter.

Chapter II
Materials and Methods

CHAPTER II

MATERIALS AND METHODS

2.1. INTRODUCTION

This chapter describes the tools and techniques used in screening of both Parkin and DJ-1(Ubiquitin E3 ligase) inducing biomolecules and in studying their interaction. Also, the experimental procedures employed in sub-culturing SH-SY5Y cell line, administering MPTPand treating cells with biomolecules have been described. All the experiments which have been carried out along with the tools and reagents used are discussed in this chapter.

2.2. *IN SILICO* TECHNIQUES

2.2.1. Data Mining

Comprehensive data mining on both Parkin and DJ-1(Ubiquitin E3 ligase) in neuronal damage was done with the keywords Parkin and DJ-1 in the NCBI database. Also, extensive literature survey was carried out. The filter criteria were set to both Parkin and DJ-1modulators in the neuronal damage under stress condition accordingly, list of 14 potential compounds were prepared. Out of 14 potential compounds, finally four compounds were filtered and selected for this study.

2.2.2. Retrieval of Ubiquitin E3 Ligase related proteins and their function recognition

The amino acid sequence of Ubiquitin E3 Ligase related Proteins Parkin and DJ-1with accession numbers 4I1H_A and 4ZGG_A respectively were retrieved from NCBI database and were 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/).

2.2.3. Phylogenetic relationship and Physico-chemical properties

For multiple sequence analysis Muscle Software (http://www.ebi.ac.uk/ Tools/msa/muscle/) was used and phylogenetic tree was constructed using Muscle

Software based on NJ (Neighbor joining) plot without distance correction. ProtParam (http://web.expasy.org/protparam/) was used to predict physicochemical properties. The parameters computed by ProtParam included the molecular weight, theoretical PI, aliphatic index and grand average of hydropathicity (GRAVY).

2.2.4. Homology modelling, visualization and quality assessment of 3D structure of Ubiquitin E3 Ligase Parkin and DJ-1

Homology modeling was used to determine the 3D structure of Parkin and DJ-1 isoforms. A BLASTP search with default parameters was performed against the Brookhaven Protein Data Bank (PDB) to find suitable templates for homology modeling. Templates with PDB ID: 4I1H and 4ZGG were retrieved for Parkin and DJ-1 proteins from Protein Data Bank (PDB). The Protein Structure Prediction Server Swiss model (Arnold K *et al.,* 2006) (http://swissmodel.expasy.org/) was used for homology model construction.

2.2.5. 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 upload in the server and it showed the ligand binding sites present in protein and the site with maximum surface area and maximum surface volume was selected and all the amino acid residues involved in binding with ligands were retrieved.

2.2.6. 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 that is backed up by three primary databases, i.e. PCsubstance, PCcompund, 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. Four compounds

(Naringenin, Quercetin, Resveratrol and Sesamol) 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 viewer.

2.2.7. 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 non-drug 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.

2.2.8. ADMET Analysis

The toxicity profiling of compounds was carried out through the online tool Swiss ADME (www.swissadme.ch/index.php). The Swiss ADME tool assessed the ligand on various parameters such as lipophilicity (logP), hydrophilic nature (logS) and BBB permeability.Additionally, two other parameters; Ghose filter and Veber rules were employed for drug-likeliness screening. The qualifying parameters of Ghose filter are (a) molecular weight 160-480 (b) number of atoms 20-70 (c) molar refractivity 40-130 and (d) polar surface area <140 (Ghose et al. 1999). The Veber rules are (a) rotatable bond count <=10 and (b) polar surface area <=140.

2.2.9. Preparation of Protein and ligand molecules

Preparation of protein involves the addition of polar hydrogen atoms, addition of charge and removal of any miscellaneous structures from the protein molecule by Autodock **4.2.1** whereas; ligand preparation involves the addition of charge.

2.2.10. Ligand optimization and Molecular docking

Prepared and optimized structures of ligands and proteins were ultimately used for molecular docking using Autodock 4.2.1 for predicting 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.

2.3. *IN VITRO* TECHNIQUES

2.3.1. Chemicals, Reagents, Drugs and Antibodies

High-purity Naringenin (NGEN), Quercetin (QUR), Resveratrol (RES), DMSO and Penicillin-streptomycin were obtained from Sigma Aldrich (Bangalore, India). Primary antibodies were purchased from Santa Cruz biotech (Santa Cruz, CA) and cell signalling (Danvers, MA). For secondary antibody, HRP conjugated anti-rabbit and anti-mouse polyclonal immunoglobulin was purchased from DAKO (Glostrup, Denmark). All other chemicals used were purchased from Merck, Sigma Aldrich and Thermo Fisher Scientific of highest grade and purity.

2.3.2. Cell line

The SH-SY5Y is a triple cloned cell line of the parent cell line SK-N-SH. It is of human origin and was sub cloned from a four year old female patient suffering from neuroblastoma (Biedler *et al.,* 1978). The cell line is adrenergic and has also been shown to possess moderate levels of dopamine beta hydroxylase activity (Ross and Biedler, 1985) and thus has served as an excellent *in vitro* model to study PD associated phenomenon. The SH-SY5Y cell line was received from NBRC Manesar, Haryana (INDIA).

2.3.3. Maintenance of the cell lines

The neuroblastoma cell line SH-SY5Y with an early passage of P4 were grown in Dulbecco's Modified Eagle's Medium/F12 (1:1) growth medium (Gibco; Thermo Fisher Scientific, USA) containing L-Glutamate, 10% heat-inactivated fetal bovine

serum, 1mM sodium pyruvate (1:100 dilution) (Sigma-Aldrich; USA), 1X non-essential amino acids (1:100 dilution) (Sigma-Aldrich; USA), sodium bicarbonate (1:50 dilution) (Sigma-Aldrich; USA) and penicillin-streptomycin (Sigma-Aldrich; USA) in T-25 flasks which were pre-coated in a humidified CO_2 incubator with 5% CO_2 and 95% air at 37^0C. The neuroblastoma cells were regularly monitored under microscope for their healthy morphology, growth and confluence.

2.3.4. Sub culturing and differentiation of cell lines

The 60-80% confluent cultures were used for sub-culturing; before trypsinization, removed complete growth media and washed the cells with 1xDPBS. Next the cells were incubated with 3ml of 0.05% trypsin-EDTA (for 75cm^2 flask) (0.05%; Gibco; Thermo Fisher Scientific, USA) for 5 minutes at 37^0C. The trypsin reaction was stopped by adding growth medium and triturating cells with pipette. Further the cells were washed twice by centrifugation at 1500 rpm for 5 minutes (10ml 1xDPBS each time). The pellet obtained at the bottom was delicately handled and supernatant removed carefully. The pellet was dissolved in residual medium. The cells were seeded ($1x10^6$ cells/mL) in fresh culture media and incubate at 37^0C in 5% CO_2 incubator. Within 3-4 days, cells were found to 60-80% confluent and were used for further experiments. Furthermore, cell density was calculated by using 10µL of this solution mixed with 10µL trypan blue solution (0.4%; Sigma-Aldrich; USA) and counting viable cells in 4 different squares of the hemocytometer. Finally, based on calculated cell density, cells were seeded in new T-25 flasks containing fresh medium and incubated in humidified CO_2 incubator with 5% CO_2 and 95% air at 37^0C till they reached 60-80% confluence and were used for further experimentation.

2.3.5. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)

The present study uses MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) (Sigma-Aldrich; USA) to mimic PD symptom in the SH-SY5Y neuroblastoma cell lines. MPTP is a pesticide, a broad spectrum pesticide which has been linked to the sporadic form of PD (Langston *et al.,* 1983). It is highly lipophilic, can cross the blood brain barrier and can directly enter cells without the aid of transporters. It

impairs mitochondrial respiration by blocking complex I activity and thus augmenting ROS generation. Further, it has also been involved in impairing proteasomal dynamics in the etiology of PD (Przedborski *et al.,* 2003).

2.3.6. MPTP Exposure

The cells were sub-cultured in 24 well plates as described above and labeled each well. Next, stock solutions of MPTP (100% purity) in DMSO (0.5 mg/ml; Sigma-Aldrich; USA) were prepared for 1mM and 1μM concentration respectively. The final concentration of DMSO wherever used in present study was always less than 0.1% to rule out any toxicity linked to DMSO. MPTP treatment was given to cells with each well containing different concentration in an increasing order ranging from 300 nM, 600 nM, 900 nM, 20 μM, 40 μM and 80 μM. The cells were incubated at 37^0C in a CO_2 incubator with 5% CO_2 and 95% air. Next, the cells were observed and photographed under microscope for changes in morphology and confluency at regular intervals of 6 hours, 12 hours and 24 hours and compared with control and SHAM. The experiment was done to determine dose-dependent and time-dependent effect of MPTP on SH-SY5Y cells and was carried out in triplicates.

2.3.7. Biomolecules treatment

After determining the LD_{50} concentration of MPTP and the time of incubation required, biomolecules were applied in an increasing dose concentration to control and MPTP treated cells. A stock solution of Naringenin, Quercetin and Resveratrol was prepared in a stock solution of 1mM in 0.1% DMSO. Naringenin and Quercetin were applied at final concentration of 10μM, 20μM, 40μM and 80μM. Further, Resveratrol was applied at final concentration of 15μM, 30μM, 60μM and 120μM. The cells were incubated at 37^0C in a CO_2 incubator with 5% CO_2 and 95% air. Further, the cells were observed and photographed under microscope for changes in morphology and confluence at regular intervals of 6 hours, 14 hours and 24 hours and compared with control and SHAM. The triplicate experimental observations were used to determine the dose-dependent and time-dependent effect of Naringenin, Quercetin and Resveratrol in MPTP induced toxicity in SH-SY5Y cells.

2.3.8. Trypan blue exclusion test

Cell viability assay was done by using trypan blue exclusion test. The SH-SY5Y was seeded at a density of $2.0x10^5$ cells/well in 24 well plates and incubated overnight at 37^0C in a CO_2 incubator with 5% CO_2 and 95% air. MPTP treatment was next given to cells for (06-24) hours. The biomolecules Naringenin, Quercetin (10-80μM) and Resveratrol (15-120μM) were added to MPTP treated cells and incubated for 24 hours. Viable cells were counted after MPTP administration and post-biomolecules treatment according to the given protocol. The cell suspension (10μL) and 10μL of trypan blue solution (0.4%; Sigma-Aldrich; USA) was mixed and loaded on the chamber underneath glass cover slip on the hemocytometer. The solution was taken up by capillary action and a clean cover slip was carefully placed. The live cells were counted as unstained and dead as blue stained cells in four different squares of hemocytometer using a cell counter under the inverted microscope. Finally, cell viability for each ml was calculated by using the formula: % Cell viability= (number of live cells/total number of cells) x100. Similarly, cell viability was determined for each concentration of MPTP and biomolecules at defined time periods in the triplicate experimental sets.

2.3.9. Preparation of cellular protein extract

Cellular protein extracts was prepared by washing SH-SY5Y cells in ice cold PBS and scraping into ice cold lysis buffer (50mM Tris-HCl, pH 7.4, 150 mM NaCl, 100 μg/ml PMSF, 0.02% NaN2, 1 μg/ml aprotinin, 1% Triton X-100) containing the protease inhibitor cocktail (Sigma-Aldrich; USA). Lysates were incubated on ice for 20 minutes before centrifugation at 14,000 rpm for 15 minutes at 4°C to remove nuclei and cellular debris. Lysates were analysed for protein concentration using the Dot-blot method and boiled in sample buffer for SDS-PAGE.

2.3.10. SDS-PAGE and Western blotting

After preparation of cellular protein extract, each protein sample (30 μg) in Lamelli buffer was heated for 10 minutes at 95^oC, separated on 10% SDS-PAGE gel and finally, electro-blotted (BioRad; USA) onto a PVDF membrane (GE Healthcare,

Piscataway, NJ). Membrane were blocked in 5% (w/v) nonfat dry milk and incubated for 3 hours at room temperature with primary antibody (1:500–1,000 dilution; Santa Cruz; USA) followed by three times washing in 1X TBST buffer. After washing, membranes were incubated in goat anti-mouse IgG or goat anti-rabbit IgG HRP-conjugated secondary antibody (1:2,000 dilutions; Santa Cruz; USA) for one hour. The autoradiography signals were visualized using ECL advance Western blotting detection kit (RPN2135) on an X-ray film (Thermo Fisher Scientific; USA) after washing the membrane three times.

2.3.11. Ponceau s stain for Western blots

Ponceau S stain was performed to check whether protein was transfer properly on PVDF membrane and were equal in all the lanes. This is a rapid and reversible staining method for locating protein bands on Western blots. Membrane was incubated with Ponceau S for 3-5 minutes, washed with TBST until stain was removed completely and background was clear before blocking the membrane in 5% milk.

2.3.12. Statistical analysis

All the data are expressed as mean + SEM. The statistical measurement was done using one way ANOVA followed by Tukey's multiple comparison test. The statistical significant values were considered for $p<0.05$.

After adopting *in silico* tools and techniques outlined in this chapter, we have further performed drug screening for targeting both Parkin and DJ-1 (Ubiquitin E3 Ligase) to explore their therapeutic potential in neuronal damage under stress condition which has been discussed in the succeeding chapter.

Chapter III

Molecular Docking study of Neuroprotective Plant-derived Biomolecules in Parkinson's Disease

CHAPTER III:

MOLECULAR DOCKING STUDY OF NEUROPROTECTIVE PLANT-DERIVED BIOMOLECULES IN PARKINSON'S DISEASE

3.1. OBJECTIVE

This chapter discusses the motor neurodegenerative disorder which states with second largest occurrence rate among all the neurodegenerative diseases. Despite intense research, the etiology of PD is still unclear. There are various causative factors, for instance; a mutation in Parkin is mainly found to be associated with PD pathogenesis. Since, Parkin possesses both ubiquitin E3 ligase and chaperone like activity and their altered expression causes advancement of PD therefore; in this chapter we have addressed the therapeutic role of biomolecules in targeting the altered expression of E3 ligase.

3.2. INTRODUCTION

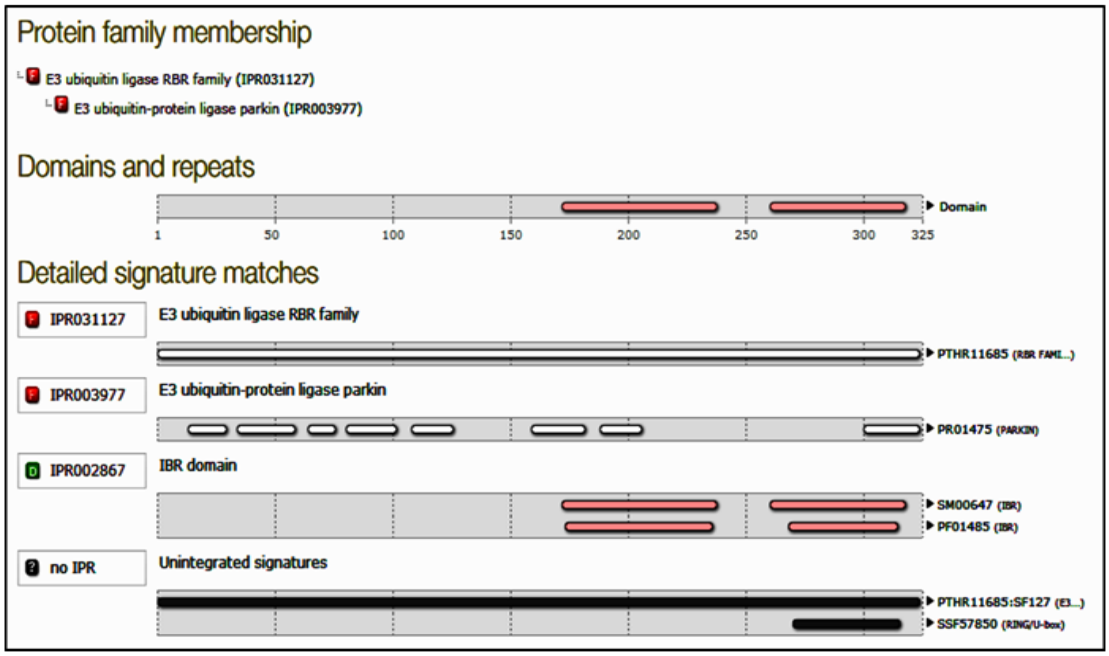
PD is characterized by progressive loss of dopaminergic neurons in the *substantia nigra*, leading to the loss of motor skills (Soto, 2003, Hardy *et al*., 2006, Manoharan *et al*., 2016 & Angeline *et al*., 2013). Several risk factors, for instance, genetic polymorphisms, aging and dietary supplements have been reportedly involved in the development and prognosis of PD. Other causative factors that might influence disease outcomes include accumulation of reactive oxygen species (ROS), selective loss of neurons, loss of mitochondrial membrane potential and ATP depletion that are also known to be associated with the PD pathogenesis (Dias *et al*., 2013). However, ubiquitin proteasome system (UPS), which is responsible for the recognition and degradation of damaged proteins, is found to be impaired in case of PD (Leroy *et al*., 1998). Parkin is a well-known component of UPS which is having pivotal role in the protein homeostasis. Conversely, under stress condition the normal functioning of Parkin is altered or down regulated and thereby leading to the prognosis of PD (Arduino *et al*., 2010). Furthermore, the connection between the UPS and neurodegeneration has been supported by the recognition of disease-causing mutations in genes coding for numerous UPS proteins in PD (Ciechanover *et al*., 2015).

Importantly, the impaired function of Parkin can be ameliorated by using different biomolecules such as, Naringenin, Quercetin, Resveratrol and Sesamol. These biomolecules are having antioxidant profile which would be a strong basis for showing neuroprotection within the brain (Barry *et al*., 1996). These biomolecules are natural compounds which are found in many fruits and vegetables and having much stronger antioxidative and neuroprotective activities (Santos *et al*., 2009). The neuroprotective properties of Naringenin Inhibited LPS induced NOS and COX2 expression and suppressed the production of NO in microglial cells. It also inhibited LPS/IFN-ϒ induced p38 and STAT-1 phosphorylation (Kannappan *et al*., 2010). However, Quercetin, improved behavioral function, suppressed oxidative stress, brain swelling, and cellular injury both *in vitro* and *in vivo* (Bavithra S *et al*., 2012). Although, Sesamol and Naringenin reversed both the rotenone/MPTP+-induced toxicity in PD rat model (Angeline *et al*., 2013).Similarly, Resveratrol (3, 5, 4 - trihydroxystilbene) is a naturally-occurring polyphenol found in peanuts, skin and seeds of grapes (Chen *et al*., 2002). It plays a pivotal role in cell proliferation and apoptosis by acting on a variety of signalling mechanisms such as, Protein kinase B (Akt), Mitogen-activated protein kinase (MAPK), and many othersignaling cascades (Soleas *et al*., 1997). Most-recent reports suggest that resveratrol can induce the Heme Oxygenase-1 (HO-1) expression in dopaminergic neuron and thus can prevent the dopaminergic cell death through autophagic flux (Lin *et al*., 2014). Additionally, resveratrol can also display neuroprotective effects against rotenone-induced neurotoxicity in dopaminergic SH-SY5Y cells by modulating HO-1 dependent autophagy (Tsu-Kung *et al*., 2014).Therapeutic interferences in neurodegenerative conditions using biomolecules are a seemingly new prospect. Therefore, a mature understanding of the mechanism impacting biomolecules mediated therapeutics could contribute towards fostering neuroprotective strategies. Here, we have focused our study towards impaired Parkin activity by using various *in silico* approaches including molecular docking analysis. Further, these biomolecules such as Naringenin, Quercetin, Resveratrol and Sesamol were selected and screened via ADMET analysis followed by molecular docking of the screened molecules towards their clinical aspects in PD. Finally, in this chapter, we have discussed the active site of Parkin protein for a potential target of these biomolecules by molecular docking that may further provide their probable clinical relevance in PD biology.

3.3. EXPERIMENTAL AND RESULTS

3.3.1. Retrieval of Ubiquitin E3 Ligase Parkin and their functional 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 Parkin on the basis of families and domains identified from Interproscan results **(Table 3.1)**. Interproscan study revealed that all homologues proteins for Parkin were belonging to E3 ubiquitin ligase RBR family (IPR031127), E3 ubiquitin-protein ligase Parkin family (IPR003977) and IBR domain (IPR002867) **(Figure 3.1).**



Retrieval of Ubiquitin E3 Ligase (Parkin) related Proteins and their functional elucidation

Figure 3.1*:* Interproscan result for Parkin

Table 3.1*:* Hunted Parkin related proteins

S.No.	Accession	Protein	Score	Identity	E Value
1	AGH62057.1	PARK2 splice variant	686	100%	0
2	BAA25751.1	Parkin	680	99%	0
3	ABN46990.1	Parkin 2	679	99%	0
4	4K95_A	Chain A, Crystal Structure Of Parkin	624	90%	0
5	Q9JK66.1	E3 ubiquitin-protein ligase Parkin	623	89%	0

3.3.2. Phylogenetic relationship and Physico-chemical properties

For multiple sequence analysis, *Muscle software* was used and found that amino acid residues were conserved in most of the isoforms of the Ubiquitin E3 ligase Parkin. Phylogenetic study of Parkin hunted proteins revealed that Parkinand PARK2 splice variant were different from others **(Figure-3.2 (a & b)).**

(a) Multiple Sequence Alignment of all Parkin isoforms

(b) Tree generation for Parkin using NJ Plot without distance correction

Figure 3.2: (a) Multiple Sequence Alignment of all Parkin isoforms and (b) Tree generation for Parkin using NJ Plot without distance correction

However, another Chain A, Crystal Structure of Parkin and E3 ubiquitin-protein ligase Parkin were in same cluster as share more homology while Parkin 2 was in another cluster. ProtParam showed that Mol. wt. of Parkin 2was 36393.5 Daltons and an isoelectric point was 7.06 which indicate that Parkin had slightly positive charged respectively. The GRAVY index of -0.372 for Parkinis indicative of hydrophilic (**Table 3.2)**.

Table 3.2: Physico-chemical properties of Parkin

Properties	Parkin
Molecular Formula	$C_{1568}H_{2420}N_{458}O_{468}S_{38}$
Molecular Weight (Daltons)	36393.5
Theoretical PI	7.06
Aliphatic Index	60.28
Grand Average of Hydropathicity (GRAVY)	-0.372

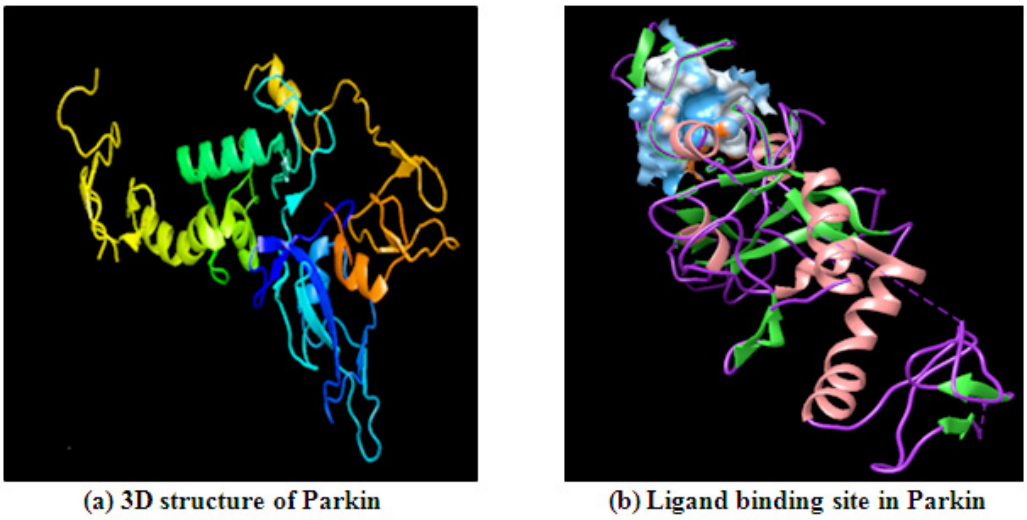
3.3.3. Homology modeling 3D structure Visualization and quality assessment of retrieved proteins

Prediction of 3D structure of proteins provides us precise functional information of how proteins interact and localize in their stable conformation. Homology modelling is one of the most common structure prediction tools in proteomics and genomics. The best matching template 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. Template showed sequence identity of 100% for Parkin isoforms. 3D structure of Parkinwas 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. SWISS MODEL has provided Z score of the template and query model. Z score of Parkin has been shown in **(Table 3.3)**, suggesting a high-quality structure for docking studies.

Table 3.3: Swiss Model server result showing template structure used in homology modelling, Sequence Identity and quality score of the model generated

Gene Name	Modeled residue range	Based on template	Sequence Identity	QMEAN Z-Score
Parkin	1-325	4I1H	100%	-0.81

3D structure of Parkin (PDB ID 4I1H) was retrieved from Protein Data Bank (PDB). Even though there were no steric clashes in the structure generated, these were assessed for geometric and energy aspects **(Figure-3.3 (a)).**



(a) 3D structure of Parkin (b) Ligand binding site in Parkin

Figure 3.3: (a) Demonstrates 3D structure of Parkin, (b) ligand binding site in Parkin.

Initial screening of the molecules was done on the basis of ADMET parameters **(Figure 3.4)**. ADMET analysis revealed that out of fourteen biomolecules four molecules (Naringenin, Quercetin, Resveratrol and Sesamol) could act like a drug as they meet the criteria of all three parameters such as Lipinski Rule of five, Ghosh and Verbe. However, the most important property required of a compound intends to be a neuroprotective agent is BBB permeability. These all four biomolecules qualified all above mentioned parameters and scored well on bioavailability, pharmacokinetics and could cross the BBB. We have validated of these finding through *in vitro* study in chapter 5.

Compounds	Physicochemical Properties				Pharmacokinetics		Lipophilicity	Drug-likeness				Water Solubility
	Mol. Weight	H-bond Acceptors	H-bond Donors	Molar Refractivity	GI Absorption	BBB Permeability	XLogP	Lipinski	Ghose	Veber	Bioavailability Score	Log S (EOSL)
Curcumin	368	6	2	102	High	No	3.2	Yes	Yes	Yes	0.55	-3.94
Valproic acid	144	2	1	42	High	No	2.75	Yes	No	Yes	0.56	-2.14
Vitamin B12	1355	20	9	351	Low	No	-3.85	No	No	No	0.17	-4.11
Telmisartan	514	4	1	157	Low	No	-3.85	No	No	Yes	0.56	-7.5
Deferoxamine	560	9	6	142	Low	No	-3.85	No	No	No	0.17	-0.14
Dabigatran	471	6	4	134	Low	No	-3.85	Yes	No	No	0.55	-3.62
SB 203580	377	4	1	105	High	No	-3.85	Yes	No	Yes	0.55	-4.56
Naringenin	**272**	**5**	**3**	**71**	**High**	**Yes**	**-3.85**	**Yes**	**Yes**	**Yes**	**0.55**	**-3.49**
Quercetin	**302**	**7**	**5**	**78**	**High**	**Yes**	**-3.85**	**Yes**	**Yes**	**Yes**	**0.55**	**-3.16**
Sesamol	**138**	**3**	**1**	**44**	**High**	**Yes**	**-3.85**	**Yes**	**No**	**Yes**	**0.55**	**-1.92**
Mimosine	198	5	3	48	High	No	-3.85	Yes	No	Yes	0.55	1.56
Vitamin D	384	1	1	125	Low	No	-3.85	Yes	No	Yes	0.55	-6.84
Orexin-B	2936	40	41	736	Low	No	-3.85	No	No	No	0.17	-1.78
Resveratrol	**228**	**3**	**3**	**67**	**High**	**Yes**	**-3.85**	**Yes**	**Yes**	**Yes**	**0.55**	**-3.62**

Figure 3.4: Drug Likeness and ADMET analysis

3.3.4. Active site prediction and molecular docking analysis of Parkin with identified molecules

CastP server was used to predict the ligand binding site. This server calculates the possible active sites from the 3D atomic coordinates of the proteins. For Parkin, residues involved in ligand binding site, site volume and volume of protein for thirty active sites were predicted. Among the thirty binding sites obtained from CastP for Parkin, site 30 was highly conserved within the active site of the protein **(Figure-3.3(b)).** The Predicted site 30 consisted 1059 Cubic angstroms site volume out of the 3741 Cubic Angstroms of protein volume. The residues in site 30 are TYR^{143}, THR^{231}, ASN^{232}, SER^{233}, ARG^{234}, ASN^{235}, ILE^{236}, THR^{237}, ARG^{256}, GLN^{400}, ARG^{402}, TRP^{403}, GLU^{404}, ALA^{405}, ALA^{406}, SER^{407}, LYS^{408}, GLU^{409}, THR^{410}, LYS^{412}, LYS^{413}, THR^{414}, LYS^{416}, LYS^{427}, GLY^{429}, GLY^{430}, MET^{432}, TRP^{447}, ASN^{448}, ASP^{464}, VAL^{465} and also illustrated in **(Table 3.5)**.

3.3.5. Molecular docking pattern of biomolecules with Parkin

Molecular docking pattern of Parkin with screened molecules (Naringenin, Quercetin, Resveratrol and Sesamol) have been identified and depicted in **(Figure 3.5)**.

3.3.5. 1. Parkin interaction with Naringenin

Free energy of binding with Naringenin was -7.12 kcal/mol and Est. Binding Constant, K_b was found to be 6.08 µM **(Figure-3.5 (a))** Intermolecular Energy was found to be -8.31 kcal/mol. VdW + Hbond + desolv Energy and Electrostatic Energy

was -8.19 kcal/mol and -0.12 kcal/mol. Total Internal Energy and Torsional Free Energy was found to be 9.71 kcal/mol and 1.19 kcal/mol.

3.3.5.2. Parkin interaction with Resveratrol

Free energy of binding with Resveratrol was -6.69 kcal/mol and Est. Binding Constant, K_b was found to be 12.43 μM **(Figure-3.5 (b))** Intermolecular Energy was found to be -8.48 kcal/mol. VdW + Hbond + desolv Energy and Electrostatic Energy was -8.47 kcal/mol and -0.02 kcal/mol. Total Internal Energy and Torsional Free Energy was found to be 17.26 kcal/mol and 1.79 kcal/mol.

3.3.5.3. Parkin interaction with Quercetin

Free energy of binding with Quercetin was -7.60 kcal/mol and Est. Binding Constant, K_b was found to be 2.67 μM. **(Figure-3.5 (c))** Intermolecular Energy was found to be -9.39 kcal/mol. VdW + Hbond + desolv Energy and Electrostatic Energy was -9.15 kcal/mol and -0.25 kcal/mol. Total Internal Energy and Torsional Free Energy was found to be 9.67 kcal/mol and 1.19 kcal/mol.

3.3.5.4. Parkin interaction with Sesamol

Free energy of binding with Sesamol was -4.99 kcal/mol and Est. Binding Constant, K_b was found to be 221.78 μM **(Figure-3.5 (d))** Intermolecular Energy was found to be -5.28 kcal/mol. VdW + Hbond + desolv Energy and Electrostatic Energy was -5.17 kcal/mol and -0.11 kcal/mol. Total Internal Energy and Torsional Free Energy was found to be 0.34 kcal/mol and 0.30 kcal/mol.

On the basis of docking analysis, interacting compounds with minimum binding constant and highest negative free energy of binding are most effective. Docking calculation of Parkin has been presented in **(Table 3.4).**

Table 3.4: Docking calculation of compounds with Parkin

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
Naringenin	-7.12 (kcal/mol)	6.08 µM	-8.31 (kcal/mol)	-8.19 (kcal/mol)	-0.12 (kcal/mol)	+9.71 (kcal/mol)	+1.19 (kcal/mol)
Quercetin	-7.60 (kcal/mol)	2.67 µM	-9.39 (kcal/mol)	-9.15 (kcal/mol)	-0.25 (kcal/mol)	+9.67 (kcal/mol)	+1.79 (kcal/mol)
Resveratrol	-6.69 (kcal/mol)	12.43 µM	-8.48 (kcal/mol)	-8.47 (kcal/mol)	-0.02 (kcal/mol)	+17.26 (kcal/mol)	+1.79 (kcal/mol)
Sesamol	-4.99 (kcal/mol)	221.78 µM	-5.28 (kcal/mol)	-5.17 (kcal/mol)	-0.11 (kcal/mol)	+0.34 (kcal/mol)	+0.30 (kcal/mol)

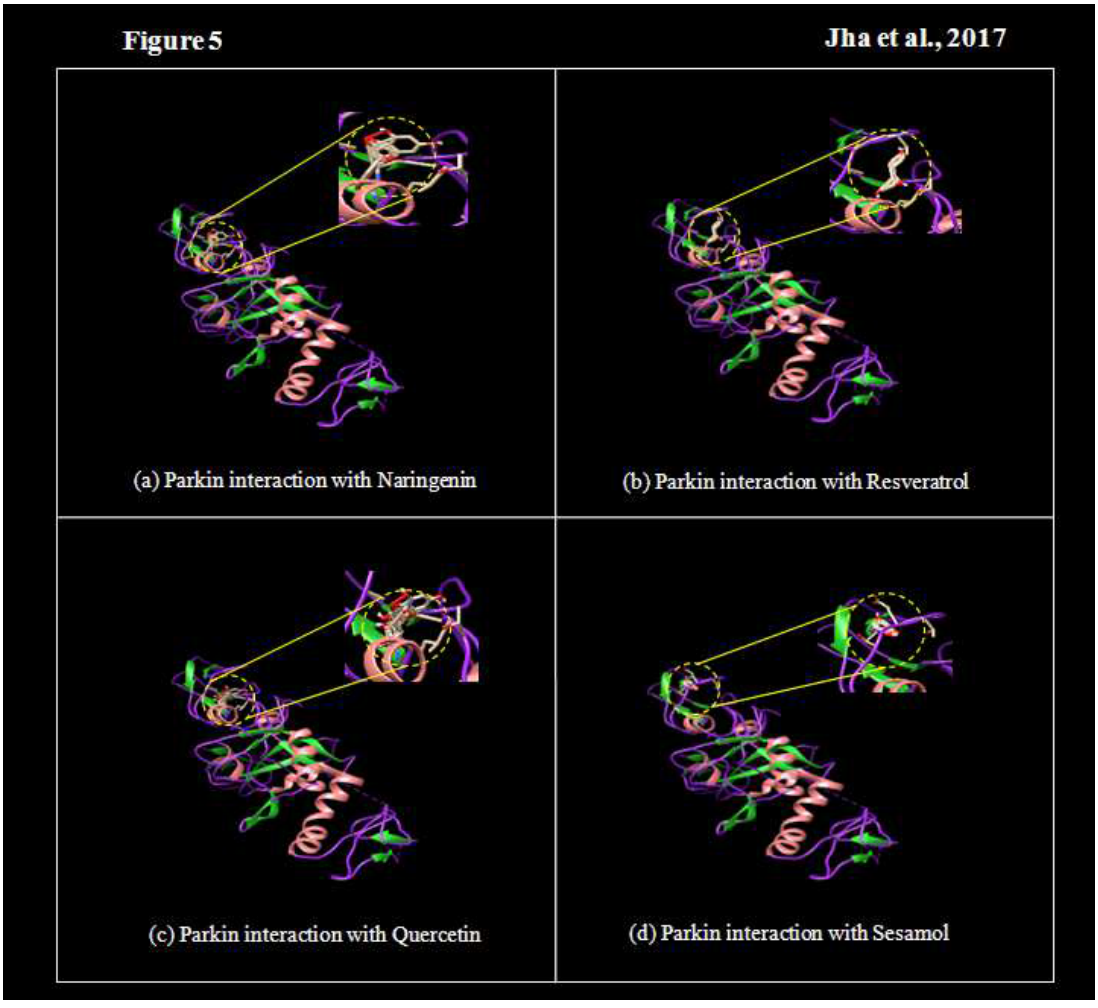


Figure 3.5: Docking study of Parkin protein with selected compounds, (a) Parkin interaction with Naringenin, (b) Parkin interaction with Resveratrol, (c) Parkin interaction with Quercetin, and (d) Parkin interaction with Sesamol.

3.3.6. Binding site of Parkin with selected compounds along with its known Stimulatory Active Site

Binding site residues of Parkin interacting with Naringenin, Quercetin, Resveratrol and Sesamol were found to be the same as the residues involved in their respective catalytic sites. Interacting residues of Parkin with Naringenin, Quercetin, Resveratrol and Sesamol along with their identified catalytic sites have been show in **(Table 3.5)** and their 2D and 3D pattern of interaction is presented in **(Figure 3.6)**. Interacting residues of Parkin with its activator were TYR143, THR231, ASN232, SER233, ARG234, ASN235, ILE236, THR237, ARG256, GLN400, ARG402, TRP403, GLU404, ALA405, ALA406, SER407, LYS408, GLU409, THR410, LYS412, LYS413, THR414, LYS416, LYS427, GLY429, GLY430, MET432, TRP447, ASN448, ASP464 and VAL465. Interacting residues in case of Naringenin **(Figure-3.6 (a & e))** were ARG234, ASN235, ILE236, THR237, GLN400, ARG402, TRP403, GLU404, ALA405, ALA406, SER407 and LYS408. Interacting residues in case of Quercetin **(Figure-3.6 (b & f))** were ARG234, ASN235, ILE236, THR237, GLN400, ARG402, TRP403, ALA405, ALA406 and SER407. Interacting residues in case of Resveratrol **(Figure-3.6 (c & g))** were ARG234, ASN235 ILE236, THR237, GLN400, SER407, LYS408 and THR410. Furthermore, Interacting residues in case of Sesamol **(Figure-3.6 (d & h))** were GLU409, THR410, LYS412, LYS413 and THR414.

Table 3.5: Parkin known binding site and selected compounds interacting residues

Compound	Interacting residues
Predicated catalytic Site	TYR^{143}, THR^{231}, ASN^{232}, SER^{233}, ARG^{234}, ASN^{235}, ILE^{236}, THR^{237}, ARG^{256}, GLN^{400}, ARG^{402}, TRP^{403}, GLU^{404}, ALA^{405}, ALA^{406}, SER^{407}, LYS^{408}, GLU^{409}, THR^{410}, LYS^{412}, LYS^{413}, THR^{414}, LYS^{416}, LYS^{427}, GLY^{429}, GLY^{430}, MET^{432}, TRP^{447}, ASN^{448}, ASP^{464} and VAL^{465}.
Naringenin	ARG^{234}, ASN^{235}, ILE^{236}, THR^{237}, GLN^{400}, ARG^{402}, TRP^{403}, GLU^{404}, ALA^{405}, ALA^{406}, SER^{407} and LYS^{408}.
Quercetin	ARG^{234}, ASN^{235}, ILE^{236}, THR^{237}, GLN^{400}, ARG^{402}, TRP^{403}, ALA^{405}, ALA^{406} and SER^{407}.
Resveratrol	ARG^{234}, ASN^{235} ILE^{236}, THR^{237}, GLN^{400}, SER^{407}, LYS^{408} and THR^{410}.
Sesamol	GLU^{409}, THR^{410}, LYS^{412}, LYS^{413} and THR^{414}.

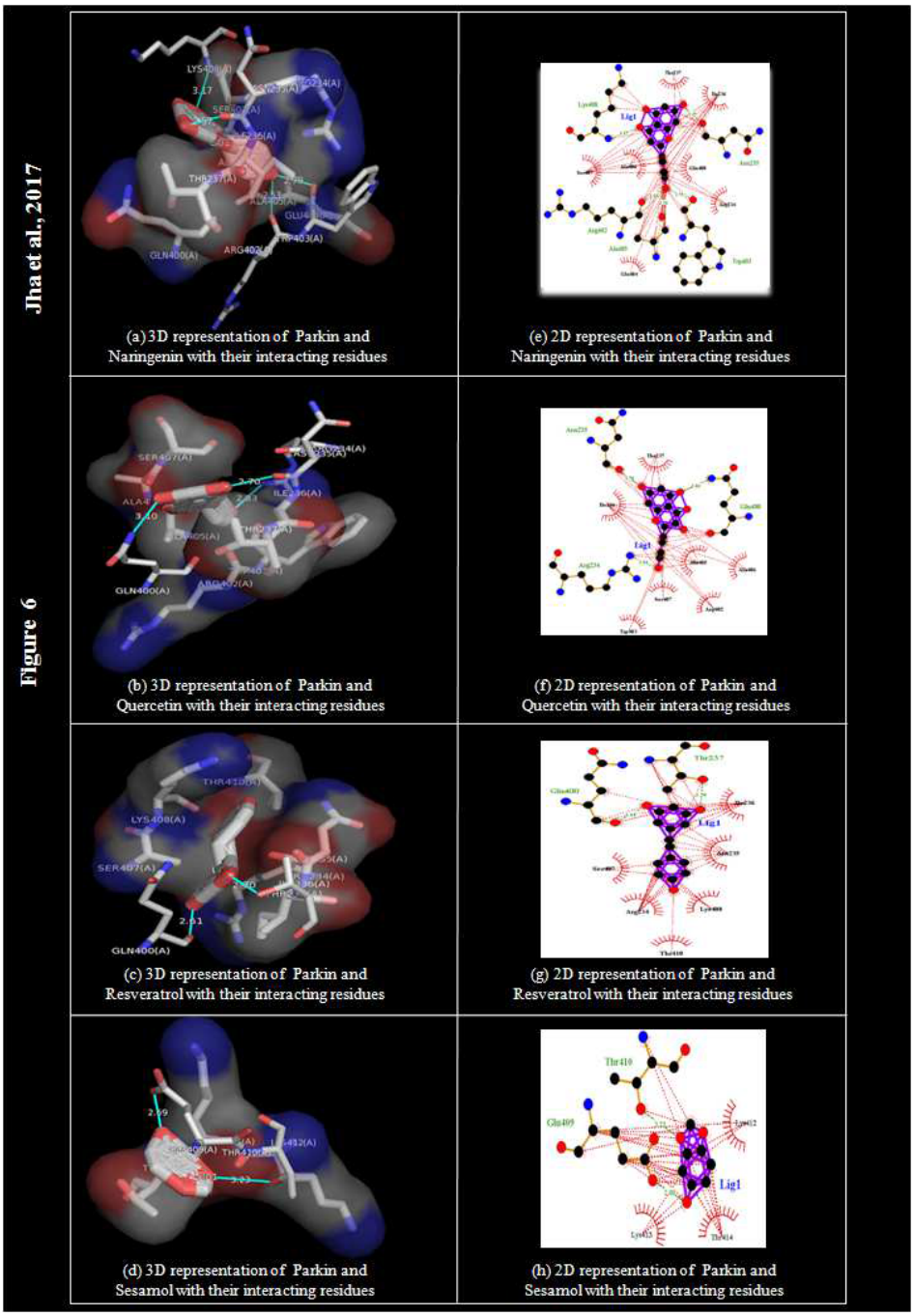


Figure 3.6: Binding site of Parkin with selected compounds along with its known stimulatory active site. (a) Three-dimensional (3D) presentation of Parkin and Naringenin with their interacting residues, (b) Quercetin with their interacting residues, (c) Resveratrol with their interacting residues, (d) Sesamol with their interacting residues, (e) Two dimensional (2D) representation of Parkin and Naringenin with their interacting residues, (f) Quercetin with their interacting residues, (g) Resveratrol with their interacting residues, and (h) Sesamol with their interacting residues

3.4. DISCUSSION

This research introduced the novel potential of biomolecules, which could be applied for therapeutic intervention in PD progression. Mutations in PD associated genes such as Parkin potentially lead to autosomal recessive PD (Gupta *et al*., 2015). Moreover, this gene displays characteristic ubiquitin E3 ligase activity. Parkin is ubiquitously expressed in a number of pathways associated with PD pathogenesis and has ubiquitin E3-ligase activity which also reduces α-synuclein aggregation (Dharani *et al*., 2016).Thus, it seems imperative to design therapeutic strategies aimed at elevating the level of Parkin to improve neuronal survival in PD. Further, various studies have advocated that several compounds of plant origin possess neuroprotective properties, however, their mode of action have not been clearly defined. Initially, we screened the compounds for ADMET and pharmacokinetics analysis. Most drugs fail on poor solubility which we have identified and selected through literature survey. Admet analysis of all the selected compounds (Naringenin, Quercetin Sesamol and Resveratrol) revealed that these compounds could act like a drug and have drug like property as these compounds meet the criteria of Admet parameters. It is evident that *in vivo* bioavailability of an orally administered drug is largely dependent on its aqueous solubility and dissolution in GI fluids (Khadka *et al*. 2014). More the water solubility and GI permeability, better the bioavailability. Similarly, lipophilicity of a drug affects various physiological properties such as the rate of metabolism, transport across cell membrane and interaction with binding sites of receptor. Further, drugs intended for CNS should have logP value less than four (Chico *et al*. 2009; Wager *et al*. 2010). All these four compounds (Naringenin, Quercetin Sesamol and Resveratrol) showed logP values of -3.85. However, the most important property required of a compound to be a protective agent is the ability to cross Blood brain barrier (BBB). As expected, most compounds failed the BBB permeability parameter. But four biomolecules namely, (Naringenin, Quercetin Sesamol and Resveratrol) could cross the BBB and combined with their high GI absorption, least violations of drug likeness and good bioavailability score, were the best candidates for targeting neuronal damage in our study. Further, based on docking study analysis, the present study provide scientific evidence that given four biomolecules namely (Naringenin, Quercetin Sesamol and Resveratrol) are interacting at the reported binding site of

Parkin. Further, Binding Constant, K_b of Naringenin, Quercetin, Resveratrol and Sesamol for Parkin were found to be 6.08 µM, 2.67 µM, 12.43 µM and 221.78 µM respectively, suggesting that all the selected compounds were effective as Ubiquitin E3 Ligase activators. Investigation of active binding sites within Parkin protein gives a better idea for a valuable drug target site and drug interaction with highest affinity. Recently, researchers reported about the different binding energy of L-Dopa with various targets. These targets molecules were α –synuclein, MAO-B, COMT, UCHL-1, α-Synuclein, Apo site, Dopamine D3 Receptor, Glycogen Synthase Kinase-3β, Parkin, and Tyrosine 3-Hydroxylase with binding energy -4.44, -4.4, -5.22, -4.24, -7.3, -5.8, -5.8, -5.3 and -6.9 kcal/mol respectively (Nagappan *et al*. 2015 & Thangarajan *et al*. 2016). While, in our study we have reported the energy value of Quercetin with Parkin is (-7.60 kcal/mol), which is highest negative free energy value compare with L-Dopa targeting Parkin (-5.8 kcal/mol). So on this background, Quercetin is showing good interaction with Parkin as compared to L-Dopa and hence could be used as a potential drug for treating PD. Additionally, in this study the most effective compound was found to be Quercetin is having minimum binding Constant, K_b and highest negative free energy of binding with maximum interacting surface area with reported highly conserved active site within Parkin protein in course of docking studies (Engels *et al*., 2017). Hence, this chapter is showing for the first-time neuroprotective effect of Quercetin with Parkin in PD pathogenesis.

The next chapter discusses the other Ubiquitin E3 ligase DJ-1can be one of the potential target for all shortlisted biomolecules solve the “targeted therapy crisis” problem in PD.

Chapter IV

An In silico study of Naringenin Mediated Neuroprotection in Parkinson's Disease

CHAPTER IV

AN *IN SILICO* STUDY OF NARINGENIN MEDIATED NEUROPROTECTION IN PARKINSON'S DISEASE

4.1. OBJECTIVE

This chapter discusses the dietary biomolecule of Naringenin with broad spectrum of activities which protects neurons from various neurotoxic insults and improves cognition and motor function in neurodegenerative diseases. DJ-1 has both, Ubiquitin E3 ligase as well as chaperonic activity and loss of ubiquitin E3 Ligase activity of DJ-1 has been found to be associated with familial PD. Naringenin induced E3 ligase activity of DJ-1 which can have possible clinical relevance in PD.

4.2. INTRODUCTION

PD is the second most common neurodegenerative disorder characterized by dopamine depletion in the striatum (Massano *et al*., 2012). The dramatic loss of neuromelanin containing dopaminergic neurons is convoyed by the presence of Lewy bodies in the remaining neurons. The hallmark of PD includes fibrillar cytoplasmic inclusions consisting of aggregated and abnormally accumulated proteins, the most prominent being α-synuclein, neurofilaments, ubiquitin, and ubiquitinated proteins (Gundersen, 2010). Additionally, at least 13 loci and nine genes have been proposed to be linked with PD, but only six genes are widely accepted to be associated with Mendelian forms of the disease (Exner *et al*., 2012). Mutations in these genes potentially lead to autosomal dominant (α - synuclein and LRRK2), or autosomal recessive PD (Parkin, PINK1, DJ1, and ATP13A2) respectively. Recent epidemiological studies have shown that less than 10% of PD cases are of familial origin with the majority being sporadic (Pirkevi *et al*., 2009). The sporadic form of PD is caused by mutated DJ1 which shows reduced nuclear localization and translocation to mitochondria (Cookson *et al*., 2012). DJ-1 is ubiquitously expressed in a number of pathways associated with PD pathogenesis and has ubiquitin E3-ligase activity which also reduces α-synuclein aggregation (Saurabh *et al*., 2009). Although mutations associated with DJ-1 lead to onset of familial PD, the exact mechanism behind the pathogenesis are still unknown (Christine *et al*., 2012). Further, various studies have

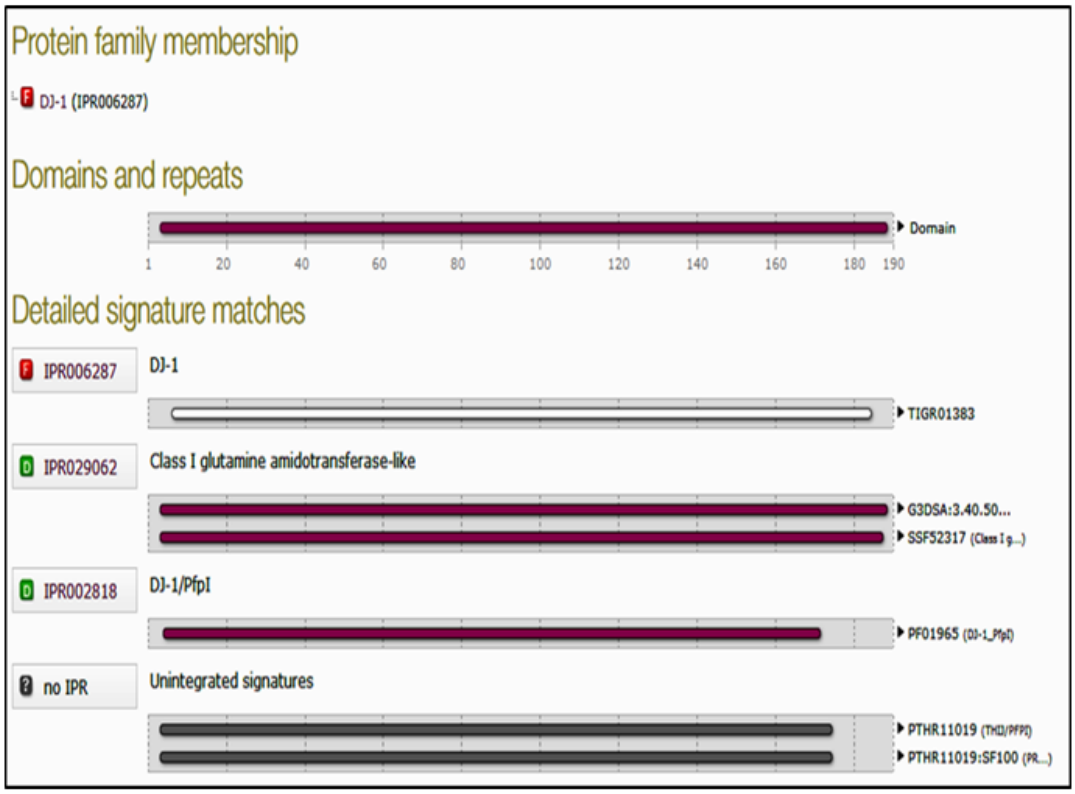
advocated that several compounds of plant origin possess neuroprotective properties, however, their mode of action have not been clearly defined (Saikat *et al*., 2010). In this study, we have initially screened four biomolecules namely Naringenin, Quercetin, Resveratrol and Sesamol based on Lipinski's rule of five. These biomolecules are found in fruits and vegetables and have various beneficial effects such as antioxidative, activation of survival genes and signalling pathways, chelation of transition metal, regulating mitochondrial function and modulating neuro inflammation. Further, these biomolecules interact with significant neuronal signalling cascades that lead to inhibition of apoptosis enhanced by the neurotoxic species and promote neuronal endurance and differentiation (Jeremy, 2007). They selectively target a number of protein kinase and lipid kinase signalling cascades, importantly, the PI3K/Akt and MAP kinase pathways which modulate pro-survival transcription factors and gene expression (Jha *et al*., 2015). Interestingly, Naringenin treatment prominently suppressed oxidative stressors, improved levels of enzymatic antioxidants and neurotransmitter significantly (Lobo *et al*., 2010). In this study, biomolecules which exhibit neuroprotective activities were subjected to docking simulations using AutoDock 4.2.1. The Preliminary investigation revealed Naringenin as the best potential biomolecule among all given four biomolecules based on minimum inhibition constant, Ki and highest negative free energy of binding with maximum interacting surface area with the active site of DJ-1 in a course of docking study. Based on *in silico* experimentation, Naringenin is a seemingly new prospect for therapeutic intervention in PD. Therefore, a comprehensive understanding of the molecular mechanism associated with Naringenin mediated therapeutics could contribute towards clinical significance in PD biology.

4.3. EXPERIMENTAL AND RESULTS

4.3.1. Retrieval of Ubiquitin E3 Ligase DJ-1Proteins and their functional 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 protein DJ-1 (**Table 4.1)** on the basis of families and domains identified from Interproscan results. Interproscan study revealed that all

homologues proteins for DJ-1, all homologous proteins were belonging to DJ-1 family (IPR006287), glutamine amido transferase-like domain (IPR029062) and DJ-1/PfpI domain (IPR002818) respectively (**Figure-4.1).**



Retrieval of Ubiquitin E3 Ligase (DJ-1) related Proteins and their functional elucidation

Figure 4.1: Interproscan result for DJ-1

Table 4.1: Hunted DJ-1 related proteins

S.No.	Accession	Protein	Score	Identity	E Value
1	NP_009193.2	protein DJ-1	368	98%	5.00e-127
2	3BWE_A	Chain A, Crystal Structure Of Aggregated Form Of DJ-1	365	100%	6.00e-126
3	4OQ4_A	Chain A, Crystal Structure Of E18a Human DJ-1	361	97%	2.00e-124
4	1J42_A	Chain A, Crystal Structure Of Human DJ-1	363	97%	2.00e-125
5	ADQ32403.1	Parkinson disease (autosomal recessive, early onset) 7	364	97%	1.00e-125

4.3.2. Phylogenetic relationship and Physico-chemical properties

For multiple sequence analysis, *Muscle software* was used and found that amino acid residues were conserved in most of the isoforms of the Ubiquitin E3 ligase DJ-1. Phylogenetic study of DJ-1 hunted proteins revealed that Parkinson disease (autosomal recessive, early onset) 7 and protein DJ-1 were differing from others (**Figure-4.2(a & b)).** However, another Chain A (Crystal Structure of Aggregated Form of DJ-1 Chain A) and Chain A (Crystal Structure of E18a Human DJ-1) were in same cluster as share more homology while Crystal Structure of Human DJ-1 was in another cluster. ProtParam showed that Mol. wt. of DJ-1 was 19848.7 Daltons and an isoelectric point of DJ-1 was 6.37 which indicate that DJ-1 was negatively charged respectively. Furthermore, GRAVY index of -0.47 for DJ-1 is indicative of hydrophilic (**Table 4.2)**.

Figure 4.2: (a) Multiple Sequence Alignment of all DJ-1 isoforms and (b) Tree generation for DJ-1 using NJ Plot without distance correction

Table 4.2: Physico-chemical properties of DJ-1

Properties	**DJ-1**
Molecular Formula	$C_{874}H_{1448}N_{242}O_{268}S_8$
Molecular Weight (Daltons)	19848.7
Theoretical PI	6.37
Aliphatic Index	99.11
Grand Average of Hydropathicity (GRAVY)	-0.047

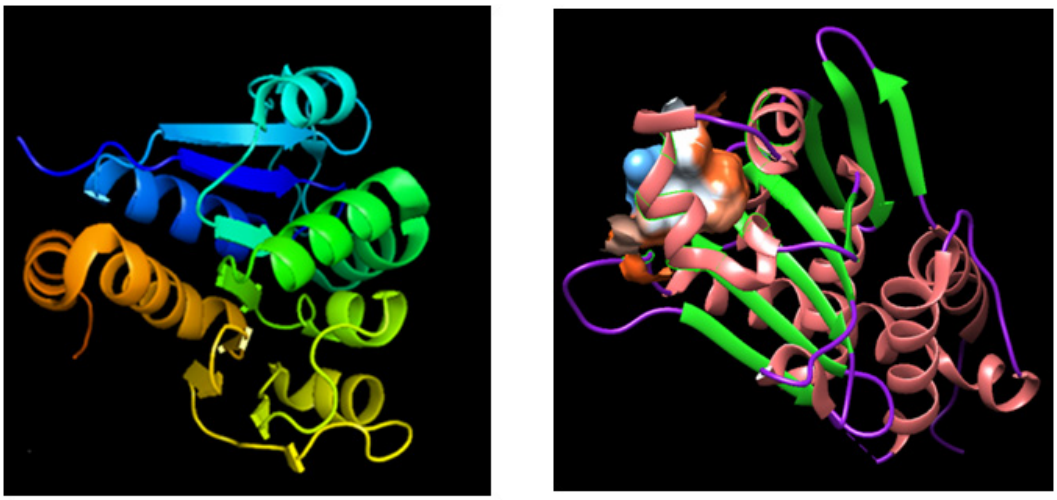
4.3.3. Homology modelling, 3D structure Visualization and quality assessment of retrieved proteins

Prediction of 3D structure of proteins provides us precise functional information of how proteins interact and localize in their stable conformation. Homology modelling is a structural prediction tools in proteomics and genomics. The best matching template 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. Template showed sequence identity of 100% for DJ-1 isoforms. 3D structure of DJ-1 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. SWISS MODEL has provided Z score of the template and query model. Further, Z score for DJ-1 has been shown in (**Table 4.3)**, suggesting a good structure.

Table 4.3: Swiss Model server result showing template structure used in homology modelling, Sequence Identity and quality score of the model generated.

Gene Name	**Modeled residue range**	**Based on template**	**Sequence Identity**	**QMEAN Z-Score**
DJ-1	2-185	4ZGG	100%	-0.42

3D structure of DJ-1(PDB ID 4ZGG) was retrieved from Protein Data Bank (PDB). Even though there were no steric clashes in the structure generated, these were assessed for geometric and energy aspects (**Figure-4.3 (a)).**

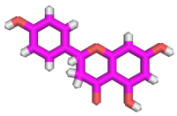
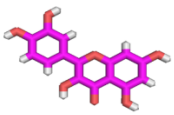
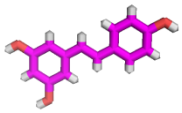
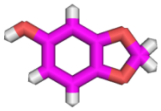


(a) 3D structure of DJ-1 (b) Ligand binding site in DJ-1

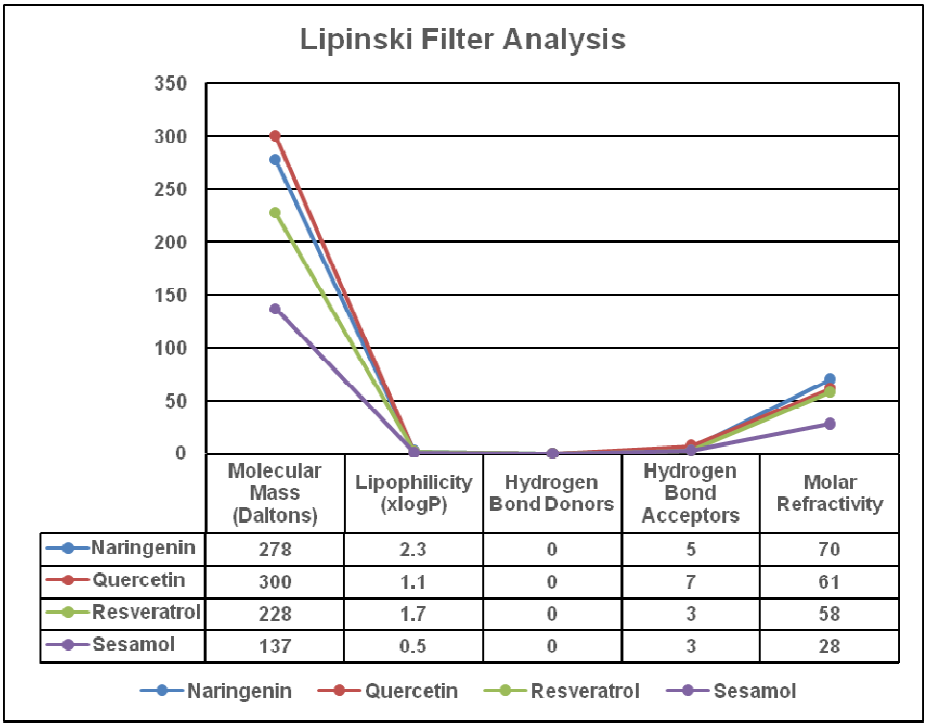
Figure 4.3: (a) Demonstrates 3D structure of DJ-1, (b) ligand binding site in DJ-1.

4.3.4. Physico-chemical Properties and Lipinski Filter Analysis retrieved of ligands

Table 4.4: Physico-chemical Properties of ligands

Characteristics	Naringenin	Quercetin	Resveratrol	Sesamol
Molecular weight	272.25 g/mol	302.23 g/mol	228.24 g/mol	138.12 g/mol
Molecular Formula	$C_{15}H_{12}O_5$	$C_{15}H_{10}O_7$	$C_{14}H_{12}O_3$	$C_7H_6O_3$
Molecular Structure				
IUPAC Name	5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	5-[(E)-2-(4-hydroxyphenyl) ethenyl]benzene-1,3-diol	1,3-benzodioxol-5-ol
xLogP	2.4	1.5	3.1	1.2
Hydrogen Bond Donor	3	5	3	1
Hydrogen Bond Acceptor	5	7	3	3
Rotatable Bond Count	1	1	2	0
Topological Polar Surface Area	$87A^2$	$127A^2$	$60.7A^2$	$38.7A^2$
Heavy Atom Count	20	22	17	10
Complexity	363	488	246	126
Covalently Bonded Unit Count	1	1	1	1

Initial screening of the molecules was done on the basis of Lipinski's rule of five (**Figure 4.4)**. Lipinski filter analysis revealed that all these molecules (Naringenin, Quercetin, Resveratrol and Sesamol) could act like a drug as they meet the criteria of Lipinski Rule of five.



	Molecular Mass (Daltons)	Lipophilicity (xlogP)	Hydrogen Bond Donors	Hydrogen Bond Acceptors	Molar Refractivity
Naringenin	278	2.3	0	5	70
Quercetin	300	1.1	0	7	61
Resveratrol	228	1.7	0	3	58
Sesamol	137	0.5	0	3	28

Figure 4.4: Drug Likeness prediction using Lipinski Filter Analysis

4.3.5. Active site prediction and molecular docking analysis of DJ-1 with identified molecules

CastP server was used to predict the ligand binding site. This server calculates the possible active sites from the 3D atomic coordinates of the proteins.For DJ-1, residues involved in ligand binding site, site volume and volume of protein for thirty three active sites were predicted (**Figure 4.3 (b)).** Among the thirty three binding sites obtained from CastP for DJ-1, site thirty three was highly conserved within the active site of the protein. The Predicted site thirty three consisted 435.6 Cubic angstroms site volume out of the 1723 Cubic Angstroms of protein volume. The residues in site 33 are PRO^{109}, THR^{110}, LEU^{112}, LEU^{113}, ALA^{114}, GLU^{116}, VAL^{123}, THR^{125}, PRO^{127}, ALA^{129}, LYS^{130}, LYS^{132}, ASN^{135}, GLY^{137}, HIS^{138}, TYR^{139}, TYR^{141}, GLU^{143} ARG^{156} and also shown in (**Table 4.6)**.

4.3.6. Molecular docking pattern of biomolecules with DJ-1

Molecular docking pattern of DJ-1 with screened molecules (Naringenin, Quercetin, Resveratrol and Sesamol) have been identified and depicted in (**Figure 4.5)**.

4.3.6.1. DJ-1 interaction with Naringenin

Free energy of binding with Naringenin was -4.19 kcal/mol and Est. Binding Constant, K_b was found to be 851.70 μM. **(Figure-4.5 (a))** Intermolecular Energy was found to be -5.38 kcal/mol. VdW + Hbond + desolv Energy and Electrostatic Energy was -5.25 kcal/mol and -0.13 kcal/mol. Total Internal Energy and Torsional Free Energy was found to be 9.69 kcal/mol and 1.19 kcal/mol.

4.3.6.2. DJ-1 interaction with Resveratrol

Free energy of binding with Resveratrol was -3.24 kcal/mol and Est. Binding Constant, K_b was found to be 4.25 mM. **(Figure-4.5 (b))**Intermolecular Energy was found to be -5.03 kcal/mol. VdW + Hbond + desolv Energy and Electrostatic Energy was -4.91 kcal/mol and -0.12 kcal/mol. Total Internal Energy and Torsional Free Energy was found to be 17.09 kcal/mol and 1.79 kcal/mol.

4.3.6.3. DJ-1 interaction with Quercetin

Free energy of binding with Quercetin was -3.97 kcal/mol and Est. Binding Constant, K_b was found to be 1.24 mM. **(Figure-4.5 (c))** Intermolecular Energy was found to be -5.76 kcal/mol. VdW + Hbond + desolv Energy and Electrostatic Energy was -5.54 kcal/mol and -0.22 kcal/mol. Total Internal Energy and Torsional Free Energy was found to be 9.44 kcal/mol and 1.79 kcal/mol.

4.3.6. 4. DJ-1 interaction with Sesamol

Free energy of binding with Sesamol was -3.08 kcal/mol and Est. Binding Constant, K_b was found to be 5.51 mM. **(Figure-4.5 (d))**Intermolecular Energy was found to be -3.38 kcal/mol. VdW + Hbond + desolv Energy and Electrostatic Energy was -3.12 kcal/mol and -0.26 kcal/mol. Total Internal Energy and Torsional Free Energy was found to be 0.32 kcal/mol and 0.30 kcal/mol.

On the basis of docking analysis, interacting compounds with minimum binding constant and highest negative free energy of binding are most effective. Docking calculation of DJ-1 has been presented in (**Table 4.5).**

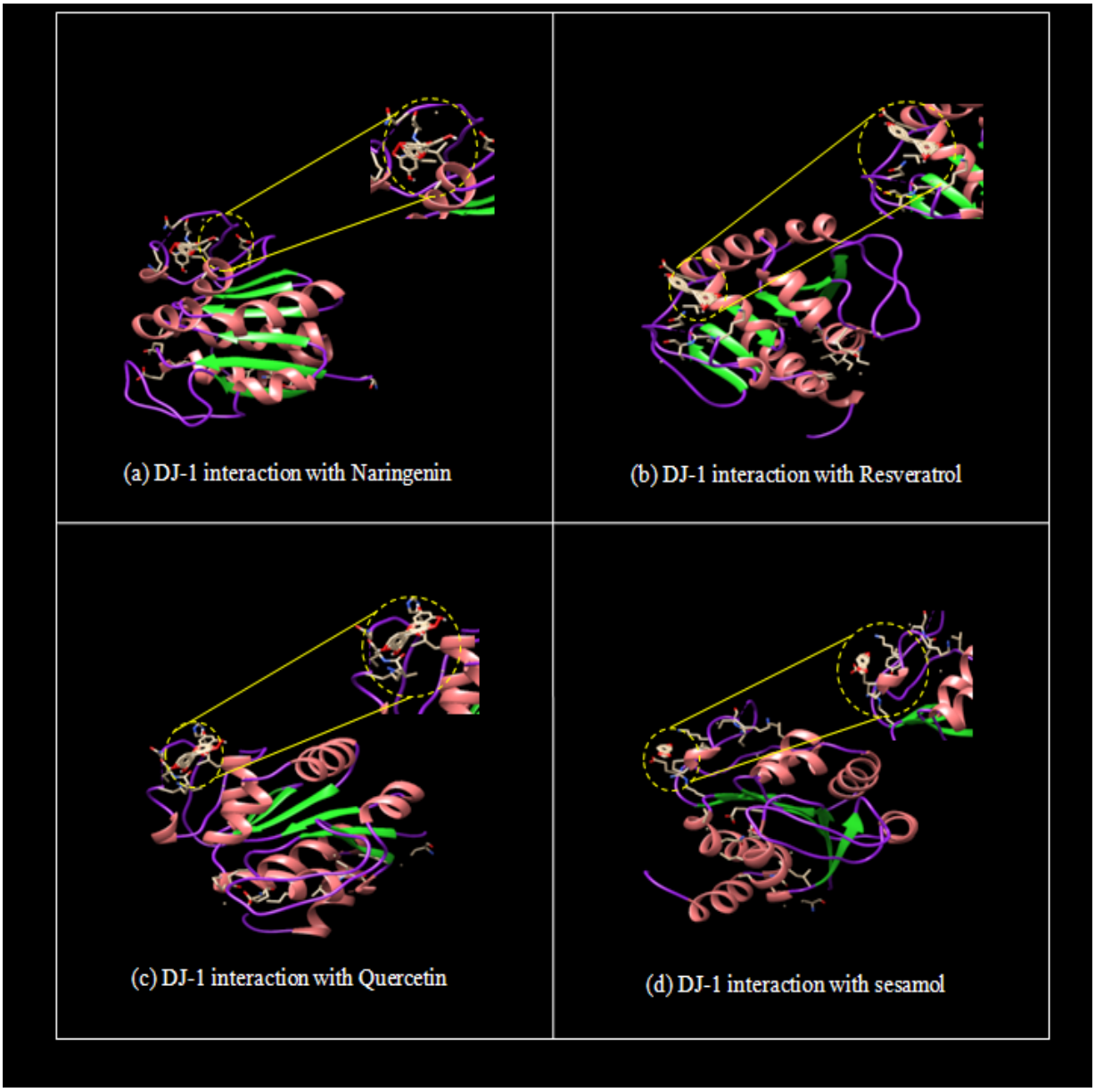


Figure 4.5: Docking study of DJ-1 protein with selected compounds, (a) DJ-1 interaction with Naringenin, (b) DJ-1 interaction with Resveratrol, (c) DJ-1 interaction with Quercetin, and (d) DJ-1interaction with Sesamol.

Table 4.5: Docking calculation of compounds with DJ-1

Compound Name	Est. Free Energy of Binding	Est. Binding Constant	Est. Inter molecular Energy	vdW+Hbond+ desolv Energy	Electrostatic Energy	Est. Internal Energy	Torsional Free Energy
Naringenin	-4.19 (kcal/mol)	851.70 µM	-5.38 (kcal/mol)	-5.25 (kcal/mol)	-0.13 (kcal/mol)	+9.69 (kcal/mol)	+1.19 (kcal/mol)
Quercetin	-3.97 (kcal/mol)	1.24 mM	-5.76 (kcal/mol)	-5.54 (kcal/mol)	-0.22 (kcal/mol)	+9.44 (kcal/mol)	+1.79 (kcal/mol)
Resveratrol	-3.24 (kcal/mol)	4.25 mM	-5.03 (kcal/mol)	-4.91 (kcal/mol)	-0.12 (kcal/mol)	+17.09 (kcal/mol)	+1.79 (kcal/mol)
Sesamol	-3.08 (kcal/mol)	5.51 mM	-3.38 (kcal/mol)	-3.12 (kcal/mol)	-0.26 (kcal/mol)	+0.32 (kcal/mol)	+0.30 (kcal/mol)

4.3.7. Binding site of DJ-1 with selected compounds along with its Known Stimulatory Active Site

Binding site residues of DJ-1 interacting with Naringenin, Quercetin, Resveratrol and Sesamolwere found to be the same as the residues involved in their respective catalytic sites. Interacting residues of DJ-1 with Naringenin, Quercetin, Resveratrol and Sesamolalong with their identified catalytic sites have been show in (**Table 4.6)** and their 2D and 3D pattern of interaction is presented in (**Figure 4.6)**. Interacting residues of DJ-1 with its activator were PRO^{109}, THR^{110}, LEU^{112}, LEU^{113}, ALA^{114}, GLU^{116}, VAL^{123}, THR^{125}, PRO^{127}, ALA^{129}, LYS^{130}, LYS^{132}, ASN^{135}, GLY^{137}, HIS^{138}, TYR^{139}, TYR^{141}, GLU^{143} and ARG^{156}. Interacting residues in case of Naringenin **(Figure-4.6 (a & e))** were THR110, LEU113, ALA114, GLU116, LYS132 and ASN135. Interacting residues in case of Quercetin **(Figure-4.6 (b & f))** were LEU113, ALA114, LYS132, ASN135 and HIS138. Interacting residues in case of Resveratrol **(Figure-4.6 (c & g))** were LEU^{113}, GLU^{116} and ASN^{135}. Furthermore, Interacting residues in case of Sesamol **(Figure-4.6 (d & h))** were PRO127, LYS130, TYR141, GLU143 and ARG156.

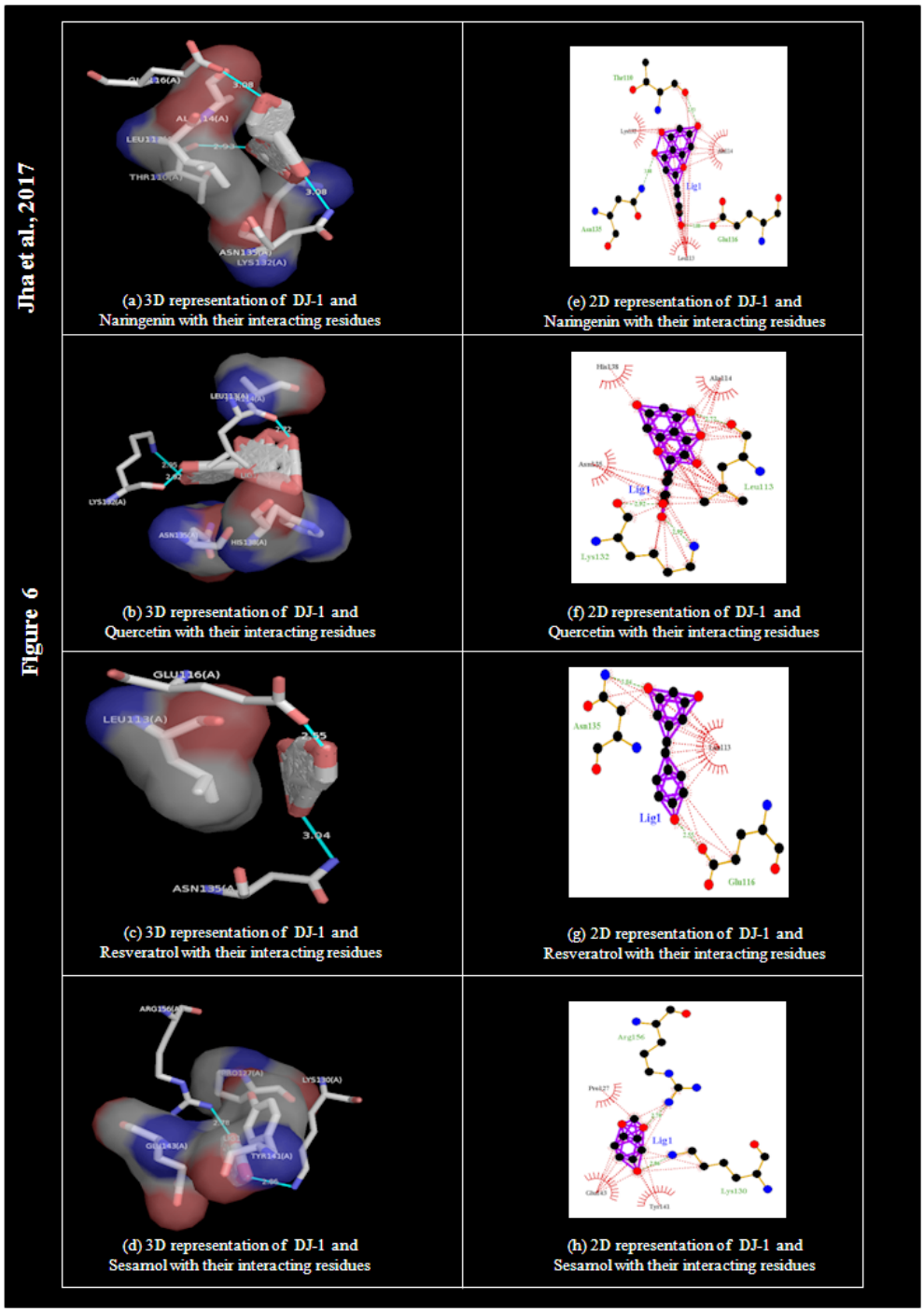


Figure 4.6: Binding site of DJ-1 with selected compounds along with its known stimulatory active site. (a) Three-dimensional (3D) presentation of DJ-1 and Naringenin with their interacting residues, (b) Quercetin with their interacting residues, (c) Resveratrol with their interacting residues, (d) Sesamol with their interacting residues, (e) Two dimensional (2D) representation of DJ-1 and Naringenin with their interacting residues, (f) Quercetin with their interacting residues, (g) Resveratrol with their interacting residues, and (h) Sesamol with their interacting residues.

Table 4.6: DJ-1 known binding site and selected compounds interacting residues

Compound	Interacting residues
Predicated catalytic Site	PRO^{109}, THR^{110}, LEU^{112}, LEU^{113}, ALA^{114}, GLU^{116}, VAL^{123}, THR^{125}, PRO^{127}, ALA^{129}, LYS^{130}, LYS^{132}, ASN^{135}, GLY^{137}, HIS^{138}, TYR^{139}, TYR^{141}, GLU^{143} and ARG^{156}.
Naringenin	THR^{110}, LEU^{113}, ALA^{114}, GLU^{116}, LYS^{132} and ASN^{135}.
Quercetin	LEU^{113}, ALA^{114}, LYS^{132}, ASN^{135} and HIS^{138}.
Resveratrol	LEU^{113}, GLU^{116} and ASN^{135}.
Sesamol	PRO^{127}, LYS^{130}, TYR^{141}, GLU^{143} and ARG^{156}.

4.4. DISCUSSION

Despite the knowledge of various factors which contribute in the occurrence and progression of PD, the exact cause and cure remains elusive. Mutations in PD associated genes potentially lead to autosomal dominant (α -synuclein and LRRK2), or autosomal recessive PD (Parkin, PINK1, DJ1, and ATP13A2) respectively (Wang *et al*., 2010). Moreover, these genes display characteristic ubiquitin E3 ligase activity. DJ-1 is ubiquitously expressed in a number of pathways associated with PD pathogenesis and has ubiquitin E3-ligase activity which also reduces α-synuclein aggregation (Mizunoet *et al*., 2006). Thus, it seems imperative to design therapeutic strategies aimed at elevating the level of DJ-1 to improve neuronal survival in PD. Further, various studies have advocated that several compounds of plant origin possess neuroprotective properties, however, their mode of action have not been clearly defined (Kumar *et al*., 2012). Initially, we screened the compounds for Lipinski's rule of five. Most drugs fail on poor solubility which we have identified and selected through literature survey. Lipinski's analysis of all the selected compounds (Naringenin, Quercetin Sesamol and Resveratrol) revealed that these compounds could act like a drug and have drug like property as these compounds meet the criteria of Lipinski's rule of five parameters. (Khadka *et al*. 2014). All these four compounds (Naringenin, Quercetin Sesamol and Resveratrol) showed Molecular mass less than 500kda and xlogP values of less than 5. However, the most important property required of a compound to be a protective agent is the ability to cross Blood brain barrier (BBB). As expected, most compounds failed the BBB permeability

parameter. But four biomolecules namely, (Naringenin, Quercetin Sesamol and Resveratrol) could cross the BBB on these parameter and the best candidates for targeting neuronal damage in our study. Furthe, based on docking study analysis, the present study provide scientific evidence that given four biomolecules namely Naringenin, Quercetin, Resveratrol and Sesamol are interacting at the reported binding site of DJ-1.Further, binding Constant, K_b of Naringenin, Quercetin, Resveratrol and Sesamol for DJ-1 was found to be 851.70 µM, 1.24 mM, 4.25 mM and 5.51 mM respectively, suggesting that all the selected compounds might be effective as activators of E3 ligase activity of DJ-1. Furthermore, investigation of binding sites within DJ-1 gives a better idea for a valuable drug target site with highest binding and interaction affinity. Based on *in vivo* experimentation, the most effective compound in modulating E3 ligase activity of DJ-1 was found to be Naringenin having minimum binding Constant K_b and highest negative free energy of binding with maximum interacting surface area in a course of docking studies (Azam *et al*., 2012, Gupta et al., 2015 & Dharani *et al*., 2016).

The *in silico* results obtained and discussed in the previous two chapters have shown very promising potential of ubiquitin E3 Ligase Parkin and DJ-1 activating biomolecules in pathogensis of PD.

This encouraged us to undertake *in vitro* studies to confirm the findings of virtual screening. Therefore, in an attempt to study the efficacy of biomolecules in alleviating neurotoxin induced toxicity, we have carried out *in vitro* experiments in the SH-SY5Y neuroblastoma cell line in the succeeding chapter.

Chapter V

Biomolecules Prevents MPTP-Induced Neurotoxicity in Dopaminergic SH-SY5Y Cell Line in Parkinson's Disease

CHAPTER V

BIOMOLECULES PREVENTS MPTP-INDUCED NEUROTOXICITY IN DOPAMINERGIC SH-SY5Y CELL LINE IN PARKINSON'S DISEASE

5.1. OBJECTIVE

The previous chapters discussed very promising neuroprotective potential of biomolecules and altered expression of E3 ligase such as Parkin and DJ-1 in PD. Currently there is no cure and researchers continue to probe the therapeutic prospect in *in silico* study, cell cultures and animal models of PD. Owing to the limitations of sample availability of PD patients and scarcity of animals, the current research scenario has shifted focus on cellular models of PD which are an excellent source of large drug screening and are easy to maintain. Firstly, we have done *In silico* studied, which have been discussed in Chapter *II* and *III* and used cell culture model to study the toxic effects of a neurotoxin; MPTP in SH-SY5Y neuroblastoma cell line. Thus, in an attempt to verify the *in silico* screening results, we carried out various studies to check the efficacy of E3 ligase such as Parkin and DJ-1 inducing biomolecule in ameliorating neuronal cell death in SH-SY5Y cell line. Also, changes in cell viability subsequent to the effect of toxin and upon biomolecules treatment have been assessed. Moreover, the expression level of various protective protein markers subsequent to MPTP administration and after biomolecules treatment has been described in this chapter.

5.2 DOSE-DEPENDENT AND TIME-DEPENDENT TOXICITY ASSAY OF MPTP

A stock of MPTP in DMSO was prepared and from this various gradients of MPTP were prepared in increasing concentration of 300nM, 600nM, 900nM, 20μM, 40μM and 80μM. The effect of each of these doses on SH-SY5Y cells was studied in a time period of 6 hours, 12 hours and 24 hours. The results obtained were evaluated through changes in cell morphology, cell viability assay, protein profiling or western blot and finally, statistical calculations and are summarised in succeeding sections.

5.2.1. MPTP exerts dose-dependent and time-dependent toxicity in SH-SY5Y cells

The results obtained in present study clearly show that MPTP induces toxicity in SH-SY5Y cells in a dose-dependent and time-dependent manner. The change in cell morphology upon MPTP administration in comparison to control cells is clearly evident in (**Figure-5.1 (h)**).

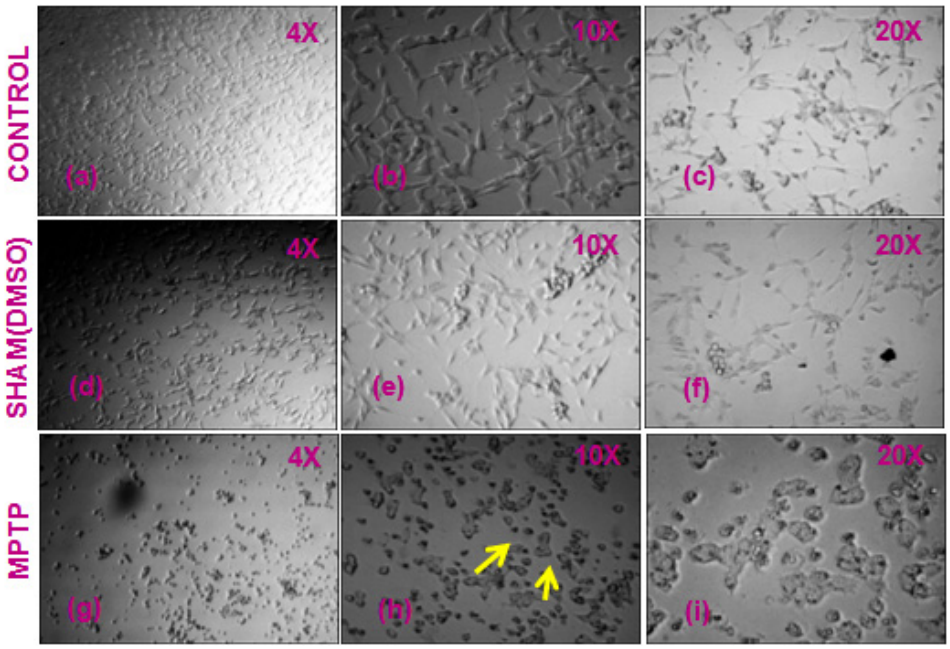


Figure 5.1: MPTP changes cell morphology (g-i) compared to both control (a-c) and SHAM (d-f)

5.2.2. MPTP exerts mild toxicity at 300nM-900nM

The SH-SY5Y neuroblastoma cells exhibited nearly normal morphology when compared with control and SHAM (DMSO) post 300nM-900nM MPTP administration after 6-12 hours. Most of the cells were alive at these concentrations even after 24 hours. The viability was only slightly decreased at 900nM in comparison to 300nM concentration (**Figure-5.2 (a-i)**)

5.2.3. MPTP exerts moderate toxicity at 900nM-20µM

The 900nM concentration of MPTP exerted some toxicity after 6 hours. Further, although cells were mostly alive, toxicity at this concentration was visibly apparent after 12 hours. After 24 hours, the confluence of cells was markedly decreased

though; the morphology of cells was somewhat retained (**Figure-5.2 (h-i)**). However, the toxicity of 20µM MPTP on neuroblastoma cells was very apparent after 6 hours as cell morphology was visibly distorted. As expected, the morphology degraded after 12 hours and clumps of morphologically abnormal cells were seen after 24 hours of treatment (**Figure-5.2 (k-l)**).

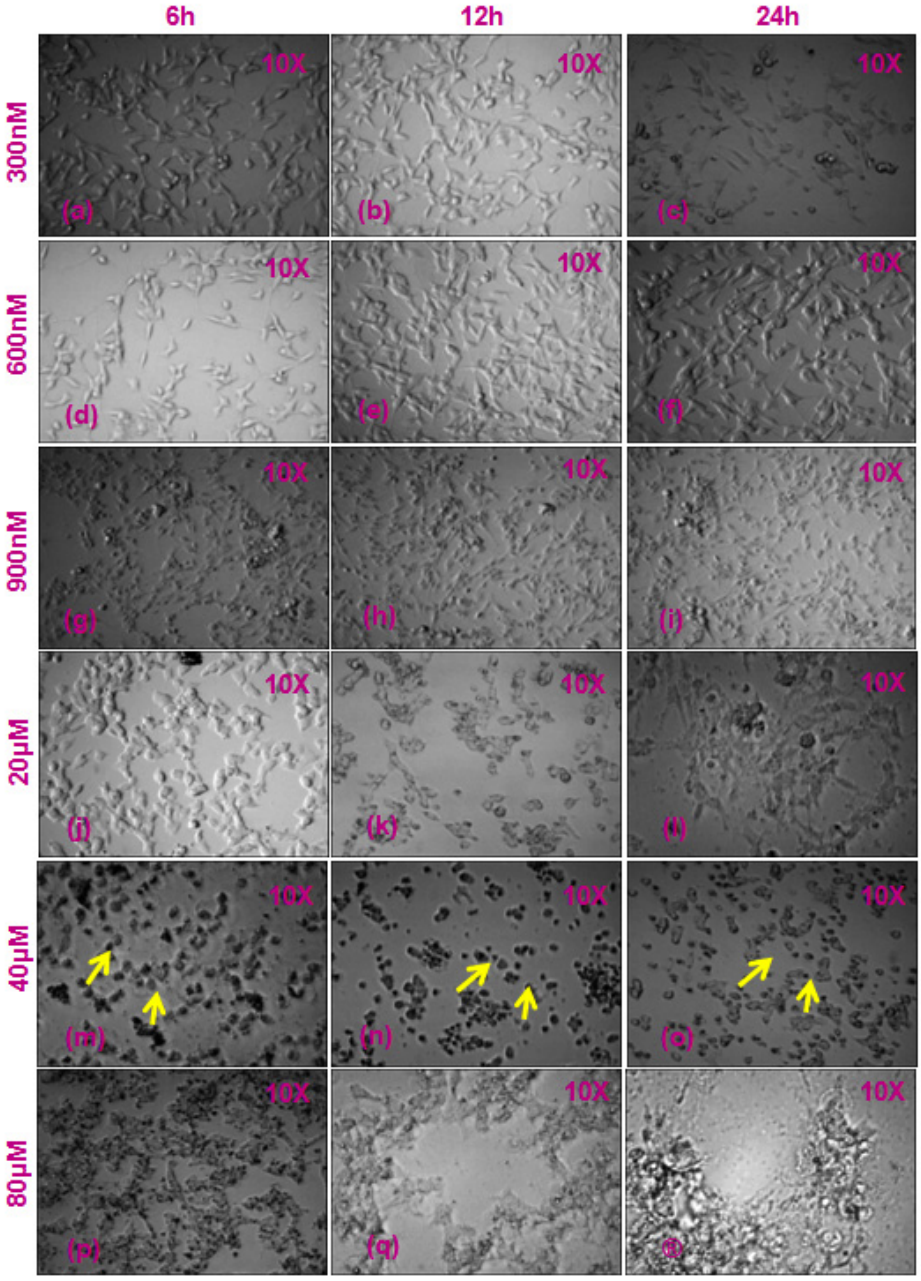


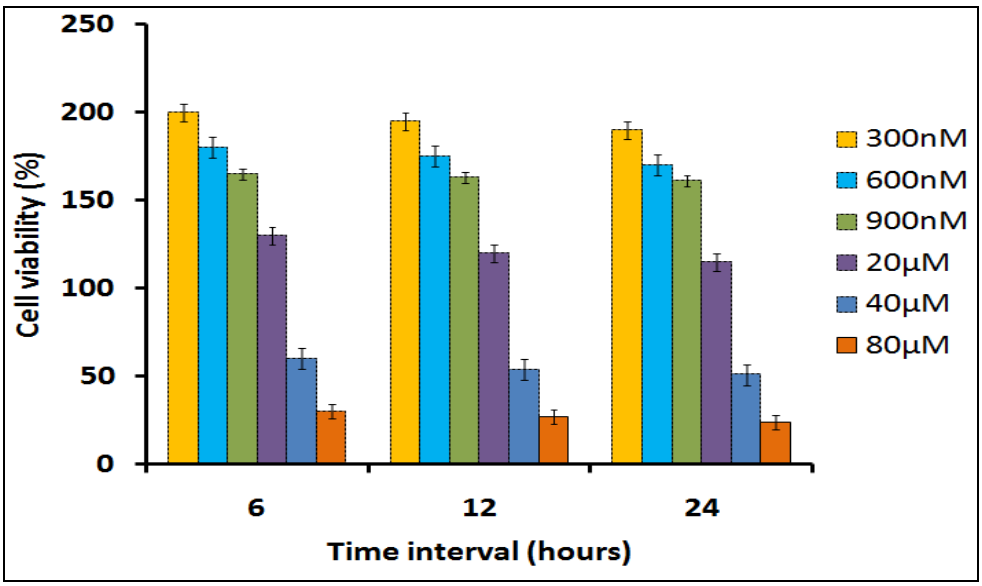
Figure 5.2: MPTP exerts mild toxicity at 300nM-900nM (a-i upper panel). Further, the moderate toxicity was observed at 900nM-20µM (g-l) and MPTP induced heavy toxicity at 40µM (m-o)

5.2.4. LD_{50} concentration of MPTP is 40µM

The 40µM concentration of MPTP exhibited heavy toxicity on SH-SY5Y cells which was clearly evident after 6 hours of administration in the form of almost complete distortion of neuronal cell morphology. Further, cell morphology was lost after 12 hours and about 50% cells were dead post 24 hours of 40µM dose administration. Thus, after repeating the experiment in triplicates thrice, 40µM concentration of MPTP was taken as the LD_{50} value in SH-SY5Y cells. Furthermore, the MPTP toxicity was extremely high at 40µM-80µM concentration and immediate deformation of neuronal cells with cell death was visible. Moreover, the morphology and viability was completely lost at 12 hours and cells were floating after 24 hours (**Figure-5.2 (m-o)**).

5.2.5. Effect of MPTP Dose on Cell Viability

To determine the effect of MPTP on cell viability, the trypan blue exclusion test was performed in all the above mentioned sets of experiments. Accordingly, the number of live (unstained) and dead (stained) cells were counted in a hemocytometer and percent cell viability determined in triplicate set of experiments. The results in the form of statistically calculated values were plotted on a graph. The cell viability was found to decrease with increasing concentration of MPTP, further validating the dose-dependent effect of rotenone toxicity on SH-SY5Y cells (**Graph 5.1**).



Graph 5.1: Dose-dependent effect of MPTP toxicity on SH-SY5Y cells. As the dose of MPTP was increased, cell viability attenuated significantly as concluded from trypan blue exclusion test (n=3)

Next, we checked the neuroprotective potential of biomolecules (Naringenin, Quercetin and Resveratrol) which have been described in both the *Chapter III* and *Chapter IV* as these molecules alleviate MPTP induced altered expression of E3 ligase such as Parkin and DJ-1 in PD and to check its efficacy on cell viability and on the expression level of various protective protein markers subsequent to MPTP administration. The results are summarized in the next section.

5.3. BIOMOLECULES REVERSE MPTP-INDUCED TOXICITY IN SH-SY5Y CELL LINE

5.3.1 Dose-dependent effect of Naringenin on MPTP -induced toxicity in SH-SY5Y Cells

The concentration gradient of Naringenin was prepared in increasing order of 10μM, 20μM, 40μM and 80μM respectively. Next, the LD_{50} value of MPTP which was determined as 40μM in preceding experiment was administered to SH-SY5Y cells for 24 hours. The cell death incurred upon MPTP administration at 40μM was determined through trypan blue cell viability assay and observed morphological deformations. Next, the varying concentrations of Naringenin were used to study its protective effect on MPTP induced toxicity on SH-SY5Y cells in period of 24 hours. The results obtained are summarized below.

5.3.1.1. Naringenin protects against MPTP-induced toxicity at 20μM concentration

Naringenin restored almost 50% cells which were non-viable post MPTP induced toxicity in SH-SY5Y cells. The rounded morphology of MPTP -treated cells was transformed into well defined neurite-like structures upon 20μM Naringenin treatment (**Figure-5.3 (g-i)**). Further, the cell viability was greatly enhanced. Therefore, the present study observed well marked protective effect of 20μM Naringenin against MPTP induced toxicity in SH-SY5Y cells.

5.3.1.2 Naringenin reverses MPTP -induced toxicity at 40μM concentration

The concentration of Naringenin at which there was almost complete reversal of MPTP - induced toxicity was found to be 40μM. The neuronal morphology was very

well defined and long neurites could be seen after treatment with this dose (**Figure-5.3 (j-l)**). Moreover, these results are corroborated with cell viability assays. Thus, Naringenin rescued neuronal cells from MPTP toxicity at 40µM.

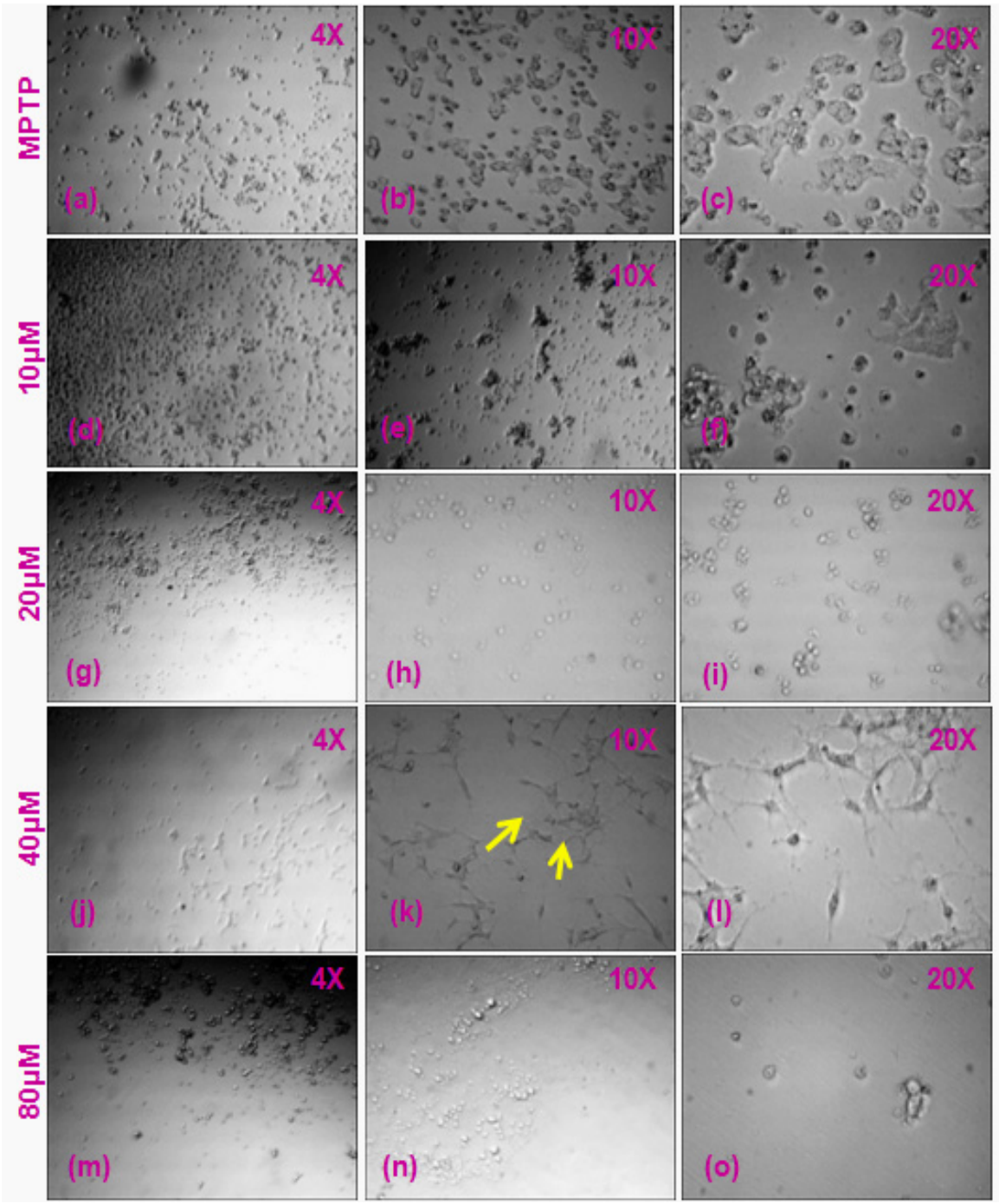
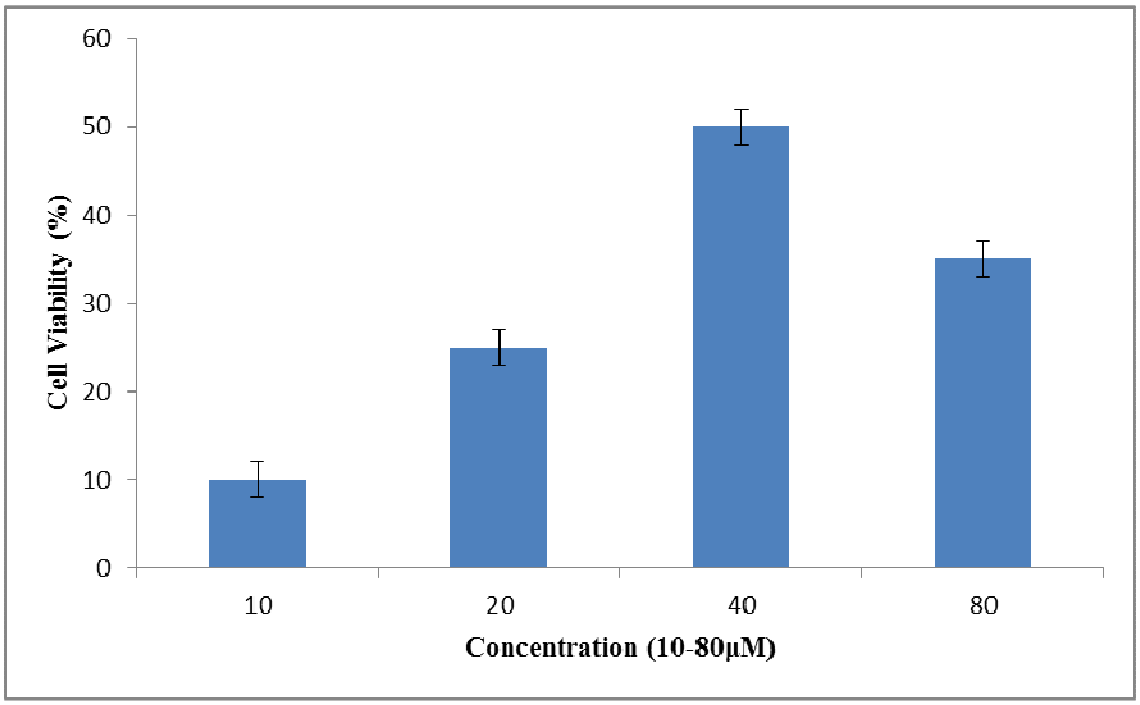


Figure 5.3: Naringenin is protective at 40µM (j-l) and revived more than 50% cells at this dose

5.3.1.3. Effect of dose of Naringenin on cell viability

The protective effect of Naringenin on cell viability in MPTP induced toxicity was determined through trypan blue exclusion test. Post 24 hours of MPTP administration, cells were treated with Naringenin for 24 hours. Next, the trypan blue test was performed for each experimental and control group. Accordingly, the number of live (unstained) and dead (stained) cells were counted in a hemocytometer and percent cell viability determined in triplicate set of experiments. The results in the form of statistically calculated values were plotted on a graph **(Graph 5.2)**. To summarise, Naringenin treatment increased cell viability in a dose-dependent manner in MPTP induced toxicity in SH-SY5Y cells.

Further, we wanted to check the effect of Quercetin and Resveratrol on MPTP induced toxicity in SH-SY5Y cells. For this purpose we performed same set of experiments and the results obtained are summarized in the succeeding section.



Graph 5.2: Naringenin increased cell viability in a dose-dependent manner against MPTP toxicity. At 40µM Naringenin reversed cell toxicity and greatly enhanced cell viability (n=3)

5.3.2. Dose-dependent effect of Quercetin in MPTP-induced toxicity

The concentration gradient of Quercetin was prepared in increasing order of 10μM, 20μM, 40 μM and 80μM respectively. Next, 40μM MPTP was administered to SH-SY5Y cells for 24 hours. The cell death incurred upon MPTP administration was determined through trypan blue cell viability assay. Next, the effect of varying concentrations of Quercetin on MPTP induced toxicity at 40μM was studied and obtained results are summarized below.

5.3.2.1. Quercetin does not attenuate MPTP induced toxicity at 10μM concentration

MPTP treated cells were incubated with 10μM Quercetin for 6-24 hours. The effect of Quercetin was observed after 12 hours and 24 hours. However, Quercetin had no effect on MPTP induced toxicity at 40 μM in SH-SY5Y cells at 10μM concentration (**Figure-5.4 (d-f)**). The morphology of cells as well as the cell viability assessed through trypan blue test and remained unchanged on treatment with 10μM Quercetin (**Graph 5.3**).

5.3.2.2. Quercetin reverses MPTP-induced toxicity at 20μM concentration

The dose of Quercetin was further increased to study its effect in MPTP induced toxicity. Accordingly, 20μM Quercetin was used to treat MPTP administered cells. After 24 hours it was observed that these dose of Quercetin it reversed MPTP induced toxicity in SH-SY5Y cell line (**Figure-5.4 (g-i)**). Moreover, a very few viable cells were observed in the total population of MPTP induced dead cells. Further, the cell viability was found to be increased at 20μM Quercetin concentration. Interestingly, on further increasing the dose of Quercetin to 40-80μM, improved cell viability as compared to the preceding dose was observed (**Figure-5.4 (j-o)**).

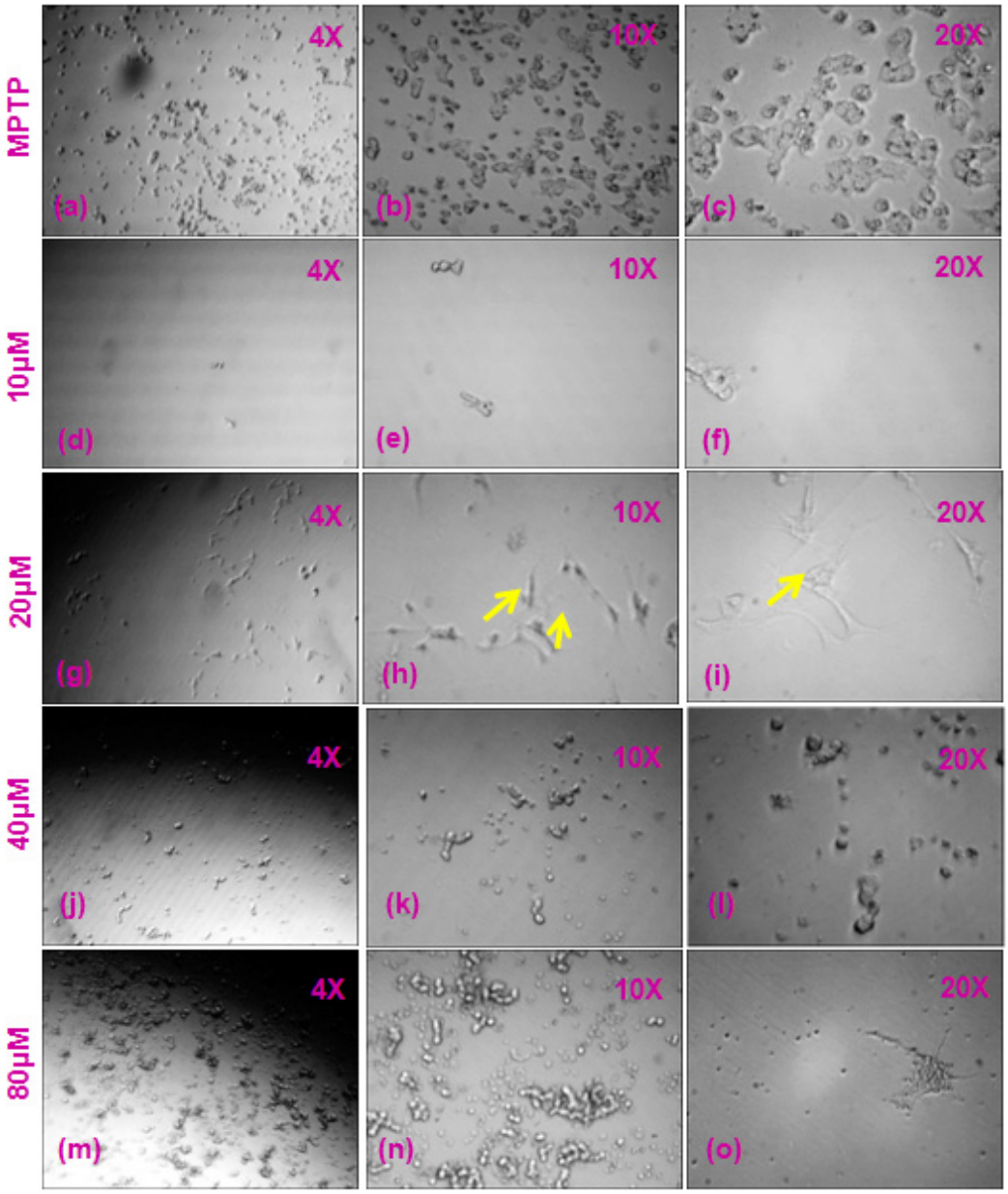
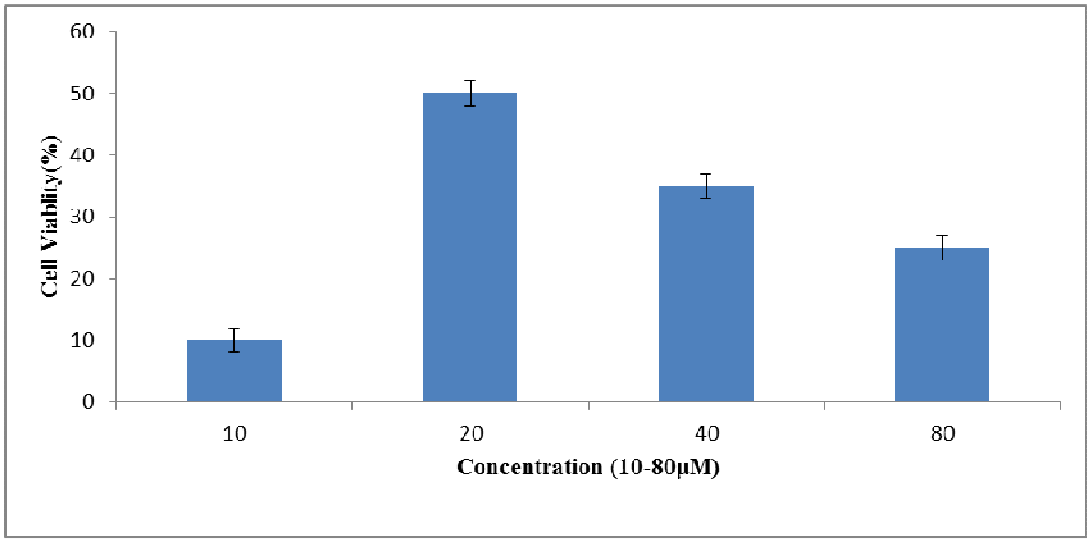


Figure 5.4: Quercetin is protective at 20µM (g-i) and revived more than 50% cells at this dose

5.3.2.3. Effect of dose of Quercetin on cell viability

The protective effect of Quercetin on cell viability in MPTP induced toxicity was determined through trypan blue exclusion test. MPTP treated cells were incubated with varying concentration of Quercetin. Next, the trypan blue test was performed for each experimental and control group. Further, the number of live (unstained) and dead (stained) cells were counted in a hemocytometer and percent cell viability determined in triplicate set of experiments. The results in the form of statistically calculated

values were plotted on a graph (**Graph 5.3**). To summarize, Quercetin treatment also exhibited significant increase in cell viability in MPTP toxicity at 20µM induced toxicity in SH-SY5Y cells.



Graph 5.3: Quercetin increased cell viability in a dose-dependent manner against MPTP toxicity. At 20µM Quercetin reversed cell toxicity. Cell viability as a measure of Quercetin doses showing significant results; (n=3)

5.3.3. Dose-dependent effect of Resveratrol in MPTP-induced toxicity

The concentration gradient of Resveratrol was prepared in increasing order of 15µM, 30µM, 60 µM and 120µM respectively. Next, 40µM MPTP was administered to SH-SY5Y cells for 24 hours. The cell death incurred upon MPTP administration was determined through trypan blue cell viability assay. Next, the effect of varying concentrations of Resveratrol on MPTP induced toxicity at 40µM was studied and obtained results are summarized below.

5.3.3.1. Resveratrol does not attenuate MPTP induced toxicity at 15-30µM concentration

MPTP treated cells were incubated with 15-30µM Resveratrol for 6-24 hours. The effect of Resveratrol was observed after 12 hours and 24 hours. However, Resveratrol had no effect on MPTP induced toxicity at 40Mm in SH-SY5Y cells at 15-30µM concentration (**Figure-5.5 (d-i)**). The morphology of cells as well as the cell viability assessed through trypan blue test and remained unchanged on treatment with 15-30µM Resveratrol (**Graph 5.4**).

5.3.3.2. Resveratrol reverses MPTP-induced toxicity at 60 µM concentration

The dose of Resveratrol was further increased to study its effect in MPTP induced toxicity. Accordingly, 60µM Resveratrol was used to treat MPTP administered cells. After 24 hours it was observed that these dose of Resveratrol it reversed MPTP induced toxicity in SH-SY5Y cell line (**Figure-5.5 (j-l)**). Moreover, viable cells were observed in the total population of MPTP induced dead cells. Interestingly, increasing the dose of Resveratrol to from 60 µM to 120µM, improved cell viability as compared to the preceding dose was observed (**Figure-5.5 (m-o)**).

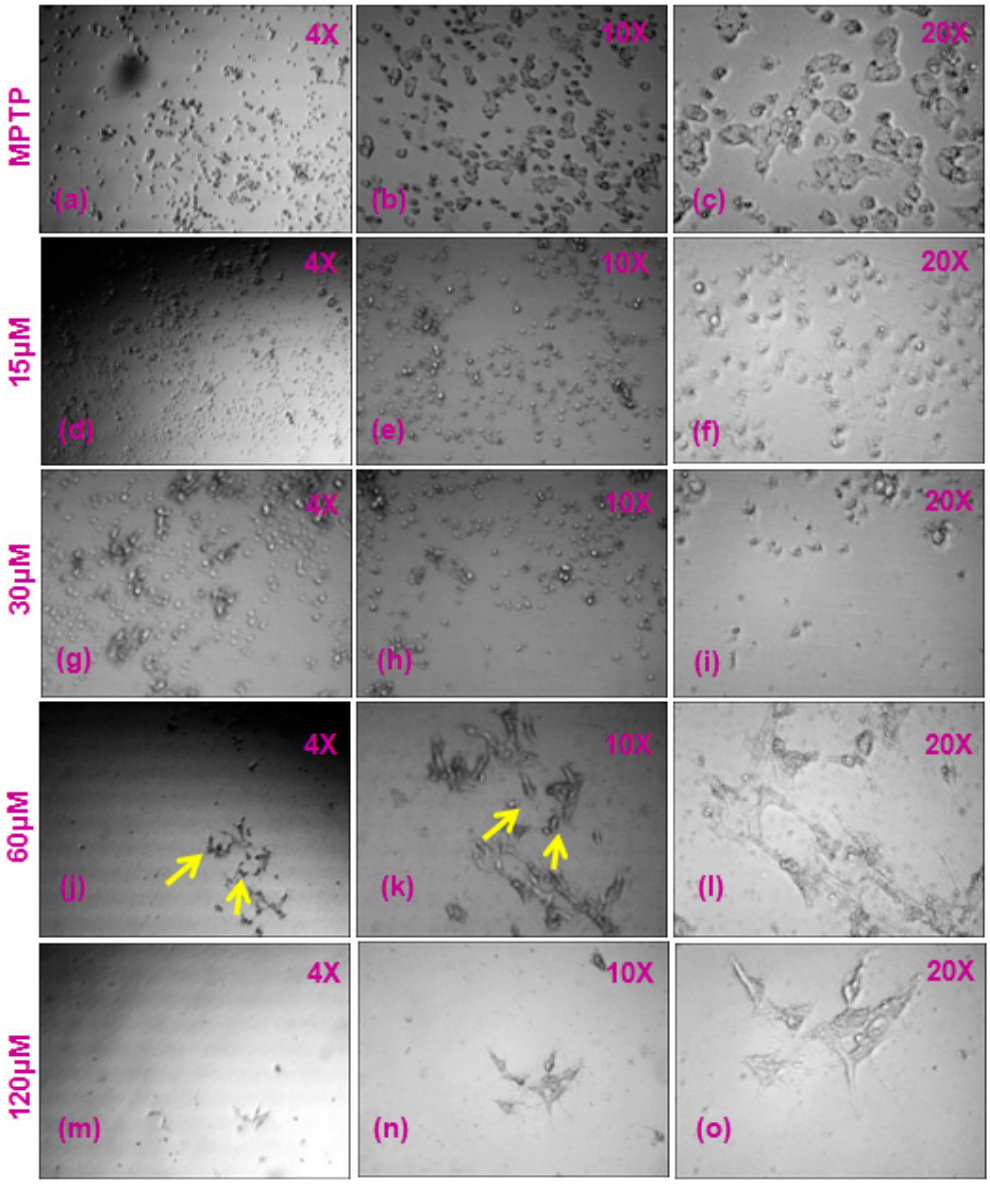
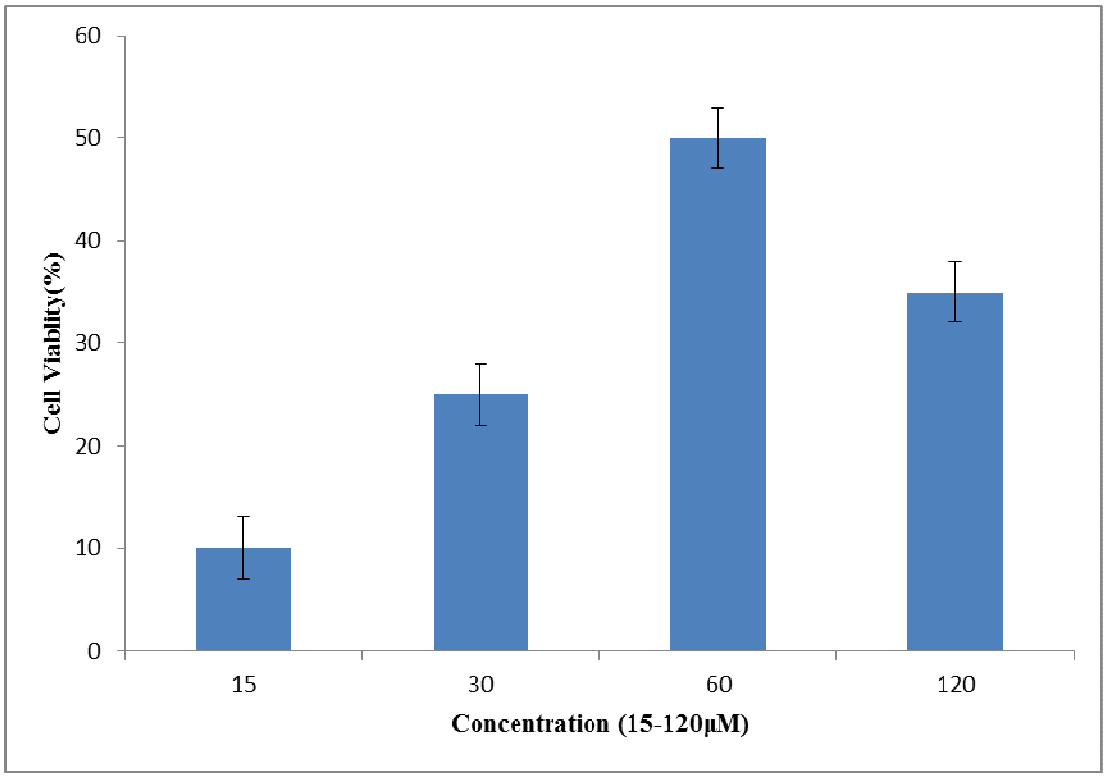


Figure 5.5: Resveratrol is protective at 60µM (j-l) and revived more than 50% cells at this dose

5.3.3.3. Effect of dose of Resveratrol on cell viability

The protective effect of Resveratrol on cell viability in MPTP induced toxicity was determined through trypan blue exclusion test. MPTP treated cells were incubated with varying concentration of Resveratrol. Next, the trypan blue test was performed for each experimental and control group. Further, the number of live (unstained) and dead (stained) cells were counted in a hemocytometer and percent cell viability determined in triplicate set of experiments. The results in the form of statistically calculated values were plotted on a graph (**Graph 5.4**). To summarize, Resveratrol treatment also exhibited significant increase in cell viability in MPTP toxicity at 60 µM induced in SH-SY5Y cells.



Graph 5.4: Resveratrol increased cell viability in a dose-dependent manner against MPTP toxicity. At 60µM Resveratrol reversed cell toxicity. Cell viability as a measure of Resveratrol doses showing significant results (n=3)

5.4 WESTERN BLOT FOR VARIOUS PROTECTIVE PROTEIN AND PD RELATED MARKERS

5.4.1. MPTP reduced the expression of Parkin and DJ-1 in SH-SY5Y cell line

Both Parkin and DJ-1 are Ubiquitin E3 ligase and which losses in its activity have been linked to increase in various substrates which can have detrimental effect on cells. MPTP treatment led to attenuate the level of both Parkin and DJ-1, when compared to control neuroblastoma cells. Loss in both the Parkin and DJ-1 activity is the indication of neuronal cell death observed in MPTP treated cells compared to control cells, where the levels of both Parkin and DJ-1are high.

5.4.1.1. Naringenin and Quercetin restored both Parkin and DJ-1 activity

Naringenin and Quercetin treatment restored the levels of Parkin and DJ-1 which were downregulated in response to MPTP exposure (**Figure-5.6).** The restored in levels of these markers may be mediating significant acceleration in cell viability upon Naringenin and Quercetin treatment in SH-SY5Y cells.

5.4.1.2. MPTP induced upregulation of p38 MAPK in SH-SY5Y cell line

The fundamental role of p38 MAPK in controlling all of the above detrimental consequences, and thereby in the process upset DA neuronal homeostasis, which ultimately progresses to an advanced diseased state and incurable neurodegeneration. Upon MPTP induction, these markers get activated and trigger downstream signalling and lead to the neuronal dysfunction. However, upon MPTP treatment increased level of p38 MAPK may be contributed to accumulation of toxic protein, which ultimately leads to hamper neuronal network and cause neuronal dysfunction.

5.4.1.3. Naringenin, Quercetin and Resveratrol restored p38 MAPK in SH-SY5Y cell line

Naringenin, Quercetin and Resveratrol treatment reduced the levels of p38 MAPK which was upregulated in response to MPTP exposure (**Figure-5.6).** The decrease in

levels of these markers may be mediating significant acceleration in cell viability upon these biomolecules treatment in SH-SY5Y cells. Such observation can underline the efficacy of Resveratrol in protecting neuronal cells against MPTP induced toxicity.

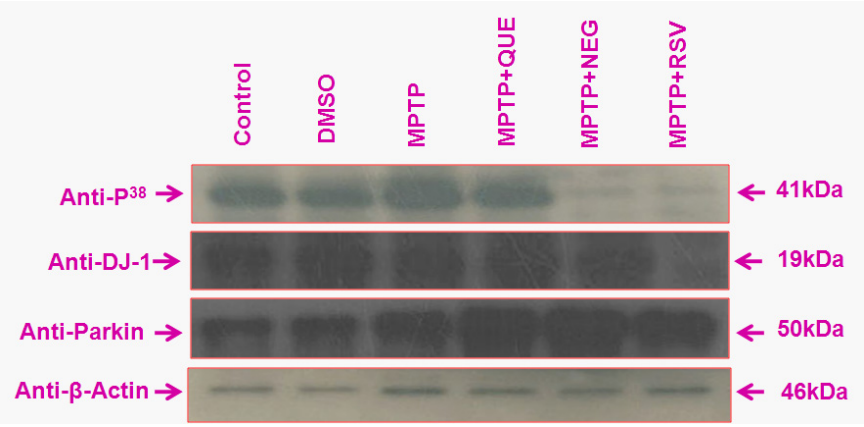


Figure 5.6: Protein expression of Parkin, DJ-1 and P38 subsequent to MPTP administration and on biomolecules treatment in SH-SY5Y cell line

5.5. DISCUSSION

The previous chapters discussed about the potential role of both Parkin and DJ-1 ubiquitin E3 ligase, regulating stress induced neuronal cell death in PD. Out of the four compounds shortlisted in *chapter III* and *chapter IV*, the protective potential role of Naringenin, Quercetin and Resveratrol in alleviating MPTP mediated cell death was evaluated in the present chapter. MPTP was the neurotoxic contaminant responsible for the effect of Parkinsonism. MPTP is highly lipophilic and it readily crosses the blood brain barrier. The MPTP, which is widely used in mimicking PD like symptom, was used to induce toxicity in SH-SY5Y cell line. The toxicity assay shows dose-dependent and time-dependent effect of MPTP in inducing neuronal cell death in SH-SY5Y cells and cell viability significantly decreased with increasing dose of MPTP. Moreover, the LD_{50} value of MPTP was determined at 40µM concentration in present study. Further, treatment with Naringenin rescued cells against MPTP induced toxicity at 40µM dose and exhibited dose-dependent and time-dependent protection in increasing cell viability. The protein expression level of both Parkin and DJ-1 ubiquitin E3 ligase subsequent to MPTP administration and on Naringenin treatment was checked through western blotting. The results show that Naringenin

restored the level of both Parkin and DJ-1, which were attenuated on MPTP treatment in SH-SY5Y cell line. Likewise, Quercetin administration rescued cells against MPTP induced toxicity at 20μM dose in SH-SY5Y cells and exhibited dose-dependent and time-dependent protection in increasing cell viability. Further, the protein expression level of both Parkin and DJ-1 to MPTP administration and on Quercetin treatment in the SH-SY5Y cell line was checked through western blotting. The results demonstrate that Quercetin restored the level of both Parkin and DJ-1 which were downregulated on MPTP treatment. Furthermore, Naringenin, Quercetin and Resveratrol treatment restored the levels of p38 MAPK which was upregulated in response to MPTP exposure**.** The decrease in levels of these markers may be mediating significant acceleration in cell viability upon these biomolecules treatment in SH-SY5Y cells. Such observation can underline the efficacy of Resveratrol in protecting neuronal cells against MPTP induced toxicity. Therefore, it can be advocated from the given results that Naringenin, Quercetin and Resveratrol may be rescuing neuronal cells against MPTP induced toxicity through altered the expression of E3 ligase such as Parkin, DJ-1 and p38 MAPK in PD. The detailed mechanism of such promising interaction can be validated through further studies.

Chapter VI
Summary Discussion and Future Perspectives

CHAPTER VI

SUMMARY, DISCUSSION AND FUTURE PERSPECTIVE

Neurodegenerative disorders are characterized by gradual and progressive loss of neurons that leads to deficient nervous system output. These disorders can affect daily activities like abstract thinking, movements, emotional feelings, cognition, memory and other abilities (Soto, 2003). Worldwide, approximately 2% of the populations are victims of such detrimental outcomes (Hardy and Orr, 2006). Most common neurodegenerative diseases include AD, PD, HD, ALS and MS several risk factors for instance, genetic polymorphisms aging, dietary supplements that play their parts in modulating development and prognosis of these disorders. Other causative factors that might influence disease outcomes includes gender, poor education, endocrine conditions, oxidative stress, inflammation, stroke, hypertension, diabetes, smoking, head trauma, depression, infection, tumors, vitamin deficiencies, immune and metabolic conditions, and chemical exposure. In addition to this mitochondrial dysfunction can also regulate pathogenesis in several neurodegenerative challenges such as PD and HD. These disorders are characterized by the accumulation of reactive oxygen species (ROS), selective loss of neurons, loss of mitochondrial membrane potential, and ATP depletion. Altogether, these factors al modulate aberrant brain functioning and subsequently neurodegeneration (Kieper *et al*., 2010).

PD is the second most prevalent neurodegenerative disease worldwide and is currently incurable. PD is characterized by the progressive loss of a subset of midbrain dopaminergic neurones in the *substantia nigra* leading to loss of motor skill (Angeline *et al*., 2013). Disease causing mechanisms of PD are poorly understood and can be attributed in part to the growing number of genetic defects that might define PD pathological outcomes. PD pathology is also characterized by progressive polymerization and development of altered conformation of soluble native neuronal protein, resulting in intracellular aggregation. Alpha-synuclein has been identified as the major constituent of Lewy bodies in sporadic PD. Lewy bodies are the alpha synuclein – immunoreactive inclusions made up of a number of neurofilament proteins together with proteins responsible for proteolysis (Davie, 2008). Ubiquitin

Proteosome System (UPS) maintains the intracellular proteolysis and viability of cells by removing the unwanted proteins that are no longer required by the cell. The link between UPS and neurodegeneration has been worked out by the discovery of mutation in genes coding for many ubiquitin-proteosome pathway proteins in PD. Identification of Parkin and DJ-1 gene revealed the first evidence of a direct role of UPS in neurodegeneration. Parkin encodes the E3 ubiquitin ligase with the characteristic two RING finger domain separated by an IBR (in-between ring) domain (Mata *et al*., 2004). Normal functional levels Parkin are observed in healthy neurons and any deviation in normal operation leads to aberrant Parkin levels thus contributing towards PD progression (Smidt, 2011). Further, DJ-1 is a ubiquitous redox-responsive cytoprotective protein with diverse functions. It may act as a sensor for oxidative stress, and it apparently protects neurons against oxidative stress and cell death (Kumaran *et al.,* 2009). Loss-of-function and mutations in DJ1 can cause early-onset of PD. This leads to the characteristic selective neurodegeneration of nigrostriatal dopaminergic neurons, which accounts for PD symptoms.

The etiology of PD still remains mysterious, whereas various investigations have revealed that its pathogenesis involves a multifactorial cascade of deleterious factors. Most insights into PD pathogenesis arise from the wide range of research that was performed using experimental models of PD, especially those produced by neurotoxins (Bove *et al.,* 2005). The four most popular PD neurotoxins are namely 6-OHDA, MPTP, rotenone, and paraquat. Each neurotoxin has its own mechanism of action to induce the disease. Among the various neurotoxins, MPTP is convenient for mimic the symptom like PD. MPTP is an insecticide that has the potential to cause PD. MPTP toxicity is also caused by mitochondrial complex I inhibition, depletion of cellular and oxidative damage. These processes cause loss of midbrain dopaminergic neurons, leading to depletion of dopamine in the brain. Being a lipophilic molecule, it can easily penetrate all types of membranes and can induce neurodegeneration. (Lapointe *et al.,* 2004). It can also cause selective dopaminergic cell degeneration *in vitro*. Recent discoveries about Parkinson's disease, its diagnosis and treatments have opened up many paths for finding novel therapeutic targets to treat PD (Savitt *et al.,* 2006). The main goal for future treatment of Parkinson's disease is the discovery and

development of neuroprotective drugs to lessen or rather to halt the disease progression (Chen and Le, 2006). It is important to consider the relationship between disease progression and the beneficial and pharmacological properties of the drug during the development of neuroprotective drugs for the treatment of Parkinson's disease. Levodopa a well known medication for PD was a great advancement in the treatment of PD, by itself it was not very effective in the PD patients, and as well it did not reduce the symptoms of the disease (Olanow and Koller, 1998). Wide range of research in the field of neuroprotection induced an interest in using natural compounds to treat the disease and offer neuroprotection to the patients. Biomolecules are known to have antioxidant, anti inflammatory and free radical scavenging activity (Meng et al., 2010). The use of biomolecules have shown betterments in cognition function by protecting susceptible neurons from neurodegeneration, maintenance of motor control, reduction in motor complications.

In the present work, we first aimed to explore for both the Parkin and DJ-1 modulating compounds in neuronal damage under stress condition. For this, we carried out extensive literature survey with the keywords Parkin and DJ-1 associated regulatory biomolecules and PD. Further we prepared a list of fourteen compounds on the basis of extensive literature survey. Next, we employed various *in silico* tools and techniques for screening of these compounds on drug-likeliness parameters. The compounds were assessed using three parameters namely; Lipinski filter, Ghose and Veber rules and those which passed these filters were then analyzed for pharmacokinetic properties. Based on various pharmacokinetic parameters such as aqueous solubility, lipophilicity, GI permeability, bioavailability and BBB permeability scores, the final candidates amongst fourteen compounds were obtained. These compounds which passed all the filters and showed good pharmacokinetics included Naringenin, Quercetin, Sesamol and Resveratrol. Next, we performed structural analysis of target proteins Parkin and DJ-1 for adding therapeutics potential of these screened compounds. Naringenin, Quercetin, Sesamol and Resveratrol depicted crucial role in regulating both the Parkin and DJ-1 levels based on interaction energies of molecular docking study. These results led to speculations that these compounds may have strong potential to ameliorate the function of both Parkin and DJ-1 as impaired expression of these proteins lead to

neuronal damage under deprived stress. Further, the protective action of these compounds in ameliorating neuronal damage may be mediated through the Parkin and DJ-1.

Finally, we tested the protective potential of three of these shortlisted biomolecules; Naringenin Quercetin and Resveratrol in SH-SY5Y neuroblastoma cell line. The neurotoxin MPTP which is widely used to mimic PD symptom was used to induce toxicity in the given cell line. Firstly, we tested the dose-dependent and time-dependent effect of MPTP toxicity on cell viability and determined the LD_{50} value of MPTP which was found to be 40μM in 24 hour time period in the given study. Moreover, we also found that MPTP exerts toxicity in a dose dependent manner and cell viability decreases significantly and markedly with increasing dose. Next, we studied the dose-dependent and time-dependent efficacy of Naringenin against MPTP induced toxicity in SH-SY5Y cell line. Accordingly, our study outlined protective role of Naringenin at a dose of 40μM, where reversal of MPTP induced toxicity in SH-SY5Y cells was observed. Similarly we studied the dose-dependent and time-dependent efficacy of Quercetin against MPTP induced toxicity in SH-SY5Y cell line. Accordingly, our study outlined protective role of Quercetin at a dose of 20μM and at this particular dose reversal of MPTP induced toxicity in neuroblastoma cell line was noticed. Accordingly, 60μM Resveratrol was used to treat MPTP administered cells. After 24 hours it was observed that these doses of Resveratrol had also restored MPTP induced toxicity in SH-SY5Y cell line. Further, Naringenin Quercetin and Resveratrol exhibited dose dependent efficacy to a greater extent and cell viability was significantly enhanced with increasing dose of these biomolecules.

Further, the levels of these protective markers were checked subsequent to MPTP administration and on treatment with biomolecules in SH-SY5Y cells. Our results showed that Naringenin and Quercetin restored the level of Parkin and DJ-1 to some extent thus showing protective role in neuronaL damage under neurotoxin. Furthermore, Naringenin, Quercetin and Resveratrol treatment restored the levels of p38 MAPK which was upregulated in response to MPTP exposure**.** To summarize, Naringenin, Quercetin and Resveratrol may be rescuing neuronal cells against MPTP induced toxicity through altered the expression of Parkin, DJ-1 and p38 MAPK in PD.

These observations have opened up a new avenue of therapeutics for further exploration through *in vivo* PD models in neuronal damage. The biomolecules, Naringenin, Quercetin and Resveratrol with various beneficial properties are found to be potential neuroprotective agents against the underlying pathology associated with PD. The present study can be extended to check the expression of different HSPs and proteins involved in PD at mRNA level.

References

REFERENCES

A. Almeida, Regulation of APC/C-Cdh1 and its function in neuronal survival, Mol Neurobiol. 46 (2012) 547-54.

A. Ciechanover, Y.T. Kwon, Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies, Experimental & Molecular Medicine. 47 (2015) e147.

A. Cuadrado, A.R.Nebreda, Mechanisms and functions of p38 MAPK signaling, Biochem J 429 (2010)403-17.

A. Faizul, M.M. Arwa, I. A. Hamed, Molecular Docking and Prediction of Pharmacokinetic Properties of Dual Mechanism Drugs that Block MAO-B and Adenosine A2A Receptors for the Treatment of Parkinson's Disease, J Y P. 4 (2012) 184-192.

A. L. Sônia, Corrêa, L. E. Katherine, The Role of p38 MAPK and Its Substrates in Neuronal Plasticity and Neurodegenerative Disease, Journal of Signal Transduction. (2012) 649079:12.

A. Migdalska-Richards, A.H. Schapira, The relationship between glucocerebrosidase mutations and Parkinson disease, J Neurochem. (2016).

A. Reynolds, C. Laurie, R.L. Mosley, et al. Oxidative stress and the pathogenesis of neurodegenerative disorders, Int Rev Neurobiol. 82 (2007) 297-325.

A.B. Pérez-Oliva, C. Olivares, C. Jiménez-Cervantes, J.C. García-BorrónMahogunin ring finger-1 (MGRN1) E3 ubiquitin ligase inhibits signaling from melanocortin receptor by competition with Galphas, J Biol Chem.284 (2009) 31714–25.

A.C. Guerra de Souza, R.D. Prediger, H. Cimarosti, Mitochondrial dysfunction and SUMOylation targets in Parkinson's disease, J Neurochem. (2016) 13599.

A.C. Sirianni, J. Jiang, J. Zeng, L.L. Mao, S. Zhou, P. Sugarbaker, X. Zhang, W. Li, R.M. Friedlander, X. Wang, N-acetyl-l-tryptophan, but not N-acetyl-d-tryptophan,rescues neuronal cell death in models of amyotrophic lateral sclerosis, J Neurochem. 134 (2015) 956-68.

A.L. DeStefano, L.I. Golbe, M.H. Mark, A.M. Lazzarini, N.E. Maher, M. Saint-Hilaire et al., Genome-wide scan for Parkinson's disease: the GenePD Study, Neurology. 57 (2001) 1124-6.

A.M. Pickrell, M. Pinto, A. Hida, C.T. Moraes, Striatal dysfunctions associated with mitochondrial DNA damage in dopaminergic neurons in a mouse model of Parkinson's disease, J Neurosci. 31 (2011) 17649-58.

A.Y. Chan, J.H. Yeunget al., Subthalamic nucleus deep brain stimulation for Parkinson's disease: evidence for effectiveness and limitations from 12 years' experience, Hong Kong Med J. (2014) 24.

A.Y. Seo, A.M. Joseph, D. Dutta, J.C. Hwang, J.P. Aris, C. Leeuwenburgh, New insights into the role of mitochondria in aging: mitochondrial dynamics and more, J Cell Sci. 123 (2010) 2533-42.

B. Halliwell. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence, Lancet. 344 (1994) 721–724.

B. Rotblat, A.L. Southwell, D.E. Ehrnhoefer, N.H. Skotte, M. Metzler, S. Franciosi, G. Leprivier, S.P. Somasekharan, A. Barokas, Y. Deng et al., HACE1 reduces oxidative stress and mutant Huntingtin toxicity by promoting the NRF2 response, Proc Natl Acad Sci U S A. 111(2014) 3032-7.

B. Su, X. Wang, L. Zheng, G. Perry, M.A. Smith, X. Zhu, Abnormal mitochondrial dynamics and neurodegenerative diseases, BiochimBiophysActa. 1802 (2010) 135-42.

B. Thrash-Williams, S.S. Karuppagounder, D. Bhattacharya, M. Ahuja, V. Suppiramaniam, M. Dhanasekaran, Methamphetamine-induced dopaminergic toxicity prevented owing to the neuroprotective effects of salicylic acid, Life Sci. (2016) 30122-9.

B. Uttara, A.V. Singh, P. Zamboni et al., Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options, Current Neuropharmacology. 7 (2009) 65-74.

B.A. Hemmings, D.F. Restuccia, PI3K-PKB/Akt pathway, Cold Spring HarbPerspect Biol. 4 (2012) 89.

B.P.Yu, Cellular defenses against damage from reactive oxygen species, Physiological Reviews. 74 (1994) 139–162.

B.Y. Li, Y.H. Yuan, J.F. Hu et al., Protective effect of Bu-7, a flavonoid extracted from Clausenalansium, against rotenone injury in PC12 cells, ActaPharmacol Sin 32 (2011) 1321-6.

C. Bartholomeusz, A.M. Gonzalez-Angulo, Targeting the PI3K signaling pathway in cancer therapy, Expert OpinTher Targets. 16 (2012) 121-30.

C. Klein, W. Ana, Genetics of Parkinson ’s disease, Cold Spring HarbPerspect Med. (2012) a008888.

C. Pacelli, D. De Rasmo, A. Signorile, I. Grattagliano, G. di Tullio, A. D'Orazio, B. Nicoet al., Mitochondrial defect and PGC-1α dysfunction in parkin-associated familial Parkinson's disease, BiochimBiophysActa. 1812 (2011) 1041-53.

C. Pirkevi, S. Lesage, A. Brice, A. NazliBaşak,From genes to proteins in Mendelian Parkinson's disease: an overview, The Anatomical Record. (2009) 1893-901.

C. Soto, Unfolding the role of protein misfolding in neurodegenerative diseases, Nat. Rev. Neurosci. 4 (2003) 49-60.

C. Stockburger, D. Miano, M. Baeumlisberger, T. Pallas, T.N. Arrey, M. Karas, K. Friedland, W.E. Müller, A Mitochondrial Role of SV2a Protein in Aging and Alzheimer's Disease: Studies with Levetiracetam, J Alzheimers Dis. 50 (2015) 201-15.

C.C. Goulet, K.A. Volk, C.M. Adams, L.S. Prince, J.B. Stokes, P.M. Snyder, Inhibition of the epithelial Na+ channel by interaction of Nedd4 with a PY motif deleted in Liddle's syndrome, J Biol Chem. 273(1998) 30012–7.

C.K. Oh , B.S. Han, W.S. Choi et al., Translocation and oligomerization of Bax is regulated independently by activation of p38 MAPK and caspase-2 during MN9D dopaminergic neurodegeneration, Apoptosis. 16 (2011) 1087-100.

C.L. Chao, C.S.Weng, N.C. Chang et al., Naringenin more effectively inhibits inducible nitric oxide synthase and cyclo- oxygenase-2 expression in macrophages than in microglia, Nutr Res. 30 (2010)858-864.

C.M. Chong, M. Shen, Z.Y. Zhou et al., Discovery of a benzofuran derivative (MBPTA) as a novel ROCK inhibitor that protects against MPP(+)-induced oxidative stress and cell death in SH-SY5Y cells, Free RadicBiol Med. 74 (2014) 283-93.

C.M. Chong, Z.Y. Zhou, V. Razmovski-Naumovskiet al.,Danshensu protects against 6-hydroxydopamine-induced damage of PC12 cells in vitro and dopaminergic neurons in zebrafish, NeurosciLett.543 (2013) 121-5.

C.W. Zhang, H.B. Adeline, B.H. Chai, E.T. Hong, C.H. Ng, K.L. Lim, Pharmacological or Genetic Activation of Hsp70 Protects against Loss of Parkin Function, Neurodegener Dis. (2016).

Cookson, R. Mark, Parkinsonism Due to Mutations in PINK1, Parkin, and DJ-1 and Oxidative Stress and Mitochondrial Pathways, Cold Spring HarbPerspect Med. 2 (2012) 9:a009415.

D. Brancho, N. Tanaka, A. Jaeschkeet al., Mechanism of p38 MAP kinase activation *in vivo*, Genes Dev.17 (2003) 1969-78.

D. Chhangani, A. Mishra,Mahogunin ring finger-1 (MGRN1) suppresses chaperone-associated misfolded protein aggregation and toxicity,Sci Rep. 3 (2013)1972.

D. Colle, D.B. Santos, J.M. Hartwig, M. Godoi, D.F. Engel, A.F. De Bem, A.L. Braga, M. Farina Succinobucol, A Lipid-Lowering Drug, Protects Against 3-Nitropropionic Acid-Induced Mitochondrial Dysfunction and Oxidative Stress in SH-SY5Y Cells via Upregulation of Glutathione Levels and Glutamate Cysteine Ligase Activity, MolNeurobiol. 53 (2016) 1280-95.

D. Heras-Sandoval, J.M. Pérez-Rojas, J. Hernández-Damián et al., The role of PI3K/AKT/mTOR pathway in the modulation of autophagy and the clearance of protein aggregates inneurodegeneration, Cell Signal.(2014) 0898-6568.

D. Tomar, P. Prajapati, L. Sripada, K. Singh, R. Singh, A.K. Singh, R. Singh, TRIM13 regulates caspase-8 ubiquitination, translocation to autophagosomes and activation during ER stress induced cell death, BiochimBiophysActa. 1833 (2013) 3134-44.

D.M. Arduino, A.R. Esteves, C.R. Oliveira, S.M. Cardoso, Mitochondrial metabolism modulation: a new therapeutic approach for Parkinson's disease, CNS Neurol. Disord. Drug Targets. 9 (2010)105–119.

E. Floor, M.G. Wetzel, Increased protein oxidation in human substantianigra pars compacta in comparison with basal ganglia and prefrontal cortex measured with an improved dinitrophenylhydrazine assay, Journal of Neurochemistry. 70(1998) 268–275.

E. Leroy, R. Boyer, G. Auburger et al., The ubiquitin pathway in Parkinson's disease, Nature. 395 (1998) 451–452.

E. Leroy, R. Boyer, G.Auburger et al., The ubiquitin pathway in Parkinson's disease, Nature. 395 (1998) 451-452.

E. Maida, B. S. Balaji, S. Divakar, G. Geetha, Ligand based pharmacophore modeling, virtual screening and molecular docking studies to design novel pancreatic lipase inhibitors, Int J Pharm PharmSci.9 (2017) 48-64.

E.A. Liedhegner, K.M. Steller, JJ.Mieyal, Levodopa activates apoptosis signalling kinase 1 (ASK1) and promotes apoptosis in a neuronal model: implications for the treatment of Parkinson's disease, Chem Res Toxicol.24 (2011) 1644-52.

E.C. Hirsch, S.Hunot, Neuroinflammation in Parkinson's disease: a target foneuroprotection, Lancet Neurol.8 (2009) 382-97.

E.K. Butler, A. Voigt, A.K. Lutz, J.P. Toegel, E. Gerhardt, P. Karsten, B., Reinartz A. Falkenburger, K.F. Winklhofer, J.B. Schulz, The mitochondrial chaperone protein TRAP1 mitigates α-Synuclein toxicity, PLoS Genet. 8 (2012) e1002488.

E.K. Kim, E.J. Choi, Pathological roles of MAPK signaling pathways in human diseases, BiochimBiophysActa (2010)1802396-405.

E.M. Valente, A.R.Bentivoglio, P.H. Dixon et al., Localization of a novel locus for autosomal recessive early-onset parkinsonism, PARK6, on human chromosome 1p35-p36, Am J Hum Genet. 68 (2001) 895-900.

F. Cicchetti, R.A. Barker, The glial response to intracerebrally delivered therapies for neurodegenerative disorders: is this a critical issue, Front Pharmacol.10 (2014) 5-139.

F. Gao, D. Chen, Q. Hu, G. Wang, Rotenone directly induces BV2 cell activation via the p38 MAPK pathway,PLoS One. 20 (2013) 8-72046.

F. Saitoh, T. Araki, Proteasomal degradation of glutamine synthetase regulates schwann cell differentiation, J Neurosci. 30(2010) 1204-12.

G. Perry, A. Nunomura, K. Hirai, Is oxidative damage the fundamental pathogenic mechanism of Alzheimer's and other neurodegenerative diseases, Free Radical Biology and Medicine. 33 (2002) 1475–1479.

G. Wang, J. Pan, S.D. Chen, Kinases and kinase signaling pathways: potential therapeutic targets in Parkinson's disease,ProgNeurobiol. 98 (2012) 207-21.

G. Zhu, X. Wang, S. Wu et al.,Neuroprotective effects of puerarin on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced Parkinson's disease model in mice, Phytother Res. 28 (2014) 179-86.

G.E. Gibson, A. Starkov, J.P. Blass, R.R. Ratan, M.F. Beal, Cause and consequence: mitochondrial dysfunction initiates and propagates neuronal dysfunction, neuronal death and behavioral abnormalities in age-associated neurodegenerative diseases, BiochimBiophysActa. 1802 (2010) 122-34.

G.F. Zhang, Y. Zhang, G. Zhao, Crocin protects PC12 cells against MPP(+)-induced injury through inhibition of mitochondrial dysfunction and ER stress, Neurochem Int. 89 (2015) 101-10.

G.J. Soleas, E.P. Diamandis, D.M.Goldberg, Wine as a biological fluid: history, production, and role in disease prevention, Journal of Clinical Laboratory Analysis. 11 (1997) 287–313.

G.P. Kumar, F.Khanum,Neuroprotective potential of phytochemicals, PharmacognRev.6 (2012) 81-90.

H. Barry, Antioxidants in human health and disease, Annu. Rev. Nut. 16(1996)33–50.

H. Kettenmann, U.K. Hanisch, M. Noda, A. Verkhratsky, Physiology of microglia,Physiol Rev. 91(2011) 461-553.

H. Liu, P. Mao, J. Wang, T. Wang, C.H. Xie, Allicin Protects PC12 Cells Against 6-OHDA-Induced Oxidative Stress and Mitochondrial Dysfunction via Regulating Mitochondrial Dynamics, Cell PhysiolBiochem. 36 (2015) 966-79.

H. Mortiboys, R. Furmston, G. Bronstad, J. Aasly, C. Elliott, O. Bandmann, UDCA exerts beneficial effect on mitochondrial dysfunction in LRRK2(G2019S) carriers and in vivo, Neurology. 85 (2015) 846-52.

H.Matsumine, M. Saito, S.Shimoda-Matsubayashi et al., Localization of a gene for an autosomal recessive form of juvenile Parkinsonism to chromosome 6q25.2-27, Am J Hum Genet, 60 (1997) 588-596.

I. Russo, L. Bubacco, E.Greggio, LRRK2 and neuroinflammation: partners in crime in Parkinson's disease, J Neuroinflammation. 21(2014)11-52.

I.E. Zohn, K.V. Anderson, L. Niswander, The Hectd1 ubiquitin ligase is required for development of the head mesenchyme and neural tube closure, Dev Biol. 306 (2007) 208–21.

I.G. Onyango, J. Lu, M. Rodova, E. Lezi, A.B. Crafter, R.H. Swerdlow, Regulation of neuron mitochondrial biogenesis and relevance to brain health, BiochimBiophysActa. 1802 (2010) 228-34.

J. Elkharaz, A. Ugun-Klusek, D. Constantin-Teodosiu, K. Lawler, R.J. Mayer, E. Billett, J. Lowe, L. Bedford, Implications for oxidative stress and astrocytes following 26S proteasomal depletion in mouse forebrain neurons, BiochimBiophysActa. 1832 (2013) 1930-8.

J. Hardy, H.Orr, The genetics of neurodegenerative diseases, J. Neurochem. 7 (2006)1690-1699.

J. Kawamata, S. Suzuki, S.Shimohama, Enhancement of nicotinic receptors alleviates cytotoxicity in neurological disease models, TherAdv Chronic Dis. 2 (2011) 197-208.

J. Kim, J.W. Byun, I. Choi et al., PINK1 Deficiency Enhances Inflammatory Cytokine Release from Acutely Prepared Brain Slices. ExpNeurobiol 22 (2013) 38-44.

J. Korbecki, I. Baranowska-Bosiacka, I. Gutowskaet al., The effect of reactive oxygen species on the synthesis of prostanoids from arachidonic acid, J PhysiolPharmacol. 64(2013) 409-21.

J. LoPiccolo, G.M. Blumenthal, W.B. Bernstein et al., Targeting the PI3K/Akt/mTOR pathway: effective combinations and clinical considerations, Drug Resist Updat.11 (2008) 32-50.

J. Massano, K.P. Bhatia , Clinical Approach to Parkinson's disease: Features, Diagnosis, and Principles of Management, Cold Spring HarbPerspect Med. 2 (2012)6:a008870.

J. Massano, K.P. Bhatia, Clinical Approach to Parkinson's disease: Features, Diagnosis, and Principles of Management. Cold Spring HarbPerspect Med. 2 (2012) 6:a008870.

J. Mei, C. Niu, Alterations of Hrd1 expression in various encephalic regional neurons in 6-OHDA model of Parkinson's disease, NeurosciLett. 474 (2010) 63-8.

J. Nataraj, T. Manivasagam, A. Justin Thenmozhi, M.M. Essa, Neuroprotective effect of asiatic acid on rotenone-induced mitochondrial dysfunction and oxidative stress-mediated apoptosis in differentiated SH-SYS5Y cells, NutrNeurosci. (2016).

J. Segura-Aguilar, I. Paris, P. Muñoz, E. Ferrari, L. Zecca, F.A. Zucca, Protective and toxic roles of dopamine in Parkinson's disease, J Neurochem. 129 (2014) 898-915.

J. Sone, J. Niwa, K. Kawai, S. Ishigaki, S. Yamada, H. Adachi, M. Katsuno, F. Tanaka, M. Doyu, G. Sobue, Dorfin ameliorates phenotypes in a transgenic mouse model of amyotrophic lateral sclerosis, J Neurosci Res. 88 (2010) 123–35.

J.A. Bayliss, Z.B. Andrews, Ghrelin is neuroprotective in Parkinson's disease: molecular mechanisms of metabolic neuroprotection, TherAdvEndocrinolMetab. 4 (2013) 25-36.

J.H. Jara, D.D. Frank, P.H. Özdinler, Could dysregulation of UPS be a common underlying mechanism for cancer and neurodegeneration? Lessons from UCHL1, Cell BiochemBiophys. 67 (2013) 45-53.

J.Y. Ha, J.S. Kim, Y.H. Kang, E. Bok, Y.S. Kim, J.H. Son, Tnfaip8 l1/Oxi-β binds to FBXW5, increasing autophagy through activation of TSC2 in a Parkinson's disease model, J Neurochem. 129 (2014) 527-38.

K. Dasuri, L. Zhang, J.N. Keller, Oxidative stress, neurodegeneration, and the balance of protein degradation and protein synthesis, Free RadicBiol Med. 62 (2012) 170-85.

K. Gunjima, R. Tomiyama, K. Takakura et al., 3, 4-dihydroxybenzalacetone protects against Parkinson's disease-related neurotoxin 6-OHDA through Akt/Nrf2/glutathione pathway, J Cell Biochem. 115 (2014) 151-60.

K. Low, P. Aebischer , Use of viral vectors to create animal models for Parkinson's disease, Neurobiol Dis. 48 (2012)189-201.

K. Nakaso, N. Tajima, Y. Horikoshi et al., The estrogen receptor β-PI3K/Akt pathway mediates the cytoprotective effects of tocotrienol in a cellular Parkinson's disease model,BiochimBiophysActa. 1842 (2014) 1303-12.

K. M. Varier, S. Thangarajan, A. Chinnasamy, G. Balakrishnan, R. Paulose, Comparative analysis of potentiality of Esculin and hinokitol (B-thujaplicin) as anti-parkinsonism drugs: a pilot in silico study, Int J Pharm Pharm Sci. 9 (2017) 108-115.

K. Takeda, H.Ichijo, Neuronal p38 MAPK signalling: an emerging regulator of cell fate and function in the nervous system, Genes Cells. 7 (2002)1099-111.

K. Tanji, Y. Miki, T. Ozaki, A. Maruyama, H. Yoshida, J. Mimura, T. Matsumiya, F. Mori, T. Imaizumi, K. Itoh, A. Kakita, H. Takahashi, K. Wakabayashi, Phosphorylation of serine 349 of p62 in Alzheimer's disease brain,ActaNeuropatholCommun. 2 (2014) 50.

K. Yasuko, K. Mayumi, K. Kanaeet et al., Roles of PI3K/AKT/GSK3/mTOR Pathway in Cell Signaling of Mental Illnesses, Depression Research and Treatment. (2012) 752563:8.

K.J. Thomas, M.R. Cookson, The role of PTEN-induced kinase 1 in mitochondrial dysfunction and dynamics, Int J Biochem Cell Biol. 41 (2009) 2025-35.

K.K. Wong, J.A. Engelman, L.C.Cantley, Targeting the PI3K signaling pathway in cancer, CurrOpin Genet Dev.20 (2010) 87.

K. Sekiyama, S. Sugama, M. Fujita et al., Neuroinflammation in Parkinson's Disease and Related Disorders: A Lesson from Genetically Manipulated Mouse Models of -Synucleinopathies, Parkinson’s Disease. (2012)271732-8.

L. Cilenti, C.T. Ambivero, N. Ward, E.S. Alnemri, D. Germain, A.S. Zervos, Inactivation of Omi/HtrA2 protease leads to the deregulation of mitochondrial Mulan E3 ubiquitin ligase and increased mitophagy, BiochimBiophysActa. 1843 (2014)1295-307.

L. Tsu-Kung, C. Shang-Der, C. Yao-Chung, et al. Resveratrol Partially Prevents Rotenone-Induced Neurotoxicity in Dopaminergic SH-SY5Y Cells through Induction of Heme Oxygenase-1 Dependent Autophagy, Int.J.Mol.Sci. 15 (2014) 1625-1646.

L. Wang, H.J. Yang, Y.Y. Xia, et al., Insulin-like growth factor 1 protects human neuroblastoma cells SH-EP1 against MPP+-induced apoptosis by AKT/GSK-3β/JNK signaling, Apoptosis.15 (2010) 1470-9.

L. Zhang, L. Huang, L. Chen et al., Neuroprotection by tetrahydroxystilbeneglucoside in the MPTP mouse model of Parkinson's disease, ToxicolLett. 222 (2013) 155-63.

L.D. Mercer, B.L. Kelly, M.K. Horne, P.M.Beart, Dietary polyphenols protect dopamine neurons from oxidative insults and apoptosis investigations in primary rat mesencephalic cultures, Biochemical Pharmacology.69 (2005) 339–345.

L.F. Burbulla, C. Schelling, H. Kato, D. Rapaport, D. Woitalla, C. Schiesling, C. Schulte, M. Sharma, T. Illig, P. Bauer, S. Jung, A. Nordheim, L. Schöls, O. Riess, R. Krüger, Dissecting the role of the mitochondrial chaperone mortalin in Parkinson's disease: functional impact of disease-related variants on mitochondrial homeostasis, Hum Mol Genet. 19 (2010) 4437-52.

L.J. Zhang, Y.Q. Xue, C. Yang et al., Human albumin prevents 6-hydroxydopamine-induced loss of tyrosine hydroxylase in in vitro and in vivo,PLoS One.7 (2012) 41226.

L.M. Collins, G.W. O'Keeffe, C.M. Long-Smith et al., Mitogen-activated protein kinase phosphatase (MKP)-1 as a neuroprotective agent: promotion of the morphological development of midbrain dopaminergic neurons, Neuromolecular Med.15 (2013) 435-46.

M. Comalada, J. Lloberas, A.Celada, MKP-1: a critical phosphatase in the biology of macrophages controlling the switch between proliferation and activation, Eur J Immunol. 42 (2012) 1938-48.

M. Elstner, C.M. Morris, K. Heim et al., Expression analysis of dopaminergic neurons in Parkinson's disease and aging links transcriptional dysregulation of energy metabolism to cell death, ActaNeuropathol 122 (2011) 75-86.

M. Jaiswal, H. Sandoval, K. Zhang, V. Bayat, H.J. Bellen, Probing mechanisms that underlie human neurodegenerative diseases in Drosophila, Annu Rev Genet. 46 (2012) 371-96.

M. Jaiswal, K. Honda, A. Takeda, Oxidative damage to RNA in neurodegenerative diseases, Journal of Biomedicine and Biotechnology. 82323 (2006) 6.

M. Klinkenberg, S. Gispert, J.A. Dominguez-Bautista et al., Restriction of trophic factors and nutrients induces PARKIN expression, Neurogenetics 13(2012) 9-21.

M. Lee, E. McGeer, R. Kodela et al., NOSH-aspirin (NBS-1120), a novel nitric oxide and hydrogen sulfide releasing hybrid, attenuates neuroinflammation induced by microglial and astrocytic activation: a new candidate for treatment of neurodegenerative disorders, Glia 61 (2013) 1724-34.

M. Mylène, M. Sébastien, C.C. Sophie et al., Neurosurgery in Parkinson's disease: Social adjustment, quality of life and coping strategies, Neural Regen Res. 25 (2013) 2856-67.

M. Ouyang, X.Shen, Critical role of ASK1 in the 6-hydroxydopamine-induced apoptosis in human neuroblastoma SH-SY5Y cells, J Neurochem. 97 (2006) 234-44.

M. Vairetti, A. Ferrigno, V. Rizzo, G. Ambrosi, A. Bianchi, P. Richelmi, F. Blandini, M.T, Armentero, Impaired hepatic function and central dopaminergic denervation in a rodent model of Parkinson's disease: a self-perpetuating crosstalk?, BiochimBiophysActa. 1822 (2012) 176-84.

M.A. Alam, M.M. Rahman, Mitochondrial dysfunction in obesity: potential benefit and mechanism of Co-enzyme Q10 supplementation in metabolic syndrome, J Diabetes MetabDisord. 13 (2014) 60.

M.A. Schreiber, S.L. McIntire, A Caenorhabditiselegans p38 MAP kinase pathway mutant protects from dopamine, methamphetamine, and MDMA toxicity, NeurosciLett. 498 (2011) 99-103.

M.A. Sonia, A. Sarkar, K. Anand, R.K. Ambasta, P. Kumar, Sesamol and naringenin reverse the effect of rotenone-induced PD rat model, Neuroscience. 254 (2013) 379-94.

M.F. Beal, Mitochondria take center stage in aging and neurodegeneration, Annals of Neurology. 58(2005) 495–505.

M.H. Polymeropoulos, J.J. Higgins, L.I. Golbe, W.G. Johnson, S.E. Ide, G. Di Iorio, G.Sanges et al., Mapping of a Gene for Parkinson's Disease to Chromosome 4q21-q23, Science. 274 (1996) 1197-1199.

M.I. Santos, R.A. Tomé, G.B. Saldanha, G.C. Militão, R.M. Freitas, Oxidative stress in the hippocampus during experimental seizures can be ameliorated with the antioxidant ascorbic acid,Oxid Med Cell Longev. 2 (2009) 214-21.

M.J. Farrer, K. Gwinn-Hardy, M. Muenter, F.W. DeVrieze, R. Crook, J. Perez-Tur, S. Lincoln et al., A chromosome 4p haplotype segregating with Parkinson's disease and postural tremor, Hum Mol Genet. 8 (1999) 81–85.

M.M. Khan, A.Ahmad,T.Ishrat et al., Resveratrol attenuates 6-hydroxydopamine-induced oxidative damage and dopamine depletion in rat model of Parkinson's disease, Brain Research. 1328 (2010) 139–151.

Mizuno, Yoshikuni, et al., Progress in familial Parkinson's disease." Parkinson's Disease and Related Disorders, Springer Vienna. (2006)191-204.

N. Egawa, K. Yamamoto, H. Inoue, Hikawa et al., The endoplasmic reticulum stress sensor, ATF6α, protects against neurotoxin-induced dopaminergic neuronal death, J Biol Chem. 286 (2011) 7947-57.

N. Exner, A.K. Lutz, C. Haass, K.F.Winklhofer, Mitochondrial dysfunction in Parkinson's disease: molecular mechanisms and pathophysiological consequences, The EMBO journal. (2012) 3038-62.

N.K. Jha, S.K. Jha, R. Karet al., Role of Oxidative Stress, ER Stress and Ubiquitin Proteasome System in Neurodegeneration, MOJ Cell Sci Report.1 (2012) 00010.

N.P. Mena, O. García-Beltrán, F. Lourido, P.J. Urrutia, R. Mena, V. Castro-Castillo, B.K. Cassels, M.T. Núñez, The novel mitochondrial iron chelator 5-((methylamino)methyl)-8-hydroxyquinoline protects against mitochondrial-induced oxidative damage and neuronal death, BiochemBiophys Res Commun. 463 (2015) 787-92.

Nunomura A, Honda K, Takeda A, Oxidative damage to RNA in neurodegenerative diseases, Journal of Biomedicine and Biotechnology. (2006) 6.

Oster S, Radad K, Scheller D, et al. Rotigotine protects against glutamate toxicity in primary dopaminergic cell culture, Eur J Pharmacol. 724 (2014) 31-42.

P. Dewapriya, S.W. Himaya, Y.X. Li et al.,Tyrosol exerts a protective effect against dopaminergic neuronal cell death in in vitro model of Parkinson's disease, Food Chem.141 (2013) 1147-57.

P. Giuliani, S. Romano, P. Ballerini et al., Protective activity of guanosine in an in vitro model of Parkinson's disease,Panminerva Med. 54 (2012) 43-51.

P. Jenner, Hunot, Olanow, Oxidative stress in Parkinson's disease, Annals of Neurology 53(2003) S26–S38.

P. Liu, H. Cheng, T.M. Roberts, J.J. Zhao, Targeting the phosphoinositide 3-kinase pathway in cancer, Nat Rev Drug Discov.8 (2009) 627-44.

P. Yu, Y. Chen, D.A. Tagle, T. Cai, PJA1, encoding a RING-H2 finger ubiquitin ligase, is a novel human X chromosome gene abundantly expressed in brain, Genomics. 79 (2002) 869–74.

P.E.J. Spencer,The Interactions of Flavonoids within Neuronal Signalling Pathways, Genes & Nutrition. (2007) 257–273.

P.gupta, N. rai, P. gautam, Anticancer drugs as potential inhibitors of acrab-tolc of multidrug resistant escherichia coli: an *in silico* molecular modeling and docking study, Asian J Pharm Clin Res. 8 (2015) 351-358.

P.M. Keeney, J. Xie, R.A. Capaldi, J.P. Bennett,Parkinson's disease brain mitochondrial complex I has oxidatively damaged subunits and is functionally impaired and misassembled, Journal of Neuroscience. 26 (2006) 5256–5264.

P.P. Roux, J.Blenis, ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions, MicrobiolMolBiol Rev. 68 (2004) 320-44.

Q. Zhong, W. Gao, F. Du, X. Wang, Mule/ARF-BP1, a BH3-only E3 ubiquitin ligase, catalyzes the polyubiquitination of Mcl-1 and regulates apoptosis, Cell. 121 (2005) 1085–95.

R. D. Sri, R. Ranjitha, R. Sripathi, K.S. Alimuhammad, S.Ravi ,Docking studies in target proteins involved in antibacterial actionmechanisms: alkaloids isolated from scutellaria genus, Asian J Pharm Clin Res. 9 (2016) 121-125.

R.K. Leak, Heat shock proteins in neurodegenerative disorders and aging. J Cell Commun Signal, 8 (2014) 293-310.

R.M. Sánchez-Mora, H. Arboleda, G.Arboleda, PINK1 overexpression protects against C2-ceramide-induced CAD cell death through the PI3K/AKT pathway, J MolNeurosci 47 (2012) 582-94.

R.P. Patel, J. M.c.Andrew, H. Sellak, C.R. White, H. Jo, B.A. Freeman, V.M. Darley-Usmar, Biological aspects of reactive nitrogen species, BiochimBiophysActa. 1411 (1999) 385-400.

R.S. Chen, P.L. Wu, R.Y. Chiou, Peanut roots as a source of resveratrol, Journal of Agricultural and Food Chemistry. 50 (2002)1665–1667.

R.S. Dharani, R. Ranjitha, R. Sripathi, K.S.M. Ali, S.Ravi, Docking studies in target proteins involved in antibacterial action mechanisms: alkaloids isolated from scutellaria genus, Asian J Pharm Clin Res. 9 (2016) 121-125.

R.S. Morrison, Y. Kinoshita, M.D. Johnson et al., Neuronal Survival and Cell Death Signaling Pathways, In: Madame Curie Bioscience Database [Internet], Landes Bioscience (2000).

S. Bavithra, K. Selvakumar, P. Kumari, R. Krishnamoorthy, G.Venkataraman, P.Arunakaran, Polychlorinated biphenyl (PCBs)-induced oxidative stress plays a critical role on cerebellar dopaminergic receptor expression: ameliorative role of quercetin, Neurotox Res. 21 (2012) 149-59.

S. Gao, C. Alarcón, G. Sapkota, S. Rahman, P.Y. Chen, N. Goerner, M.J. Macias, H. Erdjument-Bromage, P. Tempst, J. Massagué, Ubiquitin ligase Nedd4L targets activated Smad2/3 to limit TGF-beta signaling,Mol Cell. 36(2009) 457–68.

S. Han, R.M. Witt, T.M. Santos, C. Polizzano, B.L. Sabatini, V. Ramesh, Pam (Protein associated with Myc) functions as an E3 ubiquitin ligase and regulates TSC/mTOR signaling, Cell Signal. 20 (2008) 1084–91.

S. Hunot, E.C. Hirsch, Neuroinflammatoryprocesses in Parkinson's disease, Ann Neurol. 53 (2003) 49-58.

S. Jana, M. Sinha, D. Chanda, T. Roy, K. Banerjee, S. Munshi, B.S. Patro, S. Chakrabarti, Mitochondrial dysfunction mediated by quinone oxidation products of dopamine: Implications in dopamine cytotoxicity and pathogenesis of Parkinson's disease, BiochimBiophysActa. 1812 (2011) 663-73.

S. Kannappan, C.V. Anuradha, Naringenin enhances insulin-stimulated tyrosine phosphorylation and improves the cellular actions of insulin in a dietary model of metabolic syndrome, Eur J Nutr. 49 (2010) 101-9.

S. Manoharan, G.J. Guillemin, R.S. Abiramasundari, M.M. Essa, M. Akbar, M.D.Akbar, The Role of Reactive Oxygen Species in the Pathogenesis of Alzheimer's disease, Parkinson's disease, and Huntington's disease, Oxid Med Cell Longev. (2016) 8590578.

S. Sen, B.W. Andrew, The Therapeutic Potential of LRRK2 and A-Synuclein in Parkinson's Disease, Antioxidants & Redox Signaling.(2009) 2167–2187.

S. Sen, et al, Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect, IJPS. (2010) 91-100.

S. Srivastav, S.K. Singh, A.K. Yadav, S. Srikrishna, Folic acid supplementation rescues anomalies associated with knockdown of parkin in dopaminergic and serotonergic neurons in Drosophila model of Parkinson's disease, BiochemBiophys Res Commun. 460 (2015) 780-5.

S. Tangvarasittichai, Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus, World J Diabetes. 6 (2015) 456-80.

S.E. Lloyd, E.G. Maytham, H. Pota, J. Grizenkova, E. Molou, J. Uphill, H. Hummerich, J. Whitfield, M.P. Alpers, S. Mead, J. Collinge, HECTD2 is associated with susceptibility to mouse and human prion disease, PLoS Genet. 5(2009) e1000383.

S.H. Kwon, S.X. Ma, S.I. Hong et al.,EucommiaulmoidesOliv. bark. attenuates 6-hydroxydopamine-induced neuronal cell death through inhibition of oxidative stress in SH-SY5Y cells, J Ethnopharmacol. 152 (2014) 173-82.

S.K. Jha, N.K. Jha, R. Kar, R.K. Ambasta, P.Kumar, p38 MAPK and PI3K/AKT SignallingCascades in Parkinson's Disease,Int J Mol Cell Med. 4 (2015) 67-86.

S.Y. Kim, M.S. Woo, J.S. Park et al., Regulation of matrix metalloproteinase-9 gene expression in MPP+- or 6-OHDA-treated human neuroblastoma SK-N-BE(2)C cells,Neurochem Int. 56 (2010) 437-42.

S.Z. Imam, W. Trickler, S. Kimura, Z.K. Binienda, M.G. Paule, W. J. Slikker, S. Li, R.A. Clark, S.F. Ali,Neuroprotective efficacy of a new brain-penetrating C-Abl inhibitor in a murine Parkinson's disease model, PLoS One 8 (2013) e65129.

T. Araki, J. Milbrandt, ZNRF proteins constitute a family of presynaptic E3 ubiquitin ligases, J Neurosci. 23 (2003) 9385–94.

T. Chen, Y. Deng, X. Liao, J. Zhao, G. Wen, G. Weng, F. Ma, Y. Zheng, Effect of curcumin on oligomer formation and mitochondrial ATP-sensitive potassium channels induced by overexpression or mutation of α-synuclein, Zhonghua Yi Xue Yi ChuanXueZaZhi. 32 (2015) 462-7.

T. Mori, Y. Li, H. Hata, H. Kochi, NIRF is a ubiquitin ligase that is capable of ubiquitinating PCNP, a PEST-containing nuclear protein, FEBS Lett. 557 (2004) 209–14.

T. Zarubin, J. Han, Activation and signaling of the p38 MAP kinase pathway, Cell Res.15 (2005)11-8.

T.K. Lin, S.D. Chen, Y.C. Chuang, H.Y. Lin, C.R. Huang, J.H. Chuang, P.W. Wang, S.T. Huang, M.M. Tiao, J.B. Chen, C.W. Liou, Resveratrol partially prevents rotenone-induced neurotoxicity in dopaminergic SH-SY5Y cells through induction of heme oxygenase-1 dependent autophagy, Int J Mol Sci. 15 (2014)1625-46.

T.M. Dawson, V.L. Dawson, Molecular pathways of neurodegeneration in Parkinson's disease. Science 302 (2003) 819–822.

Tapia-González, R.M. Giráldez-Pérez, M.I. Cuarteroet al., Dopamine and α-synuclein dysfunction in Smad3 null mice, MolNeurodegener. 13 (2011) 6-72.

V. Dias, E. Junn, M.M. Mouradian, The role of oxidative stress in Parkinson's disease, J Parkinsons Dis. 3 (2013) 461-91.

V. Dias, E. Junn, M.M. Mouradian, The Role of Oxidative Stress in Parkinson's Disease, Journal of Parkinson's Disease. 3 (2013) 461–491.

V. Gundersen, Protein aggregation in Parkinson's disease, ActaNeurolScand Suppl. (2010) 82-7.

V. Gundersen, Protein aggregation in Parkinson's disease,ActaNeurolScand Suppl. (2010) 82-87.

V. Lobo, A. Patil, A. Phatak, N.Chandra, Free radicals, antioxidants and functional foods: Impact on human health,Pharmacognosy Reviews. (2010) 118–126.

V. Srinivasan, D.W. Spence, S.R. Pandi-Perumal, G.M. Brown, D.P. Cardinali, Melatonin in mitochondrial dysfunction and related disorders, Int J Alzheimers Dis. (2011) 326320.

V.S. Van Laar, S.B. Berman, The interplay of neuronal mitochondrial dynamics and bioenergetics: implications for Parkinson's disease, Neurobiol Dis. 51 (2013) 43-55.

W. Kim, Y. Lee, N.D. McKenna et al., miR-126 contributes to Parkinson's disease by dysregulating the insulin-like growth factor/phosphoinositide 3-kinase signaling, Neurobiol Aging 35 (2014) 1712-21.

W.I. Sivitz, M.A. Yorek, Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities, Antioxid Redox Signal. (2010) 537-77.

W.L. Chien, T.R. Lee, S.Y. Hung et al., Impairment of oxidative stress-induced heme oxygenase-1 expression by the defect of Parkinson-related gene of PINK1, J Neurochem.117 (2011) 643-53.

W.S. Choi, D.S. Eom, B.S. Han et al., Phosphorylation of p38 MAPK induced by oxidative stress is linked to activation of both caspase-8- and -9-mediated apoptotic pathways in dopaminergic neurons, J Biol Chem. 279(2004) 20451-60.

Wang, Chaodong, et al., Parkin dosage mutations in patients with early-onset sporadic and familial Parkinson's disease in Chinese: an independent pathogenic role, Brain research. (2010) 30-38.

X. Hu, Z. Weng, C.T. Chu et al., Peroxiredoxin-2 protects against 6-hydroxydopamine-induced dopaminergic neurodegeneration via attenuation of the apoptosis signal-regulating kinase (ASK1) signaling cascade, J Neurosci. 31 (2011) 247-61.

X. Ye, Y. Han, L. Zhang, W. Liu, J. Zuo, MTERF4 regulates the mitochondrial dysfunction induced by MPP(+) in SH-SY5Y cells, BiochemBiophys Res Commun. 464 (2015) 214-20.

X.Q. Bao, X.C. Kong, L.B. Kong et al.,Squamosamide derivative FLZ protected dopaminergic neuron by activating Akt signaling pathway in 6-OHDA-induced in vivo and in vitro Parkinson's disease models, Brain Res. 1547 (2014) 49-57.

Y. Cabeza-Arvelaiz, R.H. Schiestl, Transcriptome analysis of a rotenone model of parkinsonism reveals complex I-tied and -untied toxicity mechanisms common to neurodegenerative diseases, PLoS One. 7 (2012) e44700.

Y. Han, P. Gao, S. Qiu, L. Zhang, L. Yang, J. Zuo, C. Zhong, S. Zhu, W. Liu, MTERF2 contributes to MPP(+)-induced mitochondrial dysfunction and cell damage, BiochemBiophys Res Commun. 471 (2016) 177-83.

Y. Lee, H.R. Park, H.J. Chun, J. Lee, Silibinin prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease via mitochondrial stabilization, J Neurosci Res. 93 (2015) 755-65.

Y. Li, T. Ozaki, H. Kikuchi, H. Yamamoto, M. Ohira, A. Nakagawara , A novel HECT-type E3 ubiquitin protein ligase NEDL1 enhances the p53-mediated apoptotic cell death in its catalytic activity-independent manner, Oncogene. 27 (2008) 3700–9.

Y. Tasaki, J. Yamamoto, T. Omuraet al., Meloxicam ameliorates motor dysfunction and dopaminergic neurodegeneration by maintaining Akt-signaling in a mouse Parkinson's disease model, NeurosciLett. 521 (2012) 15-9.

Y.D. Kwak, B. Wang, J.J. Li, R. Wang, Q. Deng, S. Diao, Y. Chen, R. Xu, E. Masliah, H. Xu, J.J. Sung, F.F. Liao, Upregulation of the E3 ligase NEDD4-1 by oxidative stress degrades IGF-1 receptor protein in neurodegeneration, J Neurosci. 32 (2012) 10971-81.

Y.J. Chen, H.Y. Zheng, X.X. Huang, S.X. Han, D.S. Zhang, J.Z. Ni, X.Y. He, Neuroprotective Effects of Icariin on Brain Metabolism, Mitochondrial Functions, and Cognition in Triple-Transgenic Alzheimer's Disease Mice, CNS NeurosciTher. 22 (2016) 63-73.

Z. Cai, W. Zeng, K. Tao, F. Lu, G. Gao, Q. Yang, Myricitrin alleviates MPP^+-induced mitochondrial dysfunction in a DJ-1-dependent manner in SN4741 cells, BiochemBiophys Res Commun. 458 (2015) 227-33.

Z. Tan, H. Xu, X. Shen, H. Jiang, Nesfatin-1 antagonized rotenone-induced neurotoxicity in MES23.5 dopaminergic cells, Peptides. 69 (2015) 109-14.

Appendices

APPENDIX I

REAGENTS AND BUFFERS

1X Phosphate-buffered saline (PBS; pH-7.4)	
NaCl	8g
KCl	0.2g
Na_2HpO_4	1.44g
KH_2PO_4	0.24g
ddH_2O	Adjust the volume to one litre and stored at room Tm
1X Phosphate-buffered saline (PBS; pH-7.2)	
NaCl	8g
KCl	0.2g
Na_2HpO_4	1.44g
KH_2PO_4	0.2g
ddH_2O	Adjust the volume to one litre and stored at room Tm
Laemmli sample buffer (SB; pH-6.8)	
SDS	1.6g
Glycerol	8mL
β-ME	4mL
Bromophenol blue	0.0016g
ddH_2O	Adjust the volume to one litre and stored at at 4ºC/-20ºC
1X Phosphate-buffered saline (PBS; pH-7.2)	
NaCl	8g
KCl	0.2g
Na_2HpO_4	1.44g
KH_2PO_4	0.2g
ddH_2O	Adjust the volume to one litre and stored at room Tm
Acrylamide mix	
Acrylamide	29g
Bis Acrylamide	1g
ddH2O	Adjust the volume to 100mL and stored at 4ºC
10% Ammonium persulfate (APS))	
APS	0.1g
ddH_2O	Adjust the volume to 1mL and stored at -20ºC

1 M Tris Cl (pH- 6.8)	
Trizma	12.114g
ddH2O	Adjust the volume to 100mL and stored at 4ºC
1.5 M Tris Cl (pH- 8.8)	
Trizma	18.17g
ddH2O	Adjust the volume to 100mL and stored at 4ºC
	10% SDS
SDS	10g
ddH2O	Adjust the volume to 100mL and stored at room Tm
Tris Glycine (pH-8.3)	
Trizma	3.02g
Glycine	18.768g
SDS	1g
ddH_2O	Adjust the volume to one litre and stored at room Tm
10% resolving gel (pH-6.8)	
ddH2O	2.1mL
30% Acrylamide mix	0.5mL
1 M Tris (pH-6.8)	0.38mL
10% SDS	0.03mL
10% APS	0.035mL
TEMED	0.004mL
5% stacking gel (pH-6.8)	
ddH2O	2.1mL
30% Acrylamide mix	0.5mL
1 M Tris (pH-6.8)	0.38mL
10% SDS	0.03mL
10% APS	0.035mL
TEMED	0.004mL

APPENDIX II

LIST OF CHEMICALS AND ANTIBODIES

S.No.	Name of Antibodies	Company
1.	β-Actin	Santacruz
2.	PARKIN	Santacruz
4.	DJ-1	Santacruz
5.	P38	Santacruz
6.	Fluorescent Secondary Antibodies (Anti- rabbit IGg)	R&D System
7.	Fluorescent Secondary Antibodies (Anti- mouse IGg)	R&D System

S.No.	Common Name	Chemical Formula	Company
1.	Acrylamide	CH_2=$CHCONH_2$	Sigma-Aldrich
2.	Acetic acid glacial	CH_3COOH	Sigma-Aldrich
3.	Ammonium persulfate (APS)	$(NH4)_2S_2O_8$	Sigma-Aldrich
4.	Quercetin	$C_{15}H_{10}O_7$ $2H_2O$	Sigma-Aldrich
5.	Bis Acrylamide	$(H_2C$=$CHCONH)_2CH_2$	Sigma-Aldrich
6.	Bovine serum albumin (BSA)	-	Sigma-Aldrich
7.	Bromophenol blue	$C_{19}H_{10}Br_4O_5S$	Sigma-Aldrich
8.	Resveratrol	$C_{14}H_{12}O_3$	Sigma-Aldrich
9.	Dimethyl sulfoxide (DMSO)	$(CH_3)_2SO$	Sigma-Aldrich
10.	Dulbecco's Modified Eagle Medium (DMEM)	-	Invitrogen
11.	Ethanol	CH_3CH_2OH	Sigma-Aldrich
12.	Ethylene glycol-bis(2-aminoethylether) -*N,N,N′,N′*-tetraacetic acid	$[CH_2OCH_2CH_2N(CH_2CO_2H)_2]_2$	Sigma-Aldrich
13.	Ethylene Diamine tetraacetic acid disodium salt dehydrate (EDTA)	$C_{10}H_{14}N_2Na_2O_8$ $2H_2O$	Sigma-Aldrich
14.	Formaldehyde solution	HCHO	Sigma-Aldrich
15.	GBX developer/replenisher	-	Sigma-Aldrich
16.	GBX fixer/replenisher	-	Sigma-Aldrich
17.	Glycerol	$C_3H_8O_3$	Sigma-Aldrich
18.	Glycine	NH_2CH_2COOH	Sigma-Aldrich

S.No.	Common Name	Chemical Formula	Company
19.	Coomassie Brilliant Blue (CBB)	$C_{45}H_{44}N_3NaO_7S_2$	Sigma-Aldrich
20.	Hydrochloric acid	HCl	Sigma-Aldrich
21.	Lithium carbonate	Li_2CO_3	Sigma-Aldrich
22.	Methanol	CH_3OH	Sigma-Aldrich
23.	*N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (TEMED)	$(CH_3)_2NCH_2CH_2N(CH_3)_2$	Sigma-Aldrich
24.	Naringenin	$C_{15}H_{12}O_5$	Sigma-Aldrich
25	Penicillin streptomycin	-	Sigma-Aldrich
26.	Phenylmethylsulfonyl fluoride (PMSF)	$C_7H_7FO_2S$	Sigma-Aldrich
27.	Ponceau S	$C_{22}H_{12}N_4Na_4O_{13}S_4$	Sigma-Aldrich
28	Potassium chloride	KCl	Sigma-Aldrich
29.	Potassium phosphate monobasic	KH_2PO_4	Sigma-Aldrich
30.	Protease inhibitor	-	Roche
31.	Skimmed milk	-	Sigma-Aldrich
32.	Sodium chloride	NaCl	Sigma-Aldrich
33.	Sodium dodecyl sulfate (SDS)	$CH_3(CH_2)_{11}OSO_3Na$	Sigma-Aldrich
34.	Sodium orthovanadate	Na_3VO_4	Sigma-Aldrich
35.	Sodium phosphate dibasic	Na_2HPO_4	Sigma-Aldrich
36.	Sodium phosphate monobasic monohydrate	NaH_2PO_4 H_2O	Sigma-Aldrich
37.	Sodium pyrophosphate tetrabasic decahydrate	$Na_4P_2O_7$ $10H_2O$	Sigma-Aldrich
38.	Triton-X 100	-	Sigma-Aldrich
39.	Trizma	$NH_2C(CH_2OH)_3$	Sigma-Aldrich
40.	Tween-20	-	Sigma-Aldrich
41.	β-Mercaptoethanol (β-ME)	$HSCH_2CH_2OH$	Sigma-Aldrich
42.	MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine	Sigma-Aldrich

APPENDIX III

LIST OF INSTRUMENTS

S.No.	Name of instruments	Company
1.	CO_2 incubator	Thermo fisher scientific
2.	Chest freezer	Blue Star CHF150B
3.	Autoradiography Hypercassette	Amersham Biosciences RPN11642
4.	Inverted laboratory microscope	Magnus
5.	Gel Rocking Shaker	Tarsons 4080. Rockymax
6.	Minispin Microcentrifuge	Eppendorf Centrifuge Z606235-
7.	Refrigerator	Blue Star
8.	SDS PAGE and Western Blot unit	BioRad Mini-PROTEAN Tetra Cell, Mini Trans-Blot Module, and PowerPac Basic Power
9.	Water bath	JULABO
10.	Biological Safety Cabinet	Esco Labculture® Class II Type A2

List of Publications

LIST OF PUBLICATIONS

PUBLICATIONS FROM THESIS

[Cumulative impact factor: 34.88; cumulative citations: 84; h-index: 5]

1. **Saurabh Kumar Jha**, Pravir Kumar*, **(2017*)*** *"Molecular docking study of neuroprotactiveplant-derived biomolecules in Parkinson's disease".* ***International Journal of Pharmacy and Pharmaceutical Sciences***. Vol 9 Issue 9, Sept 2017. **[Impact factor: 0.88]**

2. **Saurabh Kumar Jha**, Pravir Kumar*, **(2017)** *"An insilico study of Naringenin mediated neuroprotection in Parkinson Disease"*. ***Asian J Pharm Clin Res***. Vol 10, Issue 8, 2017. **[Impact factor: 0.68]**

3. **Saurabh Kumar Jha**, Niraj Kumar Jha, Dhiraj Kumar, Rashmi K. Ambasta, Pravir Kumar (**2017**), *Linking mitochondrial dysfunction, metabolic syndrome and stress signaling in Neurodegeneration*, ***Biochimica et Biophysica Acta, (BBA Molecular Basis of disease***; Biochim Biophys Acta. 2017 May;1863(5):1132-1146. doi: 10.1016/j.bbadis.2016.06.015. Epub 2016 Jun 21. Elsevier; [**Impact factor: 5.47]**

4. **Saurabh Kumar Jha**, **Niraj Kumar Jha**, Dhiraj Kumar, Renu Sharma, Abhishek Shrivastava, Rashmi K Ambasta and Pravir Kumar (**2017**), *Stress induced synaptic dysfunction and neurotransmitter release in Alzheimer's disease: Can neurotransmitter and Neuromodulator be a potential therapeutic target?*, ***Journal of Alzheimer's Disease***, J Alzheimers Dis. 2017;57(4):1017-1039. doi: 10.3233/JAD-1606232016; [**Impact factor: 3.72]**

5. **Saurabh Kumar Jha**, Niraj Kumar Jha, Rohan Kar, Rashmi K Ambasta, Pravir Kumar (**2015**), *p38 MAPK and PI3K/AKT signaling in Parkinson's disease,* ***International Journal of Molecular and Cellular Medicine*** (IJMCM), Spring 2015 2015 Spring;4(2):67-86.

OTHER PUBLICATIONS

1. **Saurabh Kumar Jha**, Niraj Kumar Jha, Pravir Kumar and Rashmi K Ambasta (**2016**), *Molecular Chaperones and Ubiquitin Proteasome System in Tumor Biogenesis: An Overview,* ***Journal of Cell Biology and Cell Metabolism***, 3: 010

2. Renu Sharma, Dhiraj Kumar, Niraj Kumar Jha, **Saurabh Kumar Jha**, Rashmi K Ambasta and Pravir Kumar (**2016**), *Re-expression of Cell cycle markers in aged Neurons and Muscles: Whether cells should divide or die?,* ***Biochimica et Biophysica Acta, (BBA Molecular Basis of disease***; Elsevier; [**Impact factor: *5.*47]**

3. Rashmi K. Ambasta, **Saurabh Kumar Jha**, Dhiraj Kumar, Renu Sharma, Niraj Kumar Jha, and Pravir Kumar (**2015**) *Comparative study of anti-angiogenic activities of luteolin, lectin and lupeol biomolecules,* Accepted September 2015**,** ***Journal of Translational Medicine*****, [Impact factor: 3.96]**

4. Niraj Kumar Jha, **Saurabh Kumar Jha**, Dhiraj Kumar, Noopur Kejariwal, Renu Sharma, Rashmi K Ambasta and Pravir Kumar (**2015**), *Impact of IDE and Neprilysin in Alzheimer's disease biology: Characterization of putative cognates for therapeutic applications*. Accepted ***Journal of Alzheimer's Disease*** 2015;48(4):891-917. doi: 10.3233/JAD-150379. 14th July 2015, [**Impact factor: 4.15]**

5. Pravir Kumar, Dhiraj Kumar, **Saurabh Kumar Jha**, Niraj Kumar Jha, Rashmi K Ambasta (**2015**), *Ion channels in neurological disorders*, Accepted October 2015, ***Advances in Protein Chemistry and Structural Biology*****,** 2016;103:97-136. doi: 10.1016/bs.apcsb.2015.10.006. Epub 2015 Nov 28. **[Impact factor: 3.03]**

6. Pravir Kumar, Niraj Kumar Jha, **Saurabh Kumar Jha**, Karunya Ramani and Rashmi K Ambasta (**2015**), *Tau phosphorylation, molecular chaperones, Ubiquitin E3 ligase: clinical relevance in Alzheimer's disease*, ***Journal of Alzheimer's Disease***, 2015;43(2):341-61; [**Impact factor: 3.72]**

7. Niraj Kumar Jha, **Saurabh Kumar Jha,** Rohan Kar, Rashmi K Ambasta and Pravir Kumar (2014), *Role of oxidative stress, ER stress and Ubiquitin Proteasome system in neurodegeneration,* **MOJ *Cell Science and Report***. 07/**2014**; 01(2).

8. Niraj Kumar Jha, **Saurabh Kumar Jha,** Renu Sharma, Dhiraj Kumar, Rashmi K Ambasta and Pravir Kumar (**2017**), *Hypoxia induced signaling activation in Neurodegenerative Diseases: Targets for new therapeutic strategies,* ***Journal of Alzheimer's Disease***, 1 July 2017, [**Under revision] [Impact factor: 3.72].**

BOOK CHAPTER

1. Niraj Kumar Jha, **Saurabh Kumar Jha**, Satyaprakash, Rohan Kar, Deepak Rathore, Rashmi K Ambasta and Pravir Kumar (2015) *Epigenetics and angiogenesis in cancer,* ***Epigenetic Advancements in Cancer***, Edited by Manoj Mishra, Kumar Bishnupuri, 12/**2015**: **BOOK CHAPTER**; Springer International publishing AG.

CONFERENCE PROCEEDINGS

1. **Saurabh Kumar Jha** and Pravir Kumar*, (**2015**), *Comparative analysis of biomolecules in Parkinson's disease therapeutics,* 29th Annual Conference of Society for Neurochemistry India and Advancement in computation Neurochemistry and Neurobiology (SNCI-ACNN), December 19-21, 2015, NEHU Shillong [**Oral presentation**]

2. **Saurabh Kumar Jha,** Niraj Kumar Jha, SatyaPrakash, M. Sonia Angeline, Rashmi K. Ambasta and Pravir Kumar (2014), In silico study of flavonones in neurodegenerative disorders, International Symposium on Translational Neuroscience and XXXII Annual Conference of the Indian Academy of Neurosciences, 01-03 November, NIMHANS Bangalore, INDIA

3. **Saurabh Kumar Jha,** Niraj Kumar Jha, Rashmi K. Ambasta and Pravir Kumar (**2013**), *Structurally and functionally analyzed new generation anti-aging neuroprotective drugs,* NCRTPSB 2013, JMI 16-18 December, INDIA Journal of Protein and Proteomics, Vol.4, No.2 p62

4. **Saurabh Kumar Jha,** Niraj Kumar Jha, Deepak Rathore, Rashmi K. Ambasta and Pravir kumar (**2013**), *Traditional FDA approved anti-cancerous drugs*

versus new anti-cancerous drugs: A computational approach, 09-11 December, Bioworld Conference, IIT Delhi, Delhi, INDIA

5. Niraj Kumar Jha, **Saurabh Kumar Jha,** Satya Prakash, M. Sonia Angeline, Rashmi K. Ambasta and Pravir Kumar (2014), *Physiological stress in neurodegeneration: Interatomic partners based on In silco study,* International Symposium on Translational Neuroscience and XXXII Annual Conference of the Indian Academy of Neurosciences, 01-03 November, NIMHANS Bangalore, INDIA

6. Niraj Kumar Jha, **Saurabh Kumar Jha,** Rashmi K. Ambasta and Pravir kumar (**2013**) *Computer assisted protein analysis in hypoxia signaling,* NCRTPSB 2013, JMI 16-18 December, INDIA Journal of Protein and Proteomics, Vol.4, No.2 p37

7. Niraj Kumar Jha, Lakshmi, Binod Koirala, **Saurabh Kumar Jha,** Renu Sharma, Rohan Kar, Dhiraj, Jitendra Singh, Rashmi K. Ambasta and Pravir kumar; (**2013**), *Identification and validation of key Ubiquitin E3 ligases in type II diabetes: An in silico work*; 09-11 December, Bioworld Conference, IIT Delhi, Delhi, INDIA

8. Alka Raina, **Saurabh Kumar Jha,** Niraj Kumar Jha, Dhiraj kumar, Rashmi K Ambasta and Pravir Kumar*(**2015**), *Putative transcription factor binding elements of ubiquitin E3 ligase in neurodegenerative disorders,* 29th Annual Conference of Society for Neurochemistry India and Advancement in computation Neurochemistry and Neurobiology (SNCI-ACNN), December 19-21, 2015, NEHU Shillong [Poster presentation]

9. Abhisekh Srivastava, Puspendramani Mishra, Dhiraj kumar, **Saurabh Kumar Jha,** Niraj Kumar Jha, Rashmi K Ambasta and Pravir Kumar* (**2015**), *Relevance of terpenoids and alkaloids in neuroprotection,* 29th Annual Conference of Society for Neurochemistry India and Advancement in computation Neurochemistry and Neurobiology (SNCI-ACNN), December 19-21, 2015, NEHU Shillong [Poster presentation]

10. Swati Sharan, Niraj Kumar Jha, **Saurabh Kumar Jha,** Dhiraj kumar, Rashmi K Ambasta and Pravir Kumar*(**2015**), *Post-translational modification mechanism in Parkinson's disease pathology,* 29th Annual Conference of Society for Neurochemistry India and Advancement in computation Neurochemistry and Neurobiology (SNCI-ACNN), December 19-21, 2015, NEHU Shillong [Poster presentation]

11. Minal Singh, Niraj Kumar Jha, **Saurabh Kumar Jha,** Dhiraj kumar, Rashmi K Ambasta and Pravir Kumar* (**2015**), *In silico characterization of holo AβPP promoter and its transactivation modules* 29th Annual Conference of Society for Neurochemistry India and Advancement in computation Neurochemistry and Neurobiology (SNCI-ACNN), December 19-21, 2015, NEHU Shillong [Poster presentation]

12. Abhishek Srivastava, Puspendra M Mishra, **Saurabh Kumar Jha,** Niraj Kumar Jha, Rashmi K Ambasta and Pravir Kumar (2015), *In silico analysis cannaboids in neurodegeneration,* 6th World congress on biotechnology, New Delhi, October 5th-7th, *J Biotechnol Biomater* 2015, 5:6

13. Dhiraj Kumar, Niraj Kumar Jha, **Saurabh Kumar Jha,** Renu Sharma, Kushi Anand, Rashmi K. Ambasta and Pravir Kumar (2014), *Anti cancerous drugs as a neuroprotectant: a therapeutic intervention in neurodegenerative disorders,* International Symposium on Translational Neuroscience and XXXII Annual Conference of the Indian Academy of Neurosciences, 01-03 November, NIMHANS Bangalore, INDIA

WORKSHOP

1. **Saurabh Kumar Jha** (**2015**), Work shop on advancement in computation Neurochemistry and Neurobiology (SNCI-ACNN), December 16-18, 2015, NEHU Shillong.

Curriculum Vitae

CURRICULUM VITAE

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EDUCATION/TRAINING (*Begin with bachelor education and include all higher education)*

INSTITUTION AND LOCATION	POSITION	YEAR(s)	FIELD OF STUDY
Delhi Technological University (Formerly DCE), Delhi-110042, (India),	Ph.D. (Senior Research Fellow) (Thesis submission: August 2017)	2013-2017	Neuroscience (Biotechnology) Mentor: Dr. Pravir Kumar
VIT University Vellore, Tamil Nadu, (India),	M.Sc Biotechnology	2011 - 2013	Biotechnology
L.N.M.U Darbhanga, Bihar, (India),	B.Sc Biotechnology	2007-2010	Biotechnology (Honours)

Honors

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2013-2015	DTU-JRF (MHRD)	Department of Biotechnology, Delhi Technological University (Formerly Delhi College of Engineering), India

B. PEER-REVIEWED PUBLICATIONS

Cumulative impact factor of all publications =**34 (approx.); h-index: 5; i10-index: 2;**

Cumulative citation index =**83**

Publication as PhD student (Department of Biotechnology, Delhi Technological University, India)

2017

1. **Saurabh Kumar Jha**, Niraj Kumar Jha, Dhiraj Kumar, Rashmi K. Ambasta, Pravir Kumar (**2017**), *Linking mitochondrial dysfunction, metabolic syndrome and stress signaling in Neurodegeneration*, ***Biochimica et Biophysica Acta, (BBA Molecular Basis of disease***; Biochim Biophys Acta. 2017 May;1863(5):1132-1146. doi: 10.1016/j.bbadis.2016.06.015. Epub 2016 Jun 21. Elsevier; [**Impact factor: 5.47]**
2. **Saurabh Kumar Jha**, **Niraj Kumar Jha**, Dhiraj Kumar, Renu Sharma, Abhishek Shrivastava, Rashmi K Ambasta and Pravir Kumar (**2017**), *Stress induced synaptic dysfunction and neurotransmitter release in Alzheimer's disease: Can neurotransmitter and Neuromodulator be a potential therapeutic target?*, ***Journal of Alzheimer's Disease***, J Alzheimers Dis. 2017;57(4):1017-1039. doi: 10.3233/JAD-1606232016; [**Impact factor: 3.72]**

3. Niraj Kumar Jha, **Saurabh Kumar Jha,** Renu Sharma, Dhiraj Kumar, Rashmi K Ambasta and Pravir Kumar (**2017**), *Hypoxia induced signaling activation in Neurodegenerative Diseases: Targets for new therapeutic strategies,* ***Journal of Alzheimer's Disease***, 1 July 2017, [**Under revision]** [**Impact factor: 3.72]**

4. Renu Sharma, Dhiraj Kumar, Niraj Kumar Jha, **Saurabh Kumar Jha**, Rashmi K Ambasta and Pravir Kumar (**2016**), *Re-expression of Cell cycle markers in aged Neurons and Muscles: Whether cells should divide or die?,* ***Biochimica et Biophysica Acta, (BBA Molecular Basis of disease***; Elsevier; 1863(1):324-336, [**Impact factor: *5*.47]** [https://www.ncbi.nlm.nih.gov/pubmed/27639832]

2016

5. **Saurabh Kumar Jha**, Niraj Kumar Jha, Pravir Kumar and Rashmi K Ambasta (**2016**), *Molecular Chaperones and Ubiquitin Proteasome System in Tumor Biogenesis: An Overview,* ***Journal of Cell Biology and Cell Metabolism***, 3: 010 [http://heraldopenaccess.us/fulltext/Cell-Biology-&-Cell-Metabolism/Molecular-Chaperones-and-Ubiquitin-Proteasome-System-in-Tumor-Biogenesis-An-Overview.php]

2015

6. **Saurabh Kumar Jha**, Niraj Kumar Jha, Rohan Kar, Rashmi K Ambasta, Pravir Kumar (**2015**), *p38 MAPK and PI3K/AKT signaling in Parkinson's disease,* ***International Journal of Molecular and Cellular Medicine*** (IJMCM), Spring 2015 2015 Spring;4(2):67-86.

7. Rashmi K. Ambasta, **Saurabh Kumar Jha**, Dhiraj Kumar, Renu Sharma, Niraj Kumar Jha, and Pravir Kumar (**2015**) *Comparative study of anti-angiogenic activities of luteolin, lectin and lupeol biomolecules,* Accepted September 2015**,** 13:307, ***Journal of Translational Medicine*****, [Impact factor: 3.96]**

8. Niraj Kumar Jha, **Saurabh Kumar Jha**, Dhiraj Kumar, Noopur Kejariwal, Renu Sharma, Rashmi K Ambasta and Pravir Kumar (**2015**), *Impact of IDE and Neprilysin in Alzheimer's disease biology: Characterization of putative cognates for therapeutic applications*. Accepted ***Journal of Alzheimer's Disease*** 2015;

48(4):891-917. doi: 10.3233/JAD-150379. 14th July 2015, [**Impact factor: 4.15]**

9. Pravir Kumar, Dhiraj Kumar, **Saurabh Kumar Jha**, Niraj Kumar Jha, Rashmi K Ambasta (**2015**), *Ion channels in neurological disorders*, Accepted October 2015, ***Advances in Protein Chemistry and Structural Biology*****,** 2016;103:97-136. doi: 10.1016/bs.apcsb.2015.10.006. Epub 2015 Nov 28. **[Impact factor: 3.03]**

2014

10. Pravir Kumar, Niraj Kumar Jha, **Saurabh Kumar Jha**, Karunya Ramani and Rashmi K Ambasta (**2015**), *Tau phosphorylation, molecular chaperones, Ubiquitin E3 ligase: clinical relevance in Alzheimer's disease*, ***Journal of Alzheimer's Disease***, 2015;43(2):341-61; [**Impact factor: 3.72]**

11. Niraj Kumar Jha, **Saurabh Kumar Jha,** Rohan Kar, Rashmi K Ambasta and Pravir Kumar (2014), *Role of oxidative stress, ER stress and Ubiquitin Proteasome system in neurodegeneration,* **MOJ *Cell Science and Report***. 07/**2014**; 01(2).

BOOK CHAPTER

1. Niraj Kumar Jha, **Saurabh Kumar Jha**, Satyaprakash, Rohan Kar, Deepak Rathore, Rashmi K Ambasta and Pravir Kumar (2015) Epigenetics and angiogenesis in cancer, Epigenetic Advancements in Cancer, Edited by Manoj Mishra, Kumar Bishnupuri, 12/**2015**: BOOK CHAPTER; **Springer International publishing AG**.

CONFERENCE, PROCEEDINGS AND SYMPOSIUM

2015

1. **Saurabh Kumar Jha** and Pravir Kumar*, (**2015**), *Comparative analysis of biomolecules in Parkinson's disease therapeutics,* 29th Annual Conference of Society for Neurochemistry India and Advancement in computation

Neurochemistry and Neurobiology (SNCI-ACNN), December 19-21, 2015, NEHU Shillong [**Oral presentation**], **INDIA**

2. Alka Raina, **Saurabh Kumar Jha,** Niraj Kumar Jha, Dhiraj kumar, Rashmi K Ambasta and Pravir Kumar*(**2015**), *Putative transcription factor binding elements of ubiquitin E3 ligase in neurodegenerative disorders,* 29th Annual Conference of Society for Neurochemistry India and Advancement in computation Neurochemistry and Neurobiology (SNCI-ACNN), December 19-21, 2015, NEHU Shillong [Poster presentation], **INDIA**

3. Abhisekh Srivastava, Puspendramani Mishra, Dhiraj kumar, **Saurabh Kumar Jha,** Niraj Kumar Jha, Rashmi K Ambasta and Pravir Kumar* (**2015**), *Relevance of terpenoids and alkaloids in neuroprotection,* 29th Annual Conference of Society for Neurochemistry India and Advancement in computation Neurochemistry and Neurobiology (SNCI-ACNN), December 19-21, 2015, NEHU Shillong [Poster presentation], **INDIA**

4. Swati Sharan, Niraj Kumar Jha, **Saurabh Kumar Jha,** Dhiraj kumar, Rashmi K Ambasta and Pravir Kumar*(**2015**), *Post-translational modification mechanism in Parkinson's disease pathology,* 29th Annual Conference of Society for Neurochemistry India and Advancement in computation Neurochemistry and Neurobiology (SNCI-ACNN), December 19-21, 2015, NEHU Shillong [Poster presentation], **INDIA**

5. Minal Singh, Niraj Kumar Jha, **Saurabh Kumar Jha,** Dhiraj kumar, Rashmi K Ambasta and Pravir Kumar* (**2015**), *In silico characterization of holo AβPP promoter and its transactivation modules* 29th Annual Conference of Society for Neurochemistry India and Advancement in computation Neurochemistry and Neurobiology (SNCI-ACNN), December 19-21, 2015, NEHU Shillong [Poster presentation], **INDIA**

6. Abhishek Srivastava, Puspendra M Mishra, **Saurabh Kumar Jha,** Niraj Kumar Jha, Rashmi K Ambasta and Pravir Kumar (2015), *In silico analysis cannaboids*

in neurodegeneration, 6th World congress on biotechnology, New Delhi, October 5th-7th, *J Biotechnol Biomater* 2015, 5:6, **INDIA**

2014

7. **Saurabh Kumar Jha,** Niraj Kumar Jha, SatyaPrakash, M. Sonia Angeline, Rashmi K. Ambasta and Pravir Kumar (2014), In silico study of flavonones in neurodegenerative disorders, International Symposium on Translational Neuroscience and XXXII Annual Conference of the Indian Academy of Neurosciences, 01-03 November, NIMHANS Bangalore, INDIA, **INDIA**

8. Niraj Kumar Jha, **Saurabh Kumar Jha,** Satya Prakash, M. Sonia Angeline, Rashmi K. Ambasta and Pravir Kumar (2014), *Physiological stress in neurodegeneration: Interatomic partners based on In silco study,* International Symposium on Translational Neuroscience and XXXII Annual Conference of the Indian Academy of Neurosciences, 01-03 November, NIMHANS Bangalore, **INDIA**

9. Dhiraj Kumar, Niraj Kumar Jha, **Saurabh Kumar Jha,** Renu Sharma, Kushi Anand, Rashmi K. Ambasta and Pravir Kumar (2014), *Anti cancerous drugs as a neuroprotectant: a therapeutic intervention in neurodegenerative disorders,* International Symposium on Translational Neuroscience and XXXII Annual Conference of the Indian Academy of Neurosciences, 01-03 November, NIMHANS Bangalore, **INDIA**

2012

10. **Saurabh Kumar Jha,** Niraj Kumar Jha, Rashmi K. Ambasta and Pravir Kumar (**2013**), *Structurally and functionally analyzed new generation anti-aging neuroprotective drugs,* NCRTPSB 2013, JMI 16-18 December, INDIA Journal of Protein and Proteomics, Vol.4, No.2 p62, **INDIA**

11. **Saurabh Kumar Jha,** Niraj Kumar Jha, Deepak Rathore, Rashmi K. Ambasta and Pravir kumar (**2013**), *Traditional FDA approved anti-cancerous drugs versus new anti-cancerous drugs: A computational approach,* 09-11 December, Bioworld Conference, IIT Delhi, **INDIA**

12. Niraj Kumar Jha, **Saurabh Kumar Jha,** Rashmi K. Ambasta and Pravir kumar (**2013**) *Computer assisted protein analysis in hypoxia signaling,* NCRTPSB 2013, JMI 16-18 December, INDIA Journal of Protein and Proteomics, Vol.4, No.2 p37, **INDIA**

13. Niraj Kumar Jha, Lakshmi, Binod Koirala, **Saurabh Kumar Jha,** Renu Sharma, Rohan Kar, Dhiraj, Jitendra Singh, Rashmi K. Ambasta and Pravir kumar; (**2013**), *Identification and validation of key Ubiquitin E3 ligases in type II diabetes: An in silico work*; 09-11 December, Bioworld Conference, IIT Delhi, Delhi, **INDIA**

2012

14. P. Asthana, A.Sarkar, S. Angeline, Kushi Anand, N. Jaiswal, **S.K. Jha**, N.K. Jha, Priya Chaterjee R. K. Ambasta, Pravir Kumar* (2012), "Protective effect of naringenin on hypoxia induced muscles degeneration". 2012-S-7580-SfN (*Poster presentatio*n in the annual meeting of *Society of Neuroscience*, Oct 17, 2012, New Orleans, Poster, **USA**.

WORKSHOP AND SEMINARS ATTENDED

2015

1. **Saurabh Kumar Jha** (2015), Work shop on advancement in computation Neurochemistry and Neurobiology (SNCI ACNN), December 16-18, 2015, NEHU Shillong, **INDIA**

2. Participated in conference on "***Brain and Brain Sciences***: Hot Spot Area in Translational Research" Society for Neurochemistry, India (**SNCI**) VIT University chapter meeting 13th April, 2012, **INDIA**

3. Participated in the special course on "***Plant Transgenic Technology***" jointly organized by the institute of plant genetics, Gottfried Wilhelm Leibniz University, Hannover, Germany and Indo-German Centre for Excellence in Biosciences(IGCEB) in SBST, VIT University 29th January, 2012, **INDIA**

4. 5th International Conference on Science, Engineering and Technology (**SET**), VIT UNIVERSITY, 8th November, 2012, **INDIA**

5. 4th International Conference on Science, Engineering and Technology (**SET**), VIT UNIVERSITY,3th May, 2012, **INDIA**

6. 3nd International Conference on Science, Engineering and Technology (**SET**), VIT UNIVERSITY, 17th November,

2011, INDIA

C. NATIONAL LEVEL EXAMS QUALIFIED

1. **Delhi Technological University (DTU),** PhD written examination and interview in Biotechnology Engineering. 2013, (DTU-JRF), Aug-2013-July-2015, (**Rank-GENERAL -01**)

2. **Madurai Kamaraj University** written test for "**Integrated M.Sc- Ph.D**" in Genomics, 2012, **(Rank-GENERAL-23)**

3. **BHU M.sc Plant Biotechnology,** 2011, **(Rank-GENERAL-54)**

4. **South Asian University** (SAU) combined entrance examination 2011 for M.Sc Biotechnology **(Rank-GENERAL-63)**

5. **Pondicherry Central University (**PCU) written test for "**Biochemistry and Molecular Biology**" in Biological Sciences, 2011 **(Rank-GENERAL-45)**

6. **Pondicherry Central University** (PCU) written test for "**Ecology and Environmental Sciences**" in Biological Sciences, 2011 **(Rank-GENERAL-22)**

D. PROFESSIONAL MEMBERSHIPS

1. Society of Neurochemistry India (SNCI), (India)-Life member

E. TECHNICAL EXPERIENCE

1. **Microbiology** – Culturing, Plating, Isolation, Identification and Preservation of Bacteria, Competent cell preparation

2. **Molecular Biology** – Isolation of Bacterial / Plant / Animal / Plasmid DNA, PCR and Restriction Enzyme Digestion, Electrophoresis ,SDS, Polymerase chain reaction (PCR), Paraformaldehyde fixation of tissues, Immunocytochemistry, Hematoxylin and Eosin staining, Chick Chorioallantoic Membrane (CAM) assay

3. **Bioinformatics** – Sequence searching and alignment (BLAST, CLUSTAL W), Auto Dock, Integrated database search (Entrez)

4. **Immunology** – Blood group typing, Widal Test, Immuno-diffusion and Immuno-electrophoresis, ELISA

5. **Biochemistry** – Qualitative and Quantitative estimation of Proteins and Carbohydrates and tests for their identification, Paper and Thin Layer Chromatography, UV Spectroscopy, Restriction digestion

6. **Cell culture-** Sub culturing of cell, Drug screening, Cell viability Assays (Trypan blue exclusion test, MTT assay), Microscopic work, SDS-PAGE and Western Blotting

7. **Animal Model Generation-**Hypoxia model, Parkinson's disease model

F. COMPUTER SKILLS

Conversant with Microsoft Windows and good computer skills with proficiency in common utilities like MS Office, Adobe Photoshop and statistics software (Prism)

G. STRENGTHS

Confidence, Team work spirit, Extrovert personality

H. PERSONAL DETAILS

Date of Birth: 27th December 1989

Languages Known: To Speak: English, Hindi & Maithili

To write: English, Hindi & Maithili

Age: 28

I. REFERENCES

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Original Article

MOLECULAR DOCKING STUDY OF NEUROPROTECTIVEPLANT-DERIVED BIOMOLECULES IN PARKINSON'S DISEASE

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ABSTRACT

Objective: The objective of this study was to explore the therapeutic role of biomolecules in targeting the altered expression of Parkin in PD pathogenesis.

Methods: We employed various *in silico* tools such as drug-likeness parameters, namely, Lipinski filter analysis, Muscle tool for phylogenetic analysis, Castp Server for active site prediction, molecular docking studies using AutoDock 4.2.1 and LIGPLOT1.4.5 were carried out.

Results: Our results show that neuroprotective activity of Quercetin to be most effective and can provide their possible clinical relevance in PD. Further, initial screenings of the molecules were done based on Lipinski's rule of five. CastP server used to predict the ligand binding site suggests that this protein can be utilized as a potential drug target. Finally, we have found Quercetin to be most effective amongst four biomolecules in modulating Parkin based on minimum inhibition constant, Ki and highest negative free energy of binding with the maximum interacting surface area in a course of docking studies.

Conclusion: This research could provide a potential therapeutic window for the treatment of PD.

Keywords: Parkinson's disease, Ubiquitin E3 ligase, Parkin, Molecular docking, Biomolecules

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INTRODUCTION

PD is characterized by progressive loss of dopaminergic neurons in the *substantia nigra*, leading to the loss of motor skills [1-4]. Several risk factors, for instance, genetic polymorphisms, aging and dietary supplements have been reportedly involved in the development and prognosis of PD. Other causative factors that might influence disease outcomes include accumulation of reactive oxygen species (ROS), selective loss of neurons, loss of mitochondrial membrane potential and ATP depletion that are also known to be associated with the PD pathogenesis [5]. However, ubiquitin proteasome system (UPS), which is responsible for the recognition and degradation of damaged proteins, is found to be impaired in case of PD [6]. Parkin is a well-known component of UPS which is having a pivotal role in the protein homeostasis. Conversely, under stress condition, the normal functioning of Parkin is altered or down regulated and thereby leading to the prognosis of PD [7]. Furthermore, the connection between the UPS and neurodegeneration has been supported by the recognition of disease-causing mutations in genes coding for numerous UPS proteins in PD [8].

Importantly, the impaired function of Parkin can be ameliorated by using different biomolecules such as, Naringenin, Quercetin, Resveratrol and Sesamol. These biomolecules are having an antioxidant profile which would be a strong basis for showing neuroprotection within the brain [9]. These biomolecules are natural compounds which are found in many fruits and vegetables and having much stronger antioxidative and neuroprotective activities [10]. The neuroprotective properties of Naringenin Inhibited LPS induced NOS and COX2 expression and suppressed the production of NO in microglial cells. It also inhibited LPS/IFN-Y induced p38 and STAT-1 phosphorylation [11]. However, Quercetin, improved behavioural function, suppressed oxidative stress, brain swelling, and cellular injury both *in vitro* and *in vivo* [12]. Although, Sesamol and Naringenin reversed both the rotenone/MPTP+-induced toxicity in PD rat model [4]. Similarly, Resveratrol (3, 5, 4-trihydroxystilbene) is a naturally-occurring polyphenol found in peanuts, skin and seeds of grapes [13]. It plays a pivotal role in cell proliferation and apoptosis by acting on a variety of signalling mechanisms such as, Protein kinase B (Akt), Mitogen-activated protein kinase (MAPK), and many other signaling cascades [14]. Most recent reports suggest that resveratrol can induce the Heme Oxygenase-1 (HO-1) expression in dopaminergic neuron and thus can prevent the dopaminergic cell death through autophagic flux. Additionally, resveratrol can also display neuroprotective effects against rotenone-induced neurotoxicity in dopaminergic SH-SY5Y cells by modulating HO-1 dependent autophagy [15].

Therapeutic interferences in neurodegenerative conditions using biomolecules are a seemingly new prospect. Therefore, a mature understanding of the mechanism impacting biomolecules mediated therapeutics could contribute towards fostering neuroprotective strategies. Here, we have focused our study towards impaired Parkin activity by using various *in silico* approaches including molecular docking analysis. Further, these biomolecules such as Naringenin, Quercetin, Resveratrol and Sesamol were selected and screened via lipinski filter analysis followed by molecular docking of the screened molecules towards their clinical aspects in PD. Finally, in this paper, we have reported the active site of Parkin protein for a potential target of these biomolecules by molecular docking that may further provide their probable clinical relevance in PD biology.

MATERIALS AND METHODS

Retrieval of ubiquitin E3 ligase Parkin and their function recognition

The amino acid sequence of Ubiquitin E3 Ligase Parkin with accession numbers 4I1H_A 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/) [16].

Phylogenetic relationship and physicochemical properties

For multiple sequence analysis Muscle Software (http://www.ebi.ac.uk/Tools/msa/muscle/) was used and a phylogenetic tree was constructed using Muscle Software based on NJ (Neighbor

joining) plot without distance correction [17]. ProtParam (http://web.expasy.org/protparam/) was used to predict physicochemical properties. The parameters computed by ProtParam included the molecular weight, theoretical PI, aliphatic index and grand average of hydropathicity (GRAVY).

Homology modelling, visualization and quality assessment of 3D structure of Ubiquitin E3 Ligase Parkin

Homology modeling was used to determine the 3D structure of Parkin isoforms. A BLASTP search with default parameters was performed against the Brookhaven Protein Data Bank (PDB) to find suitable templates for homology modeling. Templates with PDB ID: 4I1H was retrieved for Parkin proteins 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 proteins was generated, structural evaluation and stereochemical analysis were performed using RAMPAGE (http://www.mordred.bioc.cam.ac.uk/~rapper/ rampage. php) [18]. Errat server (http://nihserver.mbi.ucla.edu/ ERRATv2/) was used to find the accuracy of the structure and visualization of determined structures was performed using Pymol viewer.

Ligand optimization

Reported ligand molecules (*Naringenin*, *Quercetin*, *Resveratrol* and *Sesamol*) along with their physical and chemical properties were retrieved from PubChem Compound Database (http:// www.pubchem.ncbi.nlm.nih.gov/). 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 viewer.

Lipinski filter analysis of screened drugs

An online tool Lipinski Filter (http://www.scfbio-iitd.res.in/ software/drug design/lipinski. jsp) was used to retrieve the information about drug likeness of drugs with the help of Lipinski Rule of five [19]. Lipinski rule (or Lipinski rule of five) helps to differentiate drug and non-drug 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 log P 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.

Active site prediction

Castp Server (http://www.sts.bioe.uic.edu/castp/) was used to predict the active sites of the protein. It could also be used to measure area, the circumference of mouth openings of each binding site in a solvent and molecular accessible surface [20]. PDB file of protein was upload in the server and it showed the ligand binding sites present in protein and the site with maximum surface area and maximum surface volume was selected and all the amino acid residues involved in binding with ligands were retrieved.

Preparation of protein and ligand molecules

Preparation of protein involves the addition of polar hydrogen atoms, the addition of charge and removal of any miscellaneous structures from the protein molecule by Autodock 4.2.1 whereas; ligand preparation involves the addition of charge.

Molecular docking analysis

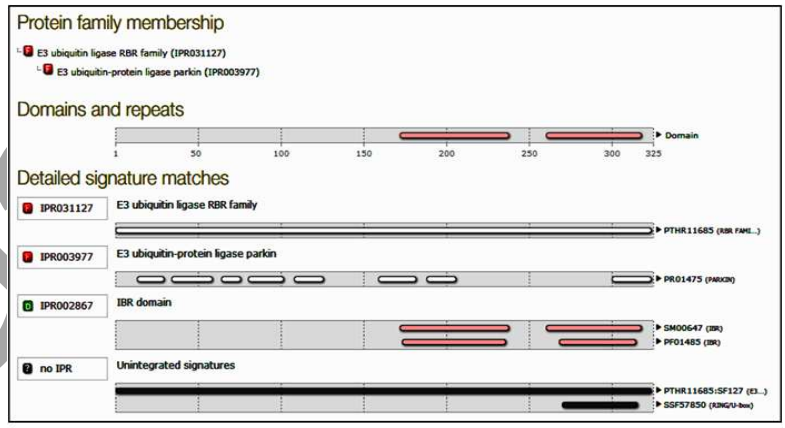
Prepared and optimized structures of ligands and proteins were ultimately used for molecular docking using Autodock 4.2.1 for predicting the possible protein–ligand interactions and the results that include the understanding of the association that involves H-bonding and hydrophobic interactions were analyzed using LIGPLOT1.4.5, a program to generate schematic diagrams of protein-ligand interactions [21].

RESULTS

Retrieval of ubiquitin E3 Ligase Parkin and their functional elucidation

Based on functional domain sequence of well-characterized gene/protein, a homology search was done using Basic Local Alignment Search Tool (BLAST). We have successfully hunted 5 isoforms of Parkin (table 1) on the basis of families and domains identified from Interproscan results. Interproscan study revealed that all homologues proteins for Parkin were belonging to E3 ubiquitin ligase RBR family (IPR031127), E3 ubiquitin-protein ligase Parkin family (IPR003977) and IBR domain (IPR002867) (fig. 1).

Figure 1 **Jha et al., 2017**



Retrieval of Ubiquitin E3 Ligase (Parkin) related Proteins and their functional elucidation

Fig. 1*:* Interproscan result for Parkin

Table 1*:* Hunted parkin related proteins

S. No.	Accession	Protein	Score	Identity	E Value
1	AGH62057.1	PARK2 splice variant	686	100%	0
2	BAA25751.1	Parkin	680	99%	0
3	ABN46990.1	Parkin 2	679	99%	0
4	4K95_A	Chain A, Crystal Structure Of Parkin	624	90%	0
5	Q9JK66.1	E3 ubiquitin-protein ligase Parkin	623	89%	0

Phylogenetic relationship and physicochemical properties

For multiple sequence analysis, *Muscle software* was used and found that amino acid residues were conserved in most of the isoforms of the Ubiquitin E3 ligase Parkin. A phylogenetic study of Parkin hunted proteins revealed that Parkin and PARK2 splice variant were different from others (fig. 2 (a and b).

However, another Chain A, Crystal Structure of Parkin and E3 ubiquitin-protein ligase Parkin were in the same cluster as share more homology while Parkin 2 was in another cluster. ProtParam showed that Mol. wt. of Parkin was 36393.5 Daltons and an isoelectric point was 7.06 which indicate that Parkin had slightly positive charged respectively. The GRAVY index of-0.372 for Parkin is indicative of hydrophilic (table 2).

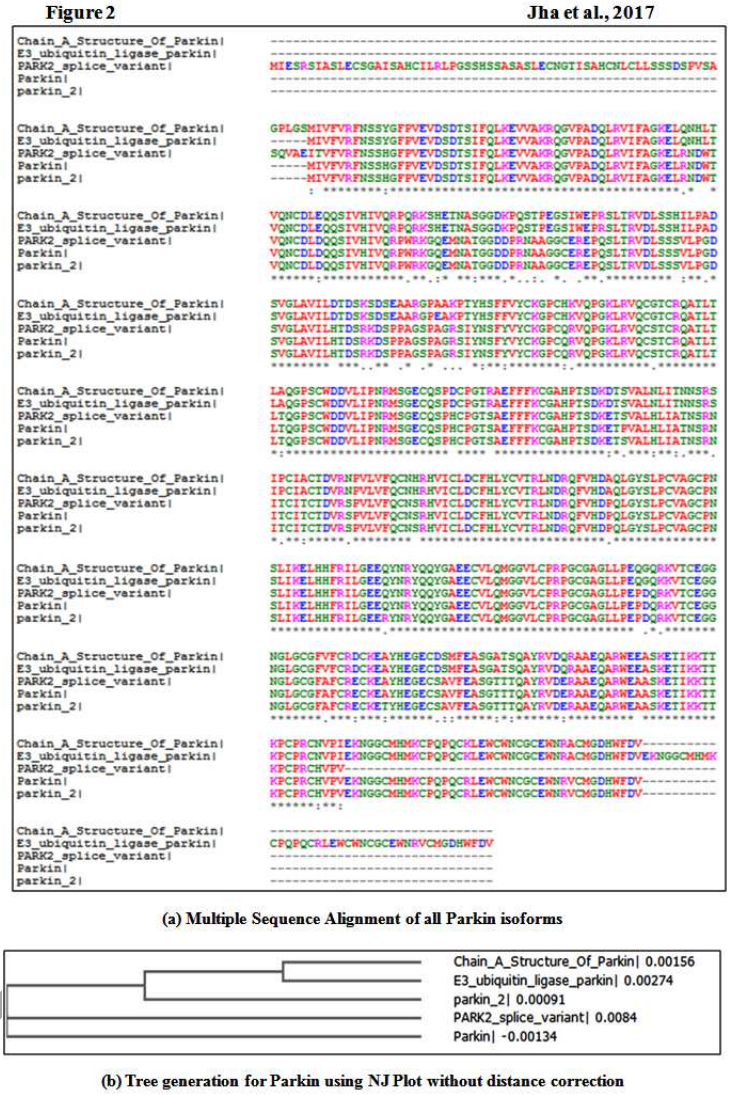


Fig. 2: (a) Multiple sequence alignment of all Parkin isoforms and (b) tree generation for parkin using NJ plot without distance correction

Table 2: Physico-chemical properties of parkin

Properties	Parkin
Molecular Formula	$C_{1568}H_{2420}N_{458}O_{468}S_{38}$
Molecular Weight (Daltons)	36393.5
Theoretical PI	7.06
Aliphatic Index	60.28
Grand Average of Hydropathicity (GRAVY)	-0.372

Homology modeling 3D structure visualization and quality assessment of retrieved proteins

Prediction of 3D structure of proteins provides us precise functional information of how proteins interact and localize in their stable conformation. Homology modelling is one of the most common structure prediction tools in proteomics and genomics. The best matching template was selected for the target protein on the basis of sequence homology using PDB Advance Blast. The template is experimentally determined 3D structure of a protein that share sequence similarity with the target sequence. Template showed a sequence identity of 100% for Parkin isoforms. 3D structure of Parkin 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. SWISS MODEL has provided Z score of the template and query model. Z score of Parkin has been shown in (table 3), suggesting a high-quality structure for docking studies.

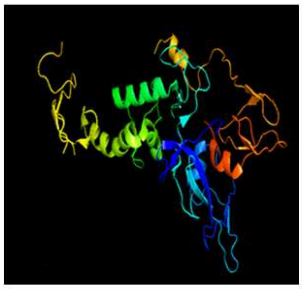
Table 3: Swiss model server result showing template structure used in homology modelling, sequence Identity and quality score of the model generated

Gene name	Modeled residue range	Based on template	Sequence identity	Q mean Z-score
Parkin	1-325	4I1H	100%	-0.81

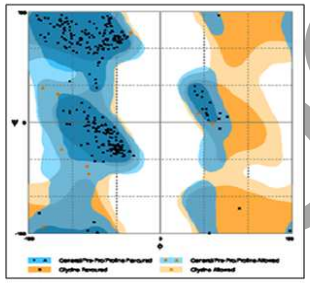
3D structure of Parkin was generated. Even though there were no steric clashes in the structure generated, these were assessed for geometric and energy aspects (fig. 3(a)). Ramachandran plot was used to check the reliability of predicted 3D structure of Ubiquitin E3 Ligase Proteins Parkin. RAMPAGE checks the stereo chemical quality of a protein structure by analyzing residue-by-residue geometry and overall structure geometry.

Further, Ramachandran plots were obtained for Parkin for quality assessment (fig. 3(b)). RAMPAGE displayed 97% of residues in the most favoured regions, 3% residues in additionally allowed and no residues in disallowed regions in the case of Parkin protein. Errat server was used to find the accuracy of the model. The result of Errat showed 93.262% accurate structure for Parkin proteins.

Figure 3 Jha et al., 2017



(a) Demonstrates 3D structure of Parkin



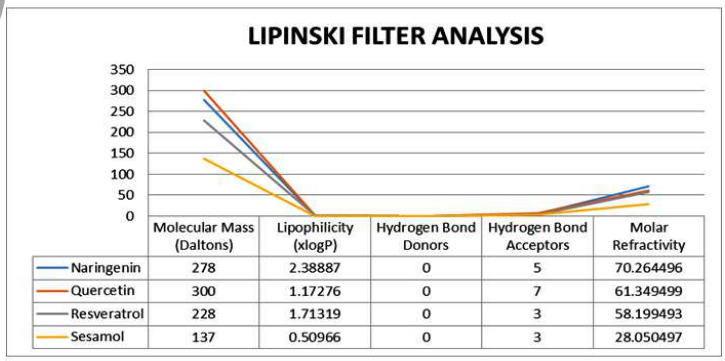
(b) Ramachandran plots was obtained for quality assessment

(c) Ligand binding site in Parkin

Fig. 3: 3D structure, Ramachandran plot and active site of parkin models

Initial screening of the molecules was done on the basis of Lipinski's rule of five (fig. 4). Lipinski filter analysis revealed that all these molecules (Naringenin, Quercetin, Resveratrol and Sesamol) could act like a drug as they meet the criteria of Lipinski Rule of five.

Figure 4 Jha et al., 2017



	Molecular Mass (Daltons)	Lipophilicity (xlogP)	Hydrogen Bond Donors	Hydrogen Bond Acceptors	Molar Refractivity
Naringenin	278	2.38887	0	5	70.264496
Quercetin	300	1.17276	0	7	61.349499
Resveratrol	228	1.71319	0	3	58.199493
Sesamol	137	0.50966	0	3	28.050497

Screening of the molecules was done on the basis of Lipinski's rule of five

Fig. 4: Drug likeness prediction using lipinski filter analysis

Active site prediction and molecular docking analysis of Parkin with identified molecules

CastP server was used to predict the ligand binding site. This server calculates the possible active sites from the 3D atomic coordinates of the proteins. For Parkin, residues involved in ligand binding site, site volume and volume of protein for thirty active sites were predicted. Among the thirty binding sites obtained from CastP for Parkin, site 30 was highly conserved within the active site of the protein (fig. 3(c)).

The Predicted site 30 consisted 1058.8 Cubic angstroms site volume out of the 3741 Cubic Angstroms of protein volume. The residues in site 30 are illustrated in (table 5).

Molecular docking pattern of Parkin with screened molecules (Naringenin, Quercetin, Resveratrol and Sesamol) have been identified and depicted in (fig. 5). On the basis of docking analysis, interacting compounds with minimum binding constant and highest negative free energy of binding are most effective. Docking calculation of Parkin has been presented in (table 4).

Table 4: Docking calculation of compounds with parkin

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
Naringenin	-7.12 (kcal/mol)	6.08 µM	-8.31 (kcal/mol)	-8.19 (kcal/mol)	-0.12 (kcal/mol)	+9.71 (kcal/mol)	+1.19 (kcal/mol)
Quercetin	-7.60 (kcal/mol)	2.67 µM	-9.39 (kcal/mol)	-9.15 (kcal/mol)	-0.25 (kcal/mol)	+9.67 (kcal/mol)	+1.79 (kcal/mol)
Resveratrol	-6.69 (kcal/mol)	12.43 µM	-8.48 (kcal/mol)	-8.47 (kcal/mol)	-0.02 (kcal/mol)	+17.26 (kcal/mol)	+1.79 (kcal/mol)
Sesamol	-4.99 (kcal/mol)	221.78 µM	-5.28 (kcal/mol)	-5.17 (kcal/mol)	-0.11 (kcal/mol)	+0.34 (kcal/mol)	+0.30 (kcal/mol)

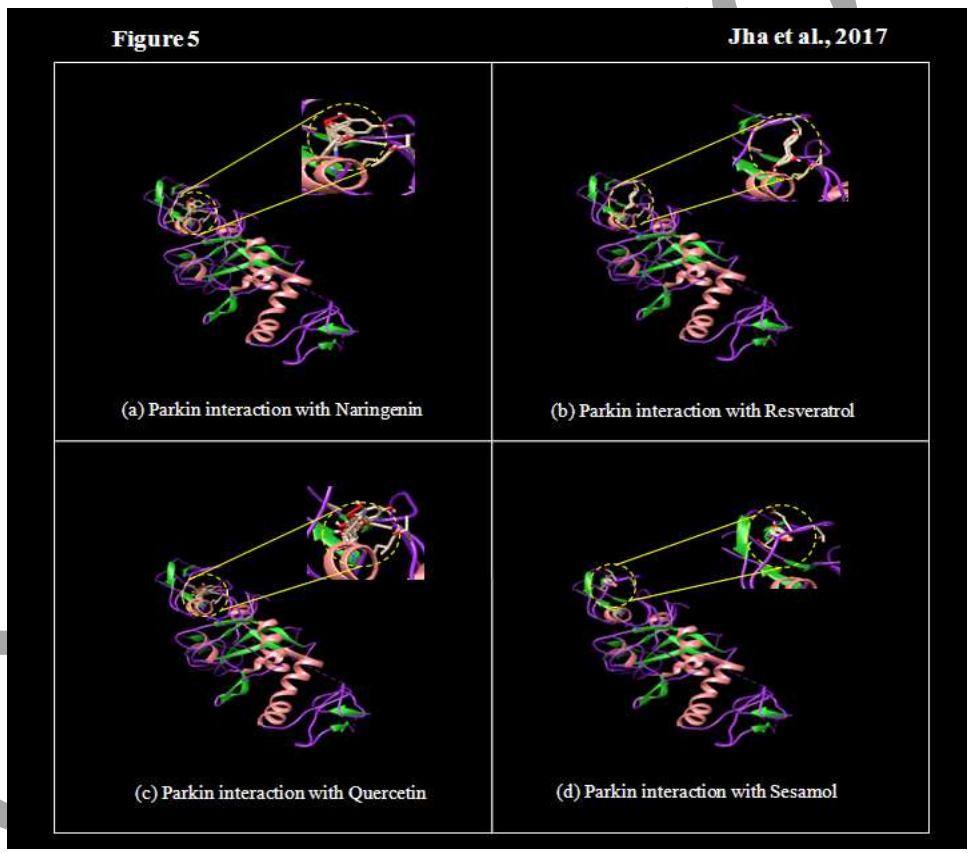


Fig. 5*:* Docking study of parkin protein with selected compounds

Table 5: Parkin known binding site and selected compounds interacting residues

Compound	Interacting residues
Reported catalytic Site	TYR^{143}, THR^{231}, ASN^{232}, SER^{233}, ARG^{234}, ASN^{235}, ILE^{236}, THR^{237}, ARG^{256}, GLN^{400}, ARG^{402}, TRP^{403}, GLU^{404}, ALA^{405}, ALA^{406}, SER^{407}, LYS^{408}, GLU^{409}, THR^{410}, LYS^{412}, LYS^{413}, THR^{414}, LYS^{416}, LYS^{427}, GLY^{429}, GLY^{430}, MET^{432}, TRP^{447}, ASN^{448}, ASP^{464} and VAL^{465}.
Naringenin	ARG^{234}, ASN^{235}, ILE^{236}, THR^{237}, GLN^{400}, ARG^{402}, TRP^{403}, GLU^{404}, ALA^{405}, ALA^{406}, SER^{407} and LYS^{408}.
Quercetin	ARG^{234}, ASN^{235}, ILE^{236}, THR^{237}, GLN^{400}, ARG^{402}, TRP^{403}, ALA^{405}, ALA^{406} and SER^{407}.
Resveratrol	ARG^{234}, ASN^{235} ILE^{236}, THR^{237}, GLN^{400}, SER^{407}, LYS^{408} and THR^{410}.
Sesamol	GLU^{409}, THR^{410}, LYS^{412}, LYS^{413} and THR^{414}.

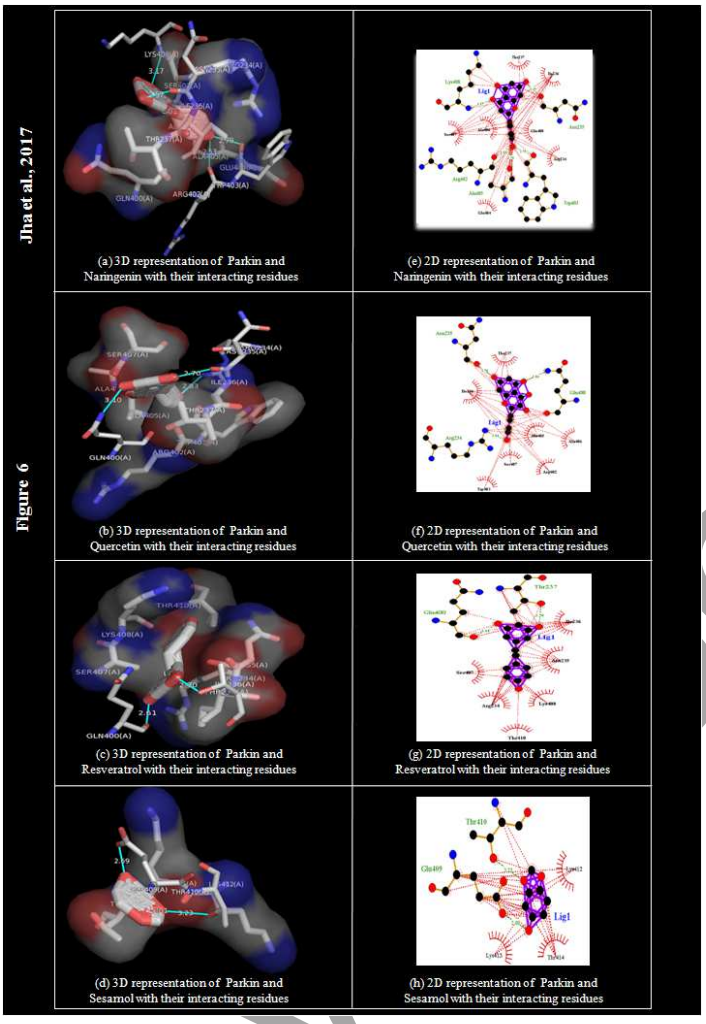


Fig. 6*:* Binding site of Parkin with selected compounds along with its reported catalytic site

Binding site residues of Parkin interacting with Naringenin, Quercetin, Resveratrol and Sesamol were found to be the same as the residues involved in their respective catalytic sites. Interacting residues of Parkin with Naringenin, Quercetin, Resveratrol and Sesamol along with their identified catalytic sites have been shown in (table 5) and their 2D and 3D pattern of interaction is presented in (fig. 6).

DISCUSSION

This research introduced the novel potential of biomolecules, which could be applied for therapeutic intervention in PD progression. Mutations in PD associated genes such as Parkin potentially lead to autosomal recessive PD 22]. Moreover, this gene displays characteristic ubiquitin E3 ligase activity. Parkin is ubiquitously expressed in a number of pathways associated with PD pathogenesis and has ubiquitin E3-ligase activity which also reduces α-synuclein aggregation 23]. Thus, it seems imperative to design therapeutic strategies aimed at elevating the level of Parkin to improve neuronal survival in PD. Further, various studies have advocated that several compounds of plant origin possess neuroprotective properties, however, their mode of action has not been clearly defined. Based on docking study analysis, the present study provides scientific evidence that given four biomolecules namely Naringenin, Quercetin, Resveratrol and Sesamol are interacting at the reported binding site of Parkin. Further, Binding Constant, K_b of Naringenin, Quercetin, Resveratrol and Sesamol for Parkin were found to be 6.08 µM, 2.67 µM, 12.43 µM and 221.78 µM respectively, suggesting that all the selected compounds were effective as Ubiquitin E3 Ligase activators. Investigation of active binding sites within Parkin protein gives a better idea for a valuable drug target site and drug interaction with the highest affinity. In this study the most effective compound was found to be Quercetin is having minimum binding Constant, K_b and highest negative free energy of binding with a maximum interacting surface area with reported highly conserved active site within Parkin protein in course of docking studies [24-27].

Hence, this manuscript is showing for the first-time neuroprotective effect of Quercetin with Parkin in PD pathogenesis.

CONCLUSION

The future of neurodegenerative therapies depends on the researchers' ability to adjust actions of circumstances and have a clear projection relating to the aberrant mechanisms that ultimately decides the fate of neurons and henceforth degeneration. Despite, tremendous advancements in the field of neurotherapeutics, still the future of such therapies hangs on morbid conjecture and fragile hopes the biomolecules present us an interesting avenue to exploit in PD. Interrupting critical interactions of the biomolecules can solve the "targeted therapy crisis" problem in neurodegeneration. In conclusion, we have found Parkin protein for a potential target of Quercetin by molecular docking studies, which will be useful for the design of novel and highly efficient drug for the treatment of PD therapeutics.

AUTHORS' CONTRIBUTION

P. K designed the manuscript. S. K. J performed the experiments and software analysis for the target molecules. S. K. J performed the docking software analysis. P. K and S. K. J analyzed, coordinated and

drafted the manuscript. Authors read and approved the final manuscript.

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CONFLICTS OF INTERESTS

The authors declare no conflict of interest

REFERENCES

1. Soto C. Unfolding the role of protein misfolding in neurodegenerative diseases. Nat Rev Neurosci 2003;4:49-60.
2. Hardy J, Orr H. The genetics of neurodegenerative diseases. J Neurochem 2006;97:1690-9.
3. Manoharan S, Guillemin GJ, Abiramasundari RS, Essa MM, Akbar M, Akbar MD. The role of reactive oxygen species in the pathogenesis of Alzheimer's disease, Parkinson's disease, and Huntington's disease: a mini review. Oxid Med Cell Longevity 2016. DOI:10.1155/2016/8590578
4. Sonia Angeline M, Sarkar A, Anand K, Ambasta RK, Kumar P. Sesamol and naringenin reverse the effect of rotenone-induced PD rat model. Neuroscience 2013;254:379-94.
5. Dias V, Junn E, Mouradian MM. The role of oxidative stress in Parkinson's disease. J Parkinson's Disease 2013;3:461–91.
6. Leroy E, Boyer R, Auburger G. The ubiquitin pathway in Parkinson's disease. Nature 1998;395:451–2.
7. Arduino DM, Esteves AR, Oliveira CR, Cardoso SM. Mitochondrial metabolism modulation: a new therapeutic approach for Parkinson's disease. CNS Neurol Disord Drug Targets 2010;9:105–19.
8. Ciechanover A, Kwon YT. Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies. Exp Mol Med 2015;47:e147.
9. Barry H. Antioxidants in human health and disease. Annu Rev Nut 1996;16:33–50.
10. Santos IM, Tomé Ada R, Saldanha GB, Ferreira PM, Militão GC, Freitas RM. Oxidative stress in the hippocampus during experimental seizures can be ameliorated with the antioxidant ascorbic acid. Oxid Med Cell Longevity 2009;2:214-21.
11. Kannappan S, Anuradha CV. Naringenin enhances insulin-stimulated tyrosine phosphorylation and improves the cellular actions of insulin in a dietary model of metabolic syndrome. Eur J Nutr 2010;49:101-9.
12. Bavithra S, Selvakumar K, Kumari P, R Krishnamoorthy, G Venkataraman, P Arunakaran J. Polychlorinated biphenyl (PCBs)-induced oxidative stress plays a critical role on cerebellar dopaminergic receptor expression: the ameliorative role of quercetin. Neurotox Res 2012;21:149-59.
13. Chen RS, Wu PL, Chiou RY. Peanut roots as a source of resveratrol. J Agric Food Chem 2002;50:1665–7.
14. Soleas GJ, Diamandis EP, Goldberg DM. Wine as a biological fluid: history, production, and role in disease prevention. J Clin Laboratory Anal 1997;11:287–313.
15. Tsu-Kung L, Shang-Der C, Yao-Chung C. Resveratrol partially prevents rotenone-induced neurotoxicity in dopaminergic SH-SY5Y cells through induction of heme oxygenase-1 dependent autophagy. Int J Mol Sci 2014;15:1625-46.
16. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215:403-10.
17. Edgar RC. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 2004;5:113.
18. 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.
19. Lipinski CA. Lead-and drug-like compounds: the rule-of-five revolution. Drug Discovery Today 2004;1:337–41.
20. 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;34:116-8.
21. 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.
22. Wang, Chaodong. Parkin dosage mutations in patients with early-onset sporadic and familial Parkinson's disease in Chinese: an independent pathogenic role. Brain Res 2010;1358:30-8.
23. Mizuno, Yoshikuni. Progress in familial Parkinson's disease. Parkinson's disease and related disorders. Springer Vienna 2006;70 Suppl:191-204.
24. Pranjaligupta, Nishant Rai, Pankaj Gautam. Anticancer drugs as potential inhibitors of acrab-tolc of multi drug resistant escherichia coli: an *in silico* molecular modeling and docking study. Asian J Pharm Clin Res 2015;8:351-8.
25. Sri dharani R, Ranjitha R, Sripathi R, Ali muhammad KS, Ravi S. Docking studies in target proteins involved in antibacterial action mechanisms: alkaloids isolated from scutellaria genus. Asian J Pharm Clin Res 2016;9:121-5.
26. Maida Engels, Se Balaji B, Divakar S, Geetha G. ligand based pharmacophore modeling, virtual screening and molecular docking studies to design novel pancreatic lipase inhibitors. Int J Pharm Pharm Sci 2017;9:48-64.
27. Rigoberto Villanueva Guerrero, Rodolfo Abarca Vargas, Vera L. Petricevich, Chemical compounds and biological activity of an extract from bougainvillea x buttiana (var. rose) holttum and standl. Int J Pharm Pharm Sci 2017;9:42-6.

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Research Article

AN *IN SILICO* STUDY OF NARINGENIN-MEDIATED NEUROPROTECTION IN PARKINSON'S DISEASE

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ABSTRACT

Objective: Naringenin is a dietary biomolecule with broad spectrum of activities which protects neurons from various neurotoxic insults and improves cognition and motor function in neurodegenerative diseases. DJ-1 has both, ubiquitin E3 ligase as well as chaperonic activity, and loss of ubiquitin E3 ligase activity of DJ-1 has been found to be associated with familial Parkinson's disease (PD). Naringenin induced E3 ligase activity of DJ-1 which can have possible clinical relevance in PD.

Methods: Various *in silico* parameters such as phylogenetic analysis, homology modeling, active site prediction, and molecular docking studies using AutoDock 4.2.1 and LIGPLOT1.4.5 were carried out.

Results: Three-dimensional structure of DJ-1 was generated and Ramachandran plot was obtained for quality assessment. RAMPAGE displayed 99.5% of residues in the most favored regions. 0% residues in additionally allowed and 0.5% disallowed regions of DJ-1 protein. Further, initial screenings of the molecules were done based on Lipinski's rule of five. CastP server used to predict the ligand binding site suggests that this protein can be utilized as a potential drug target. Finally, we have found naringenin to be most effective among four biomolecules in modulating DJ-1 based on minimum inhibition constant, Ki, and highest negative free energy of binding with maximum interacting surface area in the course of docking studies.

Conclusion: Our study suggests that based on different *in silico* parameters and molecular docking studies, naringenin can provide a new avenue for PD therapeutics.

Keywords: Parkinson's disease, Ubiquitin E3 ligase, DJ-1, Molecular docking, Naringenin.

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INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder characterized by dopamine depletion in the striatum. PD is characterized by bradykinesia, tremor, rigidity, and weakening of postural reflexes [1]. The dramatic loss of neuromelanin containing dopaminergic neurons is convoyed by the presence of Lewy bodies in the remaining neurons. The hallmark of PD includes fibrillar cytoplasmic inclusions comprising aggregated and abnormally accumulated proteins, the most prominent being α-synuclein, neurofilaments, ubiquitin, and ubiquitinated proteins [2]. In addition, at least 13 loci and nine genes have been proposed to be linked with PD, but only six genes are widely accepted to be associated with Mendelian forms of the disease [3]. Mutations in these genes potentially lead to autosomal dominant (α-synuclein and LRRK2) or autosomal recessive PD (Parkin, PINK1, DJ1, and ATP13A2). Recent epidemiological studies have shown that <10% of PD cases are of familial origin with the majority being sporadic [4]. The sporadic form of PD is caused by mutated DJ1 which shows reduced nuclear localization and translocation to mitochondria [5]. DJ-1 is ubiquitously expressed in a number of pathways associated with PD pathogenesis and has ubiquitin E3 ligase activity which also reduces α-synuclein aggregation [6]. Although mutations associated with DJ-1 lead to onset of familial PD, the exact mechanism behind the pathogenesis is still unknown [7]. Furthermore, various studies have advocated that several compounds of plant origin possess neuroprotective properties, however, their mode of action has not been clearly defined [8]. In this study, we have initially screened four biomolecules, namely, naringenin, quercetin, resveratrol, and sesamol based on Lipinski's rule of five. These biomolecules are found in fruits and vegetables and have various beneficial effects such as antioxidative, activation of survival genes and signaling pathways, chelation of transition metal, regulating mitochondrial function, and modulating neuroinflammation. Further, these biomolecules interact with significant neuronal signaling cascades that lead to inhibition of apoptosis enhanced by the neurotoxic species and promote neuronal endurance and differentiation [9]. They selectively target a number of protein kinase and lipid kinase signaling cascades, importantly, the PI3K/Akt and MAP kinase pathways which modulate prosurvival transcription factors and gene expression [10]. Interestingly, naringenin treatment prominently suppressed oxidative stressors, improved levels of enzymatic antioxidants, and neurotransmitter significantly [11]. In this study, biomolecules which exhibit neuroprotective activities were subjected to docking simulations using AutoDock 4.2.1. The preliminary investigation revealed naringenin as the best potential biomolecule among all given four biomolecules based on minimum inhibition constant, Ki, and highest negative free energy of binding with maximum interacting surface area with the active site of DJ-1 in a course of docking study. Based on *in silico* experimentation, naringenin is a seemingly new prospect for therapeutic intervention in PD. Therefore, a comprehensive understanding of the molecular mechanism associated with naringenin-mediated therapeutics could contribute toward clinical significance in PD biology.

METHODS

Retrieval of ubiquitin E3 ligase proteins DJ-1 and their function recognition

The amino acid sequence of ubiquitin E3 ligase DJ-1 with accession numbers 4ZGG_A was retrieved from NCBI database and used for homology search using basic local alignment search tool (BLAST). Protein functional elucidation was done using Interproscan server (http://www.ebi.ac.uk/interpro/search/sequence-search/) [12].

Phylogenetic relationship and physicochemical properties

For multiple sequence analysis, Muscle software (http://www.ebi.ac.uk/Tools/msa/muscle/) was used and phylogenetic tree was constructed using muscle software based on neighbor joining plot without distance correction. ProtParam (http://web.expasy.org/protparam/) was used to predict physicochemical properties [13]. The parameters computed by ProtParam included the molecular weight, theoretical PI, aliphatic index, and grand average of hydropathicity (GRAVY).

Homology modeling, visualization, and quality assessment of three-dimensional (3D) structure of ubiquitin E3 ligase DJ-1

Homology modeling was used to determine the 3D structure of DJ-1 isoforms. A BLASTP search with default parameters was performed against the Brookhaven Protein Data Bank (PDB) to find suitable templates for homology modeling. Templates with PDB ID 4ZGG were retrieved for DJ-1 proteins from PDB. The protein structure prediction server Swiss-model (http://swissmodel.expasy.org/) was used for homology model construction [14]. Once the 3D structure of proteins was generated, structural evaluation and stereochemical analysis were performed using RAMPAGE (http://www.mordred.bioc.cam.ac.uk/~rapper/rampage.php). Errat server (http://nihserver.mbi.ucla.edu/ERRATv2/) was used to find the accuracy of the structure and visualization of determined structures was performed using PyMOL viewer.

Ligand optimization

Reported ligand molecules (naringenin, quercetin, resveratrol, and sesamol) along with their physical and chemical properties were retrieved from PubChem compound database (http://www.pubchem.ncbi.nlm.nih.gov/). 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 the molecular structure of compounds was done using PyMOL viewer.

Lipinski's filter analysis of screened drugs

An online tool Lipinski's filter (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) was used to retrieve the information about druglikeness of drugs with the help of Lipinski's rule of five. Lipinski's rule (or Lipinski's rule of five) helps to differentiate drug and non-drug-like molecules [15]. It is used to identify the possibility of success or failure due to druglikeness for molecules fulfilling with two or more of the following rules: (a) Molecular mass should be <500 Da, (b) high lipophilicity (expressed as logP <5), (c) <5 hydrogen bond donors, (d) <10 hydrogen bond acceptors, and (e) molar refractivity should be between 40 and 130.

Active site prediction

CastP server (http://www.sts.bioe.uic.edu/castp/) was used to predict the active sites of protein [16]. CastP could also be used to measure area, circumference of mouth openings of each binding site insolvent, and molecular accessible surface. PDB file of protein was upload in the server and it showed the ligand binding sites present in protein and the site with maximum surface area and maximum surface volume was selected and all the amino acid residues involved in binding with ligands were retrieved.

Preparation of protein and ligand molecules

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

Molecular docking analysis

Prepared and optimized structures of ligands and proteins were ultimately used for molecular docking using Autodock 4.2.1 for predicting 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.

RESULTS

Retrieval of ubiquitin E3 ligase DJ-1 proteins and their functional elucidation

Based on functional domain sequence of well-characterized gene/protein, homology search was performed using BLAST. We have successfully hunted 5 isoforms of protein DJ-1 (Table 1) on the basis of families and domains identified from Interproscan results. Interproscan study revealed that all homologues proteins for DJ-1, all homologous proteins were belonging to DJ-1 family (IPR006287), glutamine amidotransferase-like domain (IPR029062), and DJ-1/PfpI domain (IPR002818), respectively (Fig. 1).

Phylogenetic relationship and physicochemical properties

For multiple sequence analysis, Muscle software was used and found that amino acid residues were conserved in most of the isoforms of the ubiquitin E3 ligase DJ-1. Phylogenetic study of DJ-1 hunted proteins revealed that PD (autosomal recessive, early-onset) 7 and protein DJ-1 were differing from others (Fig. 2a and b). However, another Chain A (crystal structure of aggregated form of DJ-1 Chain A) and Chain A (crystal structure of E18a human DJ-1) were in same cluster as share more homology while crystal structure of human DJ-1 was in another cluster. ProtParam showed that molecular weight of DJ-1 was 19848.7 Da and an isoelectric point of DJ-1 was 6.37 which indicate that DJ-1 was negatively charged, respectively. Furthermore, GRAVY index of -0.47 for DJ-1 is indicative of hydrophilic (Table 2).

Homology modeling, 3D structure visualization, and quality assessment of retrieved proteins

Prediction of 3D structure of proteins provides us precise functional information of how proteins interact and localize in their stable

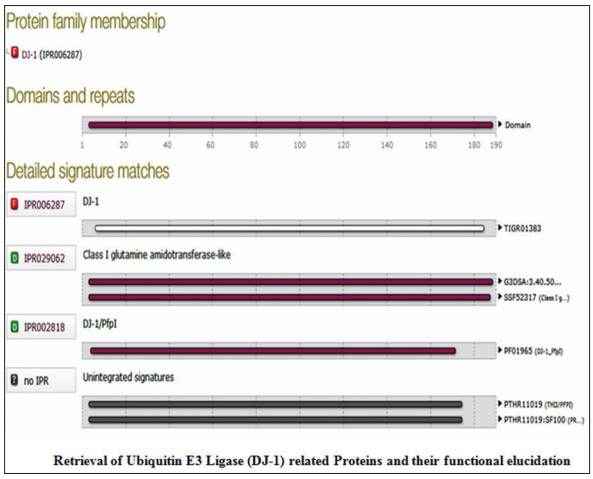


Fig. 1: Interproscan result for DJ-1

Table 1: Hunted DJ-1-related proteins

S.No.	Accession	Protein	Score	Identity (%)	E value
1	NP_009193.2	Protein DJ-1	368	98	5.00e-127
2	3BWE_A	Chain A, crystal structure of aggregated form of DJ-1	365	100	6.00e-126
3	4OQ4_A	Chain A, crystal structure of E18a human DJ-1	361	97	2.00e-124
4	1J42_A	Chain A, crystal structure of human DJ-1	363	97	2.00e-125
5	ADQ32403.1	Parkinson disease (autosomal recessive, early-onset) 7	364	97	1.00e-125

conformation. Homology or comparative modeling is one of the most common structure prediction methods in structural genomics and proteomics. The best matching template 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 shares sequence similarity with target sequence. Template showed sequence identity of 100% for DJ-1 isoforms. 3D structure of DJ-1 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 DJ-1 has been shown in Table 3, suggesting a good structure.

3D structure of DJ-1 was generated. Even though there were no steric clashes in the structure generated, these were assessed for geometric and energy aspects (Fig. 3a). Ramachandran plot was used to check the reliability of predicted 3D structure of ubiquitin E3 ligase proteins DJ-1. RAMPAGE checks the stereochemical quality of a protein structure by analyzing residue-by-residue geometry and overall structure geometry. Ramachandran plots were obtained for DJ-1 for quality assessment (Fig. 3b). RAMPAGE displayed 99.5% of residues in the most favored regions, 0% residues in additionally allowed, and 0.5% disallowed regions of DJ-1 protein. Errat server was used to find the accuracy of the model. Result of Errat showed 98.844% of accurate structure for DJ-1 proteins.

Physicochemical properties and Lipinski's filter analysis retrieved of ligands

Initial screening of the molecules was done on the basis of Lipinski's rule of five (Fig. 5). Lipinski's filter analysis revealed that all these molecules (naringenin, quercetin, resveratrol, and sesamol) could act as a drug as they meet the criteria of Lipinski's rule of five.

Active site prediction and molecular docking analysis of DJ-1 with identified molecules

CastP server was used to predict the ligand binding site. This server calculates the possible active sites from the 3D atomic coordinates of the proteins. For DJ-1, residues involved in ligand binding site, site volume, and volume of protein for 33 active sites were predicted (Fig. 3c). Among the 33 binding sites obtained from CastP for DJ-1, site 33 was highly conserved within the active site of the protein. The predicted site 33 comprised 435.6 Å^3 site volume out of the 1723.5 Å^3 of protein volume. The residues in site 33 are shown in Table 6.

Molecular docking pattern of DJ-1 with screened molecules (naringenin, quercetin, resveratrol, and sesamol) has been identified and depicted in Fig. 5. On the basis of docking analysis, interacting compounds with minimum binding constant and highest negative free energy of binding are most effective. Docking calculation of DJ-1 has been presented in Table 5.

Binding site residues of DJ-1 interacting with naringenin, quercetin, resveratrol, and sesamol were found to be the same as the residues involved in their respective catalytic sites. Interacting residues of DJ-1 with naringenin, quercetin, resveratrol, and sesamol along

Table 2: Physicochemical properties of DJ-1

Properties	DJ-1
Molecular formula	$C_{874}H_{1448}N_{242}O_{268}S_8$
Molecular weight (Da)	19848.7
Theoretical PI	6.37
Aliphatic index	99.11
GRAVY	−0.047

GRAVY: Grand average of hydropathicity

Table 3: Swiss-model server result showing template structure used in homology modeling, sequence identity, and quality score of the model generated

Gene name	Modeled residue range	Based on template	Sequence identity (%)	QMEAN Z-score
DJ-1	2-185	4ZGG	100	−0.42

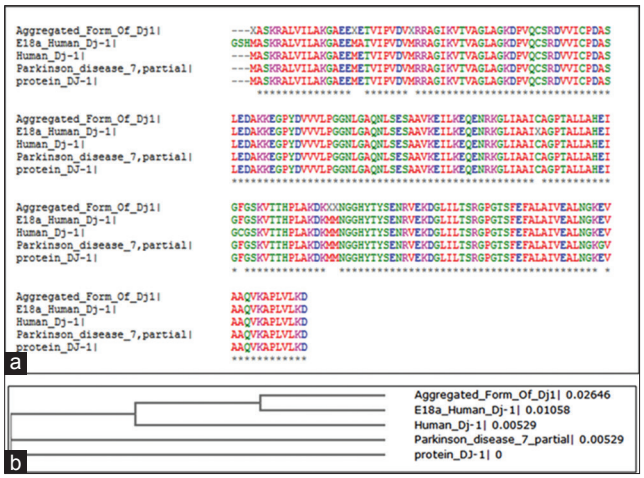


Fig. 2: (a) Multiple sequence alignment of all DJ-1 isoforms and (b) tree generation for DJ-1 using neighbor joining plot without distance correction

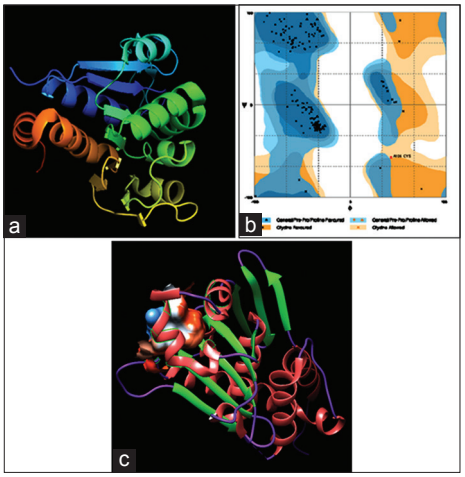


Fig. 3: Three-dimensional (3D) structure, Ramachandran plot, and active site of DJ-1 models. (a) Demonstrates 3D structure of DJ-1, (b) Ramachandran plot was obtained for quality assessment, and (c) ligand binding site in DJ-1

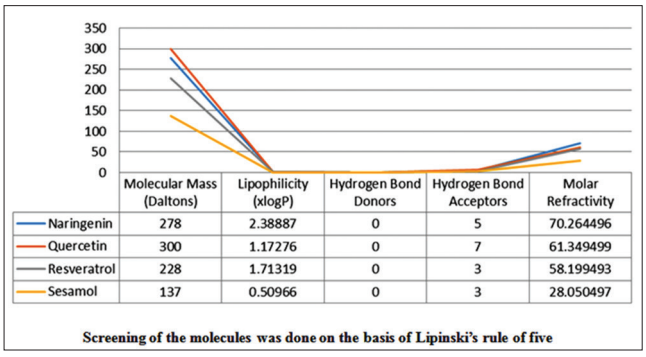


Fig. 4: Druglikeness prediction using Lipinski's filter analysis AQ3

AQ4

Table 4: Physicochemical properties of ligands

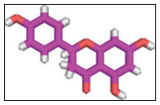
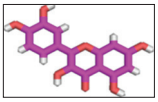
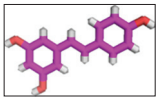
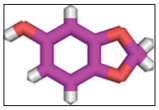
Characteristics	Naringenin	Quercetin	Resveratrol	Sesamol
Molecular weight (g/mol)	272.25278	302.2357	228.24328	138.12074
Molecular formula	$C_{15}H_{12}O_5$	$C_{15}H_{10}O_7$	$C_{14}H_{12}O_3$	$C_7H_6O_3$
Molecular structure				
IUPAC name	5,7-dihydroxy-2-(4-hydroxyphenyl) -2,3-dihydrochromen-4-one	2-(3,4-dihydroxyphenyl)-3,5,7 -trihydroxychromen-4-one	5-[(E)-2-(4-hydroxyphenyl) ethenyl] benzene-1,3-diol	1,3-benzodioxol-5-ol
xLogP	2.4	1.5	3.1	1.2
Hydrogen bond donor	3	5	3	1
Hydrogen bond acceptor	5	7	3	3
Rotatable bond count	1	1	2	0
Topological polar surface area (A^2)	87	127	60.7	38.7
Heavy atom count	20	22	17	10
Complexity	363	488	246	126
Covalently bonded unit count	1	1	1	1

Table 5: Docking calculation of compounds with DJ-1

Compound name	Estimated free energy of binding (kcal/mol)	Estimated binding constant	Estimated intermolecular energy (kcal/mol)	vdW+Hbond+desolv energy (kcal/mol)	Electrostatic energy (kcal/mol)	Estimated internal energy (kcal/mol)	Torsional free energy (kcal/mol)
Naringenin	−4.19	851.70 μM	−5.38	−5.25	−0.13	+9.69	+1.19
Quercetin	−3.97	1.24 mM	−5.76	−5.54	−0.22	+9.44	+1.79
Resveratrol	−3.24	4.25 mM	−5.03	−4.91	−0.12	+17.09	+1.79
Sesamol	−3.08	5.51 mM	−3.38	−3.12	−0.26	+0.32	+0.30

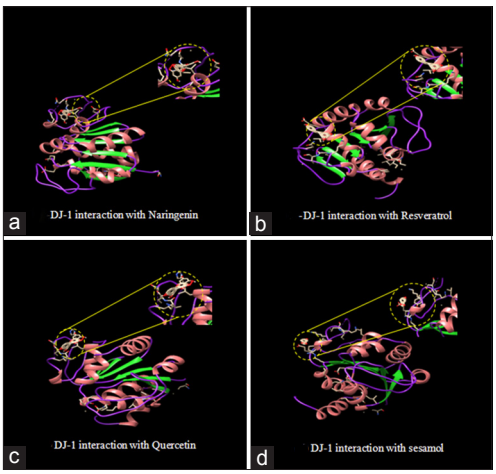


Fig. 5: Docking study of DJ-1 protein with selected compounds. (a) DJ-1 interaction with naringenin, (b) DJ-1 interaction with resveratrol, (c) DJ-1 interaction with quercetin, and (d) DJ-1 interaction with sesamol

with their identified catalytic sites have been shown in Table 6 and their two-dimensional and 3D pattern of interaction is presented in Fig. 6.

DISCUSSION

Despite the knowledge of various factors which contribute in the occurrence and progression of PD, the exact cause and cure remains elusive. Mutations in PD-associated genes potentially lead to autosomal dominant (α-synuclein and LRRK2), or autosomal recessive PD (Parkin, PINK1, DJ1, and ATP13A2), respectively [17]. Moreover, these genes display characteristic ubiquitin E3 ligase activity. DJ-1 is ubiquitously expressed in a number of pathways associated with PD pathogenesis and has ubiquitin E3 ligase activity which also reduces α-synuclein aggregation [18]. Thus, it seems imperative to design therapeutic strategies aimed at elevating the level of DJ-1 to improve neuronal survival in PD. Further, various studies have advocated that several compounds of plant origin possess neuroprotective properties, however, their mode of action have not been clearly defined [19]. Based on docking study analysis, the present study provides scientific evidence that given four biomolecules, namely, naringenin, quercetin, resveratrol, and sesamol are interacting at the reported binding site of DJ-1. Further, binding constant, K_b of naringenin, quercetin, resveratrol, and sesamol for DJ-1 was found to be 851.70 μM, 1.24 mM, 4.25 mM, and 5.51 mM, respectively, suggesting that all the selected compounds might be effective as activators of E3 ligase activity of DJ-1. Furthermore, investigation of binding sites within DJ-1 gives a better idea for a valuable drug target site with highest binding and interaction affinity. Based on *in vivo* experimentation, the most effective compound in modulating E3 ligase activity of DJ-1 was found to be naringenin

Table 6: DJ-1 known binding site and selected compounds interacting residues

Compound	Interacting residues
Reported catalytic site	Pro^{109}, Thr^{110}, Leu^{112}, Leu^{113}, Ala^{114}, Glu^{116}, Val^{123}, Thr^{125}, Pro^{127}, Ala^{129}, Lys^{130}, Lys^{132}, Asn^{135}, Gly^{137}, His^{138}, Tyr^{139}, Tyr^{141}, Glu^{143}, and Arg^{156}
Naringenin	Thr^{110}, Leu^{113}, Ala^{114}, Glu^{116}, Lys^{132}, and Asn^{135}
Quercetin	Leu^{113}, Ala^{114}, Lys^{132}, Asn^{135}, and His^{138}
Resveratrol	Leu^{113}, Glu^{116}, and Asn^{135}
Sesamol	Pro^{127}, Lys^{130}, Tyr^{141}, Glu^{143}, and Arg^{156}

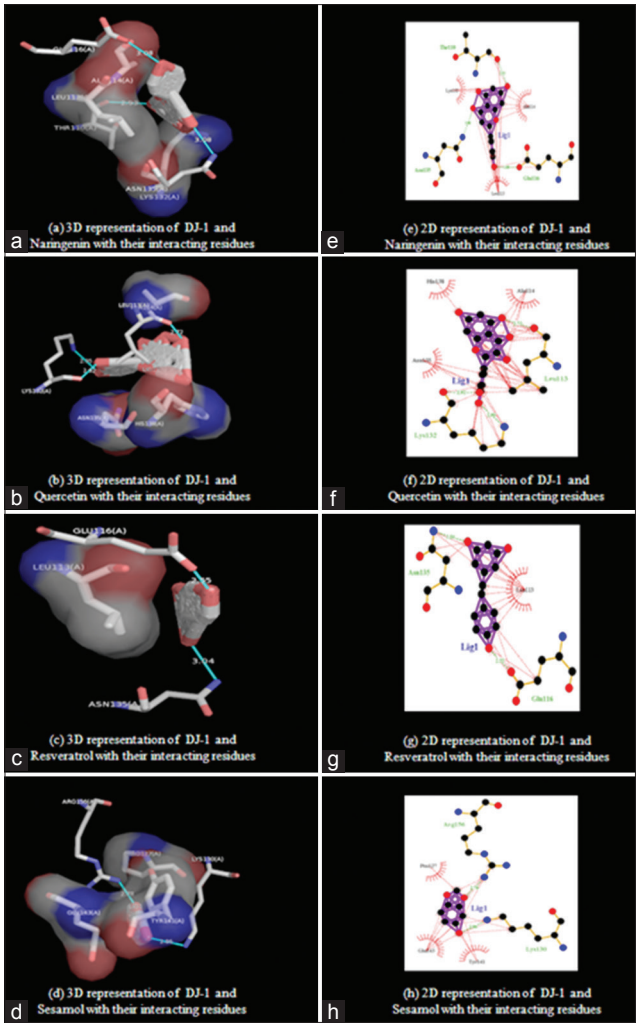


Fig. 6: Binding site of DJ-1 with selected compounds along with its known stimulatory active site. (a) Three-dimensional (3D) presentation of DJ-1 and naringenin with their interacting residues, (b) 3D representation of DJ-1 and quercetin with their interacting residues, (c) 3D representation of DJ-1 and resveratrol with their interacting residues, (d) 3D representation of DJ-1 and sesamol with their interacting residues, (e) two-dimensional (2D) representation of DJ-1 and naringenin with their interacting residues, (f) 2D representation of DJ-1 and resveratrol with their interacting residues, (g) 2D representation of DJ-1 and resveratrol with their interacting residues, and (h) 2D representation of DJ-1 and sesamol with their interacting residues

having minimum binding constant K_b and highest negative free energy of binding with maximum interacting surface area in a course of docking studies [20-25].

CONCLUSION

In this study, the sequence and structure analysis of ubiquitin E3 ligase protein DJ-1 were done by various computational tools and softwares. Molecular docking study advocated naringenin to be the most effective compound in elevating E3 ligase action of DJ-1 based on highest negative free energy of binding, minimum inhibition constant Ki, and maximum interacting surface area among the given four biomolecules. Such biomolecules can be effectively used to validate *in vitro* and *in vivo* prosurvival outcomes in PD models as well as in clinical scenario. Knowledge gained from this study can be used in broad screening of neuroprotective biomolecules and can be further implemented in designing effective therapeutics for PD.

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REFERENCES

1. Massano J, Bhatia KP. Clinical approach to Parkinson's disease: Features, diagnosis, and principles of management. Cold Spring Harb Perspect Med 2012;2(6):a008870.
2. Gundersen V. Protein aggregation in Parkinson's disease. Acta Neurol Scand Suppl 2010;190:82-7.
3. Exner N, Lutz AK, Haass C, Winklhofer KF. Mitochondrial dysfunction in Parkinson's disease: Molecular mechanisms and pathophysiological consequences. EMBO J 2012;31:3038-62.
4. Pirkevi C, Lesage S, Brice A, Basak AN. From genes to proteins in mendelian Parkinson's disease: An overview. Anat Rec (Hoboken) 2009;292(12):1893-901.
5. Cookson MR. Parkinsonism due to mutations in PINK1, parkin, and DJ-1 and oxidative stress and mitochondrial pathways. Cold Spring Harb Perspect Med 2012;2(9):a009415.
6. Saurabh S, West AB. The therapeutic potential of LRRK2 and A-synuclein in Parkinson's disease. Antioxid Redox Signal 2009;11(9):2167-87.
7. Klein C, Westenberger A. Genetics of Parkinson's disease. Cold Spring Harb Perspect Med 2012;2(1):a008888.
8. Saikat S, Chakraborty R, De B, Mazumder J. Free radicals, antioxidants, diseases and phytomedicines: Current status and future prospect. Indian J Pharm Sci 2010;3(1):91-100.
9. Jeremy PE. The interactions of flavonoids within neuronal signalling pathways. Genes Nutr 2007;2(3):257-73.
10. 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;4(2):67-86.
11. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 2010;4(8):118-26.
12. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool 1990;215(3):403-10.
13. Edgar RC. Muscle: A multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 2004;5:113.
14. Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL workspace: A web-based environment for protein structure homology modelling. Bioinformatics 2006 15;22:195-201.
15. Lipinski CA. Lead - And drug-like compounds: The rule-of-five revolution. Drug Discov Today Technol 2004;1(4):337-41.
16. 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;34:116-8.
17. Wang C, Ma H, Feng X, Xie S, Chan P. Parkin dosage mutations in patients with early-onset sporadic and familial Parkinson's disease in Chinese: An independent pathogenic role. Brain Res 2010;1358:30-8.
18. Mizuno Y, Fisher A, Hanin I. Progress in familial Parkinson's disease. Parkinson's Disease and Related Disorders. Vienna: Springer; 2006. p. 191-204.
19. Kumar GP, Khanum F. Neuroprotective potential of phytochemicals. Pharmacogn Rev 2012;6(12):81-90.
20. Azam F, Madi AM, Ali HI. Molecular docking and prediction of

pharmacokinetic properties of dual mechanism drugs that block MAO-B and adenosine A(2A) receptors for the treatment of Parkinson's disease. J Young Pharm 2012;4(3):184-92.

21. Gupta P, Rai N, Gautam P. Anticancer drugs as potential inhibitors of acrab-tolc of multidrug resistant *Escherichia coli*: An *in silico* molecular modeling and docking study. Asian J Pharm Clin Res 2015;8(1):351-8.
22. Dharani RS, Ranjitha R, Sripathi R, Alimuhammad KS, Ravi S. Docking studies in target proteins involved in antibacterial actionmechanisms: Alkaloids isolated from *Scutellaria* genus. Asian J Pharm Clin Res 2016;9(5):121-5.
23. Engels M, Balaji BS, Divakar S, Geetha G. Ligand based pharmacophore modeling, virtual screening and molecular docking studies to design novel pancreatic lipase inhibitors. Int J Pharm Pharm Sci 2017;9(4):48-64.
24. Guerrero RV, Vargas RA, Petricevich VL. Chemical compounds and biological activity of an extract from *Bougainvillea x Buttiana* (var. *Rose*) holttum and standl. Int J Pharm Pharm Sci 2017;9(4):42-6.
25. Singh N, Kumar B, Kolli MK, Kumari M, Mishra AK. Molecular docking studies of some novel thiophene carbohydrazide derivatives on enterotoxin of bacillus cereus. Int J Curr Pharm Res 2016;8(3):59-63.

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Review Article

p38 MAPK and PI3K/AKT Signalling Cascades in Parkinson's Disease

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Parkinson's disease (PD) is a chronic neurodegenerative condition which has the second largest incidence rate among all other neurodegenerative disorders barring Alzheimer's disease (AD). Currently there is no cure and researchers continue to probe the therapeutic prospect in cell cultures and animal models of PD. Out of the several factors contributing to PD prognosis, the role of p38 MAPK (Mitogen activated protein-kinase) and PI3K/AKT signalling module in PD brains is crucial because the impaired balance between the pro- apoptotic and anti-apoptotic pathways trigger unwanted phenotypes such as microglia activation, neuroinflammation, oxidative stress and apoptosis. These factors continue challenging the brain homeostasis in initial stages thereby essentially assisting the dopaminergic (DA) neurons towards progressive degeneration in PD. Neurotherapeutics against PD shall then be targeted against the misregulated accomplices of the p38 and PI3K/AKT cascades. In this review, we have outlined many such established mechanisms involving the p38 MAPK and PI3K/AKT pathways which can offer therapeutic windows for the rectification of aberrant DA neuronal dynamics in PD brains.

Key words: Parkinson's disease (PD), p38MAPK, PI3K/AKT, neuroinflammation, oxidative stress (OS), apoptosis, neurotherapeutics

Neurodegenerative disorders (NDs) continue to traumatize an aging proportion of the human population especially in the industrialized world. Aging has long been recognized as a compound process of damage accretion that ultimately leads to noticeable disruption of multiple cellular and molecular proceedings, which ultimately are translated into various chronic ailments such as Alzheimer's disease (AD), multiple sclerosis (MS), Parkinson's disease (PD), atherosclerosis and many more (1-3). Although, several NDs have a pharmacological treatment, which as in the case of AD, PD, epilepsy and MS slow down the course of the disease, and are restricted to damage limitation,

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but are not equipped enough to annul the effects or for that reason heal the infirm. Sadly though, the future of such ambitious modalities currently hangs on morbid conjecture and fragile hopes and thus the current focus of the research bevy is to primarily delve unprecedented mechanisms that shall in future restrain the cardinal effects in NDs and also presumably act as custodians of permanent cure (4, 5).

PD is a chronic, neurodegenerative state and the second most commonly observed brain disorder (the most common being AD) which impacts nearly 1% of the global population aged 65 and older. Incidentally, PD appears to be less prevalent among Asian population as compared to the Western world and it is unclear whether this is in a way allied to the extensive use of traditional medicine in the Eastern half of the planet (6). Nevertheless, the use of complementary and alternative medicine (CAM) has been reported to be as high as 76% in countries like Korea. PD is typically characterized by the progressive loss of muscle control, impaired balance, slowness, akinesia, bradykinesia, tremors, postural instability, and decline in striatal dopamine levels of the central nervous system (CNS), and rigidity observed due to the significant loss of dopaminergic (DA) neurons in the substantia nigra (SN) in the midbrain (7). Interestingly, only 10% of all PD cases are caused by genetic mutations, and animal models previously used to comprehend these mutations revealed a significant insight into the loss-of-function status of α-synuclein and LRRK2 particularly in autosomal dominant PD and PINK1/Parkin and DJ-1 in autosomal recessive cases. These findings remain crucial since they represent possible therapeutic targets, however, in the face of such advances, the precise etiology of PD still remains uncertain. Multiple lines of evidence from molecular and cellular to epidemiological studies suggest that innate and environmental factors such as aging, genetics, 1-methy l-4-pheny l-1, 2, 3, 6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA) metals, mitochondrial dysfunction induced by environmental toxins, such as mitochondrial complex I inhibitors rotenone, traumatic brain injury, and shortage of trophic factors that can play a role in PD neuropathology (8-10). In addition, lifestyle factors viz. cigarette smoking and coffee consumption, with a gender bias can also influence the onset of PD. Notably; the neurotoxin 6-hydroxydopamine (6-OHDA) is extensively used to mimic the PD associated neurodegeneration in both *in vivo* and *in vitro* experimental models. On the treatment front, the use of levodopa, dopamine agonists, herbal medicines, health supplement foods, and acupuncture are on the rise all over the world among patients afflicted with PD, however, without much benefit (9, 11, 12).

In the last 15 years, the procedure of deep brain stimulation (DBS) has emerged as touchstone to mitigate the adverse neuropathlogical symptoms witnessed in advanced forms of the disease. The procedure is mainly designated for PD victims who are dopamine-responsive but with disabling motor complications such as motor fluctuation, dyskinesia, or unendurable side-effects of anti-PD suppositories. It is well known that motor fluctuations like wearing-off and peak-dose dyskinesia are motor anomalies observed few years after patients are started on medical treatment. When these complications breach the edge of severity, despite maximal fine-tuning of pharmacological agents, DBS has been shown to be effective and safe with benefits lasting for no less than 10 years. In fact, bilateral sub thalamic nucleus DBS has arisen as a treatment of choice and proven to have an unquestionable influence on motor symptoms, countenancing the minimization of drug treatment and its side effects. Numerous other reports have demonstrated the effectiveness of neurosurgery, specifically on motor symptoms and on health related quality of life. However, a vital concern has been that most of those studies have

testified no significant perfection in social adaptation after subthalamic nucleus deep brain stimulation in some patients. Furthermore, the lack of post-operative improvement in the psychosocial dimension of health-related quality of life and its link to coping strategies is still unclear and there are contentious reports surrounding the use of DBS in the early stage of PD and most medication centers will offer this modality typically to infirm with disruptive motor complications obstinate to drug treatment (13, 14). Therefore, it is of urgency to examine novel mechanisms that can be used as a benchmark therapy of choice in patients bloated with Parkinson's in future.

It is well documented that oxidative stress (OS), impaired ROS/NO balance, microglia activation, and chronic inflammation are striking pathological features observed in PD brains. These factors altogether have detrimental effects on the integrity of dopaminergic (DA) neurons, thus, may potentially lead to neuronal apoptosis and subsequent neurodegeneration. Not surprisingly, these critical neuro determinants are under the control of an array of proteins and signalling networks, and accruing genetic studies in recent years have advocated prominently on the role of improper phosphorylation events, dysfunctional kinases, and aberrantly functioning kinases associated signalling events as few of many responsible determinants involved in a convoluted network defining PD progression and pathogenesis. One such mechanism contributing to microglia response and neuroinflammation in both inherited and sporadic forms involve the protein named LRRK2 (leucine-rich repeat kinase 2). LRRK2 can effectively moderate the neuroinflammatory ambience in traumatized neurons in response to a panel of pathological stimuli. LRRK2 is a large multi-domain protein belonging to the cohort of mammalian ROCO (Ras of complex) proteins. It can be functionally characterized by the presence of an enzymatic core, comprising of ROC/GTPase, COR (C-terminus of ROC) and serine threonine kinase domains. In addition, there are multiple protein-protein interaction domains including ankyrin and leucine-rich repeat motifs at the N-terminus, and WD40 repeats at the C-terminus. LRRK2 mutations can induce microglias through hyperpolymerization and hyperphosphorylation of cytoskeleton and vesicle components, thereby, directing these cells towards a pro-inflammatory ambience, which in turn can result in aggravated inflammation and subsequent neurodegeneration. Profound investigation into the innumerable functionalities of misregulated signalling cascades involving kinases such as p38 mitogen-activated protein (MAP) kinase, protein kinase B (AKT) kinase, and C-Jun N-terminal kinase (JNK), extracellular signal-regulated kinases (ERK), PI3K/AKT shall unravel novel mechanism for drug targeting in future. In that regard, the identification of microglia-specific kinase substrates, GTPase downstream effectors, and interactors shall reveal acute therapeutic hot spots and outline credible prototypes for the attenuation of the cardinal symptoms and motor complications in this group of disorders (8, 15, 16).

Numerous intracellular signalling cascades that congregate on MAPK exist in all eukaryotic cells and play critical roles in various cellular activities. The p38 MAPK as also can be designated as stress-activated protein kinase (SAPK), is especially triggered by a range of cytotoxic stress stimuli and cytokines. In response, p38 potentially drive crucial cellular activities such as proliferation, differentiation, survival, and stress-induced apoptosis. In the central nervous system (CNS), p38 MAPK is central towards the maintenance of synaptic plasticity, and as a result, anomalies' resulting from the deviant functioning of p38 MAPK pathway in neurons has been observed in brain diseases like AD, PD and amyotrophic lateral

sclerosis (ALS). Consistent activation of JNK or p38 MAPK is critical towards facilitating neuronal apoptosis in AD, PD and ALS brains (17-19).

On contrary, PI3K/AKT pathway modulates cellular activities like neuronal cell proliferation, migration and plasticity. The cytoprotective phenotype of PI3K/AKT provides an important signalling for neuroprotection, however, it prerequisites that the pathway is optimally activated in PD brains; this could possibly antagonize the detrimental effects of the p38 MAPK activation in degenerating DA neurons and thus, can assist in establishing a neuro-protective setting in insulted brains. In general, the activation of ERK or p42/p44 MAP kinase and the PI3K/AKT pathway encourage cell survival (cytoprotective pathways), whereas SAPK's, c-Jun N-terminal kinases (JNK's) and the p38 MAPK, moderate cell mortality (20, 21). Nevertheless, several plausible cyto-dynamics involving P38 MAPK- PI3K/AKT and their subtle contribution towards progressive neurodegenerative remains an area of active curiosity for research in PD. Moreover, active biomolecules targeting the impaired p38-PI3K/AKT balance could significantly contribute to neuroprotection in PD challenged brains.

In this review, we will focus solely on the viable contributions made by the p38 MAPK and PI3K pathways towards maintaining neuronal dynamics in PD brains. At a later stage we have enumerated a group of probable neurotherapeutics molecules, which can offer neuroprotection by mechanisms involving the likely targeting of the cell survival (PI3K/AKT) and death pathways (p38 MAPK), and actually can attenuate or prevent neurodegenerative symptoms associated with PD brains in future.

MAP Kinase pathway: At a glance

p38 MAPK

p38 MAPK (mitogen-activated protein kinase) signalling cascade provides a mechanism for cells to respond to a catalogue of external mitogens (signals) and respond accordingly by mediating a wide range of cellular effects. In fact, the diversity and specificity in cellular responses as depicted by the cascade is facilitated via a simple linear architecture, which comprises of sequentially operating core of three evolutionarily conserved protein kinases namely; MAPK, MAPK kinase (MAPKK), and MAPKK kinase (MAPKKK). MAPKKKs are serine/threonine kinases, which are activated via phosphorylation and/or because of their interaction with a small GTP-binding protein of the Ras/Rho family in response to extracellular stimulus. MAPKKK activation results in phosphorylation and activation of MAPKKs, which consequently stimulate MAPK activity through dual phosphorylation of threonine and tyrosine residues positioned in the activation loop of kinase subdomain VIII (22). The activated MAPKs now phosphorylates target substrates specifically on serine or threonine residues followed by a proline. MAPKKs such as MEK3 and MEK6 are activated by a wide range of MAPKKKs (MEKK1 to 3, MLK2/3, ASK1, Tpl2, TAK1, and TAO1/2), which themselves become activated in response to oxidative stress, UV irradiation, hypoxia, ischemia, and cytokines, including interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) (23).

At present, five different MAP kinases (MAPks) have been characterized and investigated namely; extracellular signal-regulated kinases (ERKs) 1 and 2 (ERK1/2), c-Jun amino-terminal kinases or stress-activated protein kinases (JNKs/SAPKs) 1, 2, and 3, p38 isoforms α, β, γ, and δ, ERKs 3 and 4, and ERK5. The kinase p38α (p38) was initially isolated as a 38-kDa protein which was observed to be rapidly phosphorylated at tyrosine motifs in response to LPS stimulation. Later, p38 was cloned and studied as a molecule capable of binding puridinyl imidazole derivatives; these derivatives inhibit the biosynthesis of inflammatory mediators like interleukin-1 (IL-1) and tumor-necrosis factor (TNF) in LPS activated

monocytes. p38 (also known as CSBP, mHOG1, RK, and SAPK2) kinases are more responsive towards stress stimuli such as osmotic shock, ionizing radiation, and cytokine stimulation and four different variants of p38 arising out of alternative splicing are known viz. p38α, p38β, p38γ (ERK6, SAPK3), and p38δ (SAPK4). Among these, p38 and p38β are ubiquitously expressed in tissues, whereas, p38γ and p38δ show variegated expression in a tissue specific manner (24).

Each of the p38 variants comprises of a Thr-Gly-Tyr (TGY) dual phosphorylation motif and sequence comparison performed earlier suggests that each p38 isoform shares approximately 60% identity with other members of the p38 group but only 40-45% with other MAP kinase family members. The activity of p38 is controlled and coordinated *in vitro* by three different kinases: MKK3, MKK4, and MKK6. *In vivo*, MKK3 and MKK6 are necessary for tumor necrosis factor-stimulated p38 MAPK activation whereas, ultraviolet radiation-mediated p38 MAPK activation requires MKK3, MKK4, and MKK6 (25).

p38 isoforms can also be stimulated by GPCRs and by Rho family GTPases; Rac and Cdc42. It is interesting to mention here that MAPKs catalyse the phosphorylation and activation of several protein kinases, termed MAPK-activated protein kinases (MKs), which represent an additional enzymatic step in the MAPK catalytic signaling cascade. MEK3 and MEK6 do not participate in the activation of ERK1/2 or JNK and display a high degree of specificity for p38. In addition, MEK4 (MKK4/Sek1) JNK kinase show limited MAPKK activity toward p38. MEK6 is capable of activating all the p38 isoforms, whereas, MEK3 is discerning and preferentially phosphorylates the p38α and p38β isoforms. p38 isoforms are activated as a result of MEK3/6-catalyzed phosphorylation of Thr-Gly-Tyr (TGY) motif in the p38 activation loop. The differential specificity in p38 activation results from the formation of functional complexes between MEK3/6 and different p38 isoforms and the selective recognition of the activation loop of p38 isoforms by MEK3/6. The length of the phosphorylated TGY motif and the activation loop is different in other MAPKs namely ERK2 and JNK, which likely contributes to the p38 substrate specificity. P38 substrates include cPLA2, MNK1/2, MK2/3, HuR, Bax, and Tau in the cytoplasm and ATF1/2/6, MEF2, Elk-1, GADD153, Ets1, p53, and MSK1/2 in the nucleus (26).

Emerging proofs advocate a role for the p38 MAPK and MKP-1 in the maintenance and demise of dopaminergic neurons. Mitogen-activated protein kinase phosphatase-1 (MKP-1) is a negative regulator of p38 activity and other MAPKs such as ERK, and c-Jun NH (2) -terminal kinase (JNK). MKP-1 was found to be expressed in DA neurons cultured from E14 rat ventral mesencephalon (VM) and it was reported that DA neurons when transfected to overexpress MKP-1, triggered a substantial increase in neurite length and branching with maximum upsurge observed in primary branches (27). In addition, DA neurons displaying over-expressed MKP-1 patterns are subjected to neuroprotection against the effects of PD inducing neurotoxin 6-OHDA. MKP-1 can also promote the growth and elaboration of dopaminergic neuronal processes suggesting that MKP-1 is actively involved in DA neuronal maintenance and therefore deviant MKP-1 expression is a hallmark of damaged DA neurons in PD (28). Therefore, formulating strategies aimed at augmenting MKP-1 expression to appropriate p38 activity may be advantageous in shielding dopaminergic neurons from PD induced damage (29).

PI3K/AKT/mTOR pathway

The PI3K- PKB/Akt pathway is highly

conserved, tightly controlled and a multistep signalling cascade. Since its discovery in the 1980s, lipid kinase termed phosphoinositide 3-kinases (PI3Ks) has been proven time and time again to facilitate crucial cellular dynamics viz. survival, proliferation and differentiation. PI3Ks critically operate downstream of receptor tyrosine kinases (RTKs) and G protein coupled receptors (GPCRs) and are responsible for propagating a wide array of signals arising out from numerous growth factors and cytokines into intracellular communications by generating phospholipids, which in turn activate the serine/threonine kinase AKT and several other effector pathways (30).

PI3Ks can be divided into three classes based on their structural physiognomies and substrate specificity; of these, the most commonly investigated are the class I enzymes that are activated directly by cell surface receptors. Class I PI3Ks can further be segmented into class IAs that are activated by RTKs, GPCRs and oncogenes like the small G protein Ras, and class IBs, that are entirely moderated by GPCRs. Activated receptors can directly trigger class 1A PI3Ks bound via their regulatory subunit or adapter molecules like the insulin receptor substrate (IRS) proteins. Once activated, class I PI3Ks generate the phospholipid PI(3, 4, 5)P_3 that serves as a secondary messenger, driving multiple effector pathways influencing key cellular processes. PI3K cascade is negatively regulated by the tumour suppressor PTEN (phosphatase and tensin homolog deleted from chromosome 10) and the cellular levels of PI(3,4,5)P_3 is closely regulated by the antagonizing PTEN levels. In fact, PTEN down regulates PI3K activity via its intrinsic lipid phosphatase activity that diminishes the cellular pool of PIP3 by converting PI(3,4,5)P_3 back to PI(4,5)P_2. Therefore, loss of function of PTEN will result in uncontrolled PI3K signalling thereby leading to baffling disorders like cancer and neurodegeneration (31).

AKT is a serine/ threonine kinase which is expressed as three isoforms, AKT1, AKT2 and AKT3, which are encoded by three PKB genes namely α (PKBα), β (PKBβ), and γ (PKBγ). All the three isoforms can be characterized based upon a parallel architecture comprising of an N-terminal PH domain, a central serine/threonine catalytic domain, and a small C-terminal regulatory domain. Initial AKT activation is facilitated by translocation to the plasma membrane mediated by docking of the PH domain to the membranous phospholipid PI(3,4,5)P_3, resulting a change in AKT conformational and subsequently divulging the two critical amino acid residues for phosphorylation. Phosphorylation at both the exposed sites, T308 by PDK1 and S473 by PDK2, are a prerequisite in order for the AKT to achieve full activation status. Once AKT is activated by phosphorylation at T308 and S473, it then facilitates the phosphorylation of targets viz. GSK3 (glycogen synthase kinase 3) and FOXOs (the forkhead family of transcription factors). A variety of PDKs are currently known to operate in the process, including ILK (integrin-linked kinase), PKCbII, DNA-PK (DNA-dependent protein kinase), and ATM (ataxia telangiectasia mutated), while AKT itself has PDK functionality. However, it is strongly believed that mTORC2 (the mTOR/rictor complex) is the chief source of PDK2 activity under most circumstances (32).

mTOR belongs to a group of Ser/Thr protein kinases group of more distantly related enzymes (related to class I,II and III PI3Ks) and occasionally referred to class IV PI3Ks and includes members like ATM, ATR (ataxia telangiectasia and Rad3 related), DNA-PK and SMG-1 (SMG1 homolog, phosphatidylinositol 3-`kinase-related kinase). PI3K/AKT pathway upstream of mTOR, moderates mTOR activity. mTOR is made up of two distinct complexes namely mTORC1 and mTORC2. The mTORC1 subunit is responsible for mTOR catalytic activity and harbours other components such as Raptor (regulatory associated protein of mTOR), PRAS40 (proline- rich AKT substrate 40

kDa) and the protein mLST8/GbL. Likewise, mTORC2 comprises of mTOR, Rictor (rapamycin insensitive companion of mTOR), mSIN1 (mammalian stress- activated protein kinase interacting protein 1) and mLST8/GbL. AKT is capable of activating mTOR by phosphorylating both PRAS40 and TSC2 (tuberous sclerosis complex) thereby offsetting the inhibitory effects on mTORC1. PKB/Akt binds to PIP3 at the plasma membrane in that way allowing PDK1 to access and phosphorylate the exposed T308 site in the "activation loop," thus leading to the partial activation of the PKB/Akt component. This amendment is however adequate to stimulate mTORC1 by directly phosphorylation and inactivation of PRAS40 and TSC2 as mentioned above (33). Second phosphorylation of Akt at S473 in the carboxy-terminal hydrophobic motif, either by mTOR or by DNA-PK; result in full activation of PKB/Akt. mTOR can also operate as PDK2 and phosphorylate AKT when it is bound to Rictor in the mTORC2 subunit. mTOR is pivotal to cell growth and proliferation as it monitors variables like monitoring nutrient availability, cellular energy levels, oxygen levels, and mitogenic signals. Well-characterized effector targets of mTORC1 includes 4E-BP1 (4E-binding protein), ribosomal protein S6 kinase and S6K1 (p70S6 kinase) which, in turn, phosphorylates the ribosomal protein S6 (S6/RPS6) (34).

mTOR also regulates autophagy, the failure to which leads to deficiency in the elimination of abnormal and toxic protein aggregates which subsequently trigger catastrophic cellular stress, failure and ultimately death. Autophagy is also modulated by starvation, growth factors, and cellular stressors and has long been proven to play a critical role in PD neuropathology. Yet, the cross-talk between PI3K/AKT/mTOR and autophagy is compound and the comprehensive examination of tissue from patients suffering from PD and of animal and cellular models shall provide further valuable insght (35).

p38 MAPK mediates microglial response and neuroinflammation.

Microglial cells arise from mesodermal /mesenchymal progenitors and are the resident macrophages in the CNS. Once matured, these cells are disseminated into all regions of the CNS, spread through the brain parenchyma, and attain a specific ramified morphological appearance known as "resting microglia". In the normal brain, microglia however have highly motile procedures by which they patrol their territorial domains. Additionally, these cells can communicate with macroglial cells and neurons and with other cells of the immune system, the interaction mediated by a wide range of cell signalling mechanisms. Microglial cells display characteristic receptors as labelled for brain-specific communication such as neurotransmitter receptors and immune cell-specific such as for cytokines. Interestingly, microglial cells are the most vulnerable sensors of brain homeostasis and upon any detection of anomaly such as traces of brain lesions; nervous system dysfunction or external insults (trauma, toxicants), these cells surpass a multi-staged activation prototype to transform from "resting microglia" to "activated microglia". The activated microglial cells have the enormous capacity to secrete a repertoire of molecules which can either act damaging or advantageous to the neighbouring population. Activated microglial cells migrate to the site of injury where they proliferate further and phagocytize damaged cells and cellular compartments (36, 37).

Microglial activation and neuroinflammation in PD pathology

Human brain and immune system are convolutedly involved in a crosstalk so as to maintain tissue homeostasis. A panel of evidences obtained from human and animal research have advocated the principle that neuroinflammation

significantly contributes to the neuronal loss in PD. Available reports highlight the centrality of non-cell-autonomous pathological mechanisms in PD, which in most cases are regulated by the activation of glial and peripheral immune population. Neuroinflammation in PD is a chronic mechanism that can be connected with the alteration of glial cells, including astrocytes and microglia. Microglia activation in PD brains acutely involves a panel of microglial-derived neurotoxic factors such as reactive oxygen species (ROS), inducible nitric oxide synthase (iNOS), elevated pro-inflammatory cytokine levels, and upregulated inflammatory-associated factors such as cyclooxygenase-2, which altogether cooperate to stabilize microglial response in PD brains (Figure 1). Therefore, and it is not surprising that the prolonged use of anti-inflammatory drugs can indeed reduce the risk for the disease. The neuronal response to microglia activation triggers unwanted trauma viz. oxidative stress (OS), neuroinflammation, cytokine-receptor-mediated apoptosis, which eventually contribute to DA neuronal mortality and subsequent disease progression. Interestingly, recent reports on transgenic mice related model mice were supportive of the idea that neuroinflammation in PD can be ambiguous, that is protective in the initial stages of degeneration but becomes severely damaging as the disease progresses (7, 38-40).

In vivo evidences of neuroinflammation in PD reported the upregulation of inflammatory genes in the periphery and in the CNS, the intrusion of peripheral immune cells into the CNS, and the transformed composition and phenotype of peripheral immune cells. Notably, activated microglial cells are at the heart of the neuro-inflammatory program and the hypothesis has constantly been supported by reports highlighting the neuropathology of PD brains. Initial breakthrough study by McGeer et al. first reported about the increased microglia status in the substantia nigra *parts compacta* (SNpc) region of post mortem PD brains, following which, several other novel post- mortem studies underlined the significant signs of inflammation and oxidative stress, including increased microglial activation and lipid peroxidation in PD afflicted brains. The midbrain region, which includes the SNpc houses

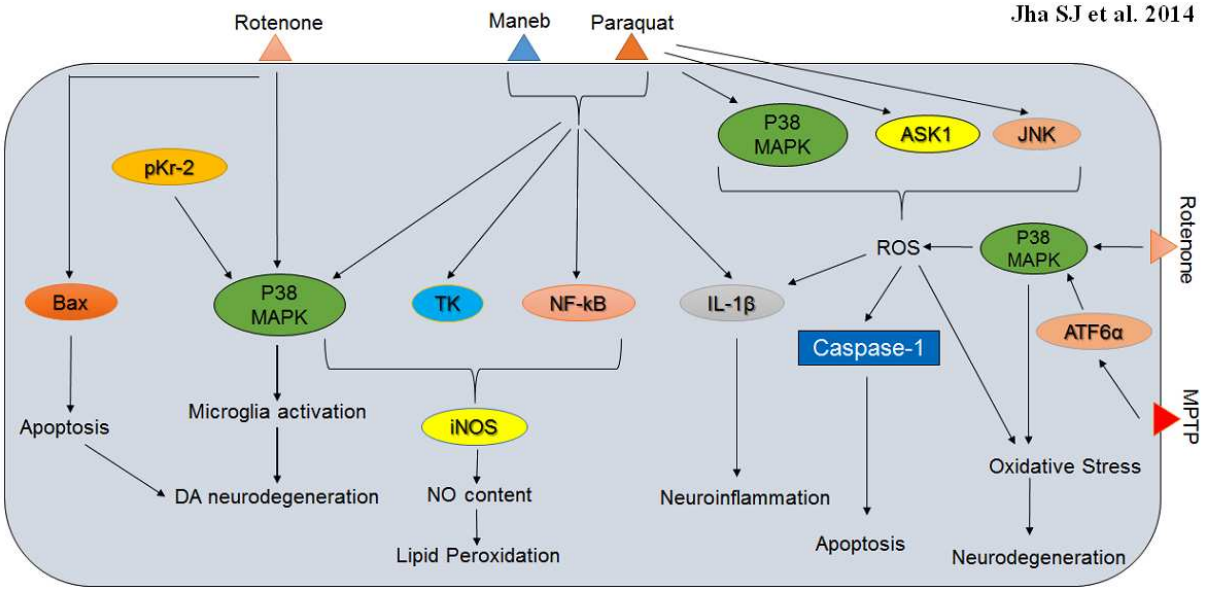

Fig. 1. p38 MAPK interactions involved in Parkinson's disease neuropathology and associated neurodegeneration. Neurotoxins viz. rotenone, maneb, paraquat and MPTP evokes numerous detrimental phenotypes in degenerating neurons and p38 MAPK is responsible for microglia activation, induction of oxidative stress, apoptosis, neuroinflammation and neurodegeneration as triggered by these toxins.

the highest proportion of microglial cells, the resident immune cells of the brain, as compared to the other regions of the brain and this could be decisive towards the heightened sensitivity of this region to inflammatory stimuli.

Further, microglias respond to a panel of signals which include bacterial and viral products, α-synuclein, complement, antibodies, cytokines, and neuronal death which can occur as a result of response to cytokines such as TNFα, ligation of death receptors such as Fas, and toxicity of reactive oxygen species. Phagocytosis may also trigger microglial activation through loss of inhibitory CD200-CD200R signalling cascade. In response to these wide range of stimuli, microglia further secrete cytokines, ROS, prostanoids that have immune modulatory properties, and chemokines which are responsible for the deployment of peripheral immune cells. Similar to all other cells with antigen-presenting features such as B lymphocytes, macrophages, monocytes, dendritic cells, activated microglia cells also harbors major histocompability class II (MHC-II) molecules on its surface, which are responsible for presenting endocytozed or lysosomal peptides to CD4 T lymphocytes for toxicity clearance. Microglial activation is therefore a major driving force behind dopaminergic neurodegeneration in PD animal models generated using neurotoxins such as rotenone, MPTP and paraquat. Nonetheless, continued microglial activation and neuroinflammation in post-mortem samples and animal models have since been long- established in living PD patients enduring PET scans with the ligand PK 11195. Therefore, reducing or preventing the sustained microglial activation might restrict inflammation and may protect the brain from neurodegenerative insults in PD patients (41-44).

p38 MAPK and associated components together mediate microglial response in PD

It is well-known for quite some time that microglial response is central to DA neuron degeneration and recent studies have proposed that p38 MAPK cascade has a critical impact on microglial activation and response. P38 MAPK together with CD200-CD200R signalling can moderate microglial dynamics in PD brains. CD200-CD200R holds the microglia cells in a quiescent state and PD associated neurodegeneration may well be concomitantly associated with disruption of CD200-CD200R and p38 MAPK signalling axis. This dual signalling axis when operating normally can promote microglia silencing in the SN, which could indeed prevent disease onset and progression. Additionally, p38 MAPK can also activate NADPH oxidase and intercede microglial response. Endogenous molecule(s) like prothrombin kringle-2 (pKr-2) is a domain of human prothrombin distinct from thrombin that has the ability to activate cultured rat brain microglia *in vitro*. Alternatively, prothrombin can trigger NO release and enhance mRNA expression levels of inducible NO synthase, IL-1β, and TNF-α in rat brain microglia. PKr-2 can imitate the effects of prothrombin in promoting NO synthesis and in the upregulation of various inflammatory mediators as mentioned above. Interestingly, both prothrombin and pKr-2 can trigger the same signalling cascades in particular involving p38 MAPK and other kinases such as extracellular signal- regulated kinase ½and c-Jun N-terminal kinase and NF-κB in an analogous manner. Increased NO levels in response to either of these molecules can be diminished by inhibitors such as PD98059 (extracellular signal regulated kinase pathway), SB203580 (p38 MAPK), N-acetylcysteine (NF-κB), Go6976, bisindolylmaleimide, and Ro31-8220 (all three against protein kinase C), and D609 and U73122 (both against phospholipase C). Further, pKr-2 can also facilitate the apoptosis of DA neurons in the SN by activating microglial cells via diverse mechanisms

involving MAPKs (45-47). Neurotoxins such as maneb and paraquat can similarly activate the microglial cells, escalate the nitrite content, and upgrade the expression levels of IL-1β, p38 MAPK, NF- kB and TK, thereby contributing to DA neurodegeneration. NO can modulate PD pathology by contributing to an increase in maneb and paraquat-induced lipid peroxidation in mouse striatum and molecules such as TK, NF-kB and p38 MAPK are well-acknowledged to modulate iNOS expression. Notably, caffeine can offer neuroprotection by diminishing nitric oxide (NO) production, neuro-inflammation and microglial activation in DA neurons (48, 49). These observations altogether propose that the expression prototype of p38 MAPK and inflammatory mediators are central towards microglial maintenance, inflammation and eventually DA neuron degeneration.

The environmental toxin rotenone, a mitochondrial complex I inhibitor can directly activate microglial cells through the p38 MAPK pathway and initiate DA neuronal damage in SNpc, which ultimately results in parkinsonism, but the exact mechanisms behind the selective degeneration of nigral DA neurons are not yet fully understood. It was observed that rotenone administration in SNpc of Lewis rats significantly downgraded the motor activity and resulted in loss of THase immunereactivity. Moreover, the degeneration of nigral DA neurons was escorted by an increase in p38 (MAPK), P-p38(MAPK), p53, and Bax expression levels. The neurotoxin exhibited similar effects in cultured PC12 cells and resulted in the upregulation of p38 (MAPK), P-p38(MAPK), p53 and Bax levels, thereby drawing some kind of parallelism between the activation prototypes *in vitro* and *in vivo*. Once activated, Bax is exported into the mitochondrial membrane where it oligomerizes and triggers the mitochondrial apoptotic signalling and the aforesaid observation strongly indicates that p38 (MAPK)/p53-associated stimulation of Bax can indeed contribute to rotenone's neurotoxicity in PD models (50). Lastly, 6-OHDA treatment of MN9D dopaminergic neuronal cells also results in translocation and oligomerization of Bax onto the mitochondria as is the case with the earlier rotenone models. Altogether, these findings suggest that there exist an independent amplification loop of Bax translocation and oligomerization mediated by caspase and p38 MAPK during ROS- mediated DA neurodegeneration (51). An exhaustive investigation is therefore obligatory so as to establish a tangible role of p38 MAPK and auxiliary components in moderating the microglial integrity and activation program and subsequent neurodegeneration in PD brains.

p38 MAPK mediates oxidative stress in diseased neurons

Neural cells suffer severe functional or sensory loss in neurodegenerative disorders and as discussed in previous sections, microglia- derived inflammatory neurotoxins play a major role in disease pathogenesis. Although, oxygen is the basis for life, defective metabolism and excess ROS production contributes to severe trauma and in contribution with several other environmental or genetic factors, oxidative stress ultimately leads to ROS accumulation in neural cells. Nevertheless, the human body's innate antioxidant defence plays a decisive role in the prevention of any loss due to ROS but imbalance in defence mechanism of antioxidants, the overproduction or incorporation of free radicals from environment to living system can lead to serious penalty and calamitous neurodegeneration. Moreover, a spectrum of environmental prompts, ER-stress, mitochondrial dysfunction, DNA injury, the accretion of the damaged misfolded proteins due to defective proteasome function, neuroinflammation, tissue damage and subsequent neural cell apoptosis also subject the brain to severe insults. These factors affect glial function thereby aiding to hasten the

cadence of the disease. Understanding the connections between oxidative stress, free radical formation, neuro-inflammation, and neurotoxicity is critical to deciphering novel disease mechanisms and the development of model neurotherapeutics to antagonize disease progression (52- 54). Oxidative stress in DA neurons can trigger the p38 MAPK pathway thus leading to the activation of both mitochondrial and extra mitochondrial apoptotic pathways PD culture models. These results suggest that oxidative stress and p38 MAPK pathways operate to balance the pro and anti- apoptotic phenotypes of DA neurons (55).

Paraquat (PQ) elicits a dose-dependent increase in ROS which results in death of SH-SY5Y neuroblastoma cells. This observation can be closely associated with the activation of ASK1 and the stress kinases p38 and JNK SH-SY5Y cells. It has recently reported that the chemical inhibition of either p38 or JNK can confer resistance from L-DOPA-induced apoptosis. Moreover, direct knockdown of ASK1 protects from L-DOPA-induced neuronal cell death. Furthermore, the suppression of the 6-OHDA- generated ROS by treating the cells with N-acetyl-L-cysteine effectively constrains the 6-OHDA- triggered activation of ASK1, p38 and JNK, and thereby protects the cells from apoptosis. It must be noted here that ROS mediated caspase-1 activation and mature IL-1β release are strictly reliant on the p38 MAPK levels in 6-OHDA model systems. These studies clearly show the path from ROS generation to the initiation of p38/JNK signalling via activation of ASK1 and subsequent apoptosis in investigated PD systems (56-57). Rotenone can also meritoriously generate ROS, the concentration levels of which can be directly correlated with the activity of p38 MAPK in the microglia populace (8, 51). These studies clearly show the path from ROS generation to initiation of p38/JNK signalling via the activation of ASK1 and subsequent apoptosis in investigated PD systems.

ATF6α is an ER-membrane-bound transcription factor in mammalian cells that is activated as a consequence of protein misfolding in the ER. ATF6α functions as a critical regulator of ER quality control. 1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), a dopaminergic neurotoxin well-known to generate OS, activates ATF6α and increases the level of ER chaperones and ER-associated degradation (ERAD) component in DA neurons. This induced oxidative stress not only stimulates phosphorylation of p38 MAPK but also augments the interaction between phosphorylated p38MAPK and ATF6α, leading to an increment in the transcriptional activity of ATF6α. This mechanism provides a credible link between oxidative stress and ER stress by underscoring the reputation of ATF6α in the protection of the DA neurons from MPTP induced neurotoxicity that occurs via OS-induced activation of ATF6α and p38MAPK- mediated enrichment of ATF6α transcriptional activity (59). Mutations in PINK1 (phosphatase and tensin homolog (PTEN)-induced putative kinase 1) gene is causative behind autosomal recessive PD. Recent studies have investigated the impact of PINK1 on HO-1 (heme oxygenase-1) activation in SH-SY5Y cell lines following H_2O_2 or 1-methyl-4-phenylpyridinium [MPP (+)] treatment. It was suggested that the H_2O_2 induced HO-1 induction was dependent on Akt and ERK phosphorylation. Moreover, in cells expressing PINK1 G309D mutant and the knockdown of tumour necrosis factor receptor-associated protein-1(TRAP1), the phosphorylation of ERK and Akt was inhibited but not p38 MAPK phosphorylation. These results identified a novel mechanism involving p38 MAPK by which the defect in PINK1 inhibits the oxidative stress-induced HO-1 production. Above all, aberrant HO-1 production following oxidative stress hastens the DA neurodegeneration and directs the brain to a

traumatic state in PD patients with PINK1 defect (60). Finally, the uncharacteristic expression of matrix metalloproteinases (MMPs) play their part in PD prognosis and contributing factors such as ROS, PI3K, NF-κB, and AP-1 are commonly involved in 6-OHDA- and MPP (+)- induced MMP-9 gene expression during PD. SK-N-BE(2)C human neuroblastoma and Cath.a mouse DA cell lines when treated with 6-OHDA and MPP(+), resulted in an induction of MMP-9 expression, where the role of p38 MAPK was found to be only differential (61).

PI3K/AKT/mTOR pathway mediates neuroprotection in PD

Accumulating evidences strongly suggest on PI3K/Akt and mTOR to being neuroprotective and hence malfunctioned in PD brains; this is actually of relevance to longevity and may present strategic targets for therapeutic improvement (62). Recent research statistics strongly advise that the vulnerability of DA neuron could arise from elevated metabolic stress levels, resulting from numerous perturbed cascades designated for the control of energy metabolism and cell survival in response to growth factors, oxidative stress, and nutrient deprivation (PI3K/AKT, mTOR, eIF4/p70S6K and Hif-1α). Altogether, these factors operate in a convoluted network thereby adding to archetypal phenotypes observed in PD patients. One of the cardinal symptom observed in diseased brains is neuroinflammation and PTEN induced putative kinase 1 (PINK1), an autosomal recessive familial PD gene, regulates the inflammatory ambience during traumatic states. Dearth in PINK1 levels expedites neuro-inflammation in PD brains through diminished AKT activation and enhanced IκB degradation in response to traumatic brain injury (63). In fact, mutations in PINK1 genes have provided a credible basis to a certain extent to meticulously monitor and comprehend the otherwise complex etiology of PD. PINK1 mutations were found to be severely damaging in C2-ceramide (neurotoxin) challenged brains thereby suggesting on the neuroprotective role of PINK1 in preventing mitochondrial dysfunction and reinforcing the anti- apoptotic and neuronal survival pathways such as Bcl-2 and PI3K/AKT (64). PINK1 and PARKIN are responsible for mitochondrial damage limitation during the active durations of stress and cooperate together in autophagy following mitochondrial injury. Examination of primary mouse cells acquired from PINK1- knockout mice directed that PARKIN induction and lysosomal translocation proceeded autonomous of PINK1. Moreover, suppression of the PI3K/AKT-mTOR pathway by therapeutic proxies can vary PARKIN expression accordingly. These results altogether validate that PARKIN and PINK1 are co-regulated during starvation and suggest a likely role of PI3K/AKT-mTOR in response to trophic signals and starvation stress (65).

PI3K/AKT pathway can also play a key role in IGF-mediated cell survival and prevention of apoptosis in MPP+ induced human neuroblastoma SH-EP1 cells. This defensive activity of AKT is principally reliant on the BIO mediated inactivation of GSK-3β, the result of which could imitate the protective influence of IGF-1 in SH-EP1 cells. Interestingly, the IGF-1 potentiated PI3K/AKT activity was found to further down regulate the JNK related apoptotic activity and this negative regulation was reported to be facilitated via AKT-dependent GSK-3β inactivation (Figure 2). Moreover, these results acknowledge that IGF-1 protects SH-EP1 cells from MPP+-induced apoptotic cell mortality via the cytoprotective PI3K/AKT/GSK-3β pathway involving GSK-3β inactivation (66-68). Most recently, it hes been studied in DA neuronal cell systems that upregulation of miR-126 impaired IGF-1 signalling and increased the susceptibility of such systems to 6-OHDA, possibly by stamping down factors involved in IGF-1/PI3K signalling, including its

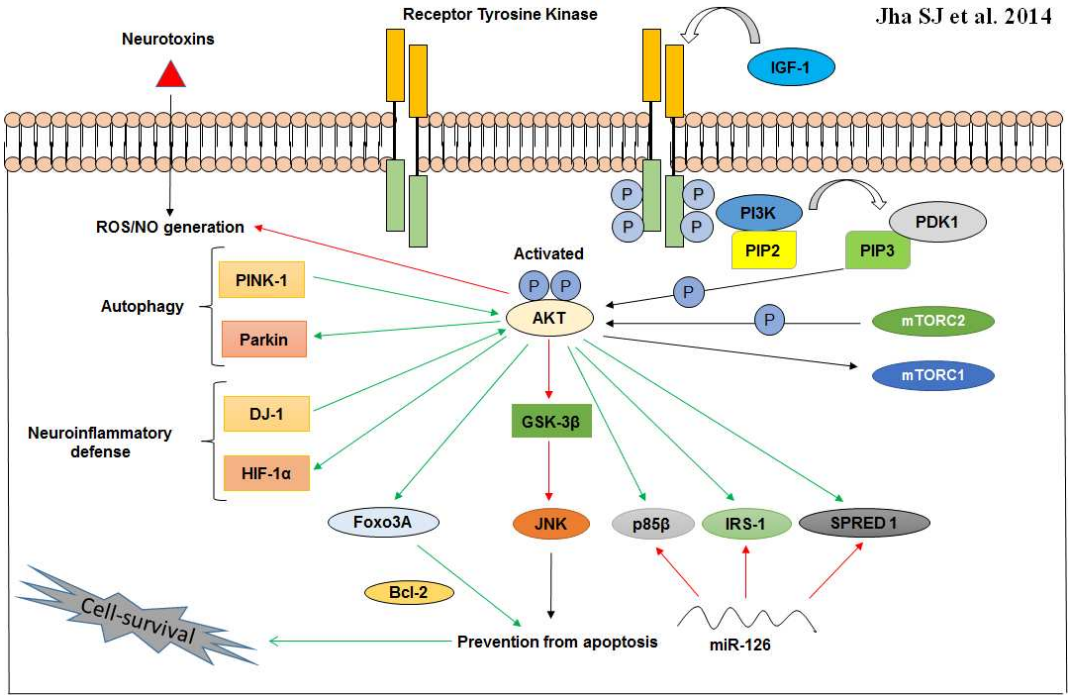

Fig. 2 Neuroprotective cross-talk involving the cytoprotective PI3K/AKT pathway. AKT when optimally activated by phosphorylation at serine and threonine residues, can interact with a spectrum of molecules to erect an anti- inflammatory (DJ-1 and HIF-1α) and anti-apoptotic (Bcl-2) ambience in vulnerable neurons. In addition, phosphorylated AKT can also promote autophagy via PINK-1 and Parkin. IRS-1 activation takes place via IGF-1/AKT cascade and other AKT targets including p85β and SPRED 1 are known to be downregulated by miR-126 in PD neurons. Activation (green arrows); prevention or suppression (red arrows).

downstream targets p85β, IRS-1, and SPRED 1. MicroRNAs (miRs/miRNAs) act as posttranscriptional regulators of gene expression and therefore, it is unsurprising that they could be critically modulating pathogenesis in PD. Notably, blocking miR-126 activity increased IGF-1 trophism and thereby combating the cataclysmic events of 6-OHDA. This result strongly ascertain the criticality IGF-1/ PI3K cascade in DA neuron maintenance and also suggests that the higher expression patterns of miR-126 may contribute towards DA neurodegeneration aided by downregulation of IGF-1/PI3K/AKT signalling (69).

Glial cell line-derived neurotrophic factor (GDNF) is necessary for DA neuronal maintenance and development. GDNF is normally found to be neuroprotective in animal models of PD, where selective DA neurodegeneration is a characteristic feature (70). GDNF can have potent neuroprotective effects in nigrostriatal DA neurons that are degraded in PD. H_2O_2 or l-3, 4-dihydroxyphenylalanine (l-DOPA) when used to injure DA neurons, prompts the release of soluble factors that signal the ventral midbrain astrocytes to upregulate GDNF concentration. Notably, PI3K pathway is central towards this mechanism of striatal GDNF up-regulation as triggered by H_2O_2. Conversely, diffusible factors released in the presence of l-DOPA- trigger GDNF expression via activation of the MAPK pathway (71). Another study attempted to decipher whether cadherin has a profound impact on PI3K/AKT activation in DA neurons mediated by the protective effects of GDNF. Cadherins are calcium-dependent adhesion proteins, and N-cadherins are expressed in DA neurons. Interestingly, the results of the investigation suggested that N-cadherin was indeed involved in PI3K/AKT activation in DA neurons triggered by GDNF (72). Aging mice which lacks DJ-1 and the GDNF-receptor Ret expression in the DA system exhibits loss of substantia nigra (SN) cell bodies, but not axons, compared to mice

compromised only in Ret. This survival requirement for DJ-1 is specific for the most vulnerable GIRK2-positive populace in the SN which projects entirely to the striatum. Study on Drosophila genetics further revealed about the constitutively active Ret and associated Ras/ERK, but not PI3K/AKT, signalling, which interact genetically with DJ-1. A better understanding of the molecular connections between trophic signalling, cellular stress and aging could uncover new targets for drug development in PD (73).

Neuroprotective molecules of relevance in p38 and PI3K/AKT mediated PD prognosis

Neurotherapeutics research in recent times have probed a spectrum of protective biomolecules which can either activate the PI3K/AKT cascade, while others assist in limiting the activation of p38 MAPK in neurons; Table 1 provides a list of neuro- protective therapeutic modalities that sum on either the misfiring p38 MAPK and/or PI3K/AKT cascades in some form or the other, en route to providing a resilient neuroprotective shield against the hastened degeneration of neurons in PD.

Table 1. Neuroprotective Biomolecules offering neuroprotection in neurotoxin challenged Parkinson's model systems presumably via p38 MAPK and/or PI3K/AKT cascade.

Biomolecules	Nature	PD Model systems	Neurotoxin	Proteins involved	Mode of action	References
Guanine based purines	anti-apoptotic	SH-SY5Y	6-OHDA	AKT, p38, JNK, and Bcl-2	Triggers an early upsurge in the phosphorylation of Akt and subsequent activation of the cytoprotective PI3K/AKT/PKB pathway; prevents the 6-OHDA intermediated activation of p38 and JNK and cause an upsurge in the expression level of the anti-apoptotic Bcl-2 protein.	(9)
Human albumin	anti-oxidant and anti-apoptotic	PC12	6-OHDA	JNK, c-Jun, ERK, and p38 MAPK	Attenuates 6-OHDA-inflicted ROS generation and apoptosis; inhibits 6-OHDA-induced activation of JNK, c-Jun, ERK, and p38 MAPK signalling.	(74)
Peroxiredoxin (PRX-2)	anti-oxidant and anti-apoptotic	MN9D DA neurons	6-OHDA	ASK1, c-Jun, p38	Inhibits 6-OHDA-induced ASK1 activation by regulating the redox properties of the endogenous ASK1 inhibitor Trx; display significant anti-apoptotic properties via suppression of ASK1-dependent activation of the c-Jun N-terminal kinase/c-Jun and p38 pro-mortality cascades; lastly, PRX2 over expression preserves Trx in a reduced state by blocking the cysteine thiol-disulfide interchange, thus preventing the dissociation of thioredoxin from ASK1.	(75, 76)
NOSH-ASA (NO- and H2S-releasing hybrid of aspirin)	anti-inflammatory	IFNγ-stimulated human astroglia and U373 cells, SH-SY5Y		TNFα, IL-6, P38 MAPK and NFκB	Results in reduced TNFα and IL-6 levels along with a concomitant deactivation of P38 MAPK and NFκB proteins.	(77)

Bu-7	anti-apototic	PC12	Rotenone	JNK,p38,p53,caspase-3,Bax,Bcl-2	Protects the cells from rotenone triggered apoptosis and subsequent death; limits the rotenone induced potential reduction in mitochondria of the treated cells, prevents the rotenone induced activation of JNK, p38, p53, cleaved caspase-3 and decreases the Bax/Bcl-2 ratio.	(78)
3,4-Dihydroxy benzalacetone (DBL)	anti-oxidant, anti-inflammatory, and anti-tumorigenic	SH-SY5Y	6-OHDA	Akt, ERK, p38 MAPK, PI3K	Induce stress-associated kinases such as Akt, ERK, and p38 MAPK, and PI3K or Akt inhibitors, but not ERK, p38, or JNK inhibitors; activates the Nrf2/glutathione cascade via PI3K/Akt, and facilitates survival of SH-SY5Y cells.	(79)
Tetrahydroxystilbene glucoside (TSG)	anti-apoptotic	PC12 and mice	MPTP	DAT, AKT, GSK3β, Bcl-2, BAD, caspase-3 and caspae-9	Protects DA neurodegeneration by averting MPTP-induced reduction of SN tyrosine hydroxylase (TH)-positive cells and striatal dopaminergic transporter (DAT) protein expression; increase in striatal Akt and GSK3β phosphorylation, up-regulation of the Bcl-2/BAD ratio, and inhibition of caspase-9 and caspase-3 activity; offers neuroprotective effects against MPP-prompted damage and apoptosis in PC12 cells, presumably through PI3K/Akt. Activation.	(80)
Tyrosol [2-(4-ydroxy-phenyl) ethanol]	anti-apoptotic	CATH.a	MPP(+)	PI3K, AKT, SOD-1, SOD-2 and DJ-1	Is neuroprotective against (MPP(+))-induced CATH.a neuronal death in a dose dependant manner by its ability to activate the PI3K/Akt signalling cascade; Tyrosol also upregulate SOD-1, SOD-2 and DJ-1.	(81)
Oxicam non-steroidal anti-inflammatory drugs (NSAIDs)	anti-inflammtory	SH-SY5Y and mice	MPTP	PI3K, AKT, and COX	Offers protection via the PI3K/Akt cascade independentally of cyclooxygenase (COX) inhibition.	(82)
Tocotrienols (T3s)	anti-oxidant	SH-SY5Y	MPP(+)	ER β and PI3K/AKT	γT3 and δT3 treatments triggers the PI3K/Akt signalling module and this could perhaps be under the control of estrogen receptor (ER) β; ER β being an upstream regulator of PI3K/Akt; T3s and, especially, γT3/δT3 in conjunction with the activation of ERβ/PI3K/Akt cascade, display not only antioxidant activity but also offers a receptor signal-mediated neuroprotection.	(83)

Danshensu (beta-3,4-dihydroxy phenyl-lactic acid)	ROS scavenger and anti-oxidant	PC12 and Zebra fish DA neurons	6-OHDA	PI3K/AKT, Nrf-2, and HO-1	Induces Akt phosphorylation, and the induced cytoprotective effects are reversed by PI3K, Akt and HO-1 inhibitors; enhances HO-1 expression in order to suppress 6-OHDA-induced oxidative stress via PI3K/Akt/Nrf2 cascade.	(84)
Puerarin	anti-oxidant	Mice	MPTP	PI3K/AKT, GSH, and GDNF	Puerarin administration enhances glutathione (GSH) activity, glial cell line-derived neurotrophic factor (GDNF) expression and activates the PI3K/Akt pathway; dampens MPTP-reduced lysosome-associated membrane protein type 2A (Lamp 2A) expression.	(85)
Eucommia ulmoides Oliv. Bark. (EUE)	anti-oxidant	SH-SY5Y	6-OHDA	JNK, PI3K/Akt, GSK-3β, and NF-κB	EUE reduces 6-OHDA-induced ROS formation, mitochondrial dysfunction, cell death and cytotoxicity; mitigates oxidative stress through induction of JNK, PI3K/Akt, GSK-3β, and NF-κB cascades.	(86)
Rotigotine	anti-oxidant, anti-apototic	primary dopaminergic cultures	glutamate	D3 receptor, AKT, and GSK-3-β	The molecule most likely stimulates the dopamine D3 receptor; abates the production and accumulation of superoxide radicals; consistent exposure to Rotigotine promotes Akt phosporylation, and results in deactivation of the pro-apoptotic component GSK-3-β.	(87)
Squamos-amide derivative FLZ		Rats	6-OHDA	PI3K/AKT, α-Syn, and TH	FLZ protects TH activity and DA neurons by diminishing α-synuclein (α-Syn) expression and the cooperation between α-Syn and TH, FLZ neuroprotection involves the PI3K/AKT cascade and blocking the cascade attenuates α-Syn expression and subsequently the protection offered by FLZ is lost.	(67)

In conclusion environmental exposures to toxic mediators lead to neurodegenerative sickness that has common pathophysiology and clinical findings with PD. It is conjectured that decisive factors like microglia activation, neuroinflam-mation, oxidative stress due to ROS accumulation, NO activity, and neuronal apoptosis resulting from all these modes are at the base of DA neuronal toxicity and subsequent damage in Parkinson's brains. However, the precise identity and functional prototypes of molecular intermediates leading to neuronal mortality still remains to be deciphered. Recent studies have highlighted the fundamental role of p38 MAPK in controlling all of the above detrimental consequences, and thereby in the process upset DA neuronal homeostasis, which ultimately progresses to an advanced diseased state and incurable neurodegeneration. Using cell systems like SH-SY5Y and PC12, several factors like inducible NO synthase, ATF6α (oxidative stress), IL-1β, and TNF-α (neuro-inflammation), and Bax and ASK1 (apoptosis) were found to be relevant in DA neuronal death. Unprecedented observations that these factors operate in unison with the p38 MAPK cascade strongly advocate the cyto-destructive nature of the cascade in

degenerating neurons. Conversely, the normal functioning of the PI3K/AKT pathway ensures that the neuroprotective defence is active in order to negate the destructive aftermath of p38 MAPK activation in degenerating neurons. In the process, the activated AKT interacts with several mediators like JNK, FoxO, GSK3β, etc., thereby to render neuroprotection by limiting apoptosis, preventing microglia activation and neuroinflammation, preventing ROS accumulation and by keeping oxidative stress levels under check. However, the pathway is misregulated in PD brains and eventually it fails to render its protective veneer in traumatized brains. It is thereby necessary to identify modalities which can repair the misbalanced p38/PI3K interactome in order to limit poor prognosis in PD patients.

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Conflict of Interests

The authors declare no conflict of interests.

References

1. Adami R, Scesa G, Bottai D. Stem cell transplantation in neurological diseases: improving effectiveness in animal models. Front Cell Dev Biol 2014; 2: 17.

2. Caulfield TR, Fiesel FC, Moussaud-Lamodiere EL, et al. Phosphorylation by PINK1 releases the UBL domain and initializes the conformational opening of the E3 ubiquitin ligase Parkin. PLoS Comput Biol 2014; 10: e1003935.

3. Ikehara S, Li M. Stem cell transplantation improves aging-related diseases. Front Cell Dev Biol 2014; 2: 16.

4. Bega D, Zadikoff C. Complementary & alternative management of Parkinson's disease: an evidence-based review of eastern influenced practices. J Mov Disord 2014; 7: 57-66.

5. Ertekin C. Electrophysiological evaluation of oropharyngeal Dysphagia in Parkinson's disease. J Mov Disord 2014; 7: 31-56.

6. Ansari R, Mahta A, Mallack E, et al. Hyperhomocysteinemia and neurologic disorders: a review. J Clin Neurol 2014; 10: 281-8.

7. Jung UJ, Kim SR. Effects of naringin, a flavanone glycoside in grapefruits and citrus fruits, on the nigrostriatal dopaminergic projection in the adult brain. Neural Regen Res 2014; 9: 1514-7.

8. Gao F, Chen D, Hu Q, et al. Rotenone directly induces BV2 cell activation via the p38 MAPK pathway. PLoS One 2013; 8: e72046.

9. Giuliani P, Romano S, Ballerini P, et al. Protective activity of guanosine in an in vitro model of Parkinson's disease. Panminerva Med 2012; 54: 43-51.

10. Low K, Aebischer P. Use of viral vectors to create animal models for Parkinson's disease. Neurobiol Dis 2012; 48: 159-201.

11. Tapia-Gonzalez S, Giraldez-Perez RM, Cuartero MI, et al. Dopamine and alpha-synuclein dysfunction in Smad3 null mice. Mol Neurodegener 2011; 6: 72.

12. Kawamata J, Suzuki S, Shimohama S. Enhancement of nicotinic receptors alleviates cytotoxicity in neurological disease models. Ther Adv Chronic Dis 2011; 2: 197-208.

13. Mylene M, Sebastien M, Sophie CC, et al. Neurosurgery in Parkinson's disease: Social adjustment, quality of life and coping strategies. Neural Regen Res 2013; 8: 2856-67.

14. Chan AY, Yeung JH, Mok VC, et al. Subthalamic nucleus deep brain stimulation for Parkinson's disease: evidence for effectiveness and limitations from 12 years' experience. Hong Kong Med J 2014; 20: 474-80.

15. Wang G, Pan J, Chen SD. Kinases and kinase signaling pathways: potential therapeutic targets in Parkinson's disease. Prog Neurobiol 2012; 98: 207-21.

16. Russo I, Bubacco L, Greggio E. LRRK2 and neuroinflammation: partners in crime in Parkinson's disease? J Neuroinflammation 2014; 11: 52.

17. Takeda K, Ichijo H. Neuronal p38 MAPK signalling: an emerging regulator of cell fate and function in the nervous system. Genes Cells 2002; 7: 1099-111.

18. Kim EK, Choi EJ. Pathological roles of MAPK signaling pathways in human diseases. Biochim Biophys Acta 2010; 1802: 396-405.

19. Correa SA, Eales KL. The Role of p38 MAPK and Its Substrates in Neuronal Plasticity and Neurodegenerative Disease. J Signal Transduct 2012; 2012: 649079.

20. Kitagishi Y, Kobayashi M, Kikuta K, et al. Roles of PI3K/AKT/GSK3/mTOR Pathway in Cell Signaling of Mental Illnesses. Depress Res Treat 2012; 2012: 752563.

21. Morrison RS, Kinoshita Y, Johnson MD, et al. Neuronal survival and cell death signaling pathways. Adv Exp Med Biol 2002; 513: 41-86.

22. Cuadrado A, Nebreda AR. Mechanisms and functions of p38 MAPK signalling. Biochem J 2010; 429: 403-17.

23. Zarubin T, Han J. Activation and signaling of the p38 MAP kinase pathway. Cell Res 2005; 15: 11-8.

24. Roux PP, Blenis J. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. Microbiol Mol Biol Rev 2004; 68: 320-44.

25. Brancho D, Tanaka N, Jaeschke A, et al. Mechanism of p38 MAP kinase activation in vivo. Genes Dev 2003; 17: 1969-78.

26. Cargnello M, Roux PP. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. Microbiol Mol Biol Rev 2011; 75: 50-83.

27. Collins LM, O'Keeffe GW, Long-Smith CM, et al. Mitogen-activated protein kinase phosphatase (MKP)-1 as a neuroprotective agent: promotion of the morphological development of midbrain dopaminergic neurons. Neuromolecular Med 2013; 15: 435-46.

28. Korbecki J, Baranowska-Bosiacka I, Gutowska I, et al. The effect of reactive oxygen species on the synthesis of prostanoids from arachidonic acid. J Physiol Pharmacol 2013; 64: 409-21.

29. Comalada M, Lloberas J, Celada A. MKP-1: a critical phosphatase in the biology of macrophages controlling the switch between proliferation and activation. Eur J Immunol 2012; 42: 1938-48.

30. Liu P, Cheng H, Roberts TM, et al. Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov 2009; 8: 627-44.

31. Hemmings BA, Restuccia DF. PI3K-PKB/Akt pathway. Cold Spring Harb Perspect Biol 2012; 4: a011189.

32. LoPiccolo J, Blumenthal GM, Bernstein WB, et al. Targeting the PI3K/Akt/mTOR pathway: effective combinations and clinical considerations. Drug Resist Updat 2008; 11: 32-50.

33. Bartholomeusz C, Gonzalez-Angulo AM. Targeting the PI3K signaling pathway in cancer therapy. Expert Opin Ther Targets 2012; 16: 121-30.

34. Wong KK, Engelman JA, Cantley LC. Targeting the PI3K signaling pathway in cancer. Curr Opin Genet Dev 2010; 20: 87-90.

35. Heras-Sandoval D, Perez-Rojas JM, Hernandez-Damian J, et al. The role of PI3K/AKT/mTOR pathway in the modulation of autophagy and the clearance of protein aggregates in neurodegeneration. Cell Signal 2014; 26: 2694-701.

36. Kettenmann H, Hanisch UK, Noda M, et al. Physiology of microglia. Physiol Rev 2011; 91: 461-553.

37. Cicchetti F, Barker RA. The glial response to intracerebrally delivered therapies for neurodegenerative disorders: is this a critical issue? Front Pharmacol 2014; 5: 139.

38. Hunot S, Hirsch EC. Neuroinflammatory processes in Parkinson's disease. Ann Neurol 2003; 53 Suppl 3: S49-58.

39. Hirsch EC, Hunot S. Neuroinflammation in Parkinson's disease: a target for neuroprotection? Lancet Neurol 2009; 8: 382-97.

40. Sekiyama K, Sugama S, Fujita M, et al. Neuroinflammation in Parkinson's Disease and Related Disorders: A Lesson from Genetically Manipulated Mouse Models of alpha-Synucleinopathies. Parkinsons Dis 2012; 2012: 271732.

41. Richardson JR, Hossain MM. Microglial ion channels as potential targets for neuroprotection in Parkinson's disease. Neural Plast 2013; 2013: 587418.

42. Kannarkat GT, Boss JM, Tansey MG. The role of innate and adaptive immunity in Parkinson's disease. J Parkinsons Dis 2013; 3: 493-514.

43. More SV, Kumar H, Kim IS, et al. Cellular and molecular mediators of neuroinflammation in the pathogenesis of Parkinson's disease. Mediators Inflamm 2013; 2013: 952375.

44. Sanchez-Guajardo V, Barnum CJ, Tansey MG, et al. Neuroimmunological processes in Parkinson's disease and their relation to alpha-synuclein: microglia as the referee between neuronal processes and peripheral immunity. ASN Neuro 2013; 5: 113-39.

45. Wang XJ, Zhang S, Yan ZQ, et al. Impaired CD200-CD200R-mediated microglia silencing enhances midbrain dopaminergic neurodegeneration: roles of aging, superoxide, NADPH oxidase, and p38 MAPK. Free Radic Biol Med 2011; 50: 1094-106.

46. Kim SR, Chung ES, Bok E, et al. Prothrombin kringle-2 induces death of mesencephalic dopaminergic neurons in vivo and in vitro via microglial activation. J Neurosci Res 2010; 88: 1537-48.

47. Ryu J, Min KJ, Rhim TY, et al. Prothrombin kringle-2activates cultured rat brain microglia. J Immunol 2002

; 168: 5805-10.

48. Yadav S, Gupta SP, Srivastava G, et al. Role of secondary mediators in caffeine-mediated neuroprotection in maneb- and paraquat-induced Parkinson's disease phenotype in the mouse. Neurochem Res 2012; 37: 875-84.

49. Gupta SP, Patel S, Yadav S, et al. Involvement of nitric oxide in maneb- and paraquat-induced Parkinson's disease phenotype in mouse: is there any link with lipid peroxidation? Neurochem Res 2010; 35: 1206-13.

50. Wu F, Wang Z, Gu JH, et al. p38(MAPK)/p53-Mediated Bax induction contributes to neurons degeneration in rotenone-induced cellular and rat models of Parkinson's disease. Neurochem Int 2013; 63: 133-40.

51. Oh CK, Han BS, Choi WS, et al. Translocation and oligomerization of Bax is regulated independently by activation of p38 MAPK and caspase-2 during MN9D dopaminergic neurodegeneration. Apoptosis 2011; 16: 1087-100.

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

53. Reynolds A, Laurie C, Mosley RL, et al. Oxidative stress and the pathogenesis of neurodegenerative disorders. Int Rev Neurobiol 2007; 82: 297-325.

54. Jha NK, Jha SK, Kar R. Role of oxidative stress, ER stress and ubiquitin proteasome system in neurodegeneration. MOJ Cell Sci Report 2014; 1: 00010.

55. Choi WS, Eom DS, Han BS, et al. Phosphorylation of p38 MAPK induced by oxidative stress is linked to activation of both caspase-8- and -9-mediated apoptotic pathways in dopaminergic neurons. J Biol Chem 2004; 279: 20451-60.

56. Liedhegner EA, Steller KM, Mieyal JJ. Levodopa activates apoptosis signaling kinase 1 (ASK1) and promotes apoptosis in a neuronal model: implications for the treatment of Parkinson's disease. Chem Res Toxicol 2011; 24: 1644-52.

57. Ouyang M, Shen X. Critical role of ASK1 in the 6-hydroxydopamine-induced apoptosis in human neuroblastoma SH-SY5Y cells. J Neurochem 2006; 97: 234-44.

58. Niso-Santano M, Gonzalez-Polo RA, Bravo-San Pedro JM, et al. Activation of apoptosis signal-regulating kinase 1 is a key factor in paraquat-induced cell death: modulation by the Nrf2/Trx axis. Free Radic Biol Med 2010; 48: 1370-81.

59. Egawa N, Yamamoto K, Inoue H, et al. The endoplasmic reticulum stress sensor, ATF6alpha, protects against neurotoxin-induced dopaminergic neuronal death. J Biol Chem 2011; 286: 7947-57.

60. Chien WL, Lee TR, Hung SY, et al. Impairment of oxidative stress-induced heme oxygenase-1 expression by the defect of Parkinson-related gene of PINK1. J Neurochem 2011; 117: 643-53.

61. Kim SY, Woo MS, Park JS, et al. Regulation of matrix metalloproteinase-9 gene expression in MPP+- or 6-OHDA-treated human neuroblastoma SK-N-BE(2)C cells. Neurochem Int 2010; 56: 437-42.

62. Elstner M, Morris CM, Heim K, et al. Expression analysis of dopaminergic neurons in Parkinson's disease and aging links transcriptional dysregulation of energy metabolism to cell death. Acta Neuropathol 2011; 122: 75-86.

63. Kim J, Byun JW, Choi I, et al. PINK1 Deficiency Enhances Inflammatory Cytokine Release from Acutely Prepared Brain Slices. Exp Neurobiol 2013; 22: 38-44.

64. Sanchez-Mora RM, Arboleda H, Arboleda G. PINK1 overexpression protects against C2-ceramide-induced CAD cell death through the PI3K/AKT pathway. J Mol Neurosci 2012; 47: 582-94.

65. Klinkenberg M, Gispert S, Dominguez-Bautista JA, et al. Restriction of trophic factors and nutrients induces PARKIN expression. Neurogenetics 2012; 13: 9-21.

66. Chong CM, Shen M, Zhou ZY, et al. Discovery of a benzofuran derivative (MBPTA) as a novel ROCK inhibitor that protects against MPP(+)-induced oxidative stress and cell death in SH-SY5Y cells. Free Radic Biol Med 2014; 74: 283-93.

67. Bao XQ, Kong XC, Kong LB, et al. Squamosamide derivative FLZ protected dopaminergic neuron by activating Akt signaling pathway in 6-OHDA-induced in vivo and in vitro Parkinson's disease models. Brain Res 2014; 1547: 49-57.

68. Wang L, Yang HJ, Xia YY, et al. Insulin-like growth factor 1 protects human neuroblastoma cells SH-EP1 against MPP+-induced apoptosis by AKT/GSK-3beta/JNK signaling. Apoptosis 2010; 15: 1470-9.

69. Kim W, Lee Y, McKenna ND, et al. miR-126 contributes toParkinson's disease by dysregulating the insulin-like growth

factor/phosphoinositide 3-kinase signaling. Neurobiol Aging 2014; 35: 1712-21.

70. Murakami M, Ito H, Hagiwara K, et al. Sphingosine kinase 1/S1P pathway involvement in the GDNF-induced GAP43 transcription. J Cell Biochem 2011; 112: 3449-58.

71. Fonseca CP, Gama S, Saavedra A, et al. H2O2- or l-DOPA-injured dopaminergic neurons trigger the release of soluble mediators that up-regulate striatal GDNF through different signalling pathways. Biochim Biophys Acta 2014; 1842: 927-34.

72. Zuo T, Qin JY, Chen J, et al. Involvement of N-cadherin in the protective effect of glial cell line-derived neurotrophic factor on dopaminergic neuron damage. Int J Mol Med 2013; 31: 561-8.

73. Aron L, Klein P, Pham TT, et al. Pro-survival role for Parkinson's associated gene DJ-1 revealed in trophically impaired dopaminergic neurons. PLoS Biol 2010; 8: e1000349.

74. Zhang LJ, Xue YQ, Yang C, et al. Human albumin prevents 6-hydroxydopamine-induced loss of tyrosine hydroxylase in in vitro and in vivo. PLoS One 2012; 7: e41226.

75. Hu X, Weng Z, Chu CT, et al. Peroxiredoxin-2 protects against 6-hydroxydopamine-induced dopaminergic neurodegeneration via attenuation of the apoptosis signal-regulating kinase (ASK1) signaling cascade. J Neurosci 2011; 31: 247-61.

76. Schreiber MA, McIntire SL. A Caenorhabditis elegans p38 MAP kinase pathway mutant protects from dopamine, methamphetamine, and MDMA toxicity. Neurosci Lett 2011; 498: 99-103.

77. Lee M, McGeer E, Kodela R, et al. NOSH-aspirin (NBS-1120), a novel nitric oxide and hydrogen sulfide releasing hybrid, attenuates neuroinflammation induced by microglial and astrocytic activation: a new candidate for treatment of neurodegenerative disorders. Glia 2013; 61: 1724-34.

78. Li BY, Yuan YH, Hu JF, et al. Protective effect of Bu-7, a flavonoid extracted from Clausena lansium, against rotenone injury in PC12 cells. Acta Pharmacol Sin 2011; 32: 1321-6.

79. Gunjima K, Tomiyama R, Takakura K, et al. 3,4-dihydroxybenzalacetone protects against Parkinson's disease-related neurotoxin 6-OHDA through Akt/Nrf2/glutathione pathway. J Cell Biochem 2014; 115: 151-60.

80. Zhang L, Huang L, Chen L, et al. Neuroprotection by tetrahydroxystilbene glucoside in the MPTP mouse model of Parkinson's disease. Toxicol Lett 2013; 222: 155-63.

81. Dewapriya P, Himaya SW, Li YX, et al. Tyrosol exerts a protective effect against dopaminergic neuronal cell death in in vitro model of Parkinson's disease. Food Chem 2013; 141: 1147-57.

82. Tasaki Y, Yamamoto J, Omura T, et al. Meloxicam ameliorates motor dysfunction and dopaminergic neurodegeneration by maintaining Akt-signaling in a mouse Parkinson's disease model. Neurosci Lett 2012; 521: 15-9.

83. Nakaso K, Tajima N, Horikoshi Y, et al. The estrogen receptor beta-PI3K/Akt pathway mediates the cytoprotective effects of tocotrienol in a cellular Parkinson's disease model. Biochim Biophys Acta 2014; 1842: 1303-12.

84. Chong CM, Zhou ZY, Razmovski-Naumovski V, et al. Danshensu protects against 6-hydroxydopamine-induced damage of PC12 cells in vitro and dopaminergic neurons in zebrafish. Neurosci Lett 2013; 543: 121-5.

85. Zhu G, Wang X, Wu S, et al. Neuroprotective effects of puerarin on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced Parkinson's disease model in mice. Phytother Res 2014; 28: 179-86.

86. Kwon SH, Ma SX, Hong SI, et al. Eucommia ulmoides Oliv. bark. attenuates 6-hydroxydopamine-induced neuronal cell death through inhibition of oxidative stress in SH-SY5Y cells. J Ethnopharmacol 2014; 152: 173-82.

87. Oster S, Radad K, Scheller D, et al. Rotigotine protects against glutamate toxicity in primary dopaminergic cell culture. Eur J Pharmacol 2014; 724: 31-42.

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Review

Stress-Induced Synaptic Dysfunction and Neurotransmitter Release in Alzheimer's Disease: Can Neurotransmitters and Neuromodulators be Potential Therapeutic Targets?

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Abstract. The communication between neurons at synaptic junctions is an intriguing process that monitors the transmission of various electro-chemical signals in the central nervous system. Albeit any aberration in the mechanisms associated with transmission of these signals leads to loss of synaptic contacts in both the neocortex and hippocampus thereby causing insidious cognitive decline and memory dysfunction. Compelling evidence suggests that soluble amyloid-β (Aβ) and hyper-phosphorylated tau serve as toxins in the dysfunction of synaptic plasticity and aberrant neurotransmitter (NT) release at synapses consequently causing a cognitive decline in Alzheimer's disease (AD). Further, an imbalance between excitatory and inhibitory neurotransmission systems induced by impaired redox signaling and altered mitochondrial integrity is also amenable for such abnormalities. Defective NT release at the synaptic junction causes several detrimental effects associated with altered activity of synaptic proteins, transcription factors, Ca^{2+} homeostasis, and other molecules critical for neuronal plasticity. These detrimental effects further disrupt the normal homeostasis of neuronal cells and thereby causing synaptic loss. Moreover, the precise mechanistic role played by impaired NTs and neuromodulators (NMs) and altered redox signaling in synaptic dysfunction remains mysterious, and their possible interlink still needs to be investigated. Therefore, this review elucidates the intricate role played by both defective NTs/NMs and altered redox signaling in synaptopathy. Further, the involvement of numerous pharmacological approaches to compensate neurotransmission imbalance has also been discussed, which may be considered as a potential therapeutic approach in synaptopathy associated with AD.

Keywords: Amyloid-β, neurotransmitters/neuromodulators, redox signaling, synaptic dysfunction, tau, therapeutics

INTRODUCTION

To maintain brain homeostasis, synapses and their associated neurotransmitters (NTs) play the role where synapses are specialized structures that form a network to transmit electrochemical signals

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or information from one neuron to another. The signal transmission involves a complex process of NT release and uptake at synaptic junctions [1] where dysregulation of the synaptic junction in response to numerous insults and aberrant NT releases or its receptors lead to synaptopathy associated with Alzheimer's disease (AD) [2]. It has been demonstrated that soluble amyloid plaques and hyperphosphorylated tau served as toxins in disrupting synaptic plasticity and NT release at synapses, thereby causing a cognitive decline in AD [3, 4]. In addition, free radicals, oxidative stress, and mitochondrial dysfunctions have also contributed significantly toward synaptic loss [5]. Redox signaling has also been shown to alter the signaling cascades associated with the pathophysiology of synaptic loss and thus cause vulnerability to neuronal cells in AD [6]. Since the exact mechanisms associated with synaptic dysfunction induced by impaired NTs and altered redox signaling remain enigmatic, their plausible association is under investigation. In this regard, the present review underlines the involvement of both defective NTs and altered redox signaling in the etiology of synaptic loss associated with AD, and also demonstrates the involvement of numerous biological compounds and recent therapeutic strategies for targeting synaptic loss induced by defective NTs and neuromodulators (NMs).

SYNAPTOPATHY IN ALZHEIMER'S DISEASE: CORRELATION BETWEEN SYMPTOMS AND SYNAPTIC FAILURE

Synapses are an alliance of specialized structures that allow a neuron to pass a chemical or an electrical signal to another neuron. This electrochemical transmission is a complex interplay among NT release at presynaptic terminals and its detection at receptors of postsynaptic terminals of a neuron [7, 8]. While any dysregulation in synaptic transmission leads to a number of chronic brain disorders, including addiction, depression, anxiety, and dementia like AD and Parkinson's disease [9]. There is numerous evidence depicting the promising role of synaptic plasticity in memory formation and its stabilization. Recent methodological advancements have uncovered the mystery behind synaptopathy and its consequent dysregulation in neural circuitry [10]. For instance, amyloid-β (Aβ) and tau proteins were reported to function normally at synaptic junctions while their overburden caused neuronal toxicity and thus synaptic loss in the case of AD [11]. Hyperphosphorylated and aggregated forms of tau are leading agents for synaptic dysfunction, behavioral impairment, and neuronal death in neurodegenerative disorders (NDDs) [12]. It is reported to directly interact with postsynaptic signaling complexes to regulate synaptic transmission [13]. On the other hand, major kinases such as glycogen synthase-3β (GSK3β), cyclic adenosine monophosphate response element-binding protein (CREB), extracellular receptor kinase (ERK), and mitogen-activated protein kinase (MAPK) are found to induce synaptic dysfunction through their dynamic association with stress-mediated abnormal hyperphosphorylated or accumulated forms of tau in the AD brain [5]. Recently, abnormal acetylation at K281 and K274 of tau protein has been reported to promote synaptic loss in the AD brain viz. reduction of AMPA receptors trafficking, damaged actin dynamics, and diminished postsynaptic KIdney/BRAin (KIBRA) signaling pathways [14]. Additionally, several groups demonstrated the pathogenic role of soluble Aβ in dendritic spine injury in cultured neurons, while its monomeric and fibrillar forms remained inert to synaptic loss [15]. The available evidence suggests that abnormally acetylated and phosphorylated forms of tau, aggregated forms of Aβ, and impaired synaptic plasticity are the key components involved in the synaptopathy of AD [16]. Unlike Aβ and tau, various other factors have also been identified to cause synaptic dysfunction in AD (Table 1). These factors significantly affect neurotransmission and correlate with the disease symptoms including cognitive decline and dementia in AD.

THE PERTINENT ROLE OF AMYLOID-β IN SYNAPTIC BIOLOGY OF ALZHEIMER'S DISEASE

Aβ is a prevalent toxic protein deposited as senile plaques and is likely to be involved in the impairment of synaptic plasticity in both sporadic and familiar forms of AD [35]. One and foremost among the numerous proposed mechanisms associated with Aβ-mediated synaptic dysfunction is toxicity due to its self-aggregation and interaction with various other membranous proteins at synaptic junctions [36]. Importantly, Aβ is found to moderately terminate mGluR-dependent synaptic long-term depression (LTD), thereby signifying its role in modulating synaptic plasticity [37]. Growing

Table 1
Causative factors and associated mechanisms of synaptic dysfunction in AD

SN	Causative Factors	Associated Mechanisms (Synaptic Dysfunctions)	Disease Involved	References
1	Factors associated with oxidative stress and mitochondrial dysfunction	Causes apoptotic cell death and elevation of caspase-3 activity associated with enhanced level of long-term depression (LTD), which further leads to synaptic loss	Alzheimer's disease	[17]
2	Ca^{2+} dyshomeostasis	Affects calcineurin (CaN) activity that causes alteration in N-methyl-D-aspartate (NMDA) receptor activity and impairment of long-term potentiation (LTP), leading to synaptic loss		[18]
3	Cholesterol and lipid depletion	Induces formation of protein aggregates that leads to the impairment of both NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which further affects glutamate transmission, alters the LTP and synaptic loss		[19]
4	Factors associated with inhibition of protein phosphatases	Increases cytoskeletal protein phosphorylation which further induces Ca^{2+} dyshomeostasis and glutamate excitotoxicity, thereby leading to loss of synaptic activity in the brain		[20]
5	$STEP_{61}$	Increased activity of $STEP_{61}$ causes inactivation of ERK1/2 signaling and alteration in NMDA receptor		[21]
6	MAPK phosphatases	Inactivate Mitogen-activated protein kinases (MAPK) and cause neuronal excitability and synaptic dysfunction		[22]
7	Dyshomeostasis of Fe^{2+}, Cu^{2+}, Zn^{2+} and other metal ions	Causes alteration in glutamate activity and modulates various ionotropic, metabotropic, and postsynaptic receptors such as NMDA and AMPA receptors		[23]
8	Factors associated with microglial dysfunction	Induces the release of proinflammatory cytokines such as IL-1β, TNFα, and TNFR1, thus causes apoptosis. Moreover, it also causes alteration in the AMPA receptors at the synapse		[24]
9	GABAergic and Glutamatergic excitotoxicity	Causes alteration in Ca^{2+} homeostasis, involved in the modulation of various receptors such as NMDA and AMPA, and also responsible for the production of free radicals thereby leading to apoptosis of neuronal cells		[4, 25]
10	Glutamate transporter 1 (GLT1)	Dysfunction of GLT1 causes excessive glutamate release and impairment of NMDA and AMPA receptors		[26]
11	Mutations in AβPP/PS1	Causes alteration in stathmin signaling, 14-3-3-mediated signaling, CREB signaling, and protein kinase A (PKA) mediated signaling pathways. Further, it also alters the activity of both NMDA receptor and glutamate release		[27]
12	Aβ	Alters acetylcholine, NMDA, and AMPA receptor activity. It also affects Wnt signaling pathway and causes alteration in glutamate, norepinephrine, and GABA release that further leads to the accumulation of tau at postsynaptic terminals		[28–30]
13	Tau	Mislocalization of hyperphosphorylated tau in dendritic spines causes both the disruption of glutamate receptor and Aβ-related synaptic excitotoxicity		[31]
14	GirK Channels	Aβ interferes with GirK channels, curtailing LTP and increasing LTD, thereby mediating synaptic dysfunction		[32]
15	IGF-1 depletion	Causes defects in both LTP and LTD and augments incorporation of glutamate receptors through the activation of PI3K/Akt signaling		[33]
16	ApoE4	Affects PKA, calcineurin, BDNF, and CREB mediated signaling pathways thereby leading to synaptic loss		[34]

evidence suggests that Aβ oligomers also interact with various NTs/NMs to inhibit synaptic transmission by dysregulation of these receptors. For instance, glutamatergic, GABAergic, and serotonergic receptors were observed with compromised expression at neuronal synaptic junctions [38, 39]. Similarly, Aβ oligomers interacted with a dozen receptors to trigger the distribution of critical synaptic proteins and induce hyperactivity in ionotropic and metabotropic glutamate receptors [40]. Likewise, there is evidence indicating the interaction of Aβ oligomers with glutamatergic receptors to either facilitate or inhibit the uptake of glutamate and thus cause Aβ-mediated synaptic loss [26]. Moreover, glutamatergic receptors (AMPA and NMDA) are found to regulate Aβ-mediated synaptic dysfunction

via aberrant redox signaling and cytoplasmic Ca^{2+} overload, which triggers downstream pathways including protein phosphatase 2A (PP2A) and Ca^{2+} dependent protein phosphatase calcineurin/PP2B [39]. Additionally, NMDA receptors insults are also found to promote the amyloidogenic processing of amyloid-β protein precursor (AβPP) to induce oligomeric Aβ production and trigger synaptic failure and memory loss [41]. Multiple studies also revealed that Aβ causes neuroinflammation via activation of microglial cells and alters the level of ERK, CaMII, and pCREB to impair long-term potentiation (LTP) and LTD. Such alterations trigger a negative feedback mechanism to deplete the regulation of GSK3β and consequent abnormal Aβ and oligomeric tau production at synapses leading to synaptic dysfunction and memory impairment [42–44]. Besides, numerous studies on hippocampal neurons explored the effects of Aβ and human amylin on LTP with the expression of amylin receptors, while its blockade led to LTP enhancement in transgenic mice to trigger Aβ burden in brain [45]. In this way, multi-disciplinary research has been carried out extensively that identified potential receptors involved in synaptic loss, which could be potential targets for therapeutic intervention.

TAU PROTEIN AS A CULPRIT OF SYNAPTOPATHY IN ALZHEIMER'S DISEASE

Tau protein is another significant pathological hallmark of AD in its hyperphosphorylated form as neurofibrillary tangles (NFTs) and is associated with cognitive decline, memory impairment, synaptic dysfunction, and neuronal loss [46]. Nevertheless, tau phosphorylation is also known for synaptic plasticity during the early stages of neuronal development, but it declines with the aging brain [47]. However, NFTs are reported in varying degrees in the brain before the onset and throughout the progression of AD, but it was not proportionate with the neuronal death. Even the neuronal death exceeded the amount of NFTs indicating that it is not a prominent cause for neuronal death [48]. Furthermore, researchers revealed that it is not the number of NFTs that are responsible for dementia, but it is the aggregated form of tau at synaptic junctions that causes synaptic dysfunction and is vulnerable to neurons [46]. Recent evidence showed that tau-mediated memory impairment is partly associated with decreased RNA translation, due to very close association of ribosomes with tau proteins in AD with respect to control brains [49]. Another study identified that impaired synthesis of postsynaptic density protein-95 (PSD-95) contributes toward the decline of synaptic plasticity that is crucial for learning and memory [50]. Similarly, earlier studies on human fetal cerebral cortical neurons reported the association of an aberrant rise in Ca^{2+} levels with tau hyperphosphorylation leading to microtubular degeneration in AD [51]. Further, chronic exposure of inorganic Arsenic compounds (iAs) and its metabolites facilitated tau hyperphosphorylation and increased AβPP expression. Besides, it also causes altered NT synthesis, increased glutamate receptors activation, and reduced glutamate transporters expression, thereby affecting synaptic transmission [52]. A study demonstrated the increase in accumulation of phosphorylated tau that triggered synaptic loss, neurite retraction, Ca^{2+} dyshomeostasis, and altered NT release (reduced acetylcholine (ACh) levels) in tau oligomer treated neurons [53]. Tau phosphorylation is also modulated by Bcl2 Associated Athanogene-2 (BAG2) expression, since it controls a functional intracellular switch between the p38-dependent functions of nicotine on tau phosphorylation levels via the α7 nicotinic receptor [54]. Furthermore, glucocorticoid (GC, stress hormones) mediated synaptic loss has been evident in AD models via tau hyperphosphorylation, missorting, and mislocation [55, 56]. Recently, abnormal acetylation of K274 and K281 sites on tau has been reported to stimulate disruption of synaptic plasticity and memory by reducing postsynaptic KIBRA (a memory-associated protein) in transgenic mice [14]. Interestingly, another variant of human tau A152T (hTau-A152T) is found to increase the risk for synaptic loss by increasing hyperphosphorylated forms of tau protein and by promoting network hyperexcitability that triggered age dependent neuronal loss at synapses in AD [57]. Apart from Aβ and tau, oxidative stress and mitochondrial dysfunction also play a key role in synaptic dysfunction.

HOW OXIDATIVE STRESS LINKED MITOCHONDRIAL DYSFUNCTION IS A CAUSE FOR SYNAPTIC LOSS

Mitochondria possess an extensive role in ATP production, reactive oxygen species (ROS) generation, Ca^{2+} homeostasis, and apoptotic signaling; while being a great source of intracellular ROS, they are mainly vulnerable to oxidative stress [17].

Nowadays, oxidative stress and subsequent damage to mitochondrial integrity has been widely implicated in various NDDs including AD, Parkinson's disease, Huntington's disease, multiple sclerosis, and amyotrophic lateral sclerosis [58]. Since neurons are energy dependent on mitochondrial activities for its critical functioning, including axonal/dendritic transport, synaptic transmission, and ion pumps and channels, oxidative stress-mediated mitochondrial damage is the predetermining factor for causing synaptic loss in neurons [59, 60]. Moreover, it has been observed that under extreme conditions of oxidative stress, mitochondrial damage takes place primarily at complex IV (cytochrome oxidase) of the electron transport system [61]. This damage ultimately leads to synaptic loss in association with oxidative alteration of the mitochondrial membrane phospholipid; cardiolipin [62]. Synapse maintenance is a prerequisite for proper communication and therefore, synapses are densely packed with mitochondria in order to fulfill their high-energy demands and Ca^{2+} buffering requirements for synaptic transmission [63]. Moreover, synaptic mitochondria are responsible for clearing Ca^{2+} either directly or by providing ATP to Na^{+}/Ca^{2+} exchangers to maintain Ca^{2+} homeostasis in order to govern normal synaptic function [64]. Defective mitochondrial buffering mediated Ca^{2+} overburden causes severe brain tissue injury in response to glutamate excitotoxicity [65]. Likewise, elevated ROS accumulation causes vulnerability to cells in response to compromised shock regulatory proteins and leads to the formation of the mitochondrial permeability transition pore (mPTP). The prolonged opening of mPTP can cause both necrosis and apoptosis via cytochrome C release and consequent activation of caspases [66]. Furthermore, oxidative stress was shown to also contribute significantly toward neuronal damage in the substantia nigra via dopamine-mediated quinone formation [67]. Nevertheless, deficits in axonal transport of mitochondria from soma to distal synapses are prevalent in NDDs. Moreover, some genetic factors have been identified that regulate mitochondrial transport; for instance, PTEN-induced putative kinase 1 (PINK1) is found to interact with Miro and Milton's protein to govern mitochondrial trafficking and distribution [68]. In another study, perturbed mitochondrial fusion/fission proteins are found to affect dendritic mitochondrial populations thereby affecting synaptic plasticity [69]. Likewise, many compounds and elements have been identified so far, which are responsible for oxidative stress/mitochondrial dysfunction mediated synaptic loss in AD (Table 2). The investigations suggest that synaptic dysfunction is presumably one of the initial events in the majority of NDDs associated with mitochondrial abnormalities or irregular mitochondrial distribution in neurons causing clinical symptoms such as motor dysfunction, cognitive decline, and memory loss.

FREE RADICALS, REACTIVE OXYGEN SPECIES, AND CELL-SIGNALING IN SYNAPTIC DYSFUNCTION

Free radicals are highly reactive chemical species having one or more unpaired electrons, and are generated in the complex I and complex III of mitochondria. The iron-sulfide centers and semiquinone or cytochrome b are believed to be likely candidates for its generation in mitochondrial complex I and III, respectively [81, 82]. The altered ROS homeostasis activates various signaling pathways underlying cell inflammation; for instance, ROS and other reactive species regulate the expression of numerous inflammatory mediators including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS), lipoxygenase (LOX), cycloxygenase-2 (COX-2), and cell adhesion molecules (VCAM-1, ICAM-1, P & E-selectin) [83–85]. Additionally, ROS has been identified as key modulators of signal transduction cascades pertaining to synaptic plasticity and memory functions without the help of GTPases, phosphatases, protein kinases, Ca^{2+}- dependent enzymes, and other transcription/translation factors [86, 87]. For instance, ROS sensitive calcineurin (PP2B) is believed to suppress LTP by inhibiting LTP-inducing kinases CaMKII and PKC. Moreover, ROS exhibits two-fold roles in LTP; one is stimulation and other is inhibition. For example, in rodent hippocampus, superoxide scavenging blocked high frequency stimulated LTP (HFS-LTP), while superoxide dismutase (SOD) mediated H_2O_2 burden caused LTP inhibition [88]. NMDA receptor is another candidate, which is directly attacked by ROS to affect synaptic plasticity and long-term memory formation. Another reactive species, nitric oxide (NO) plays a dual role in neurobiology by provoking both neuroprotection and neurodegeneration. For instance, NO boosts synaptic plasticity by evoking dendritic Ca^{2+} release via ryanodine receptor (RyR) while its aberrant production triggers synaptic loss via enhanced activation of soluble guanylyl cyclase (sGC)/protein kinase G

Table 2
Key compounds and elements involved in oxidative stress/mitochondrial dysfunction mediated synaptopathy

SN	Compounds and Elements	Mechanisms associated with Synaptic Loss	Disease Involved	References
1	Methotrexate (MTX)	MTX treatment increases the levels of phosphorylated tau, AβPP, and β-secretase thereby causing oxidative stress mediated synaptic loss	Alzheimer's disease	[70]
2	Okadaic acid (OKA)	It induces neurodegeneration by provoking tau hyperphosphorylation, GSK3β overstimulation, oxidative stress, neuroinflammation, altered neurotransmission, and neurotoxicity. Moreover, it also acts as a selective inhibitor of protein phosphatase, PP1 and PP2A		[71]
3	Acrolein	Acrolein is a peroxidation product of arachidonic acid (AA) that augments tau phosphorylation at the site recognized by PHF-1 thereby causing oxidative stress in primary neuronal cultures		[72]
4	Cocaine	Peritoneal injection of cocaine promotes Alzheimer-like hyperphosphorylation of tau and neurofilaments thereby causing oxidative stress in rat brain. Further, it also increases the levels of dopamine, as does nicotine		[73]
5	Lipopolysaccharide	Lipopolysaccharide treatment causes both synaptic alteration and learning-memory deficit by inducing paired immunoglobulin-like receptor B (PirB) expression		[74]
6	Rotenone	Rotenone treatment causes inhibition of complex I of the electron transport chain (ETC), thus induces cell death of cholinergic neurons		[75]
7	Paraquat	Paraquat exposure causes increased oxidative damage specifically in mitochondria of cerebral cortex and manifests mitochondrial dysfunction, thus leads to synaptic loss		[76]
8	1-methyl-4-phenylpyridinium (MPP(+))	Treatment with MPP (+) causes generation of ROS from inhibition of complex I of the mitochondrial respiratory chain and inactivation of aconitase. Further, it also causes ROS-mediated oxidative damage and apoptosis, thereafter lead to synaptic loss		[77]
9	Copper (Cu^{2+})	Chronic exposure of Cu^{2+} causes altered spatial memory with selective loss of synaptic proteins pre-synaptic protein synapsin 1 and post PSD-95 through the mechanisms including activation of PKR/eIF2α signaling pathway		[78]
10	Iron (Fe^{2+})	Fe^{2+} is responsible for triggering synaptic loss and apoptotic cell death by affecting mitochondrial dynamics		[79]
11	Aluminum (Al^{3+})	Al^{3+} exposure decreased the levels of NTs and acetylcholinesterase activity in the brain. Further, it promotes cross-linking of toxic Aβ thereby causing both oxidative stress and synaptic dysfunction		[80]

(PKG) pathway and RhoA/Rho kinase (ROCK) signaling pathway [89, 90]. Furthermore, ROS also directly modulates voltage-dependent Ca^{2+} channels and thus altering synaptic transmission. Additionally, the protein RanBP9 is found to elicit ROS production, mitochondrial dysfunction, and Ca^{2+} dysregulation in AD models [91]. Accumulating evidence suggests that synaptic loss is also caused by altered insulin signaling pathway. This signaling is triggered via an insulin receptor substrate (IRS) that further interacts with numerous other receptor tyrosine kinases including IGF1/2, tropomyosin-related kinase receptor B (TrkB), and ErbB. The phosphorylation of IRS1 on tyrosine residues thereafter leads to the activation of downstream signaling including, Akt, mTOR, and GSK3. Furthermore, the phosphorylation of IRS1 on multiple serine (Ser) residues inhibits IRS1 activity leading to insulin resistance (IR), which further contributes to both Aβ accumulation and tau phosphorylation associated with synaptic loss (Fig. 1). Most importantly, IR is also accountable for altered insulin degrading enzyme (IDE) and neprilysin (NEP) activity, which is induced by accumulated Aβ in AD [92, 93]. Similarly, ROS-mediated microglial activation induced by toxic Aβ is another cause for both neuroinflammation and synaptic dysfunction in AD. Because, Aβ activated microglia is responsible for synaptic loss by releasing numerous neurotoxic mediators including cytokines, interleukin, and TNF-α that propagate an inflammatory

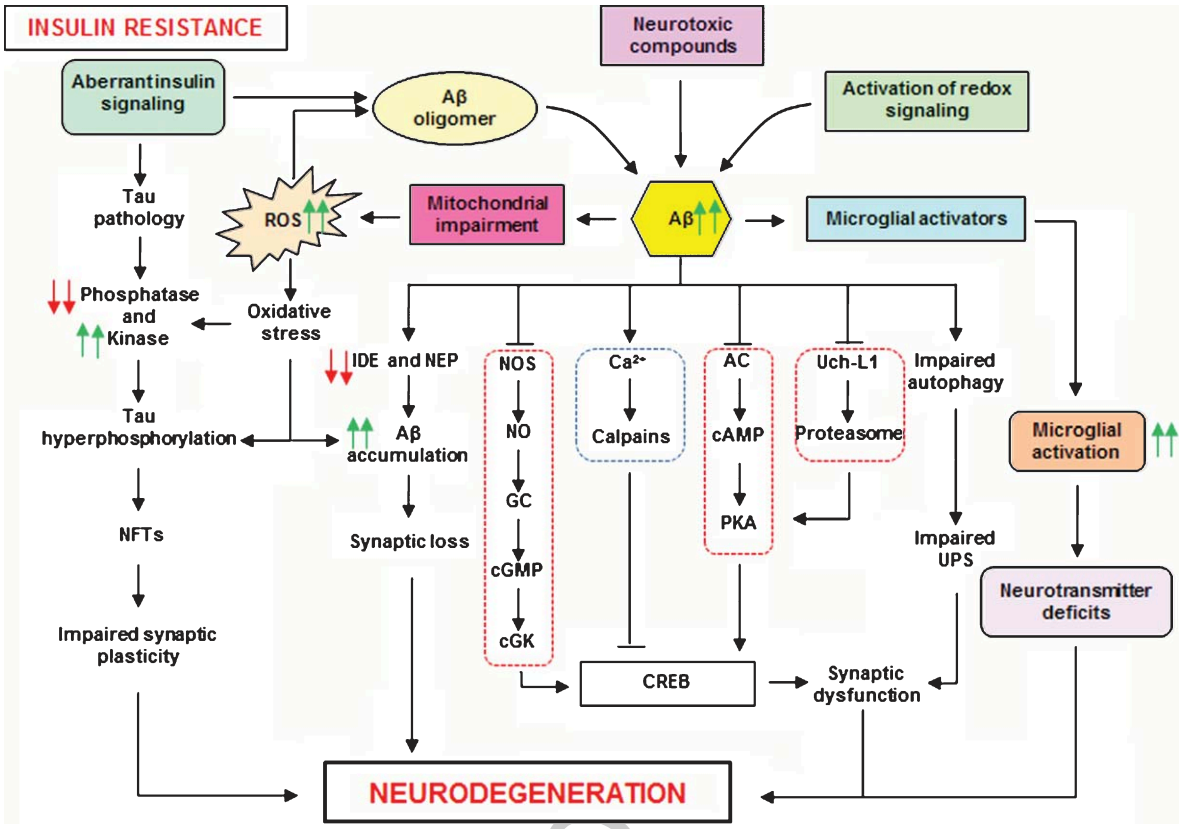

Fig. 1. Schematic illustration showing the different signaling axis that is involved in Aβ-induced synaptic dysfunction and its associated factors. ROS, reactive oxygen species; NFTs, neurofibrillary tangles; IDE, insulin-degrading enzyme; NEP, neprilysin; NOS, nitric oxide synthase; NO, nitric oxide; GC, guanylyl cyclase; cGMP, cyclic guanosine monophosphate; AC, adenylate cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; CREB, cyclic adenosine monophosphate response element-binding protein; UPS, ubiquitin proteasome system.

cycle [94]. Furthermore, CREB acts as a central converging point of diverse signaling cascades that are involved in synaptic strengthening and memory formation and is also reported to be altered by Aβ accumulation [95]. Further research is required to determine other important targets of ROS signaling to investigate their significance in synaptic transmission and neuronal homeostasis.

NEUROTRANSMITTERS/ NEUROMODULATORS: A KEY MEDIATOR OF CELLULAR HOMEOSTASIS OR SYNAPTOPATHY?

NTs are a diverse group of endogenous compounds that act as chemical messengers to transmit the electrical/chemical information throughout the body. Nerve impulses rely on synapses for the release of NTs from presynaptic axons and its detection at postsynaptic terminals [8]. These NTs normally maintain cellular homeostasis via regulation of interdependent elements/chemicals through a number of physiological processes, while its depletion may affect different processes like concentration, mood, sleep, weight, and various others leading to altered cellular homeostasis [96]. For instance, NTs like glutamate, aspartate, GABA, and ACh have been implicated in synaptic dysfunction associated with the progression of AD [4]. The mechanisms underlying synaptic dysfunction are linked to the alteration of their receptors that lead to the pathogenic events in the progression of NDDs. The mechanisms associated with NT/NM-mediated synaptic dysfunction in AD are discussed here.

Acetylcholine

ACh release is responsible for the regulation of memory storage and cognition in cortex and subcortical circuits [97]. ACh interacts with two types

of receptors; G protein coupled muscarinic acetyl cholinergic receptors (mAChR) and ionotropic nicotinic acetyl cholinergic receptors (nAChR) and triggers distinct responses to different modulators [98]. The α7 nAChR receptors are found to regulate intracellular Ca^{2+} and NT release at synaptic junctions. nAChR are also evident for synaptic plasticity by stimulating the upregulation of LTP in the hippocampus [99]. In fact, the progressive loss of cholinergic signaling is among the major breakdown mechanisms associated with the etiology of AD. For instance, dysfunctional nAChR/mAChR in the cortex resulted in cognitive decline in AD. However, this failure of cholinergic transmission is not merely a loss of ACh containing neurons but also marked by attenuated acetylcholine esterase and choline acetyl transferase activity [100]. Further, SNPs associated with nAChR genes (α3, α4, α7, and β2) have been reported to cause the pathogenesis of AD [101]. Besides, nAChRs are found to trigger synaptopathy due to its up- and downregulation at different sites in AD brain. For instance, chronic Aβ exposure enhanced the levels of α7-nAChR in the hippocampus, cortex, and amygdala both *in vivo* and *in vitro* while marked reduction in α4β2-nAChR is observed in the cortical regions of AD patients [102, 103]. Additionally, the interaction between Aβ and α7-nAChR has also been documented in postmortem AD brains [104].

Dopamine (DA)

DA is a major NT in the central nervous system (CNS) and is characterized as an important modulator of synaptic plasticity. Failure of the DA transmission system results in apathy, a negative prognostic indicator of normal aging as well as AD. The occurrences of apathy and motor dysfunctions were predictive of rapid cognitive loss and shorter lifespan in AD patients [105]. Various studies have identified a reduced level of DA at the site of Aβ plaques and NFTs in nigrostriatal regions of AD brain, depicting its prominent role in pathogenesis and impaired cognition [106]. In a study of the 3xTg AD model, Aβ-induced impaired dopaminergic neurotransmission resulted in conversion of LTP into LTD, which led to poor memory and neuroplastic insults in the basolateral amygdaloid nucleus-insular cortex pathway [107]. Moreover, Aβ favored LTD upon low frequency stimulation while restricted LTP after high frequency stimulation. It has also been observed that expression of dopaminergic receptors D1 and D2 is significantly reduced in the prefrontal cortex and hippocampus region of AD patients, while stimulation of dopaminergic transmission improved cognitive function in various animal models of AD. Recently, DA has been shown to possess anti-amyloidogenic and antioxidant effects in mice [108]. Interestingly, the administration of dopaminergic drugs exhibited better cortical plasticity and memory functions in AD patients.

Gamma-amino butyric acid (GABA)

GABA is the principal inhibitory NT in the CNS, which is synthesized by decarboxylation of glutamate with the help of glutamic acid decarboxylase enzyme. Synthesized GABA is transported into vesicles by vesicular GABA transporter at presynaptic terminals of neurons [109]. There are three distinct receptor subfamilies of GABA namely $GABA_A$, $GABA_B$, and $GABA_C$ receptors that contribute toward their inhibitory effects, where $GABA_A$ and $GABA_C$ receptors are ligand-gated chloride (Cl^-) channels, while $GABA_B$ receptors are G-protein coupled receptors [110]. The alteration in the balance between inhibitory GABA and excitatory glutamate NTs were found to be one of the pathological factors contributing toward synaptic dysfunction. Aβ fibrils were found to cause perforations in the cell membrane leading to enhanced Ca^{2+} influx mediated over-excitation and consequent epileptic seizures in the hippocampus and cortex. The increased seizures in turn cause alterations in GABAergic sprouting and synaptic inhibition as a protective mechanism to overcome the hyper-excitation of neurons [38]. Immunohistochemical study of GABAergic receptors revealed that α2, β1, and γ1 subunits of $GABA_A$ receptors get upregulated whereas the levels of α1 and γ2 subunits get downregulated in AD brains, indicating a functional remodeling of GABAergic neurotransmission in the cortex of AD patients [111]. Furthermore, elevated inhibitory function of GABAergic synapses induced by glutamate mediated NMDA receptor activation affects the processes required for LTP in dentate gyrus. Therefore, crosstalk between $GABA_A$ receptors and postsynaptic glutamate NMDA receptors are evident in AD pathology [112].

Glutamate

Glutamate is one of the important excitatory NTs that play a crucial role in neural activation with the help of its receptors localized on neuronal mem-

brane. A wide variety of glutamatergic receptors, namely NMDA and AMPA receptors, have been implicated in synaptopathy while normally they are known to regulate synaptic plasticity, neurotransmission, learning, and memory [113]. Numerous studies reported that glutamatergic neurons get lost in response to Aβ accumulation selectively at some synapses in the pathogenesis of AD. These Aβ oligomers also upregulate the extracellular concentration of glutamate in hippocampus of AD brain [39] and directly impact AMPA and NMDA receptors through various subunits including GluR2 and GluN2B. Moreover, Aβ is found to bind with GluR2 subunit via clathrin mediated activation of calcineurin and dynamin [114] and further downregulate AMPA-mediated signal transmission and synaptic plasticity via nuclear translocation of Jacob protein and induction of accompanying CREB shutoff signaling [115]. Furthermore, oligomeric Aβ exposure upregulates GluN2B containing NMDA and extrasynaptic NMDA receptors and disturbs signal transmission [116]. Another study reported an increase in STriatal-Enriched protein tyrosine Phosphatase (STEP) activity upon Aβ and tau exposure leading to GluN2B containing NMDA receptor endocytosis via dephosphorylation of Src kinases Fyn and GluN2B at tyrosine (Y1472) [117]. The increase in STEP activity further disrupts synaptic plasticity and affects cognitive functions in AD. It has also been revealed that $A\beta_{1\text{-}42}$ oligomers form clusters at synaptic junctions and trigger subsequent decreases in the mGlu5 receptor's mobility and distribution leading to intracellular Ca^{2+} release. The disrupted Ca^{2+} homeostasis triggers mitochondrial dysfunction mediated ATP loss and ROS generation, ultimately causing LTD via GSK3β and calcineurin over-stimulation [5]. Furthermore, Aβ-induced reduction in LTP specifically involves caspase 3 activation and subsequent Akt cleavage in AD patients [118].

Histamine (HA)

HA is a NT that directs crucial physiological functions such as sleep cycle, synaptic plasticity, cognition, and movement. The hypothalamic tuberomammillary nucleus (TMN) is the site in the adult mammalian brain where somas of HA producing neurons are located and extends throughout the CNS [119]. The action of HA is mediated by the activation of four G coupled protein receptors, namely H1R, H2R, H3R, and H4R, which are widespread in the brain. Specifically, H1 receptors were reported to be reduced in the frontal and temporal regions of AD brain. The key association between HA and AD can be ascertained from the fact that the level of HA is markedly elevated in different regions of CNS in AD patients [120]. HA is also found to regulate neuroinflammation along with TNF-α and IL-1β in hippocampal neurons, which is responsible for poor cognition and impaired cerebrovascular functions in AD. Moreover, an association between microglial activation and APOE has been reported in AD patients, where HA levels were found to correlate with APOE; for instance, patients carrying the APOE-4 alleles had lowest HA levels in the brain [121]. Furthermore, it has been identified that HA-containing neurons in the TMN get reduced in association with accumulated NFTs while its level was found to be upregulated in cerebrospinal fluid and serum of AD patients [122].

Norepinephrine

Norepinephrine is a catecholamine, synthesized through a cascade of enzymatic reactions where dopamine is converted into norepinephrine through the action of dopamine β-hydroxylase. Norepinephrine either can act on target receptors (α1, α2, and β) or can be re-uptaken into presynaptic neurons via Na/K-dependent norepinephrine transporters. The primary function of noradrenergic transmission includes regulation of spatial working memory, neuroinflammation, and cellular metabolism [123]. Moreover, norepinephrine also regulates neuroinflammation through adrenergic receptors present in astrocytes and glial cells where any aberration in adrenergic signaling leads to the progression of AD [124]. A recent study evidenced the administration of a selective neurotoxin DSP-4 against noradrenergic neurons that caused enrichment of Aβ deposition, altered spatial memory, and impaired receptor binding sites of α1, α2, and β and upregulation of hyperphosphorylated tau in a transgenic mice model of AD. Furthermore, several studies have also reported impaired LTP and cognition in norepinephrine-compromised hybrid AD mice models [125]. A few reports have linked polymorphisms in the dopamine β-hydroxylase gene leading to reduced norepinephrine production in selective Caucasian populations with AD [126]. In another experiment, the endogenous α2A receptors are shown to contribute in the cascade for AD progression [127]. Likewise, Aβ activates β2 receptors to trigger the

hyperphosphorylation of tau via protein kinase-A and c-Jun N-terminal kinase (PKA-JNK) signaling in AβPP/PS1 mice model [128].

Serotonin

Serotonin is a biogenic monoamine, which regulates important physiological functions in CNS such as mood, pain, anger, aggression, sleep, and appetite [129], and it serves as both NT as well as NM [130]. In general, serotonin regulates crucial mechanisms like learning and memory both in healthy as well as in aged individuals. This is the reason that neurological disorders such as AD are marked by aberrant serotonergic signaling and altered 5-hydroxytryptamine (5-HT) metabolism in the CNS [131]. A specific class of receptors called the 5-HT receptors orchestrates the activity of serotonin. Though a number of 5HTRs (5HT2AR, 5HT2CR, 5HT4R etc.) are involved in AβPP processing, 5HT4R has gained attention by reinstating a neuroprotective environment by inducing non-amyloidogenic AβPP cleavage mediated release of soluble AβPPα [132, 133]. The investigations reported reduced levels of serotonergic neurons and 5HT metabolites in the raphe nuclei of AD postmortem brains [134, 135]. Likewise, Aβ plaques in the projection site of serotonergic neurons triggered 5HT neuronal apoptosis accompanied by loss of neuronal cell bodies in an AβPP transgenic mice model [136]. Additionally, a link between tau phosphorylation and 5HTRs have further strengthened the notion that 5HTRs are closely associated with AD via Fyn mediated ERK1/2 activation [137]. Moreover, tau hyperphosphorylation in the raphe nuclei is also evident to induce 5HT-mediated neuronal cell death in AD brain [138]. The altered NTs/NMs and their associated factors in the etiology of AD have been depicted in Fig. 2.

OTHER NEUROTRANSMITTERS/NEUROMODULATORS

Additionally, various other NTs/NMs such as Reelin, Interleukin-33 (IL-33), Purinergic receptors, and TRP channels are identified that contribute significantly in the synaptopathy of AD. For instance, disruption in the activity of Reelin causes aberrant hyperphosphorylation of tau by altering the signaling cascades associated with GSK3β activity [139]. Another NT, interleukin-33 (IL-33), is found to regulate inflammation in neuronal cell since IL-33 depletion released IL-1β and TNF-α that contributed well in neuroinflammatory synaptic loss in AD brain [140]. Likewise, dysfunction of purinergic receptors also causes neuroinflammation, ATP release, and alteration in Ca^{2+} influx and thus induces LTD at synaptic junctions [141]. There are several TRP channels (TRPC, TRPV, TRPM, TRPP, TRPML, and TRPA) that are involved in the alteration of Ca^{2+} influx, modulation of the PSD95 pathway, and GSK3β phosphorylation mediated synaptic plasticity [142, 143]. Herein, the potential mechanisms of synaptopathy associated with NTs/NMs have been described in (Table 3).

CORRECTION MECHANISMS TO TARGET PERTURBED NEUROTRANSMITTERS/NEUROMODULATORS IN SYNAPTOPATHY

Presently, treatment against defective NT/NM-mediated synaptopathy in AD has become a demanding task for neurobiologists since there is not a single factor responsible for such abnormalities but a massive numbers of factors associated with widely dispersed signaling cascades. Nevertheless, defective NT release at the synaptic junction causes several detrimental effects, which are associated with the altered activity of synaptic proteins, transcription factors, Ca^{2+} homeostasis, and other molecules critical for neuronal plasticity [168]. These detrimental effects further disrupt the neuronal homeostasis and thereby cause the synaptic insults. In order to overcome such complications, numerous therapeutic strategies are currently being devised that alleviate the toxicity associated with defective NTs/NMs. For instance, numerous biological compound-based therapies have been designed to overcome the problems associated with these defective NTs/NMs in AD. Moreover, current therapeutic approaches have also been discussed here that can slow down the pathophysiology behind defective NT/NM-mediated synaptic dysfunction.

Biological compound-mediated therapy for altered neurotransmitters/neuromodulators in synaptopathy

The reduction in cholinergic neurotransmission in AD has led to the development of numerous compounds as the first-line of treatment for the pathological phenomenon of this disease. The clinical

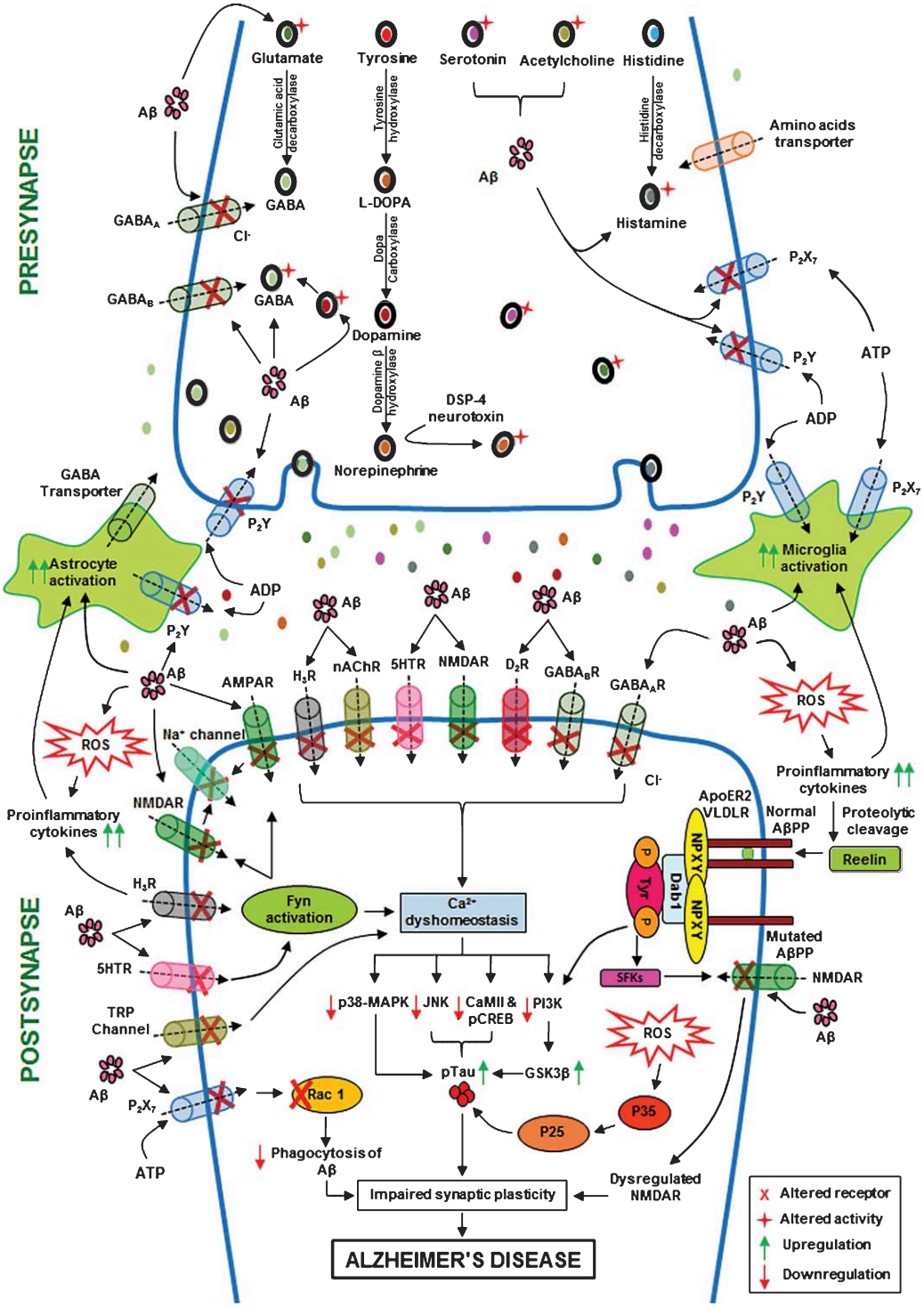

Fig. 2. Molecular mechanisms associated with defective neurotransmitters/neuromodulators (NTs/NMs) in Alzheimer's disease and their associated factors. GABA, γ-Aminobutyric acid; L-DOPA, L-3,4-dihydroxyphenylalanine P_2X_7, purinoceptor 7, P_2Y, purinoceptor; DSP-4, neurotoxin; NMDAR, N-methyl-D-aspartate receptor; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; H_3R, histamine receptor, nAChR, nicotinic acetylcholine receptor; 5HTR, 5-hydroxytryptamine (serotonin receptor); D_2R, dopamine receptor D2; AβPP, amyloid-β protein precursor; ATP, adenosine triphosphate; ADP, adenosine diphosphate; ROS, reactive oxygen species; VLDLR, very-low-density-lipoprotein receptor; TRP, transient receptor potential.

Table 3
List of neurotransmitters/neuromodulators and their mechanism associated with synaptopathy in AD

Neurotransmitters/ Neuromodulators	Involved causative factors/receptors	Involved mechanisms in synaptopathy	Principle phenotype	References
Acetylcholine (ACh)	Aβ, tau, cholesterol depletion, metal ion dyshomeostasis, and oxidative stress	Abnormalities in AChR causes downregulation of both MAPK-CREB and PI3K-Akt signaling cascade and also causes alteration in the level of glutamate that affects synaptic plasticity		[144, 145]
Glutamate	Factors associated with inhibition of protein phosphatases, Aβ, tau, cholesterol depletion, metal ion dyshomeostasis, IGF-1 depletion, altered level of dopamine, and elevated level of glucose	Glutamate toxicity causes alteration in the level of intracellular Na^+, K^+, and Ca^{2+}. It also causes impairment in NMDA, AMPA, and mGlu receptor and ultimately impairs LTP and LTD thus induces synaptic loss associated cognitive decline		[39, 114, 146–149]
Gamma Amino Butyric Acid (GABA)	KNCQ Channels, AβPP, Aβ, mitochondrial dysfunction, lipid depletion, and altered level of dopamine	GABAergic dysfunction causes alteration in Ca^{2+} channels and block μ-opioid receptors thus impairs synaptic plasticity		[38, 150–153]
Noradrenaline/ Norepinephrine	Microglia dysfunction, involvement of complement system, usage of DSP-4 and reserpine, and knockout of DβH enzyme	Norepinephrine dysfunction causes alteration in PKA-JNK pathway, microglia activation, and impairs LTP and LTD, thereby leading to synaptic loss associated with cognitive decline		[123, 154, 155]
Serotonin	Aβ and tau	Dysfunction of serotonin impairs cholinergic receptors, diminishes arachidonic acid (AA) uptake and causes axonal dysfunction thereby leading to the degeneration of raphe monoamine neurons and triggers synaptic loss associated with memory loss	Alzheimer's Disease	[138, 156, 157]
Histamine (HA)	Impact of GABAergic and glutamatergic receptors and dysregulation of mast cells	HA dysfunction impairs cholinergic and NMDA receptors, leading to synaptic dysfunction		[158]
Reelin	APOER2, VLDLR, DAB1 dysfunction, and Aβ	Depletion of Reelin causes alteration of signaling pathways that regulates GSK3β activity and enhances the level of hyperphosphorylated tau thereby leading to dysfunction of synapses		[159–161]
Purinoceptors	Loss of $P2X_7$ and $P2Y_2$, microglia activation, and Aβ	Loss of $P2X_7$ and $P2Y_2$ receptors causes neuroinflammation via activation of microglia and dysfunction of Rac1 protein which thereafter causes dysregulation of Aβ phagocytosis that ultimately leads to synaptic loss		[162–164]
Interleukin-33 (IL-33)	Microglia activation, Aβ, increased level of IL-1β and TNF-α	IL-33 is proinflammatory in CNS and activates inflammatory cells thereby leading to the disruption of synapses		[140, 165]
TRP Channels	Factors associated with oxidative stress and mitochondrial dysfunction, Aβ, mutated PSEN1 and AβPP	TRP channels disrupt Ca^{2+} homeostasis and alters various signaling cascade leading to synaptic loss		[166]
Dopamine (DA)	Aβ, Ca^{2+}, degeneration of cholinergic neurons, and inactivation of p38-MAPK pathways	DA dysfunction causes alteration in glutamatergic and cholinergic transmission, impairs physiological cell metabolism, activates various enzymatic pathways, leading to apoptosis and synaptic loss		[106, 167]

Table 4
List of potent biological compounds involved in alleviating the neurotoxic effect of altered neurotransmitters/neuromodulators in synaptopathy

SN	Biological compounds	Associated protective mechanisms against synaptic loss	Principle phenotype	References
1	Anthocyanine (ANT)	It regulates ion pump activity and cholinergic neurotransmission. Further, it also enhances memory and act as an anxiolytic compound		[174]
2	Oridonin (Ori)	Augments the expression of PSD-95 and synaptophysin and also promotes mitochondrial dynamics in the synaptosomes of AD mice thereby leading to attenuate synaptic loss and cognitive deficits. Moreover, it also activates the BDNF/TrkB/CREB signaling pathway in the hippocampus of AD mice		[175]
3	Asiaticoside	It prevents spatial learning and memory decline by scavenging free radicals, decreasing the level of Aβ, upregulating the activity of antioxidant enzymes, ameliorating dysfunction in synaptic plasticity and reversing abnormal changes in ACh level and AChE activity		[176]
4	Cotinine	The beneficial effects of cotinine are accompanied by an increase in the expression of the active form of PKB and the PSD-95 in the hippocampi and frontal cortices of Tg6799 mice. This PSD95 plays a crucial role in promoting brain plasticity by modulating NMDA receptor signaling. Additionally, it also increases 5-HT levels in the rat brains thereby stimulating the serotonergic activity in AD brain		[177]
5	Piracetam	It is a derivative of the neurotransmitter GABA and modulates neurotransmission in a range of transmitter systems (including cholinergic and glutamatergic), thus improves neuroplasticity. It also significantly restores impaired synaptic function by attenuating numerous mitochondrial dysfunctions such as, mitochondrial membrane potential and ATP production, induced by Aβ in PC12 cells		[178]
6	Naringin	It improves long-term learning and memory ability by both enhancing the autophosphorylation of CaMKII and increasing the phosphorylation of the AMPA receptor at a CaMKII-dependent site	Alzheimer's disease	[179]
7	7, 8-dihydroxyflavone (7,8-DHF)	Inhibits the loss of hippocampal synapses, restores synapse number and synaptic plasticity, and prevents memory deficits induced by impaired BDNF/TrkB signaling pathway in the hippocampus of AD mice		[180]
8	Memantine	A non-competitive NMDA receptor antagonist attenuates cognitive impairment and synaptic dysfunction by restoring protein phosphatase-2A activity via decreasing its demethylation at Leu309 selectively in adeno-associated virus vector-1-I1PP2A rats. Further, it also alleviates glutamate mediated cell excitotoxicity by excessive stimulation of NMDA receptors in astrocytes and neurons		[181]
9	Propofol	Acts as a $GABA_A$ receptor agonist which reduces Aβ generation and accelerates Aβ degradation. Further, it ameliorates cognitive function and attenuates caspase-3, caspase-9 activation in AD mice model		[112]
10	Metformin	It alleviates the increase of total tau, phospho-tau and activated JNK. Further, it also attenuates the reduction of synaptophysin, a synaptic protein in the db/db mouse hippocampus		[182]
11	Cannabidiol (CBD)	It shows neuroprotection by normalizing the caspase 3 activity, the main integral transmembrane protein of synaptic vesicles (synaptophysin) and mitochondrial fission protein DNM1L expression levels in rats with brain iron overload. CBD treatment has also been reported to improve dopamine neurotransmission in in a mouse model of tauopathy		[79, 183]

(*Continued*)

Table 4
(*Continued*)

SN	Biological compounds	Associated protective mechanisms against synaptic loss	Principle phenotype	References
12	Dizocilpine (MK801)	MK801 alleviates glutamate mediated cell excitotoxicity by excessive stimulation of NMDA receptors in astrocytes and neurons		[184]
13	UBP141	UBP141, an astroglial NMDA receptors antagonist plays a potential therapeutic role against synapses loss		[185]
14	Levetiracetam	Chronic levetiracetam administration reduces glutamate excitotoxicity and enhances the levels of inhibitory neurotransmission thereby attenuating cognitive abnormalities in AD		[186]
15	Muscimol	Selective $GABA_A$ receptor agonist provides protection against Aβ-induced neurotoxicity in hippocampal, retinal and cortical neurons in rodents		[187]
16	Etazolate (EHT-0202)	The $GABA_A$ receptor modulator protects neurons against Aβ-induced toxicity thereby displaying anti-inflammation effect after traumatic brain injury and improves cognition in mice models		[188]
17	Pentobarbital	Stimulation of GABA receptors by pentobarbital restores neuronal maturation and neurogenesis in apolipoprotein E4 (APOE4) knocking mice. APOE4 is known as a genetic risk factor for early onset AD perhaps by accelerating Aβ plaque formation, or by impairing neuron repair		[112]
18	Flumazenil or picrotoxin	Chronic systemic treatment with $GABA_A$ antagonists restores memory loss in AD		[189]
19	EVP-6124	EVP-6124, a selective agonist of the α7 nAChR, exerts an excitatory effect on the postsynaptic neuron thereby leading to LTP and memory formation		[190]
20	D-serine acid oxidase	Treatment with D-serine acid oxidase attenuates NMDA-induced excitotoxicity		[191]
21	5,7-dicholorokynurenic acid	The physiological glycine site antagonist kynurenic acid protects against NMDA-induced excitotoxicity		[191]
22	Methyl-4-carboxyphenylglycine (MCPG)	MCPG, a non-selective group I/II mGluR antagonist, prevents the reduction induced by Aβ in miniature excitatory post-synaptic cells		[192]
23	2-methyl-6-(phenylethynyl)-pyridine	An mGluR5 antagonist rescues LTP facilitation in murine hippocampal slices induced by oligomers of Aβ extracted from AD brains		[192]
24	SIB1757	An mGluR5 antagonist prevents LTD facilitation in rat hippocampal slices induced by oligomers of Aβ extracted from AD brains		[193]
25	Melatonin	It shows neuroprotection against Aβ induced neurotoxicity, mediated by activation of GABA receptors and the resulting hyper-polarization of the neurons		[194]
26	6,7-dinitroquinoxaline-2,3-dione	An AMPA receptor and kainate receptor antagonist attenuates excitotoxic cell death induced by Aβ retinal cell cultures		[192]

advantages of these compounds include significant improvements in NT release, improvement in altered synaptic plasticity, and attenuation of memory loss and cognitive failure. Several compounds have been identified so far to target altered NT/NM activity in AD. Curcumin, a major active component of turmeric, has been found to regulate the levels of dopamine, norepinephrine, serotonin, and glutamate in the brain and thus significantly reduce behavioral symptoms of AD. Moreover, it also acts as an inhibitor of mono-amine oxidase (MAO)-A and MAO-B enzyme, which is also crucial for depleting dopamine and serotonin [169]. Similarly, galantamine, an acetylcholinesterase inhibitor and an allosteric regulator of nAChR, has been reported in the treatment of severe dementia associated with AD. Additionally, it also influences diverse other NT systems, possibly modulating the activity of dopamine, serotonin, glutamate, and GABA in certain nerve tracts [170]. Further, numerous other chemical compounds, including donepezil (E2020), rivastigmine, and tacrine have been introduced for

the inhibition of acetylcholinesterase activity in AD [171]. Currently, nicotine has also been reported to reduce Aβ toxicity through the activation of α7 nicotinic acetylcholine receptor/phosphatidylinositol 3-kinase (α7nAChR/PI3K) signaling pathway and its cross-talk with the Wnt signaling pathway [172]. In a study, the effects of natural cannabinoids (Sativex®) have been reported to improve dopamine neurotransmission [173]. Similarly, many other biological compounds have been identified and implicated so far to target altered NT activity in the AD brain, which have been outlined in Table 4. These compounds bind to NTs and their specific receptors thereby reducing the severity of disease atmosphere.

Recent therapeutic strategies for targeting perturbed neurotransmitters/neuromodulators in synaptopathy

Although the significant role of distinct chemical compounds has been explored extensively to target altered NTs/NMs in synaptopathy, there are numerous other new therapeutic strategies that have been identified so far to target such alterations. For instance, the neuroprotective effect of Nanodiamond against memory deficits has currently been reported, where it showed a protective role by modulating NF-kB and STAT3 signaling cascade, the effects mediated by the regulation of NMDARs [195]. Further, treatment using vitamin D3 has shown a

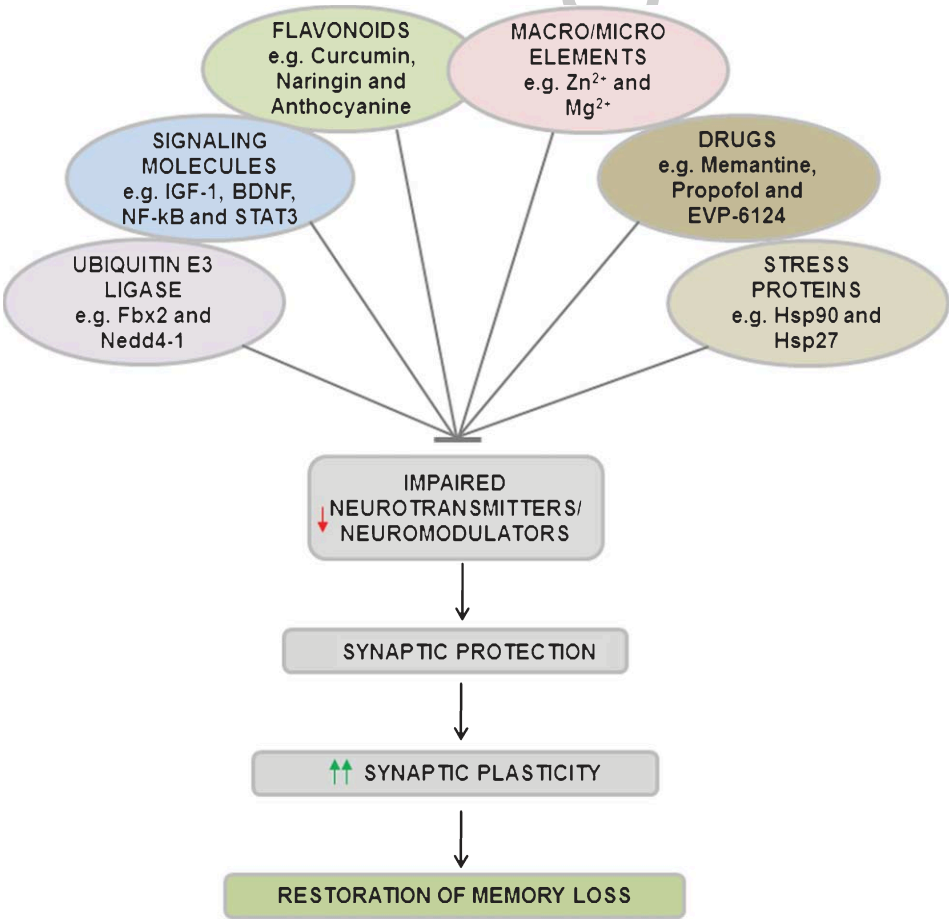

Fig. 3. Pictorial representation showing the involvement of different therapeutic approaches against synaptic loss associated with impaired neurotransmitters/neuromodulators (NTs/NMs). Fbx2, F-Box Protein 2; IGF-1, insulin-like growth factor 1; brain-derived neurotrophic factor; NF-kB, Nuclear factor-κB; STAT3, signal transducer and activator of transcription 3; Hsp90, heat shock protein 90; Hsp27, heat shock protein 27.

protective role against synaptic loss via significantly increasing the levels of ACh in neurons [196]. Treatment with zinc ion (Zn^{2+}) showed enhanced levels of NT release in aluminum (Al^{3+})-treated animals, thereby showing their protective role against synaptic injury, since Al^{3+} exposure decreases the level of NTs and acetylcholinesterase activity in brain and leads to the neuronal dysfunction [197]. Similarly, elevated levels of magnesium ion (Mg^{2+}) also exert substantial synaptoprotective effects in AD brains where it controls the synaptic density/plasticity bt preventing the onset of impaired NMDA receptor signaling pathway [198]. Interestingly, insulin is being implicated nowadays to attenuate the cognitive dysfunctions through its regulatory effect on the expression of NMDA receptors and on the associated insulin signaling cascade in AD [199]. Another therapeutics strategy to ameliorate Aβ-mediated synaptic loss is using Substance P, which is a member of the tachykinin family, distributed widely in the CNS and acts as a NT/NM as well as neurotrophic factor. Further, Substance P is able to provoke non-amyloidogenic AβPP processing, thereby curtailing the feasibility of Aβ peptides production in the brain [200]. Additionally, several studies have highlighted the role of the ubiquitin-proteasome system as a therapeutic approach to target synaptic loss induced by altered release of NTs at synapses. The ubiquitin-proteasome system is found to modulate NTs/NMs, synaptic proteins, transcription factors, and other molecules critical for neuronal plasticity. For instance, altered NMDA receptors are retro-translocated and degraded by a F-box protein called Fbx2, advocating that SCF-type ligases targets NMDA receptors for ubiquitination [201]. Another protein Nedd4-1, known as a HECT E3 ubiquitin ligase, has also found to target Aβ-induced reductions in surface AMPARs, dendritic spine density, and synaptic strength [202]. Likewise, heat shock protein (HSP)-based therapeutic approach has also been currently addressed in numerous studies. For example, HSP90 is being used nowadays to modulate NT release at the presynaptic terminals independently by mediating the continuous cycling of synaptic AMPA receptors [203]. Moreover, the hypothetical representation of numerous pharmacological approaches to compensate neurotransmission imbalance has been depicted in Fig. 3. Nevertheless, several other suitable approaches still need to be devised in the near future for effective treatment against synaptic dysfunctions mediated by altered NTs/NMs.

CONCLUSION

In this review, we have discussed the pertinent role of synaptic plasticity in memory formation, its stabilization, and associated abnormalities due to Aβ accumulation and tau phosphorylation. For instance, increased levels of toxic Aβ and tau oligomers at synaptic junctions are responsible for neuronal toxicity, which is associated with synaptic loss in AD. Similarly, involvement of oxidative stress, activation of redox signaling, and subsequent damage to mitochondrial integrity in synaptic alteration has also been elucidated. Additionally, the altered activity of various NTs/NMs including glutamatergic, GABAergic, and acetylcholinergic receptors with respect to Aβ accumulation and tau phosphorylation has also been extensively reviewed, since NTs/NMs in their normal form play a crucial role in maintaining neuronal homeostasis in the brain. However, any alterations in their proper functioning cause several neurotoxic effects associated with altered activity of synaptic proteins, transcription factors, Ca^{2+} homeostasis, and other molecules critical for neuronal plasticity. These factors under the diseased state disrupt normal homeostasis of neurons, thereby causing synaptic loss. Furthermore, in order to target the malfunctioning NTs/NMs or reverse their associated chronic effects, numerous biological compound-mediated therapeutic strategies have been discussed to obviate the disease symptoms of AD. Additionally, recent therapeutic strategies for targeting synaptic loss induced by defective NTs/NMs have been addressed. Finally, this review accentuates the savvy of altered redox signaling and impaired neurotransmission in synaptic dysfunction during synaptopathy that could unveil mechanism-based therapeutics and ameliorated inferential strategies.

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REFERENCES

[1] Parikh V, Bernard CS, Naughton SX, Yegla B (2014) Interactions between Aβ oligomers and presynaptic cholinergic signaling: Age-dependent effects on attentional capacities. *Behav Brain Res* **274**, 30-42.

[2] Zarate CA Jr, Manji HK (2008) The role of AMPA receptor modulation in the treatment of neuropsychiatric diseases. *Exp Neurol* **211**, 7-10.
[3] Guerrero-Muñoz MJ, Gerson J, Castillo-Carranza DL (2015) Tau oligomers: The toxic player at synapses in Alzheimer's disease. *Front Cell Neurosci* **9**, 464.
[4] Nava-Mesa MO, Jiménez-Díaz L, Yajeya J, Navarro-Lopez JD (2014) GABAergic neurotransmission and new strategies of neuromodulation to compensate synaptic dysfunction in early stages of Alzheimer's disease. *Front Cell Neurosci* **8**, 167.
[5] Kamat PK, Kalani A, Rai S, Swarnkar S, Tota S, Nath C, Tyagi N (2016) Mechanism of oxidative stress and synapse dysfunction in the pathogenesis of Alzheimer's disease: Understanding the therapeutics strategies. *Mol Neurobiol* **53**, 48-61.
[6] Butterfield DA, Perluigi M, Reed T, Muharib T, Hughes CP, Robinson RA, Sultana R (2012) Redox proteomics in selected neurodegenerative disorders: From its infancy to future applications. *Antioxid Redox Signal* **17**, 1610-1655.
[7] Missler M, Südhof TC, Biederer T (2012) Synaptic cell adhesion. *Cold Spring Harb Perspect Biol* **4**, a005694.
[8] Henstridge CM, Pickett E, Spires-Jones TL (2016) Synaptic pathology: A shared mechanism in neurological disease. *Ageing Res Rev* **28**, 72-84.
[9] Duric V, Clayton S, Leong ML, Yuan LL (2016) Comorbidity factors and brain mechanisms linking chronic stress and systemic illness. *Neural Plast* **2016**, 5460732.
[10] Takeuchi T, Duszkiewicz AJ, Morris RG (2013) The synaptic plasticity and memory hypothesis: Encoding, storage and persistence. *Philos Trans R Soc Lond B Biol Sci* **369**, 20130288.
[11] Hamley IW (2012) The amyloid beta peptide: A chemist's perspective. Role in Alzheimer's and fibrillization. *Chem Rev* **112**, 5147-5192.
[12] Ballatore C, Lee VM, Trojanowski JQ (2007) Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nat Rev Neurosci* **8**, 663-672.
[13] Mondragón-Rodríguez S, Trillaud-Doppia E, Dudilot A, Bourgeois C, Lauzon M, Leclerc N, Boehm J (2012) Interaction of endogenous tau protein with synaptic proteins is regulated by N-Methyl-d-aspartate receptor-dependent tau phosphorylation. *J Biol Chem* **287**, 32040-32053.
[14] Tracy TE, Sohn PD, Minami SS, Wang C, Min SW, Li Y, Zhou Y, Le D, Lo I, Ponnusamy R, Cong X, Schilling B, Ellerby LM, Huganir RL, Gan L (2016) Acetylated tau obstructs KIBRA-mediated signaling in synaptic plasticity and promotes tauopathy-related memory loss. *Neuron* **90**, 245-260.
[15] Shankar GM, Walsh DM (2009) Alzheimer's disease: Synaptic dysfunction and Abeta. *Mol Neurodegener* **4**, 48.
[16] Overk CR, Masliah E (2014) Pathogenesis of synaptic degeneration in Alzheimer's disease and Lewy body disease. *Biochem Pharmacol* **88**, 508-516.
[17] Moreira PI, Carvalho C, Zhu X, Smith MA, Perry G (2010) Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology. *Biochim Biophys Acta* **1802**, 2-10.
[18] Woods NK, Padmanabhan J (2012) Neuronal calcium signaling and Alzheimer's disease. *Adv Exp Med Biol* **740**, 1193-1217.
[19] Rushworth JV, Hooper NM (2010) Lipid rafts: Linking Alzheimer's amyloid-β production, aggregation, and toxicity at neuronal membranes. *Int J Alzheimers Dis* **2011**, 603052.
[20] Malchiodi-Albedi F, Petrucci TC, Picconi B, Iosi F, Falchi M (1997) Protein phosphatase inhibitors induce modification of synapse structure and tau hyperphosphorylation in cultured rat hippocampal neurons. *J Neurosci Res* **48**, 425-438.
[21] Jang SS, Royston SE, Lee G, Wang S, Chung HJ (2016) Seizure-induced regulations of amyloid-β, STEP61, and STEP61 substrates involved in hippocampal synaptic plasticity. *Neural Plast* **2016**, 2123748.
[22] Abdul Rahman NZ, Greenwood SM, Brett RR, Tossell K, Ungless MA, Plevin R, Bushell TJ (2016) Mitogen-activated protein kinase phosphatase-2 deletion impairs synaptic plasticity and hippocampal-dependent memory. *J Neurosci* **36**, 2348-2354.
[23] Maynard CJ, Bush AI, Masters CL, Cappai R, Li QX (2005) Metals and amyloid-beta in Alzheimer's disease. *Int J ExpPathol* **86**, 147-159.
[24] Wang WY, Tan MS, Yu JT, Tan L (2015) Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. *Ann Transl Med* **3**, 136.
[25] Rudy CC, Hunsberger HC, Weitzner DS, Reed MN (2015) The role of the tripartite glutamatergic synapse in the pathophysiology of Alzheimer's disease. *Aging Dis* **6**, 131-148.
[26] Mookherjee P, Green PS, Watson GS, Marques MA, Tanaka K, Meeker KD, Meabon JS, Li N, Zhu P, Olson VG, Cook DG (2011) GLT-1 loss accelerates cognitive deficit onset in an Alzheimer's disease animal model. *J Alzheimers Dis* **26**, 447-455.
[27] Kempf SJ, Metaxas A, Ibáñez-Vea M, Darvesh S, Finsen B, Larsen MR (2016) An integrated proteomics approach shows synaptic plasticity changes in an APP/PS1 Alzheimer's mouse model. *Oncotarget*. doi: 10.18632/oncotarget.9092
[28] Tu S, Okamoto S, Lipton SA, Xu H (2014) Oligomeric Aβ-induced synaptic dysfunction in Alzheimer's disease. *Mol Neurodegener* **9**, 48.
[29] Marttinen M, Kurkinen KM, Soininen H, Haapasalo A, Hiltunen M (2015) Synaptic dysfunction and septin protein family members in neurodegenerative diseases. *Mol Neurodegener* **10**, 16.
[30] Ross JA, McGonigle P, Van Bockstaele EJ (2015) Locus coeruleus, norepinephrine and Aβ peptides in Alzheimer's disease. *Neurobiol Stress* **2**, 73-84.
[31] Sheng M, Sabatini BL, Südhof TC (2012) Synapses and Alzheimer's disease. *Cold Spring Harb Perspect Biol* **4**, a005777.
[32] Nava-Mesa MO, Jiménez-Díaz L, Yajeya J, Navarro-Lopez JD (2013) Amyloid-β induces synaptic dysfunction through G protein-gated inwardly rectifying potassium channels in the fimbria-CA3 hippocampal synapse. *Front Cell Neurosci* **7**, 117.
[33] Deak F, Sonntag WE (2012) Aging, synaptic dysfunction, and insulin-like growth factor (IGF)-1. *J Gerontol A Biol Sci Med Sci* **67**, 611-625.
[34] Liu DS, Pan XD, Zhang J, Shen H, Collins NC, Cole AM, Koster KP, Ben Aissa M, Dai XM, Zhou M, Tai LM, Zhu YG, LaDu M, Chen XC (2015) APOE4 enhances age-dependentdecline in cognitive function by down-regulating an NMDA receptor pathway inEFAD-Tg mice. *Mol Neurodegener* **10**, 7.
[35] Ardiles AO, Tapia-Rojas CC, Mandal M, Alexandre F, Kirkwood A, Inestrosa NC, Palacios AG (2012) Postsynaptic dysfunction is associated with spatial and object recognition memory loss in a natural model of

Alzheimer's disease. *Proc Natl Acad Sci U S A* **109**, 13835-13840.
[36] Mucke L, Selkoe DJ (2012) Neurotoxicity of amyloid β-protein: Synaptic and network dysfunction. *Cold Spring Harb Perspect Med* **2**, a006338.
[37] Parihar MS, Brewer GJ (2010) Amyloid-β as a modulator of synaptic plasticity. *J Alzheimers Dis* **22**, 741-763.
[38] Palop JJ, Mucke L (2010) Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: From synapses toward neural networks. *Nat Neurosci* **13**, 812-818.
[39] Danysz W, Parsons CG (2012) Alzheimer's disease, β-amyloid, glutamate, NMDA receptors and memantine–searching for the connections. *Br J Pharmacol* **167**, 324-352.
[40] Viola KL, Klein WL (2015) Amyloid β oligomers in Alzheimer's disease pathogenesis, treatment, and diagnosis. *Acta Neuropathol* **129**, 183-206.
[41] Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, Sisodia S, Malinow R (2003) APP processing and synaptic function. *Neuron* **37**, 925-937.
[42] Malm TM, Jay TR, Landreth GE (2015) The evolving biology of microglia in Alzheimer's disease. *Neurotherapeutics* **12**, 81-93.
[43] Mandrekar-Colucci S, Landreth GE (2010) Microglia and Inflammation in Alzheimer's disease. *CNS Neurol Disord Drug Targets* **9**, 156-167.
[44] Mosher KI, Wyss-Coray T (2014) Microglial dysfunction in brain aging and Alzheimer's disease. *Biochem Pharmacol* **88**, 594-604.
[45] Kimura R, MacTavish D, Yang J, Westaway D, Jhamandas JH (2012) Beta amyloid-induced depression of hippocampal long-term potentiation is mediated through the amylin receptor. *J Neurosci* **32**, 17401-17406.
[46] Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT (2011) Neuropathological alterations in Alzheimer disease. *Cold Spring Harb Perspect Med* **1**, a006189.
[47] Khan SS, Bloom GS (2016) Tau: The center of a signaling nexus in Alzheimer's disease. *Front Neurosci* **10**, 31.
[48] Eckert A, Schmitt K, Götz J (2011) Mitochondrial dysfunction – the beginning of the end in Alzheimer's disease? Separate and synergistic modes of tau and amyloid-β toxicity. *Alzheimers Res Ther* **3**, 15.
[49] Meier S, Bell M, Lyons DN, Rodriguez-Rivera J, Ingram A, Fontaine SN, Mechas E, Chen J, Wolozin B, LeVine H, 3rd, Zhu H, Abisambra JF (2016) Pathological tau promotes neuronal damage by impairing ribosomal function and decreasing protein synthesis. *J Neurosci* **36**, 1001-1007.
[50] Nagura H, Ishikawa Y, Kobayashi K, Takao K, Tanaka T, Nishikawa K, Tamura H, Shiosaka S, Suzuki H, Miyakawa T, Fujiyoshi Y, Doi T (2012) Impaired synaptic clustering of postsynaptic density proteins and altered signal transmission in hippocampal neurons, and disrupted learning behavior in PDZ1 and PDZ2 ligand binding-deficient PSD-95 knockin mice. *Mol Brain* **5**, 43.
[51] Mietelska-Porowska A, Wasik U, Goras M, Filipek A, Niewiadomska G (2014) Tau protein modifications and interactions: Their role in function and dysfunction. *Int J Mol Sci* **15**, 4671-4713.
[52] Usenovic M, Niroomand S, Drolet RE, Yao L, Gaspar RC, Hatcher NG, Schachter J, Renger JJ, Parmentier-Batteur S (2015) Internalized tau oligomers cause neurodegeneration by inducing accumulation of pathogenic tau in human neurons derived from induced pluripotent stem cells. *J Neurosci* **35**, 14234-14250.
[53] Zhou Y, Zhao Y, Xie H, Wang Y, Liu L, Yan X (2015) Alteration in amyloid β42, phosphorylated tau protein, interleukin 6, and acetylcholine during diabetes-accelerated memory dysfunction in diabetic rats: Correlation of amyloid β42 with changes in glucose metabolism. *Behav Brain Funct* **11**, 24.
[54] De Oliveira AS, Santiago FE, Balioni LF, Ferrari Mde F, Almeida MC, Carrettiero DC (2016) BAG2 expression dictates a functional intracellular switch between the p38-dependent effects of nicotine on tau phosphorylation levels via the α7 nicotinic receptor. *Exp Neurol* **275**, 69-77.
[55] Sotiropoulos I, Sousa N (2016) Tau as the converging protein between chronic stress and alzheimer's disease synaptic pathology. *Neurodegener Dis* **16**, 22-25.
[56] Lopes S, Vaz-Silva J, Pinto V, Dalla C, Kokras N, Bedenk B, Mack N, Czisch M, Almeida OF, Sousa N, Sotiropoulos I (2016) Tau protein is essential for stress-induced brain pathology. *Proc Natl Acad Sci U S A* **113**, E3755-E3763.
[57] Maeda S, Djukic B, Taneja P, Yu GQ, Lo I, Davis A, Craft R, Guo W, Wang X, Kim D, Ponnusamy R, Gill TM, Masliah E, Mucke L (2016) Expression of A152T human tau cause age-dependent neuronal dysfunction and loss in transgenic mice. *EMBO Rep* **17**, 530-551.
[58] Kovacic P, Somanathan R (2012) Redox processes in neurodegenerative disease involving reactive oxygen species. *Curr Neuropharmacol* **10**, 289-302.
[59] Arun S, Liu L, Donmez G (2016) Mitochondrial biology and neurological diseases. *Curr Neuropharmacol* **14**, 143-154.
[60] Gandhi S, Abramov AY (2012) Mechanism of oxidative stress in neurodegeneration. *Oxid Med Cell Longev* **2012**, 428010.
[61] Srinivasan S, Avadhani NG (2012) Cytochrome c oxidase dysfunction in oxidative stress. *Free Radic Biol Med* **53**, 1252-1263.
[62] Pope S, Land JM, Heales SJ (2008) Oxidative stress and mitochondrial dysfunction in neurodegeneration; cardiolipin a critical target? *Biochim Biophys Acta* **1777**, 794-799.
[63] Vos M, Lauwers E, Verstreken P (2010) Synaptic mitochondria in synaptic transmission and organization of vesicle pools in health and disease. *Front Synaptic Neurosci* **2**, 139.
[64] Zenisek D, Matthews G (2000) The role of mitochondria in presynaptic calcium handling at a ribbon synapse. *Neuron* **25**, 229-237.
[65] Walker KR, Tesco G (2013) Molecular mechanisms of cognitive dysfunction following traumatic brain injury. *Front Aging Neurosci* **5**, 29.
[66] Rao VK, Carlson EA, Yan SS (2014) Mitochondrial permeability transition pore is a potential drug target for neurodegeneration. *Biochim Biophys Acta* **1842**, 1267-1272.
[67] Santiago-López D, Bautista-Martínez JA, Reyes-Hernandez CI, Aguilar-Martínez M, Rivas-Arancibia S (2010) Oxidative stress, progressive damage in the substantia nigra and plasma dopamine oxidation, in rats chronically exposed to ozone. *Toxicol Lett* **197**, 193-200.
[68] Sheng ZH (2014) Mitochondrial trafficking and anchoring in neurons: New insight and implications. *J Cell Biol* **204**, 1087-1098.
[69] Chen H, Chan DC (2009) Mitochondrial dynamics-fusion, fission, movement, and mitophagy-in neurodegenerative diseases. *Hum Mol Genet* **18**, 69-76.

[70] Yoon SY, Choi HI, Choi JE, Sul CA, Choi JM, Kim DH (2007) Methotrexate decreases PP2A methylation and increases tau phosphorylation in neuron. *Biochem Biophys Res Commun* **363**, 811-816.
[71] Kamat PK, Nath C (2015) Okadaic acid: A tool to study regulatory mechanisms for neurodegeneration and regeneration in Alzheimer's disease. *Neural Regen Res* **10**, 365-367.
[72] Gómez-Ramos A, Díaz-Nido J, Smith MA, Perry G, Avila J (2003) Effect of the lipid peroxidation product acrolein on tau phosphorylation in neural cells. *J Neurosci Res* **71**, 863-870.
[73] Liu SJ, Fang ZY, Yang Y, Deng HM, Wang JZ (2003) Alzheimer-like phosphorylation of tau and neurofilament induced by cocaine *in vivo*. *Acta Pharmacol* **24**, 512-518.
[74] Deng XH, Ai WM, Lei DL, Luo XG, Yan XX, Li Z (2012) Lipopolysaccharide induces paired immunoglobulin-like receptor B (PirB) expression, synaptic alteration, and learning-memory deficit in rats. *Neuroscience* **209**, 161-170.
[75] Ullrich C, Humpel C (2009) Rotenone induces cell death of cholinergic neurons in an organotypic co-culture brain slice model. *Neurochem Res* **34**, 2147-2153.
[76] Chen L, Yoo SE, Na R, Liu Y, Ran Q (2012) Cognitive impairment and increased Aβ levels induced by paraquat exposure are attenuated by enhanced removal of mitochondrial H (2)O(2), *Neurobiol Aging* **33**, 432.e15-26.
[77] Shang T, Kotamraju S, Kalivendi SV, Hillard CJ, Kalyanaraman B (2004) 1-Methyl-4-phenylpyridinium-induced apoptosis in cerebellar granule neurons is mediated by transferrin receptor iron-dependent depletion of tetrahydrobiopterin and neuronal nitric-oxide synthase-derived superoxide. *J Biol Chem* **279**, 19099-19112.
[78] Ma Q, Ying M, Sui X, Zhang H, Huang H, Yang L, Huang X, Zhuang Z, Liu J, Yang X (2015) Chronic copper exposure causes spatial memory impairment, selective loss of hippocampal synaptic proteins, and activation of PKR/eIF2α pathway in mice. *J Alzheimers Dis* **43**, 1413-1427.
[79] Da Silva VK, de Freitas BS, da Silva Dornelles A, Nery LR, Falavigna L, Ferreira RD, Bogo MR, Hallak JE, Zuardi AW, Crippa JA, Schröder N (2014) Cannabidiol normalizes caspase 3, synaptophysin, and mitochondrial fission protein DNM1L expression levels in rats with brain iron overload: Implications for neuroprotection. *Mol Neurobiol* **49**, 222-233.
[80] Kawahara M, Kato-Negishi M (2011) Link between aluminum and the pathogenesis of Alzheimer's disease: The integration of the aluminum and amyloid cascade hypotheses. *Int J Alzheimers Dis* **8**, 276393.
[81] Lenaz G, Fato R, Genova ML, Bergamini C, Bianchi C, Biondi A (2006) Mitochondrial Complex I: Structural and functional aspects. *Biochim Biophys Acta* **1757**, 1406-1420.
[82] Dröse S, Brandt U (2012) Molecular mechanisms of superoxide production by the mitochondrial respiratory chain. *Adv Exp Med Biol* **748**, 145-169.
[83] Hsieh HL, Yang CM (2013) Role of redox signaling in neuroinflammation and neurodegenerative diseases. *Biomed Res Int* **2013**, 484613.
[84] von Bernhardi R, Eugenín J (2012) Alzheimer's disease: Redox dysregulation as a common denominator for diverse pathogenic mechanisms. *Antioxid Redox Signal* **16**, 974-1031.
[85] Hochstrasser T, Weiss E, Marksteiner J, Humpel C (2010) Soluble cell adhesion molecules in monocytes of Alzheimer's disease and mild cognitive impairment. *Exp Gerontol* **45**, 70-74.
[86] Massaad CA, Klann E (2011) Reactive oxygen species in the regulation of synaptic plasticity and memory. *Antioxid Redox Signal* **14**, 2013-2054.
[87] Jang SS, Chung HJ (2016) Emerging link between Alzheimer's disease and homeostatic synaptic plasticity. *Neural Plast* **2016**, 7969272.
[88] Belmeguenai A, Hansel C (2005) A role for protein phosphatases 1, 2A, and 2B in cerebellar long-term potentiation. *J Neurosci* **25**, 10768-10772.
[89] Chakroborty S, Kim J, Schneider C, West AR, Stutzmann GE (2015) Nitric oxide signaling is recruited as a compensatory mechanism for sustaining synaptic plasticity in Alzheimer's disease mice. *J Neurosci* **35**, 6893-6902.
[90] Sunico CR, González-Forero D, Domínguez G, García-Verdugo JM, Moreno-López B (2010) Nitric oxide induces pathological synapse loss by a protein kinase G-, Rho kinase-dependent mechanism preceded by myosin light chain phosphorylation. *J Neurosci* **30**, 973-984.
[91] Roh SE, Woo JA, Lakshmana MK, Uhlar C, Ankala V, Boggess T, Liu T, Hong YH, Mook-Jung I, Kim SJ, Kang DE (2013) Mitochondrial dysfunction and calcium deregulation by the RanBP9-cofilin pathway. *FASEB J* **27**, 4776-4789.
[92] Bedse G, Di Domenico F, Serviddio G, Cassano T (2015) Aberrant insulin signaling in Alzheimer's disease: Current knowledge. *Front Neurosci* **9**, 204.
[93] Jha NK, Jha SK, Kumar D, Kejriwal N, Sharma R, Ambasta RK, Kumar P (2015) Impact of insulin degrading enzyme and neprilysin in Alzheimer's disease biology: Characterization of putative cognates for therapeutic applications. *J Alzheimers Dis* **48**, 891-917.
[94] Solito E, Sastre M (2012) Microglia functions in Alzheimer's disease. *Front Pharmacol* **3**, 14.
[95] Teich AF, Nicholls RE, Puzzo D, Fiorito J, Purgatorio R, Fa' M, Arancio O (2015) Synaptic therapy in Alzheimer's disease: A CREB-centric approach. *Neurotherapeutics* **12**, 29-41.
[96] Faulconbridge LF, Hayes MR (2011) Regulation of energy balance and body weight by the brain: A distributed system prone to disruption. *Psychiatr Clin North Am* **34**, 733-745.
[97] Hasselmo ME (2006) The role of acetylcholine in learning and memory. *Curr Opin Neurobiol* **16**, 710-715.
[98] Resende RR, Adhikari A (2009) Cholinergic receptor pathways involved in apoptosis, cell proliferation and neuronal differentiation. *Cell Commun Signal* **7**, 20.
[99] Placzek AN, Zhang TA, Dani JA (2009) Nicotinic mechanisms influencing synaptic plasticity in the hippocampus. *Acta Pharmacol Sin* **30**, 752-760.
[100] Mufson EJ, Counts SE, Perez SE, Ginsberg SD (2008) Cholinergic system during the progression of Alzheimer's disease: Therapeutic implications. *Expert Rev Neurother* **8**, 1703-1718.
[101] Tammimäki A, Horton WJ, Stitzel JA (2011) Recent advances in gene manipulation and nicotinic acetylcholine receptor biology. *Biochem Pharmacol* **82**, 808-819.
[102] Feduccia AA, Chatterjee S, Bartlett SE (2012) Neuronal nicotinic acetylcholine receptors: Neuroplastic changes underlying alcohol and nicotine addictions. *Front Mol Neurosci* **5**, 83.

[103] Posadas I, López-Hernández B, Ceña V (2013) Nicotinic receptors in neurodegeneration. *Curr Neuropharmacol* **11**, 298-314.

[104] Ni R, Marutle A, Nordberg A (2013) Modulation of α7 nicotinic acetylcholine receptor and fibrillar amyloid-β interactions in Alzheimer's disease brain. *J Alzheimers Dis* **33**, 841-851.

[105] Beaulieu JM, Gainetdinov RR (2011) The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev* **63**, 182-217.

[106] Martorana A, Koch G (2014) Is dopamine involved in Alzheimer's disease? *Front Aging Neurosci* **6**, 252.

[107] Moreno-Castilla P, Rodriguez-Duran LF, Guzman-Ramos K, Barcenas-Femat A, Escobar ML, Bermudez-Rattoni F (2016) Dopaminergic neurotransmission dysfunction induced by amyloid-β transforms cortical long-term potentiation into long-term depression and produces memory impairment. *Neurobiol Aging* **41**, 187-199.

[108] Koch G, Di Lorenzo F, Bonnì S, Giacobbe V, Bozzali M, Caltagirone C, Martorana A (2014) Dopaminergic modulation of cortical plasticity in Alzheimer's disease patients. *Neuropsychopharmacology* **39**, 2654-2661.

[109] Liang SL, Carlson GC, Coulter DA (2006) Dynamic regulation of synaptic GABA release by the glutamate-glutamine cycle in hippocampal area CA1. *J Neurosci* **26**, 8537-8548.

[110] Winsky-Sommerer R (2009) Role of GABAA receptors in the physiology and pharmacology of sleep. *Eur J Neurosci* **29**, 1779-1794.

[111] Limon A, Reyes-Ruiz JM, Miledi R (2012) Loss of functional GABA (A) receptors in the Alzheimer diseased brain. *Proc Natl Acad Sci U S A* **109**, 10071-10076.

[112] Li Y, Sun H, Chen Z, Xu H, Bu G, Zheng H (2016) Implications of GABAergic Neurotransmission in Alzheimer's disease. *Front Aging Neurosci* **8**, 31.

[113] Zhuo M (2009) Plasticity of NMDA receptor NR2B subunit in memory and chronic pain. *Mol Brain* **2**, 4.

[114] Guntupalli S, Widagdo J, Anggono V (2016) Amyloid-β-induced dysregulation of AMPA receptor trafficking. *Neural Plast* **2016**, 3204519.

[115] Behnisch T, Yuanxiang P, Bethge P, Parvez S, Chen Y, Yu J, Karpova A, Frey JU, Mikhaylova M, Kreutz MR (2011) Nuclear translocation of jacob in hippocampal neurons after stimuli inducing long-term potentiation but not long-term depression. *PLoS One* **6**, e17276.

[116] Pozueta J, Lefort R, Shelanski ML (2013) Synaptic changes in Alzheimer's disease and its models. *Neuroscience* **251**, 51-65.

[117] Xu J, Kurup P, Nairn AC, Lombroso PJ (2012) Striatal-enriched protein tyrosine phosphatase in Alzheimer's disease. *Adv Pharmacol* **64**, 303-325.

[118] Snigdha S, Smith ED, Prieto GA, Cotman CW (2012) Caspase-3 activation as a bifurcation point between plasticity and cell death. *Neurosci Bull* **28**, 14-24.

[119] Blandina P, Munari L, Provensi G, Passani MB (2012) Histamine neurons in the tuberomamillary nucleus: A whole center or distinct subpopulations? *Front Syst Neurosci* **6**, 33.

[120] Nuutinen S, Panula P (2010) Histamine in neurotransmission and brain diseases. *Adv Exp Med Biol* **709**, 95-107.

[121] Cacabelos R, Torrellas C, Fernández-Novoa L, López-Muñoz F (2016) Histamine and immune biomarkers in CNS disorders. *Mediators Inflamm* **2016**, 1924603.

[122] Medhurst AD, Roberts JC, Lee J, Chen CP, Brown SH, Roman S, Lai MK (2009) Characterization of histamine H3 receptors in Alzheimer's disease brain and amyloid over-expressing TASTPM mice. *Br J Pharmacol* **157**, 130-138.

[123] Gannon M, Che P, Chen Y, Jiao K, Roberson ED, Wang Q (2015) Noradrenergic dysfunction in Alzheimer's disease. *Front Neurosci* **9**, 220.

[124] Marien MR, Colpaert FC, Rosenquist AC (2004) Noradrenergic mechanisms in neurodegenerative diseases: A theory. *Brain Res* **45**, 38-78.

[125] Hammerschmidt T, Kummer MP, Terwel D, Martinez A, Gorji A, Pape HC, Rommelfanger KS, Schroeder JP, Stoll M, Schultze J, Weinshenker D, Heneka MT (2013) Selective loss of noradrenaline exacerbates early cognitive dysfunction and synaptic deficits in APP/PS1 mice. *Biol Psychiatry* **73**, 454-463.

[126] Combarros O, Warden DR, Hammond N, Cortina-Borja M, Belbin O, Lehmann MG, Wilcock GK, Brown K, Kehoe PG, Barber R, Coto E, Alvarez V, Deloukas P, Gwilliam R, Heun R, Kölsch H, Mateo I, Oulhaj A, Arias-Vásquez A, Schuur M, Aulchenko YS, Ikram MA, Breteler MM, van Duijn CM, Morgan K, Smith AD, Lehmann DJ (2010) The dopamine β-hydroxylase -1021C/T polymorphism is associated with the risk of Alzheimer's disease in the Epistasis Project. *BMC Med Genet* **11**, 162.

[127] Chen Y, Peng Y, Che P, Gannon M, Liu Y, Li L, Bu G, van Groen T, Jiao K, Wang Q (2010) α (2A) adrenergic receptor promotes amyloidogenesis through disrupting APP-SorLA interaction. *Proc Natl Acad Sci U S A* **111**, 17296-17301.

[128] Wang D, Fu Q, Zhou Y, Xu B, Shi Q, Igwe B, Matt L, Hell JW, Wisely EV, Oddo S, Xiang YK (2013) β2 adrenergic receptor, protein kinase A (PKA) and c-Jun N-terminal kinase (JNK) signaling pathways mediate tau pathology in Alzheimer disease models. *J Biol Chem* **288**, 10298-10307.

[129] Arreola R, Becerril-Villanueva E, Cruz-Fuentes C, Velasco-Velázquez MA, Garcés-Alvarez ME, Hurtado-Alvarado G, Quintero-Fabian S, Pavón L (2015) Immunomodulatory effects mediated by serotonin. *J Immunol Res* **2015**, 354957.

[130] Fidalgo S, Ivanov DK, Wood SH (2013) Serotonin: From top to bottom. *Biogerontology* **14**, 21-45.

[131] Bantick RA, Deakin JF, Grasby PM (2001) The 5-HT1A receptor in schizophrenia: A promising target for novel atypical neuroleptics? *J Psychopharmacol* **15**, 37-46.

[132] Thathiah A, De Strooper B (2011) The role of G protein-coupled receptors in the pathology of Alzheimer's disease. *Nat Rev Neurosci* **12**, 73-87.

[133] Cochet M, Donneger R, Cassier E, Gaven F, Lichtenthaler SF, Marin P, Bockaert J, Dumuis A, Claeysen S (2013) 5-HT4 receptors constitutively promote the non-amyloidogenic pathway of APP cleavage and interact with ADAM10. *ACS Chem Neurosci* **4**, 130-140.

[134] Kovacs GG, Klöppel S, Fischer I, Dorner S, Lindeck-Pozza E, Birner P, Bötefür IC, Pilz P, Volk B, Budka H (2003) Nucleus-specific alteration of raphe neurons in human neurodegenerative disorders. *Neuroreport* **14**, 73-76.

[135] Garcia-Alloza M, Gil-Bea FJ, Diez-Ariza M, Chen CP, Francis PT, Lasheras B, Ramirez MJ (2005) Cholinergic-serotonergic imbalance contributes to cognitive and behavioral symptoms in Alzheimer's disease. *Neuropsychologia* **43**, 442-449.

[136] Hoozemans JJ, Chafekar SM, Baas F, Eikelenboom P, Scheper W (2006) Always around, never the same:

Pathways of amyloid beta induced neurodegeneration throughout the pathogenic cascade of Alzheimer's disease. *Curr Med Chem* **13**, 2599-2605.

[137] Yun HM, Kim S, Kim HJ, Kostenis E, Kim JI, Seong JY, Baik JH, Rhim H (2007) The novel cellular mechanism of human 5-HT6 receptor through an interaction with Fyn. *J Biol Chem* **282**, 5496-5505.

[138] Hendricksen M, Thomas AJ, Ferrier IN, Ince P, O'Brien JT (2004) Neuropathological study of the dorsal raphe nuclei in late-life depression and Alzheimer's disease with and without depression. *Am J Psychiatry* **161**, 1096-1102.

[139] Stranahan AM, Erion JR, Wosiski-Kuhn M (2013) Reelin signaling in development, maintenance, and plasticity of neural networks. *Ageing Res Rev* **12**, 815-822.

[140] Xiong Z, Thangavel R, Kempuraj D, Yang E, Zaheer S, Zaheer A (2014) Alzheimer's disease: Evidence for the expression of interleukin-33 and its receptor ST2 in the brain. *J Alzheimers Dis* **40**, 297-308.

[141] Guzman SJ, Gerevich Z (2016) P2Y receptors in synaptic transmission and plasticity: Therapeutic potential in cognitive dysfunction. *Neural Plast* **2016**, 1207393.

[142] Venkatachalam K, Montell C (2007) TRP channels. *Annu Rev Biochem* **76**, 387-417.

[143] Wang J, Jackson MF, Xie YF (2016) Glia and TRPM2 channels in plasticity of central nervous system and Alzheimer's diseases. *Neural Plast* **2016**, 1680905.

[144] Marcello E, Epis R, Saraceno C, Di Luca M (2012) Synaptic dysfunction in Alzheimer's disease. *Adv Exp Med Biol* **970**, 573-601.

[145] Kihara T, Shimohama S (2004) Alzheimer's disease and acetylcholine receptors. *Acta Neurobiol Exp* **64**, 99-105.

[146] Giuffrida ML, Tomasello F, Caraci F, Chiechio S, Nicoletti F, Copani A (2012) Beta-amyloid monomer and insulin/IGF-1 signaling in Alzheimer's disease. *Mol Neurobiol* **46**, 605-613.

[147] Vaarmann A, Kovac S, Holmström KM, Gandhi S, Abramov AY (2013) Dopamine protects neurons against glutamate-induced excitotoxicity. *Cell Death Dis* **4**, e455.

[148] Zhang Y, Li P, Feng J, Wu M (2016) Dysfunction of NMDA receptors in Alzheimer's disease. *Neurol Sci* **37**, 1039-1047.

[149] Akhtar MW, Sanz-Blasco S, Dolatabadi N, Parker J, Chon K, Lee MS, Soussou W, McKercher SR, Ambasudhan R, Nakamura T, Lipton SA (2016) Elevated glucose and oligomeric β-amyloid disrupt synapses via a common pathway of aberrant protein S-nitrosylation. *Nat Commun* **7**, 10242.

[150] Accardi MV, Daniels BA, Brown PM, Fritschy JM, Tyagarajan SK, Bowie D (2014) Mitochondrial reactive oxygen species regulate the strength of inhibitory GABA-mediated synaptic transmission. *Nat Commun* **5**, 3168.

[151] Chadwick W, Brenneman R, Martin B, Maudsley S (2010) Complex and multi dimensional lipid raft alterations in a murine model of Alzheimer's disease. *Int J Alzheimers Dis* **2010**, 604792.

[152] Koch G, Di Lorenzo F, Bonnì S, Ponzo V, Caltagirone C, Martorana A (2012) Impaired LTP- but not LTD-like cortical plasticity in Alzheimer's disease patients. *J Alzheimers Dis* **31**, 593-599.

[153] Fidzinski P, Korotkova T, Heidenreich M, Maier N, Schuetze S, Kobler O, Zuschratter W, Schmitz D, Ponomarenko A, Jentsch TJ (2015) KCNQ5 K^+ channels control hippocampal synaptic inhibition and fast network oscillations. *Nat Commun* **6**, 6254.

[154] Hong S, Beja-Glasser VF, Nfonoyim BM, Frouin A, Li S, Ramakrishnan S, Merry KM, Shi Q, Rosenthal A, Barres BA, Lemere CA, Selkoe DJ, Stevens B (2016) Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science* **352**, 712-716.

[155] Liu Y, Yoo MJ, Savonenko A, Stirling W, Price DL, Borchelt DR, Mamounas L, Lyons WE, Blue ME, Lee MK (2008) Amyloid pathology is associated with progressive monoaminergic neurodegeneration in a transgenic mouse model of Alzheimer's disease. *J Neurosci* **28**, 13805-13814.

[156] Meltzer CC, Smith G, DeKosky ST, Pollock BG, Mathis CA, Moore RY, Kupfer DJ and Reynolds CF 3rd. (1998) Serotonin in aging, late-life depression, and Alzheimer's disease: The emerging role of functional imaging. *Neuropsychopharmacology* **18**, 407-430.

[157] Zecharia AY, Yu X, Götz T, Ye Z, Carr DR, Wulff P, Bettler B, Vyssotski AL, Brickley SG, Franks NP, Wisden W (2012) GABAergic inhibition of histaminergic neurons regulates active waking but not the sleep-wake switch or propofol-induced loss of consciousness. *J Neurosci* **32**, 13062-13075.

[158] Panula P, Rinne J, Kuokkanen K, Eriksson KS, Sallmen T, Kalimo H and Relja M (1998) Neuronal histamine deficit in Alzheimer's disease. *Neuroscience* **82**, 993-997.

[159] Lussier AL, Weeber EJ, Rebeck GW (2016) Reelin proteolysis affects signaling related to normal synapse function and neurodegeneration. *Front Cell Neurosci* **10**, 75.

[160] Herring A, Donath A, Steiner KM, Widera MP, Hamzehian S, Kanakis D, Kölble K, ElAli A, Hermann DM, Paulus W, Keyvani K (2012) Reelin depletion is an early phenomenon of Alzheimer's pathology. *J Alzheimers Dis* **30**, 963-979.

[161] Cuchillo-Ibañez I, Balmaceda V, Mata-Balaguer T, Lopez-Font I, Sáez-Valero J (2016) Reelin in Alzheimer's disease, increased levels but impaired signaling: When more is less. *J Alzheimers Dis* **52**, 403-416.

[162] Sanz JM, Chiozzi P, Ferrari D, Colaianna M, Idzko M, Falzoni S, Fellin R, Trabace L, Di Virgilio F (2009) Activation of microglia by amyloid beta requires P2X7 receptor expression. *J Immunol* **182**, 4378-4385.

[163] Parvathenani LK, Tertyshnikova S, Greco CR, Roberts SB, Robertson B, Posmantur R (2003) P2X7 mediates superoxide production in primary microglia and is up-regulated in a transgenic mouse model of Alzheimer's disease. *J Biol Chem* **278**, 13309-13317.

[164] Erb L, Cao C, Ajit D, Weisman GA (2015) P2Y receptors in Alzheimer's disease. *Biol Cell* **107**, 1-21.

[165] Fu AK, Hung KW, Yuen MY, Zhou X, Mak DS, Chan IC, Cheung TH, Zhang B, Fu WY, Liew FY, Ip NY (2016) IL-33 ameliorates Alzheimer's disease-like pathology and cognitive decline. *ProcNatl Acad Sci U S A* **113**, E2705-E2713.

[166] Yamamoto S, Wajima T, Hara Y, Nishida M, Mori Y (2007) Transient receptor potential channels in Alzheimer's disease. *Biochim Biophys Acta* **1772**, 958-967.

[167] Martorana A, Mori F, Esposito Z, Kusayanagi H, Monteleone F, Codecá C, Sancesario G, Bernardi G, Koch G (2009) Dopamine modulates cholinergic cortical excitability in Alzheimer's disease patients. *Neuropsychopharmacology* **34**, 2323-2328.

[168] West AE, Greenberg ME (2011) Neuronal activity-regulated gene transcription in synapse development and cognitive function. *Cold Spring Harb Perspect Biol* **3**, 6.

[169] Kulkarni SK, Dhir A (2010) An overview of curcumin in neurological disorders. *Indian J Pharm Sci* **72**, 149-154.
[170] Pohanka M (2014) Inhibitors of acetylcholinesterase and butyrylcholinesterase meet immunity. *Int J Mol Sci* **15**, 9809-9825.
[171] Grossberg GT (2003) Cholinesterase inhibitors for the treatment of Alzheimer's disease: Getting on and staying on. *Curr Ther Res Clin Exp* **64**, 216-235.
[172] Inestrosa NC, Godoy JA, Vargas JY, Arrazola MS, Rios JA, Carvajal FJ, Serrano FG, Farias GG (2013) Nicotine prevents synaptic impairment induced by amyloid-β oligomers through α7-nicotinic acetylcholine receptor activation. *Neuromolecular Med* **15**, 549-569.
[173] Fernández-Ruiz J, Hernández M, Ramos JA (2010) Cannabinoid-dopamine interaction in the pathophysiology and treatment of CNS disorders. *CNS Neurosci Ther* **16**, e72-e91.
[174] Gutierres JM, Carvalho FB, Schetinger MR, Marisco P, Agostinho P, Rodrigues M, Rubin MA, Schmatz R, da Silva CR, de P, Cognato G, Farias JG, Signor C, Morsch VM, Mazzanti CM, Bogo M, Bonan CD, Spanevello R (2014) Anthocyanins restore behavioral and biochemical changes caused by streptozotocin-induced sporadic dementia of Alzheimer's type. *Life Sci* **96**, 7-17.
[175] Wang S, Yu L, Yang H, Li C, Hui Z, Xu Y, Zhu X (2016) Oridonin attenuates synaptic loss and cognitive deficits in an Aβ1-42-induced mouse model of Alzheimer's disease. *PLoS One* **11**, e0151397.
[176] Wang S, Huang R, Zhang S, Wei L, Zhuo L, Wu X, Tang A, Huang Q (2013) Beneficial effects of asiaticoside on cognitive deficits in senescence-accelerated mice. *Fitoterapia* **87**, 69-77.
[177] Patel S, Grizzell JA, Holmes R, Zeitlin R, Solomon R, Sutton TL, Rohani A, Charry LC, Iarkov A, Mori T, Echeverria Moran V (2014) Cotinine halts the advance of Alzheimer's disease-like pathology and associated depressive-like behavior in Tg6799 mice. *Front Aging Neurosci* **6**, 162.
[178] Winblad B (2005) Piracetam: A review of pharmacological properties and clinical uses. *CNS Drug Rev* **11**, 169-182.
[179] Wang DM, Yang YJ, Zhang L, Zhang X, Guan FF, Zhang LF (2013) Naringin enhances CaMKII activity and improves long-term memory in a mouse model of Alzheimer's disease. *Int J Mol Sci* **14**, 5576-5586.
[180] Zhang Z, Liu X, Schroeder JP, Chan CB, Song M, Yu SP, Weinshenker D, Ye K (2014) 7, 8-dihydroxyflavone prevents synaptic loss and memory deficits in a mouse model of Alzheimer's disease. *Neuropsychopharmacology* **39**, 638-650.
[181] Wang X, Blanchard J, Grundke-Iqbal I, Iqbal K (2015) Memantine attenuates Alzheimer's disease-like pathology and cognitive impairment. *PLoS One* **10**, e0145441.
[182] Li J, Deng J, Sheng W, Zuo Z (2012) Metformin attenuates Alzheimer's disease-like neuropathology in obese, leptin-resistant mice. *Pharmacol Biochem Behav* **101**, 564-574.
[183] Casarejos MJ, Perucho J, Gomez A, Muñoz MP, Fernandez-Estevez M, Sagredo O, Fernandez Ruiz J, Guzman M, de Yebenes JG, Mena MA (2013) Natural cannabinoids improve dopamine neurotransmission and tau and amyloid pathology in a mouse model of tauopathy. *J Alzheimers Dis* **35**, 525-539.
[184] Gupta K, Hardingham GE, Chandran S (2013) NMDA receptor-dependent glutamate excitotoxicity in human embryonic stem cell-derived neurons. *Neurosci Lett* **543**, 95-100.
[185] Palygin O, Lalo U, Pankratov Y (2011) Distinct pharmacological and functional properties of NMDA receptors in mouse cortical astrocytes. *Br J Pharmacol* **163**, 1755-1766.
[186] Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, Devidze N, Ho K, Yu GQ, Palop JJ, Mucke L (2012) Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. *Proc Natl Acad Sci U S A* **109**, E2895-E2903.
[187] Lee BY, Ban JY, Seong YH (2005) Chronic stimulation of GABAA receptor with muscimol reduces amyloid beta protein (25-35)-induced neurotoxicity in cultured rat cortical cells. *Neurosci Res* **52**, 347-356.
[188] Marcade M, Bourdin J, Loiseau N, Peillon H, Rayer A, Drouin D, Schweighoffer F, Désiré L (2008) Etazolate, a neuroprotective drug linking GABA (A) receptor pharmacology to amyloid precursor protein processing. *J Neurochem* **106**, 392-404.
[189] Yoshiike Y, Kimura T, Yamashita S, Furudate H, Mizoroki T, Murayama M, Takashima A (2008) GABA (A) receptor-mediated acceleration of aging-aassociated memory decline in APP/PS1 mice and its pharmacological treatment by picrotoxin. *PLoS One* **3**, e3029.
[190] Prickaerts J, van Goethem NP, Chesworth R, Shapiro G, Boess FG, Methfessel C, Reneerkens OA, Flood DG, Hilt D, Gawryl M, Bertrand S, Bertrand D, König G (2012) EVP-6124, a novel and selective α7 nicotinic acetylcholine receptor partial agonist, improves memory performance by potentiating the acetylcholine response of α7 nicotinic acetylcholine receptors. *Neuropharmacology* **62**, 1099-1110.
[191] Paul P, de Belleroche J (2014) The role of D-serine and glycine as co-agonists of NMDA receptors in motor neuron degeneration and amyotrophic lateral sclerosis (ALS). *Front Synaptic Neurosci* **6**, 10.
[192] Paula-Lima AC, Brito-Moreira J, Ferreira ST (2013) Deregulation of excitatory neurotransmission underlying synapse failure in Alzheimer's disease. *J Neurochem* **126**, 191-202.
[193] Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ (2008) Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* **14**, 837-842.
[194] Paula-Lima AC, Louzada PR, De Mello FG, Ferreira ST (2003) Neuroprotection against Abeta and glutamate toxicity by melatonin: Are GABA receptors involved? *Neurotox Res* **5**, 323-327.
[195] Alawdi SH, El-Denshary ES, Safar MM, Eidi H, David MO, Abdel-Wahhab MA (2016) Neuroprotective effect of nanodiamond in Alzheimer's disease rat model: A pivotal role for modulating NF-κB and STAT3 signaling. *Mol Neurobiol*. doi: 10.1007/s12035-016-9762-0
[196] Mohameda AR, Solimana GY, Ismailb CA, Mannaac HF (2015) Neuroprotective role of vitamin D3 in colchicine-induced Alzheimer's disease in rats. *Alexandria J Med* **51**, 127-136.
[197] Singla N, Dhawan DK (2016) Zinc improves cognitive and neuronal dysfunction during aluminium-induced neurodegeneration. *Mol Neurobiol*. doi: 10.1007/s12035-015-9653-9

[198] Li W, Yu J, Liu Y, Huang X, Abumaria N, Zhu Y, Huang X, Xiong W, Ren C, Liu XG, Chui D, Liu G (2013) Elevation of brain magnesium prevents and reverses cognitive deficits and synaptic loss in Alzheimer's disease mouse model. *J Neurosci* **33**, 8423-8441.

[199] Duarte AI, Moreira PI, Oliveira CR (2012) Insulin in central nervous system: More than just a peripheral hormone. *J Aging Res* **2012**, 384017.

[200] Severini C, Petrella C, Calissano P (2016) Substance P and Alzheimer's disease: Emerging novel roles. *Curr Alzheimer Res* **13**, 964-972.

[201] Hegde AN (2010) The ubiquitin-proteasome pathway and synaptic plasticity. *Learn Mem* **17**, 314-327.

[202] Rodrigues EM, Scudder SL, Goo MS, Patrick GN (2016) Aβ-induced synaptic alterations require the E3 ubiquitin ligase Nedd4-1. *J Neurosci* **36**, 1590-1595.

[203] Gerges NZ, Tran IC, Backos DS, Harrell JM, Chinkers M, Pratt WB, Esteban JA (2004) Independent functions of hsp90 in neurotransmitter release and in the continuous synaptic cycling of AMPA receptors. *J Neurosci* **24**, 4758-4766.

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Linking mitochondrial dysfunction, metabolic syndrome and stress signaling in Neurodegeneration☆

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ABSTRACT

Mounting evidence suggests a link between metabolic syndrome (MetS) such as diabetes, obesity, non-alcoholic fatty liver disease in the progression of Alzheimer's disease (AD), Parkinson's disease (PD) and other neurodegenerative diseases (NDDs). For instance, accumulated Aβ oligomer is enhancing neuronal Ca^{2+} release and neural NO where increased NO level in the brain through post translational modification is modulating the level of insulin production. It has been further confirmed that irrespective of origin; brain insulin resistance triggers a cascade of the neurodegeneration phenomenon which can be aggravated by free reactive oxygen species burden, ER stress, metabolic dysfunction, neuorinflammation, reduced cell survival and altered lipid metabolism. Moreover, several studies confirmed that MetS and diabetic sharing common mechanisms in the progression of AD and NDDs where mitochondrial dynamics playing a critical role. Any mutation in mitochondrial DNA, exposure of environmental toxin, high-calorie intake, homeostasis imbalance, glucolipotoxicity is causative factors for mitochondrial dysfunction. These cumulative pleiotropic burdens in mitochondria leads to insulin resistance, increased ROS production; enhanced stress-related enzymes that is directly linked MetS and diabetes in neurodegeneration. Since, the linkup mechanism between mitochondrial dysfunction and disease phenomenon of both MetS and NDDs is quite intriguing, therefore, it is pertinent for the researchers to identify and implement the therapeutic interventions for targeting MetS and NDDs. Herein, we elucidated the pertinent role of MetS induced mitochondrial dysfunction in neurons and their consequences in NDDs. Further, therapeutic potential of well-known biomolecules and chaperones to target altered mitochondria has been comprehensively documented. This article is part of a Special Issue entitled: Oxidative Stress and Mitochondrial Quality in Diabetes/Obesity and Critical Illness Spectrum of Diseases - edited by P. Hemachandra Reddy.

1. Introduction

For the maintenance of energy metabolism and cellular homeostasis mitochondria is an important organelle which is also known as the power house of cells and predominantly required for determining many cellular functions ranging from metabolic to catabolic activities. Mitochondria performs numerous crucial functions within the cell, which include cellular ATP production, Ca^{2+} buffering, regulation of apoptotic process and involvement in the synthesis of key metabolites. Nevertheless, it also acts as a primary source of endogenous reactive oxygen species (ROS) under oxidative stress. Additionally, mitochondria provides most of the ATP for the metabolic and cellular reaction within the cell, which is mainly coupled with electron transport system (ETS) [1,2]. However, research in the past few decades has recognized various factors, such as mutations in mitochondrial DNA and environmental toxins causing homeostatic imbalances, consequently leading to the damage of normal mitochondrial dynamics. Such alterations include altered mitophagy, decelerated ATP production, disturbed Ca^{2+} homeostasis, reduced mitochondrial membrane potential and compromised mitochondrial respiration [3]. Since, the potential mechanistic role played by altered mitochondria and their associated risk factors in MetS and NDDs remain unsettled, and their possible interlinking is still needed to be investigated. This review extensively covers the involvement of mitochondrial dysfunction in both MetS and neuronal dysfunction. Further, implementation of several biomolecules and chaperones for targeting MetS and NDDs induced by mitochondrial dysfunction has also been elaborated.

☆ This article is part of a Special Issue entitled: Oxidative Stress and Mitochondrial Quality in Diabetes/Obesity and Critical Illness Spectrum of Diseases - edited by P. Hemachandra Reddy.

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2. Metabolic syndrome and mitochondrial dysfunction: A complex interplay

Metabolic syndrome (MetS) is a cluster of aberrations of metabolic origin that augments the risk for cardiovascular disease, stroke, morbidity and mortality from type-2 diabetes (T2D) [4]. Characteristic features of these complex pathological aberrations include lipid accumulation, impaired glucose metabolism and hypertension [5]. Furthermore, obesity is also one of the most critical factors that have been linked with metabolic disturbances to facilitate tissue stress and dysfunction [6]. The obese individuals are having a greater risk for prolonged diseases and usually manifest clinical features of MetS including hypertension, insulin resistance (IR), hyperglycemia and systemic markers of chronic low-grade inflammation [7]. The central pathophysiological mechanism behind MetS is IR, which is closely related to mitochondrial dysfunctioning. However, mitochondrial dysfunction is a complicated process, but it is known to be triggered by genetic factors from both mitochondrial and nuclear genome. Additionally, mitochondrial DNA polymorphisms are also associated with the components of MetS [8]. Further, environmental risk factors, for instance, life style (food intake and physical activity), various chemicals and drugs, glucolipotoxicity, and homeostatic imbalances are also responsible for MetS associated with mitochondrial dysfunctioning and IR as well (Fig. 1) [3]. Recently, it has been reported that serum levels of many mitochondrial toxins, for instance, persistent organic pollutants (POPs) are associated with MetS. However, further investigation to reveal its precise mechanism is still unclear [9]. Likewise, deficiency of Co-enzyme Q10 (Co-Q10), an important element of the mitochondrial electron transport chain has been reported in a couple of clinical outcomes, including hypertension, heart failure, obesity, which all together leads to MetS [10]. Increasing evidence suggests that an altered or excessive glucocorticoid secretion and oxidative stress strongly lead to IR associated with MetS [11]. Furthermore, oxidative stress is also a key player in the progression of MetS, which is caused due to the loss of redox homeostasis which leads to pro-inflammatory and profibrotic pathways. These events enhance the destructions in insulin signaling, diminished endothelial-mediated vaso-relaxation and associated renal and cardiovascular risks [12]. Similarly, acute hypoxia in the body is accumulating extracellular and cytosolic irons that damage the cardiovascular tissue which is also a reason of redox homeostatic imbalance that leads to hypertension and progression of metabolic dysregulation [13].

Since, regulation of cellular energy depends on complex signaling networks that act in response to fuel availability and metabolic demands. Any imbalance in the cellular energy network also paves the way for metabolic diseases, including obesity and MetS [14]. Currently, chronic sympathetic nerve activation has been reported to promote

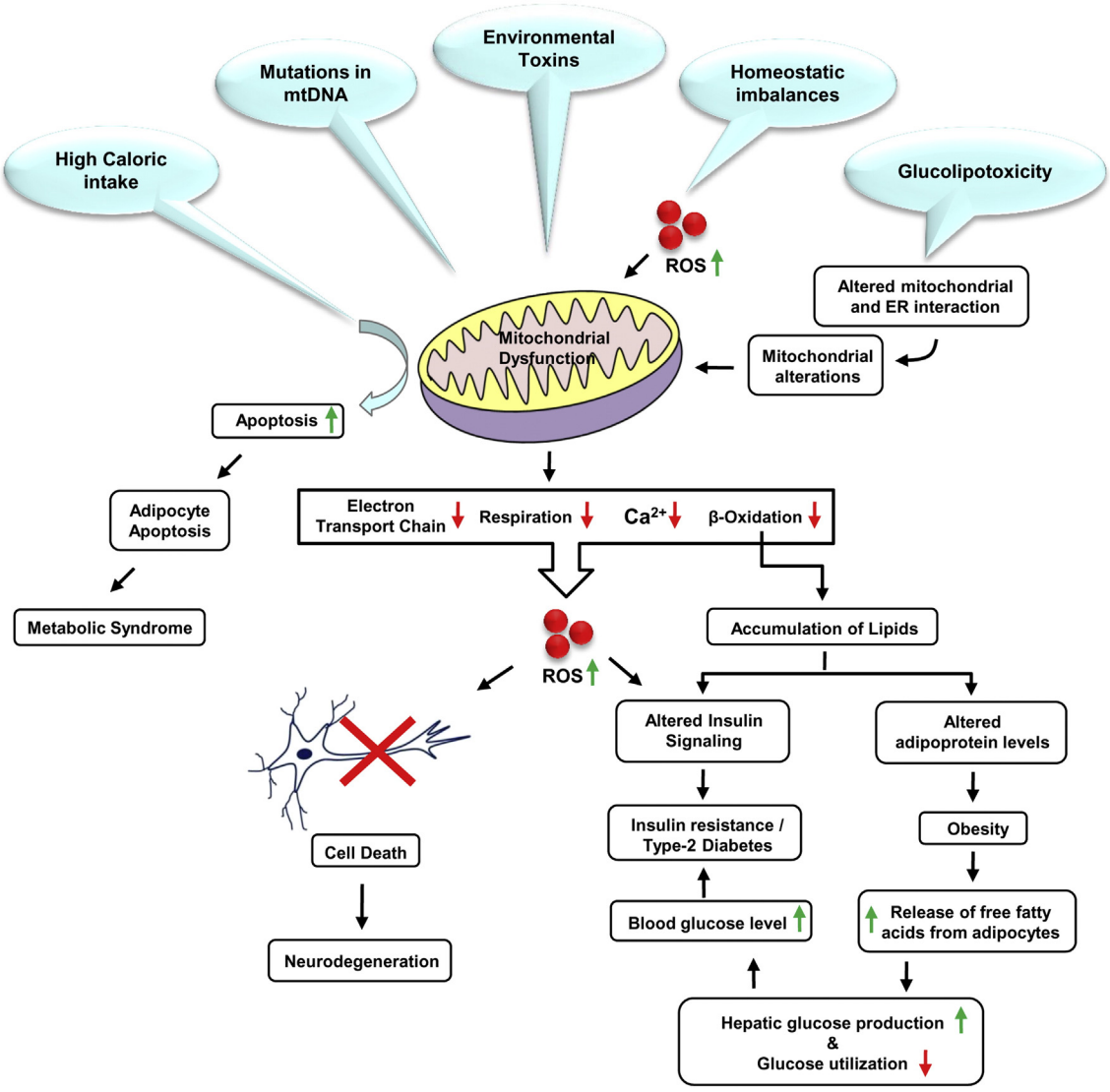

Fig. 1. Schematic illustration showing the association of mitochondrial dysfunction with clinical symptoms of metabolic syndrome as well as its associated factors.

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MetS and raised levels of plasma hormone, including glucocorticoids, noradrenaline, leptin, insulin and angiotensin-II (Ang-II). This sympatho-excitatory response is primarily triggered by the activation of the paraventricular nucleus (PVN) of the hypothalamus which leads to increase in circulating Ang-II levels and further, activates Angiotensin 1 (AT1) receptors in the circumventricular organs (CVOs), mainly in the subfornical organ (SFO). Activation of AT1 receptors thereafter induces an intracellular increase in reactive oxygen species (ROS) that leads to the pathogenesis of MetS [15]. Mutation is another causative factor for MetS progression, for example, inactivation or deficiency of Ataxia telangiectasia mutated (ATM), a cardinal multifunctional serine/threonine protein kinase due to mutation lead to the pathological hallmark for DNA damage, mitochondrial dysfunction, oxidative stress and neurodegeneration [16]. Furthermore, high-fructose/sucrose and high-fat diets have also been reported to induce MetS *in vitro*, through a HepG2 cells' model [17]. Similarly, cells of immune system such as activated leukocytes and dysfunctional metabolic tissues have been found to be associated with the clinical symptoms of obesity and MetS related chronic diseases. Conversely, it is also reported that obesity and MetS is involved in numerous immune related processes, including alterations in leukocyte activity, disruption of lymphoid tissue integrity and the coordination of innate and adaptive immune responses which, all together lead to cause chronic disease progression, altered vaccine efficacy and impaired immunity [18]. Moreover, in various studies, it has been reported that any alteration in the normal functioning of endothelial cells (ECs) due to metabolic imbalances, hyperglycemia, oxidative stress and environmental stimuli caused endothelial inflammation, compromised vascular tone, boosted permeability, advanced cell proliferation and finally cell death. These promising features lead to aggravate cardio metabolic risk factors responsible for MetS and also involved in pathologies like ischemia [19]. In a similar fashion, substantial evidence shows the role of oxidative stress and mitochondrial dysfunction not only in MetS but also in the neurodegenerative disorders (NDDs) associated with aging.

3. Intervening role of stress and mitochondrial dysfunction in neurodegenerative disorders

In order to maintain the normal brain function, the immense amount of energy (ATP) is required. Among all the essential tissues of the body, including, brain, heart and skeletal muscle, brain is the most energy-dependent tissue. The main source of energy which is predominantly required for the normal functioning of the brain is mainly produced by normal activity of mitochondrial cells. In mitochondria, ATP production is mainly associated with an ETS where, the passage of electrons through diverse electron carriers is coupled with the transport of protons from the mitochondrial matrix into the inner-membrane space, and thereafter these protons re-enter into the mitochondrial matrix by dint of ATP synthase for the generation of ATP [1,2]. However, mitochondrial metabolism is also accountable for the ROS production in cells when unpaired electrons leave the ETS and react with molecular oxygen and thus generate superoxide. This superoxide further interacts with lipids, DNA and proteins and plays a crucial role in numerous signaling processes, which is associated with the disease phenomenon of both aging and NDDs [20]. Moreover, ROS can also react with nitric oxide (NO) to produce reactive nitrogen species (RNS) [21]. Furthermore, apart from altered energy and increased ROS production, mitochondrial dysfunction is also responsible for altered Ca^{2+} buffering, apoptosis and senescence that may lead to the progressive decline of post mitotic cells and consequently, results into aging and NDDs [22]. There are numerous NDDs identified so far, that have been reported to be associated with stress and mitochondrial dysfunctioning, which include Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and Amyotrophic Lateral Sclerosis (ALS), and their underlying mechanism is depicted in Fig. 2.

3.1. Alzheimer's disease

AD is an irreversible, progressive brain disorder that is primarily characterized by the presence of extracellular Aβ accumulations as senile plaques and intracellular tau-containing neurofibrillary tangles (NFTs) in the brain, which contribute to the progressive neuronal loss and cognitive decline. A growing body of evidence has reported that mutations in Aβ precursor protein (AβPP), presenilin 1 and 2 are the main causative factor for the early-onset of familial form of AD [23,24]. However, any damage to both the structure and components of mitochondria, increased oxidative stress and diverse other stresses are also accountable for AD pathophysiology. For instance, deficiency in various key enzymes of the tricarboxylic acid cycle (TCA) that are involved in oxidative metabolism, such as pyruvate dehydrogenase complex (PDHC) and α-ketoglutarate dehydrogenase complex (KGDHC) and enzymes involved in the rate-limiting step of the TCA cycle are accountable for reducing molecular oxygen and are responsible for consistent defect in mitochondrial components in AD brain [25]. Likewise, impairment in the functional expression of Peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α), a master regulator of mitochondrial biogenesis is also involved in AD progression [26]. Furthermore, mitochondrial axonal transport is profoundly affected by mutations in Presenilin 1, most likely by affecting phosphorylation of kinesin light chain through effects on GSK3β [27]. Moreover, accumulation of Aβ in the protein import channels of mitochondria has currently been reported in AD brains that are responsible for mitochondrial dysfunction that potentiate neuronal and synaptic stress thereby increasing the severity of disease [28]. Altered cytochrome oxidase activity is another cause of AD, which is associated with altered mitochondrial dynamics [29]. Further, altered Ca^{2+} homeostasis, another causative factor for AD is also reported in AD cybrids made from mtDNA of AD subject [30]. It has been reported that sporadic mtDNA rearrangement and other sporadic mutation in the mtDNA control region is considerably raised in AD brains [31]. Moreover, oxidative stress and mitochondrial dysfunction involved in early onset of AD have been confirmed by using *in vivo* electron paramagnetic resonance (EPR) imaging where methoxycarbamyl-proxyl (MCP) is used as a redox-sensitive probe [32]. Mutations in presenilin (PS1 or PS2) gene have also been reported to cause mitochondrial dysfunction in the familial form of AD. Since, PS mutants cause altered intracellular Ca^{2+} homeostasis by increasing the gating of inositol trisphosphate (IP_3) receptor (IP_3R) Ca^{2+} release channel on the endoplasmic reticulum thereby leading to cause excessive Ca^{2+} release into the cytoplasm. This excessive Ca^{2+} release or altered Ca^{2+} signaling to contribute to the mitochondrial dysfunction and neuronal death [33]. Similarly, glutamate-induced calcium signaling is also accountable for altered mitochondrial dynamics and their associated neuronal death [34]. Recently, white matter degeneration in aging brain has found to be associated with mitochondrial dysfunction, underlying the greatest risk of AD. Moreover, it also causes reduced mitochondrial respiration and increased mitochondrial H_2O_2 productions [35]. In the same way, accumulation of discriminant metabolites such as acylcarnitines has also reported to involve in impaired mitochondrial function [36]. Additionally, toxic Aβ associated with over-expressed voltage-dependent anion channel 1 (VDAC1) has also involved in altered mitochondrial function and apoptosis induction [37]. Growing evidence suggests that AD neurons are having a considerably lower percentage of normal mitochondria with significant changes in their size depicting that a likely change in mitochondrial dynamics is involved in disease progression, while normal neurons contain a significantly higher percentage of the mitochondria with broken cristae [1]. These all findings suggest the involvement of mitochondrial dynamics in AD pathogenesis.

3.2. Parkinson's disease

Parkinson's disease (PD) is one of the most common progressive disorders of the nervous system characterized by rigidity, resting tremor,

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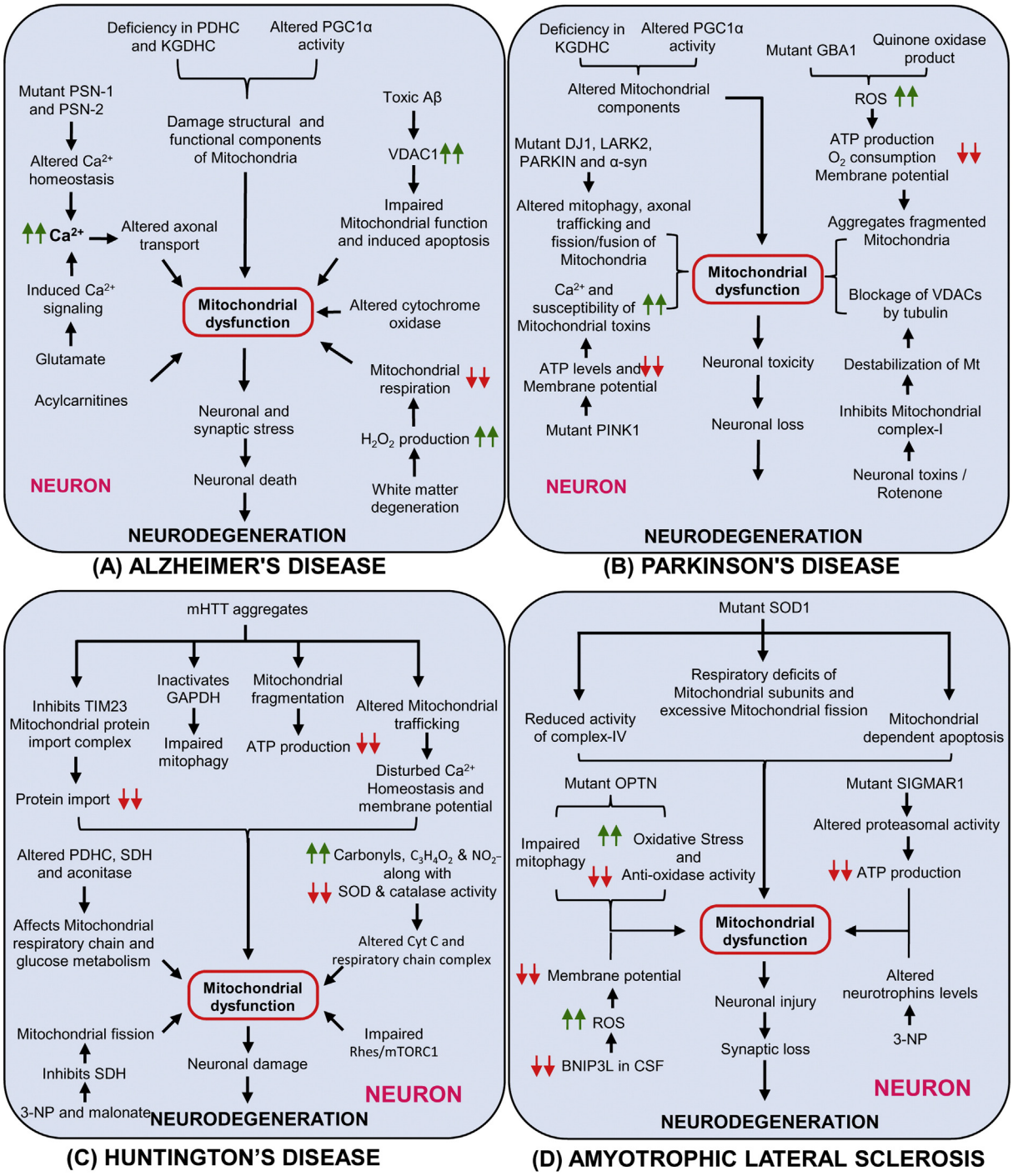

Fig. 2. Molecular mechanism involved in mitochondrial dysfunction mediated pathophysiology of neurodegenerative disorders. PDHC, pyruvate dehydrogenase complex; KGDHC, ketoglutarate dehydrogenase complex; PGC1α, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PSN-1 & PSN-2, Presenilin-1 & Presenilin-2; VDAC1, voltage dependent anion channel 1; GBA1, glucocerebrosidase 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PINK1, PTEN-induced putative kinase 1; TIM23, translocase of the inner membrane 23; SDH, succinate dehydrogenase; 3-NP, 3-Nitropropionic Acid; SOD1, superoxide dismutase; OPTN, optineurin; SIGMAR1, sigma-1 receptor (Sig-1R); BNIP3L, pro-apoptotic mitochondrial proteins; CSF, cerebrospinal fluid.

postural instability and bradykinesia [38]. Its cause remained mysterious, although numerous evidences support mitochondrial dysfunctions and oxidative stress to be associated with the disease pathophysiology. For instance, mitochondrial DNA damage in dopaminergic neurons has been reported as one of the causing factors of striatal dysfunctions in a PD mouse model [39]. However, Glucocerebrosidase 1 (GBA1) gene mutations are identified as another causative factor responsible for perturbing normal mitochondrial function by increasing ROS production and by decreasing oxygen consumption, adenosine triphosphate (ATP) production and membrane potential. GBA1 mutations are also accountable for agglomerating dysfunctional and fragmented mitochondria [40]. Furthermore, quinine oxidation products responsible for ROS production have also been evidenced to be associated with mitochondrial dysfunction and dopamine cytotoxicity [41]. Similarly, the selective loss of alpha-ketoglutarate dehydrogenase complex (KGDHC) and mitochondrial complex I in the nigral neurons is another reason of PD pathogenesis. Such selective loss causes mitochondrial respiratory system failure and lead to cause oxidative damage in neurons.

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These abnormalities can trigger apoptotic cell death as well [42]. Further, the pesticide rotenone which is known to inhibit mitochondrial complex I in PD is also responsible for microtubule (MT) destabilization that contributes to the blockage of mitochondrial voltage-dependent anions channels (VDACs) by tubulin and thus leads to PD [43]. Upcoming research identified hepatic mitochondrial dysfunctions in a rodent model of PD that arise as a result of nigrostriatal degeneration and interference in CNS-liver interaction due to thyroid hormone [44]. Likewise, deficiency in Phosphatase and tensin homolog (PTEN) induced putative kinase 1 causes Ca^{2+} accumulation in mitochondria that lead to both ROS production and intrinsic cell death in PD. It has revealed that mutations in PTE*N*-induced putative kinase 1 (PINK1) lead to a recessive form of PD. Since, PINK1 is responsible for mitochondrial quality control and its partial knock-down provokes numerous mitochondrial dysfunctions, including reduced membrane potential, decreased cellular ATP levels and enhanced susceptibility against mitochondrial toxins [45]. Apart from PINK1, mutations in numerous other PD associated genes have also been reported to be involved in impaired mitochondrial dynamics. Such genes include DJ-1, α-Synuclein, parkin and LRRK2, which caused several defects in mitochondrial dynamics, such as altered fission/fusion, biogenesis, trafficking in retrograde and anterograde directions and impaired mitophagy process [46]. Impairment in the function and expression of PGC-1α, a key regulator of mitochondrial biogenesis is also responsible for PD [47]. Further, mitochondrial transcription termination factor 2 and 4 (MTERF2 and MTERF4) has been identified in 1-methyl-4-phenylpyridinium (MPP+)-induced PD brain that is responsible for mitochondrial dysfunction [48,49]. Recently, SUMOylation, an epigenetic modification has also been found to be involved in mitochondrial dysfunction associated with PD progression [50]. Numerous studies have reported that the brain of PD patients manifests similar cellular and functional changes with signs of reduced mitochondrial function, increased oxidative stress, reduced glucose uptake and augmented peroxidation of cellular membranes [51].

3.3. Huntington's disease

Huntington's disease is a genetic neurodegenerative disorder manifested by emotional imbalance, cognitive decline and loss of movement co-ordination caused due to an autosomal dominant mutation in huntingtin gene (Htt), which is accountable for CAG repeat expansion thereby causing the synthesis of polyglutamine tract [52]. The CAG repeat expansion is mainly caused due to mitochondrial dysfunctions, oxidative and metabolic stress and altered ion-channels. Several evidence reports that any alteration in mitochondrial activity propels the pathogenesis of HD; for instance, mtDNA damage and deletion have been reported in HD brains [53]. Significant defects in the enzymes associated with mitochondrial respiratory chain and glucose metabolism, including pyruvate dehydrogenase complex (PDHC), aconitase and succinate dehydrogenase (SDH) have been reported in HD patients [54]. Furthermore, SDH inhibitions by malonate and 3-nitropropionic acid have also reported to cause excessive mitochondrial fission and neuronal death associated with clinical and pathological features of HD [55]. Recently, an altered activity of PGC1α protein due to mHtt has been observed that resulted in an overall reduction of acetyl-CoA and ATP synthesis, thus contribute to impaired cholesterol metabolism associated with HD [56]. Further, mutant Htt agglomerates associated with mitochondria that directly affect mitochondrial trafficking in axon, calcium handling and mitochondrial membrane potential as well. It is also found to be associated with mitochondrial fragmentation along with reduced ATP levels that mediates neuronal dysfunction and leads to motility deficit [57]. mHtt is also reported to cause altered Ca^{2+} homeostasis associated with mitochondrial dysfunction [58]. Exclusively, mHtt directly inhibits mitochondrial protein import complex TIM23 *in vitro*, signifying that deficient protein import is an early event in HD [59]. Moreover, altered mitophagy process has also been reported in the presence of mHtt. Since, mitophagy is accountable for maintaining the healthy population of functional mitochondria by eliminating defective mitochondria with the help of lysosomal machinery. This process is mainly triggered by glyceraldehyde-3-phosphate dehydrogenase (GAPDH), that gets inactivated by mHtt and thus losses its selective association with damaged mitochondria. Further, abnormal interaction of mitochondrial GAPDH with mHtt impeded GAPDH-mediated mitophagy, contributing to accumulation of damaged mitochondria and increased cell death [60]. Recent studies have reported that mHtt also curtails the maturation of SREBP and up regulates the expression of LXR and LXR-targeted genes (ABCG1, ABCG4, SREBP, ApoE and HMGCoA reductase) which further lowers both the synthesis and transport of cholesterol from astrocytes to neurons via ApoE [56]. Similarly, impaired Rhes/mTORC1 activity underlies numerous metabolic phenotypes including; mitochondrial dysfunction, striatal atrophy, aberrant cholesterol homeostasis and impaired dopamine signaling have also been reported in HD [61]. Furthermore, increased levels of protein carbonyls, malondialdehyde ($C_3H_4O_2$) and nitrite (NO^{2-}) along with reduced Mn-superoxide dismutase (Mn-SOD) and catalase activity is involved in impaired mitochondrial dynamics associated with altered cytochrome C levels and reduced mRNA expression of respiratory chain complexes in HD brain [62]. In addition, caspase-3 and −9 activity coupled with altered expression of apoptotic proteins such as Bim, AIF, Bad and Bax has also been reported to be involved in cognitive impairment leading to HD [63].

3.4. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a chronic motor neurodegenerative disorder, manifested by a substantial collapse of the large pyramidal neurons in the motor cortex, spinal cord and brain stem resulting in fasciculation, gradual muscle weakness, speech deficit and paralysis that eventually leads to neuronal death [64]. However, the precise mechanism for ALS is still unknown, but numerous mechanisms have been identified that is associated with motor neuronal death in ALS. These include mitochondrial dysfunction, metabolic dysfunction, protein aggregation, astrocyte- and microglial toxicity, glutamate-mediated excitotoxicity, oxidative stress and transition-metal-induced toxicity. Mitochondrial defects associated with decreased complex IV activity in the spinal cord and motor neurons have been reported in ALS pathology. Such defects have also been observed in peripheral tissues from ALS brains [65]. In mutant SOD1 transgenic mice of ALS, the aberrancy in mitochondrial functions have been observed, which is associated with respiratory deficits of mitochondrial subunits [66]. Most importantly, increased levels of mitochondrial base deletion (common 5- kb deletion) have also been observed in the skeletal muscle and motor cortex of ALS patients [67]. The presence of mitochondrial morphological abnormalities in animal models of ALS and ALS patients has also been reported by several lines of studies. Previous studies revealed the appearance of heavy mitochondrial conglomerates in neurons of the lumbar spinal cord, and the occurrence of mitochondria agglomerates in the subsarcolemmal region of muscles of ALS patients. Moreover, in ALS mutant SOD1 transgenic mice, dilated and vacuolated mitochondria with unorganized cristae has been observed in the motor neurons lacking any apoptotic features. This in turn suggests that excessive mitochondrial fission is responsible for selective susceptibility of motoneuronal mitochondria to SOD1 mutation [68–70]. Further, mitochondrial dependent cell apoptosis has been reported due to accumulation of mutant SOD1 inside the intermembrane space and deposition of misfolded SOD1 onto the outer mitochondrial membrane, leading to cluster the transport across mitochondrial membranes. Substantial *in vivo* and *in vitro* evidence has also demonstrated that localization of mutant SOD1 in mitochondrial intermembrane space inevitably contributes to impaired mitochondrial functions [71]. Currently, ALS associated optineurin (OPTN) mutants (Q398X and E478G) have been found to be associated with severe mitochondrial dysfunction and neuronal injury due to increased oxidative stress and reduced antioxidase activity

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in SH-SY5Y cells [72]. Similarly, mutations in OPTN have also been reported to associate with the altered mitophagy process in ALS. Mitophagy is responsible for degradation of damaged mitochondria by autophagosomes thereby leading to maintain the normal functioning of mitochondria [73]. Another cause of mitochondrial dysfunctioning in ALS is the administration of 3-nitropropionic acid (3-NP). Systemic administration of 3-NP is sufficient to change neurotrophin levels in the brain, leading to damage striatal neurons [74]. Recently, missense mutation in SIGMAR1 (c.304G > C), which encodes sigma-1 receptor (Sig-1R) has been reported in familial ALS where, mutant Sig-1R(E102Q) protein has found to be linked with reduced mitochondrial ATP production, altered proteasome activity coupled with mitochondrial injury and aggravating ER stress-induced neuronal death in neuro2A cells [75]. Likewise, impairment in the expression levels of fused in sarcoma (FUS) protein has been observed to cause ALS in gain-of-function/loss-of-function manners. Overexpression of FUS protein provokes death in primary cortical neurons and motor neuronal NSC34 cells via the mitochondrial apoptotic pathway which is independent of transactive response DNA-binding protein-43 [76]. Recently, sALS-CSF induced overexpression of the pro-apoptotic mitochondrial protein BNIP3L has also been reported to be associated with induced neurotoxicity, elevated ROS and lowered mitochondrial membrane potential, which collectively contribute to the pathogenesis of ALS [77].

4. How metabolic syndrome is a causative agent for neuronal dysfunction?

Metabolic syndrome, an alliance of medical disorders that greatly increase the risk for developing metabolic and neurodegenerative disorders, has reached epidemic levels in various areas of today's world. In spite of this alarming situation, previous studies have uncovered the root mechanisms of MetS associated with neuronal dysfunction are still limited, therefore, exploring the associated mechanisms behind such an aberrancy has become essentially needed nowadays. A growing body of evidence reported that multiple targets and signaling pathways are involved in metabolic abnormalities/syndromes associated with neurodegeneration (Fig. 3). Meanwhile, recent studies have indicated that MetS is linked with an increased risk of type-2 diabetes (T2D) and cardiovascular disease, which have been found to be associated with AD [78]. It is also ascertained that insulin resistance (IR) is one of the possible reasons for linkup between AD and MetS, since it modulates Aβ aggregation, i.e. the primary constituent of senile plaques. Moreover, insulin also inhibits the activity of glycogen synthase kinase-3β (GSK-3β) and thus tau phosphorylation thereby inhibiting the formation of neurofibrillary tangles (NFTs) in AD [79]. It has been proposed that Ca^{2+} dysregulation is another factor for metabolic alterations of glucose and insulin that establishes the association between

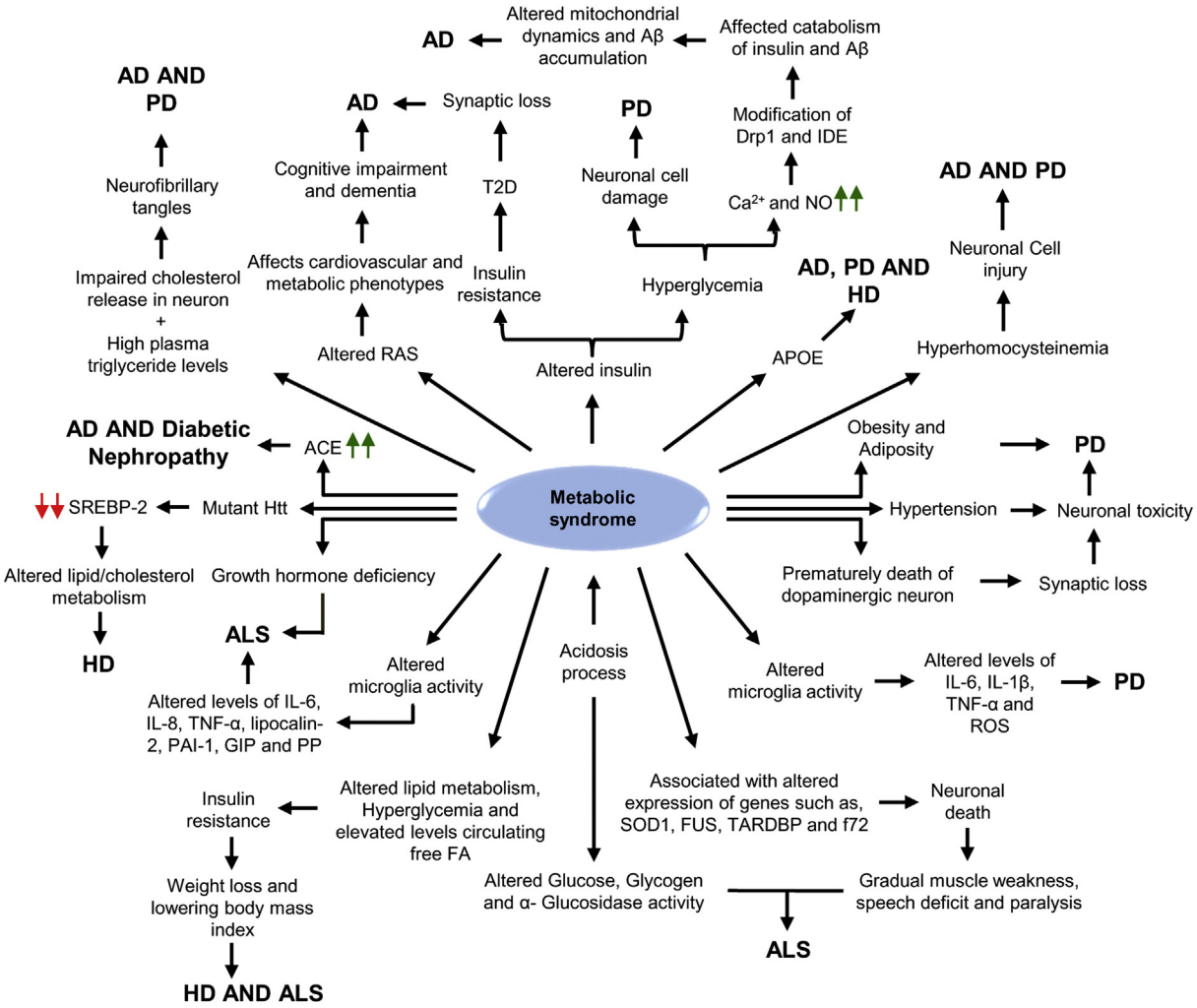

Fig. 3. Interlink between metabolic syndrome and pathophysiology of neurodegenerative disorders. AD, Alzheimer's disease; PD, Parkinson's disease; HD, Huntington's disease; ALS, Amyotrophic lateral sclerosis; RAS, Ranin-angiotensin system; ACE, angiotensin converting enzyme; APOE, apolipoprotein E; NO, nitric oxide; SREBP-2, sterol regulatory element binding protein −2; IL, interlukin; TNF-α, tumor necrosis factor-α; PAI-1, plasminogen activator inhibitor-1 ; GIP, gastric inhibitory peptide; PP, pancreatic polypeptide ; SOD1, superoxide dismutase 1; FUS, fused in sarcoma; TARDBP, transactive response DNA binding protein; ROS, reactive oxygen species.

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T2D and AD [80]. Further, elevated glucose as evidenced in MetS/T2D and Aβ peptide coordinately enhanced neuronal Ca^{2+} and nitric oxide (NO) in an NMDA receptor-dependent manner in AD brain. Thus, elevated NO level in AD causes S-nitrosylation of dynamin-related protein 1 (Drp1) and insulin-degrading enzyme (IDE), thus inhibits insulin and Aβ catabolism and hyperactivating mitochondrial fission machinery. These alterations subsequently result into compromised mitochondrial dynamics and elevated oligomeric Aβ that lead to synaptic loss and dysfunctional synaptic plasticity in cortical and hippocampal neurons [81]. Interestingly, both T2D and AD exhibit similar defects in mitochondria that impair their ability to modulate oxidation in the neuronal cell. Compelling evidence has reported about the involvement of mammalian target of rapamycin (mTOR) signaling axis in cellular senescence and age-related NDDs. Indeed, this signaling is a downstream signal of insulin signaling and regulating insulin signaling by a negative feedback mechanism that shows a valid linkup between MetS and pathogenesis of NDDs. mTOR modulates cell growth and cellular metabolism in response to growth factors, nutrients and cellular energy conditions. However, Loss of mTOR signaling in the brain disrupts multiple downstream signaling associated with glucose metabolism (insulin signaling), energy production, mitochondrial dynamics/function, cell growth and mitophagy/autophagy. These all crucial events thereafter lead to the pathogenesis of NDDs [82,83]. Further, plasma triglycerides and HDL cholesterol is found to be another possible reason for the association between AD and MetS. Since, HDL cholesterol act as a focal transporter of cholesterol in the neuronal cell, and it has been evidenced that any drop in the HDL cholesterol level causes impairment of cholesterol release in neurons thereby leading to the formation of NFTs and senile plaques [84]. Moreover, it is also reported that a low plasma HDL concentration and a high plasma triglyceride concentration cause vascular changes in the brains of AD patients [85]. Not only that, currently, the renin angiotensin system (RAS) which is crucial for fluid and blood pressure regulation has found to be associated with both MetS and AD. At one hand, genetic alterations of the RAS affect cardiovascular and metabolic phenotypes causing hypertension and cerebrovascular diseases while at another hand sustained activation of RAS augments age-related organ injury and results in cognitive impairment and dementia. Similarly, the higher levels of angiotensin-converting enzyme (ACE) confer increased risk of diseases such as congestive heart failure, diabetic nephropathy and AD [86].

A growing number of studies have been completed to confirm that the components of MetS also act as a risk factor for PD etiology. For instance, hyperglycemia exacerbates PD pathogenesis in aging brain via neuronal cell damage. It is also reported that hyperglycemia induced cell death is sustained through oxidative stress and mitochondrial superoxide generation [87]. Some evidence shows that the PD associated genes are also found to be associated with alter the insulin level through ATP-sensitive potassium ion channels (KATP channels) and extracellular kinases signaling to boost glucose level [88]. Like hyperglycemia, hyperhomocysteinemia is a risk factor for endothelial dysfunction has currently been reported in both AD and PD. Many studies have been carried out and revealed that high homocysteine associated with endothelial dysfunction is responsible for hydrogen peroxide release that caused cellular oxidative stress and inflammations mediated *in vitro* cell injury [89]. Further, it has been established that activated microglia is an important key mediator of proinflammatory and neurotoxic factors involved in advancement of PD. These proinflammatory and neurotoxic factors include cytokines such as, interleukin-6 (IL-6) and interleukin-1β (IL-1β), tumor-necrosis factor-α (TNF-α) and reactive oxygen species (ROS) [90]. Moreover, evidence indicates that both ER stress and inflammation coordinate the etiology of PD [91]. Many studies have shown that metabolic stress also causes dopamine-releasing neurons to prematurely die along with the cell death, leading to neuronal toxicity and subsequent onset of Parkinson's symptoms [92]. Further, a growing number of studies revealed that MetS like obesity in middle age individuals also augment the risk of PD and decrease life expectancy [93]. Additionally, adiposity and body mass index along with other risk factors also lead in the advancement of PD [94]. On the contrary, it has been reported that high-fat diet can also lower the risk for advancing PD via decreasing HSP27 phosphorylation, affecting glucose transport and degradation of IκBα in the nigrostriatal system. Moreover, increasing inflammatory molecules, mitochondrial dysfunction, oxidative stress and lipid metabolism has all been shown to occur due to high-fat feeding. Most importantly, hypertension as a component of MetS is less frequent in PD, suggesting the need for exploring the possible link between PD and hypertension [93]. Furthermore, abnormalities in cholesterol homeostasis or lipid metabolism have also been associated with the neurodegenerative process of PD, and such abnormalities are occurred due to metabolic imbalances, injuries, therapeutics and autoimmune diseases [95].

An additional research which focuses on the extremely important aberrancy associated with lipid metabolism or altered cholesterol metabolism has been reported to cause neuronal dysfunction and degeneration in HD. This alteration has been attributed to the pertinent action of mHtt on sterol regulatory element binding proteins that cause a reduction to the level of active sterol regulatory element response protein-2 (SREBP-2) and such depletion are pathogenic to the brain [96]. Moreover, insulin resistance (IR) appears to act as a metabolic stressor, which is critical for advancement in disease progression. An association between HD and reduced insulin secretion has also been suggested by the R6/2 transgenic mouse model characterized by CAG repeats and this pattern of IR such as catabolic state results in weight loss and in lowering body mass index in case of HD patients. Further, this state of IR is manifested by abnormal lipid metabolism, elevated concentrations of circulating free fatty acids and high blood-glucose levels in peripheral tissues and contribute to an increased risk for developing T2D [97]. Currently, the polymorphic variants of apolipoprotein E (APOE) have also been found to be associated with pathophysiology of several NDDs including, AD, PD, HD, vascular dementia, cerebrovascular diseases and lewy body dementia. Since, APOE is a multifunctional protein of a lipoprotein transport system often involved in metabolism of dietary lipids, therefore, any metabolic imbalances due to improper nutrient and fluid supply are accountable for etiology for such abnormalities [98]. Some evidence shows that, glucose intolerance, growth hormone deficiency, IR, hyperlipidemia, reduced body mass index (BMI), both hypometabolism and hypermetabolism are signs of the progressive dysregulated metabolic homeostasis in ALS [99]. Further, altered expression of ALS associated genes, including, C9 or f72, SOD1, FUS and TARDBP in humans and mice are often coupled with metabolic imbalances [100]. It is reported that familial mouse model of ALS goes through a progressive state of acidosis that is linked to several metabolic abnormalities. For instance, the acidosis process causes changes in glycogen, glucose and α-glucosidase activity in the spinal cord of ALS patients and is responsible for lipolysis [101]. Further, the altered levels of IL-6, IL-8, TNF-α, lipocalin-2, plasminogen activator inhibitor-1 (PAI-1), ghrelin, gastric inhibitory peptide (GIP) and pancreatic polypeptide (PP) that is associated with metabolic syndrome, have also been reported in the plasma of ALS patients [102]. It is also revealed that proteins which are responsible for disturbing carbohydrate metabolism are associated with altered glucose homeostasis, that link HD and ALS to T2D. Moreover, numerous other stresses apart from oxidative and ER stress like mitochondrial dysfunctions and microRNAs (miRNA29a/b and miRNA-9) interconnect these NDDs with T2D [103].

5. Therapeutics approaches for targeting mitochondrial dysfunction

Nowadays, treatment against mitochondrial mediated neuronal dysfunctions and metabolic syndromes emerges as a challenging mission for the neurobiologists. Till date, numerous factors associated with multiple targets and signaling pathways of altered mitochondrial dynamics have been reported, which include deficient mitochondrial enzymes, certain chemicals and drugs, genetic factors (mitochondrial

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DNA polymorphisms) and environmental risk factors. Thus, altered mitochondrial dynamics subsequently, causes compromised mitochondrial respiration, limited ATP production, disturbed Ca^{2+} homeostasis and altered mitophagy [104]. In order to overcome such challenges numerous therapeutic strategies are currently being devised to slow down or alleviate the toxicity associated with defective mitochondrial activity. For instance, numerous biomolecules based therapy has been designed to overcome the problem associated with metabolic syndromes and associated neurodegenerative disorders. Similarly, molecular chaperones are also being implicated these days to reverse the disease pathology associated with altered mitochondrial dynamics. Moreover, various other therapeutic approaches have also been formulated recently to slow down the pathophysiology behind defective mitochondria mediated neuronal dysfunction and metabolic syndrome that have been discussed here.

5.1. Biomolecules mediated therapy for altered mitochondrial dynamics

Since, there is not a single cause responsible for such alterations but diverse factors are conducing to such abnormalities. Therefore, it is decisive to implicate different biomolecules in multiple conditions in order to regain the normal function against altered mitochondrial dynamics. For this reason, these days, several biomolecules are being utilized for targeting defective mitochondria and its associated disease phenomenon in NDDs and MetS. For instance, vitamin Co-Q10 supplementation is used during the treatment of obesity, oxidative stress (T2DM) and the inflammatory process in MetS. Co-Q10 served as an antioxidant by acting as a cofactor and activator of mitochondrial uncoupling proteins, responsible for the reduction in ROS production, endothelial dysfunction and hypertension [105,106]. Similarly, numerous other vitamins with antioxidant properties have also been developed to treat MetS induced by oxidative stress and mitochondrial dysfunctions. These vitamins are α-lipoic acid, vitamin E, vitamin C, N-acetylcysteine (NAC) and inducers of the heme oxygenase, which are responsible for reduced ROS production [107]. Further, treatment with antiepileptic drug, Levetiracetam has been found to be associated with ameliorating numerous aspects of mitochondrial dysfunction such as alterations of fission-fusion balance in a cell model for aging and early/late-onset AD [108]. Likewise, another biomolecule Icariin has also benefitted against mitochondrial fragmentation associated with $A\beta_{1-42}$ accumulation in 3 × Tg-AD neurons [109]. Similarly, Salicylic acid has currently reported to provide neuroprotection against mitochondrial dysfunction, oxidative stress and apoptosis in PD brain due to its antioxidant and anti-inflammatory effects on neuronal cell [110]. Further, potential protective effect of Succinobucol against mitochondrial metabolic impairment and oxidative stress induced by 3-NP has been reported in HD brain [111]. Another biomolecule, N-acetyl-l-tryptophan (L-NAT) has been presently reported as an inhibitor of mitochondrial cytochrome c release and thereby acting as a possible neuroprotective agent for ALS [112]. Similarly, many other biomolecules have been identified and implicated so far to target altered mitochondrial dynamics in the brain which have been outlined in Table 1. These biomolecules act by binding to their specific targets and reduce the severity of neurological outcomes associated with altered mitochondrial dynamics.

5.2. Chaperones mediated therapy for altered mitochondrial dynamics

It is another worthwhile proposal besides using distinct biomolecules to target mitochondrial dysfunction. For this purpose, nowadays different chaperones are identified and implicated against such alterations. For instance, HSP60 along with HSP10 has been reported to provide protection against intracellular Aβ aggregation through the maintenance of mitochondrial oxidative phosphorylation and functionality of TCA enzymes. Notably, both alone and combined effect of HSPs such as HSP90, HSP70, HSP60 and HSP10 has also been studied and found to be associated with reduced ROS production, preserved ATP production, reduced cytochrome C release and lowered caspase-9 activity, which all together contribute towards protection against Aβ induced neuronal dysfunction in AD. Further, HSP60 also neutralizes the toxic effect of Aβ associated with altered complex IV activity and thereby maintaining the integrity of ETS [169]. Moreover, HSPs have also been reported to protect against increased oxidative stress and apoptosis caused by mitochondrial dysfunction in PD. For instance, in PC12 cell lines of PD, α-synuclein at nanomolar concentration is associated with increased HSP70 expression that curtails α-synuclein accumulation and toxicity. Since, inducible expression of mutant α-synuclein in PC12 cell lines can result in greater sensitivity to proteasomal alteration, leading to altered mitochondrial dynamics and neuronal cell death [170]. Interestingly, mitochondrial HSP70 is also known to play a crucial role in the mitochondrial import complex and in the mitochondrial biogenesis, which is induced in response to stimuli such as glucose deprivation and oxidative injury [171]. Similarly, mitochondrial HSP90 has also been reported to mitigate α-synuclein toxicity associated with mitochondrial dysfunction. Since, serum levels of HSP90 are raised in the brain of PD patients, and HSP90 is also found in Lewy bodies in this case. However, serum levels of HSP90 have been spotted to be decreased in AD patients and also reported to be downregulated in the temporal lobe of AD brain [171]. Recently, the mitochondrial chaperone protein, tumor necrosis factor receptor associated protein-1 (TRAP1) has also been found to be associated with reduce α-synuclein toxicity [172]. Another important chaperone, HSP27 has been currently reported to target mitochondrial pathway leading to inhibition of apoptosis. HSP27 inhibits apoptosis through blocking the entry of tBID into the mitochondria and curtails second mitochondria-derived activator of caspase (SMAC) and cytochrome C release from mitochondria [170]. Likewise, HSP40/Hdj-1, HSP70/Hsc70 and HSP27 have also been suggested to reduce poly(Q)-mediated cell death either by binding to cytochrome C and thus inhibiting the mitochondrial death pathway or by protecting against ROS [173]. Moreover, HSP40/Hdj-1 has also been proposed to have a protective role against obesity, IR and maintenance of pancreatic β-cell functions by stopping the accumulation of β-cell peptide (human amylin) [174]. Further, overexpression of the molecular chaperone regulator-1 (BAG-1) has also been identified to protect against poly(Q) induced cell death in HD. Interestingly, overexpression of BAG1 also improves the effects of HSP70 by reducing the accumulation of mHtt in cultured cells and consequently, leads to recuperate neurite outgrowth in Htt-transfected PC12 cells [175]. In case of ALS, Macrophage migration inhibitory factor (MIF) as a chaperone has been found to prevent the accumulation of misfolded SOD1 thereby leading to protect against altered mitochondrial dynamics. On the contrary, other chaperones such as HSP70/Hsc70, cyclophilin-A, aB-crystallin and glutathione peroxidase have no effect in blocking SOD1 misplacing onto the mitochondrial outer membrane which consequently, leads to damaged mitochondrial integrity [176]. Recently, reduced expression of HSP60 has exhibited the protective role against mitochondrial damage in ALS because interaction between mitochondrial chaperonin, HSP60 and FUS protein causes its translocation to mitochondria and leads to cell damage [177]. Further, it has been reported that small HSPs, including the HSP27, HSP25 and α-crystallin family also impart neuroprotective and cardioprotective effect against secondary complications of T2D [178]. Nevertheless, more research is required to explore the potential therapeutic role of chaperones against altered mitochondrial dynamics in both NDDs and MetS.

Moreover, potential protective mechanisms of numerous chaperones against altered mitochondrial dynamics in both NDDs and MetS have been summarized in Table 2.

5.3. Emerging therapies for altered mitochondrial dynamics

However, the crucial role of biomolecules and chaperones has been addressed extensively for altered mitochondrial dynamics but there

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Table 1
List of biomolecules, mitigating the effect of mitochondrial dysfunctions in both metabolic syndrome and neurodegenerative disorders.

Biomolecules	Function related to mitochondria	NDDS caused due to mitochondrial dysfunction	References
Peroxiredoxin 5	Prevents Aβ oligomer-induced neuronal cell death by inhibiting ERK-Drp1-mediated mitochondrial fragmentation	AD	[113]
Methazolamide (MTZ)	Protects against Aβ-mediated mitochondrial dysfunction and caspase activation in a model of acute amyloid-mediated toxicity. Moreover, it is proved to be neuroprotective in HD and stroke models	AD and HD	[114]
Melatonin	Improves the ultrastructure of mitochondria in the neurons of the CA1 region and prevents the decrease in the mitochondria-occupied portion of the neuronal volume in AD	AD	[115]
Mori Fructus (ME)	Decreases mitochondria depolarization, Cyt c release from mitochondria and caspase-3 activation induced by Aβ (25-35) toxicity in the mouse hippocampus	AD	[116]
Erythropoietin (EPO)	Alleviates the Aβ-induced mitochondrial dysfunction and apoptosis in brain	AD	[117]
Linagliptin	Attenuates Aβ-induced mitochondrial dysfunction and intracellular ROS production, mediated by the activation of 5' AMP-activated protein kinase (AMPK)-Sirt1 signaling	AD	[118]
Estrogen	Improves the Aβ-induced defects in mitochondria, mediated by mitochondrial signaling cascade involving ERβ, AKAP and Drp1	AD	[119]
Donepezil	Alleviates Aβ-induced mitochondrial dysfunction and reduces mitochondrial Aβ agglomeration in both *in vivo* and *in vitro*, mediated through AMPK/PGC-1α signaling	AD	[120]
Gracilins	Act as antioxidants through mitochondrial targeting and through the induction of Nrf2 translocation	AD	[121]
Orientin (Ori)	Attenuates mitochondrial dysfunction induced by Aβ and subsequently inhibits the mitochondrial apoptotic pathway	AD	[122]
Di-O-demethylcurcumin	Protects SK-N-SH cells against mitochondrial and ER-mediated apoptotic cell death induced by $A\beta_{25-35}$	AD	[123]
Naringin	Restores cognitive deficits in ICV-STZ rat coupled with alleviation of mitochondrial dysfunction directed oxido-nitrosative stress and cytokine release. Further, it also attenuates mitochondrial dysfunction induced by 3-NP through the modulation of Nrf2 signaling pathway in PC12 cells	AD and HD	[124,125]
Paeoniflorin	Attenuates $A\beta_{25-35}$-induced neurotoxicity in PC12 cells through preventing mitochondrial dysfunction	AD	[126]
Xanthoceraside	Ameliorates learning and memory impairment, promote the function of mitochondria, reduce ROS production and inhibit oxidative stress	AD	[127]
l-3-n-Butylphthalide	Attenuates Aβ induced neurotoxicity in neuroblastoma SH-SY5Y cells by regulating mitochondrion-mediated apoptosis and MAPK signaling	AD	[128]
Hesperidin	Alleviates cognitive impairment, mitochondrial dysfunction and oxidative stress in a mouse model of AD	AD	[129]
Nicotinamide and 3-aminobenzamide	Reduces lipid peroxidation, ROS generation and protein oxidation. Moreover, it also ameliorates the mitochondrial reduction capacity against Aβ	AD	[130]
Lycopene	Involves in attenuation of mitochondrial-oxidative damage, memory retention, reduced neuro-inflammation and restoration of BDNF level in $A\beta_{1-42}$ treated rats	AD	[131]
Geniposide	Exerts protective effects on mitochondrial dysfunction in APP/PS1 mice through increasing the mitochondrial membrane potential, suppressing the mitochondrial oxidative damage and activity of cytochrome c oxidase	AD	[132]
Resveratrol (RSV)	Reduces the Aβ burden and contributing to the modulation of autophagy and an increase of mitochondrial biogenesis in mouse brain mediated through increased activation of Sirtuin 1 (Sirt1) and AMP-activated protein kinase (AMPK) pathways. It also protects against diet-induced obesity, improves insulin resistance, induces genes for oxidative phosphorylation, and activates PGC-1α	AD, ALS and MetS	[133,134]
Thiazolidinediones	Activates PPAR-γ to reduce insulin resistance (IR) in T2D	AD and T2D	[133]
Dimebon	An antihistamine drug, inhibit mitochondrial permeability transition pore and rescue neuronal mitochondria from mutant proteins such as Aβ, mHtt and other mitochondrial toxic insults	AD and HD	[133]
Quercetin	Ameliorates mitochondrial dysfunction by restoration of mitochondrial membrane potential and maintain ATP levels in the mitochondria	AD	[135]
Bajijiasu	Enhances antioxidant capacity, energy metabolism and prevent free radical damage. Moreover, it also inhibits neuronal apoptosis	AD	[136]
Taurine	Attenuates Aβ-induced mitochondrial dysfunction by activating of SIRT1 in SK-N-SH cells	AD	[137]
SS31	A small molecule antioxidant peptide attenuates Aβ elevation, mitochondrial/synaptic deterioration and cognitive deficit in SAMP8 mice	AD	[138]
STX	A novel membrane estrogen receptor ligand, attenuates mitochondrial dysfunction, cell death, dendritic simplification and synaptic loss induced by Aβ	AD	[139]
MitoQ	Reduces oxidative stress, free radicals and Ca (2+)-burden in mitochondria that causes decreased ATP production. Further, altered Mitochondrial dynamic and trafficking are also targeted by MitoQ	AD and MetS	[140,141]
Oryzanols, Tocopherols and Tocotrienols	Elevates ATP production and respiratory rates as well as PGC1α protein levels in PC12APPsw cells, thereby ameliorating the altered mitochondrial function	AD	[142]
Astragaloside IV	Augments the viability of neuronal cells, reduces apoptosis and the generation of intracellular ROS. Moreover, it also reduces mitochondrial superoxide in the presence of $A\beta_{1-42}$	AD	[143]
Asiatic acid (AA)	Protects against glutamate toxicity, rotenone-induced mitochondrial dysfunction and oxidative stress-mediated apoptosis in differentiated SH-SY5Y cells	PD	[144]
Ghrelin	Protects dopaminergic neurons against rotenone via reinstating mitochondrial dysfunction and inhibiting mitochondrial dependent apoptosis	PD	[145]
Ursodeoxycholic acid (UDCA)	Exerts a beneficial effect on mitochondrial dysfunction and neuronal dysfunction *in vivo*	PD	[146]
Curcumin	In order to protect against apoptosis induced by mutation of α-synuclein it stabilizes mitochondrial membrane potential and helps in opening of mito-KATP channel	PD	[147]
Crocin	Protects PC12 cells against MPP (+)-induced injury through inhibition of ER stress and mitochondrial dysfunction	PD	[148]
Allicin	Inhibits ROS production, reduces lipid peroxidation linked to suppressed mitochondrial dysfunction and promotes the mitochondrial Ca^{2+} buffering capacity	PD	[149]
5-((methylamino)methyl)-8-hydroxyquinoline	Protects against mitochondrial-induced oxidative damage and neuronal death	PD	[150]
Nesfatin-1	Attenuates rotenone-induced mitochondrial membrane potential collapse, ROS production and the subsequent caspase-3 activation and restore the function of mitochondrial respiratory chain complex I	PD	[151]
Folic acid	Alleviates the behavioral defects, oxidative stress, and represses mitochondrial dysfunction as well	PD	[152]
Lutein	Protects dopaminergic cells against MPTP-induced apoptotic death and motor dysfunction by improving mitochondrial dysfunction and oxidative stress. Further, it is also having a potential protective effect against mitochondrial metabolic impairment and oxidative stress induced by 3-NP	PD and HD	[153,154]

(continued on next page)

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Table 1 (*continued*)

Biomolecules	Function related to mitochondria	NDDS caused due to mitochondrial dysfunction	References
Silibinin	Protects dopaminergic cells against MPTP-induced apoptotic death and motor dysfunction by improving mitochondrial membrane disruption and oxidative stress	PD	[155]
Myricitrin	Alleviates MPP^+-induced mitochondrial dysfunction and increases cell viability in a DJ-1-dependent manner in SN4741 cells	PD	[156]
Amitriptyline (AMI)	Promotes mitochondrial integrity and functionality and also responsible for reducing mHtt aggregation and potentiation of the BDNF-TrkB signaling system	HD	[157]
Agomelatine and Vanillin	Both show potential protective effect against mitochondrial metabolic impairment and oxidative stress induced by 3-NP	HD	[158]
Trichostatin A or sodium butyrate	HDAC inhibitors, reduces striatal atrophy and motor deficits, involve in amelioration of mitochondrial physiology	HD	[159]
Escitalopram	Shows potential neuroprotection against behavioural, mitochondrial and oxidative dysfunction induced by 3-NP	HD	[160]
Paliperidone	Attenuates quinolinic acid (QA)-induced mitochondrial dysfunction	HD	[161]
Rosiglitazone	Synergizes the neuroprotective effects of valproic acid against QA-induced neurotoxicity by targeting PPARγ and HDAC signaling cascade	HD	[162]
Vinpocetine, Nicorandil and Tetrabenazine	Treatment with these drugs reduced brain striatum oxidative and nitrosative stress, acetylcholinesterase activity, inflammation and mitochondrial dysfunctions induced by 3-NP	HD	[163]
Caffeine	Shows neuroprotective effect against QA induced mitochondrial dysfunction and oxidative stress	HD	[164]
N-acetylcysteine (NAC)	Reduces both excitotoxicity and oxidative stress through its actions on glutamate reuptake and antioxidant capacity. Moreover, it also slows the onset of motor deficits in the R6/1 model of HD by improving mitochondrial dysfunction	HD	[165]
Melatonin	Shows Neuroprotective effect against mitochondrial dysfunction induced by oxidative stress	PD, AD and HD	[166]
Dichloroacetate	Inhibits pyruvate dehydrogenase kinase 4 activity to improve mitochondrial dysfunction and ameliorating muscle denervation	ALS	[167]
Riluzole	Reduces glutamate-induced excitotoxicity to improve mitochondrial dysfunction, oxidative stress and protein aggregation	ALS	[168]

are numerous other emerging therapeutic strategies that have been identified currently to target such alterations. For instance, a neuroprotective effect of Lithium has been reported via modulation of mitochondrial dysfunction, which is likely achieved by impeding GSK-3 and inositol-145 triphosphate (IP_3) in AD [183]. Similarly, Acupuncture combined with moxibustion has benefited the hippocampal neurons in AD rats presumably via the regulation of altered mitochondrial dynamics (imbalance between fission and fusion proteins) and improvement in the mitochondrial damage in the hippocampus [184]. Further, GAPDH-siRNA treatment has also benefited against Aβ induced mitochondrial dysfunction in AD [185]. Not only that, Cocaine- and amphetamine-regulated transcript (Exogenous CART treatment) in APP/PS1 mice has obstructed depolarization of the mitochondrial membrane and provoke mitochondrial complex, I and II activities, thereby leading to an increase in ATP levels. Further, CART treatment also reduces ROS and 4-HNE level that mitigate oxidative DNA damage in AD brain [186]. In case of PD, MicroRNA-7 (miR-7) has been found to elicit a protective role in cellular models of PD. Moreover, miR-7 overexpression inhibits mitochondrial depolarization, mitochondrial fragmentation, cytochrome C release, ROS production and release of mitochondrial Ca^{2+} in response to MPP+ in SH-SY5Y cells. Similarly knockdown of VDAC1 also led to a decrease in intracellular ROS production and subsequent cellular protection against MPP+. Thus, the protective effect of miR-7 is partly influenced through provoking mitochondrial function by targeting VDAC1 expression [187]. Further, a Parkin-AMPK alliance has recently been reported to be involved in neuroprotection against mitochondrial dysfunction. Since, Parkin along with energy sensor AMP-activated protein kinase (AMPK) critically involved in all stages of the mitochondrial life cycle (i.e., from biogenesis to its exit from the cell via mitophagy), advocating that these two

Table 2
List of chaperones, mitigating the effect of mitochondrial dysfunctions in both metabolic syndrome and neurodegenerative disorders.

Chaperones	Molecular mechanisms associated with mitochondria	Principle phenotypes	References
HSP60 and HSP10	Provides protection against intracellular Aβ aggregation through the maintenance of mitochondrial oxidative phosphorylation and functionality of TCA enzymes	AD	[169]
HSP70	Hsp70 overexpression protect neuronal cells against proteolytic and mitochondrial stress in a manner which is similar to that brought about by parkin overexpression	PD	[179]
HSP70 and HSP90	Both HSP70 and mitochondrial HSP90 mitigate the toxic effect of α-synuclein associated with mitochondrial dysfunction. Further, mitochondrial HSP70 is also known to play a crucial role in the mitochondrial import complex and in mitochondrial biogenesis	PD	[170,171]
Mortalin	Mitochondrial chaperone mortalin maintains mitochondrial homeostasis through mitochondrial protein quality control	PD	[180]
TRAP1	TRAP1 mitigates the toxic effect of α-synuclein associated with mitochondrial dysfunction. Moreover, it also works downstream of Pink1 and in parallel with parkin in Drosophila, and that increasing its function, which lead to improve mitochondrial dysfunction	PD	[172,181]
HSP40/Hdj-1, HSP70/Hsc70 and HSP27	Reduces poly(Q)-mediated cell death either by binding to cytochrome-c and thus inhibiting the mitochondrial death pathway or by protecting against ROS	HD	[173]
HSP40/Hdj-1	It shows protective role against obesity, IR and maintenance of pancreatic β-cell function by stopping the agglomeration of β-cell peptide	obesity and insulin resistance (MetS)	[174]
F(0)F(1)-ATP synthase alpha (HSP60 family member)	Suppresses Htt poly(Q) aggregation and toxicity in transfected SH-SY5Y cell	HD	[182]
BAG-1	Protects against poly(Q) induced cell death in HD. Interestingly, overexpression of BAG1 also improves the effects of HSP70 by reducing accumulation of mHtt in cultured cells and consequently lead to improve neurite outgrowth in Htt-transfected PC12 cells	HD	[175]
MIF	Protects against altered mitochondrial dynamics by preventing the accumulation of misfolded SOD1	ALS	[176]
HSP60	Reduced expression of HSP60 shows protective role against mitochondrial damage	ALS	[177]
HSP27, HSP25 and α-crystallin	Impart their potential neuroprotective and cardioprotective effect against secondary complications of T2D	T2D (MetS)	[178]

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proteins might act in a functionally convergent manner to preserve the quality of cellular mitochondria [188]. Recently, the gene transfer-based experiments have led to the finding of potential therapeutic targets that could ameliorate mitochondria in HD (such as mitochondrial complex II or the PGC-1α). However, preclinical studies are yet required to deduce whether it is feasible to regulate these systems *in vivo* or not [159]. Further, using RNA interference (RNAi) and the p53 inhibitor pifithrin-α is another strategy to mitigate the mitochondrial dysfunction in HD [159]. Recently, Lentiviral vector delivery of antioxidant gene such as PRDX3/NRF2 has been found to be an important approach for targeting mitochondrial dysfunction in NSC34 cells expressing the human $SOD1^{G93A}$ in ALS [189]. Similarly, stem cell therapy has identified as another possible therapeutic approach for targeting altered mitochondria dynamics in ALS [190]. Additionally, lifestyle modification, such as exercise and diet has been currently reported to decrease the risk for developing T2D, whilst physical activity has found to be associated with improve glucose tolerance. Indeed, exercise helps in improving ETS, increasing mitochondrial biogenesis through PGC-1α activity and improving sensitivity to insulin. Moreover, it has also been observed to be involved in activating AMPK, which ameliorates both glucose and fat oxidation [191,192]. Nonetheless, more approaches are needed to be devised soon for effective treatments against both MetS and NDDs induced by altered mitochondrial dynamics.

5.4. Metabolic compounds mediated attenuation in neurodegeneration: current therapies

Albeit, the involved therapeutics for treatment of both MetS and NDDs have been discussed extensively, but there are numerous metabolic compounds have been currently identified, that have been used to attenuate the pathological atmosphere of both MetS and NDDs. For instance, increasing brain insulin levels have been reported to ameliorate cognitive function and reduce cognitive decline. Further, increased insulin signaling inhibits the kinases activity associated with tau hyperphosphorylation and also triggers downstream signaling crucial for neuronal plasticity. On the contrary, any malfunctioning in this signaling cascade or declines in insulin levels lead to the pathogenesis of both MetS and NDDs [193]. Similarly, nowadays both metformin (*anti*-diabetic drug) and lovastatin (cholesterol lowering drug) are being used in treating Multiple Sclerosis (MS). Treatment with these drugs in combination attenuates demyelination and axonal loss as evident from decreased levels of myelin and neurofilament proteins in the spinal cords of autoimmune encephalomyelitis (EAE) animals [194]. Further, separately and synergistically administration of metformin and thymoquinone has also been shown the protective role against ethanol-induced neuronal apoptosis in primary rat cortical neurons. Since, ethanol exposure significantly reduces mitochondrial membrane potential and increases the levels of free cytosolic Ca^{2+}, which further stimulates the release of cytochrome C from mitochondria thereby causing neurotoxicity [195]. Likewise, treatment with fat-derived hormone leptin has currently been shown to minimize neuronal damage induced by both neurotoxins and pro-apoptotic factors. It also protects against obesity and MetS via regulation of glucose homeostasis and energy intake. Leptin administration has also been reported to modulate synaptic plasticity via increasing *N*-Methyl-*D*-aspartate (NMDA) receptor function in AD. Further, neuroprotective roles for leptin have been identified in experimental models of stroke where leptin administration protects against both middle cerebral artery occlusion and oxygen-glucose deprivation. Similarly, it also shows protective role in both *in vitro* and *in vivo* models of PD [196,197]. Recently, the protective role of another *anti*-diabetes drug pioglitazone (actos) has been reported in case of AD via inhibiting the Cdk5 activity. More importantly, pioglitazone treatment also corrects impaired long-term potentiation (LTP) induced by Aβ exposure [198]. Additionally, pioglitazone administration is also found to ameliorate cognitive performance in a rodent dementia model induced by intracerebroventricular (ICV) injection of streptozotocin. Similar to pioglitazone, numerous other *anti*-diabetes drugs including, rosiglitazone (avandia) and troglitazone (rezulin) which are also known as TZDs have also been involved in delaying of neurodegenerative process. Since, these drugs are having *anti*-inflammatory, anti-amyloidogenic and insulin sensitizing role as well. Troglitazone are believed to have their connection in rescuing memory loss and decreasing plasma $A\beta_{40-42}$ levels but the exact mechanisms are still needs to be investigated further. Similarly, Rosiglitazone is reported to attenuate neuronal IR induced by Aβ oligomers. It is also reported to ameliorate cognitive function in mild to moderate AD patients. Moreover, chronic administration of TZDs, in general has no effect on risk of developing AD [80]. Despite these all findings, there are huge gaps which are still required to be investigated in order to identify the proper treatments for both MetS and NDDs.

6. Conclusion

In this review, we have addressed the pertinent role of altered mitochondrial dynamics in the pathogenesis of MetS and NDDs. Moreover, the intricate interplay between mitochondrial dysfunction and disease phenomenon of MetS has been elucidated in order to identify the linkup between MetS and NDDs. Furthermore, an interlink between MetS viz. obesity, hypertension, cardiovascular risk factors and T2D in the exacerbation of NDDs has also been extensively shed light on. The disease pathology of AD, PD, HD and ALS has also been addressed with respect to altered mitochondrial dynamics. The environmental toxins, high caloric intake, homeostatic imbalances and mutations in mtDNA are causatives for mitochondrial dysfunction, which consequently leads to decelerate ATP production, altered mitophagy, disturbed Ca^{2+} homeostasis, reduced mitochondrial membrane potential and mitochondrial respiration. These pathological changes are amenable for perturbations in the normal physiological processes and brain homeostasis thereby, resulting into fatal complications linked with neuronal dysfunction and metabolic syndromes. Additionally, in order to target the malfunctioning mitochondria or reverse the chronic effects of altered mitochondrial dynamics, numerous biomolecules and chaperones mediated therapeutic strategies have also been discussed to obviate the disease symptoms of both MetS and NDDs. Further, it is reasonable to enunciate that the current knowledge's on the involvement of altered mitochondrial dynamics in the pathogenesis of both MetS and NDDs are still at an elementary stage. However, in light of its significant advancement from both clinical research and therapeutic application perspectives, we look ahead major research efforts being drawn to this field and more approaches being formulated soon. We also presume that finally these findings will be translated into effective and novel treatments against both MetS and NDDs induced by mitochondrial dysfunctions.

Transparency Document

The Transparency document associated with this article can be found, in online version.

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References

[1] B. Su, X. Wang, L. Zheng, G. Perry, M.A. Smith, X. Zhu, Abnormal mitochondrial dynamics and neurodegenerative diseases, Biochim. Biophys. Acta 1802 (2010) 135–142.
[2] I.G. Onyango, J. Lu, M. Rodova, E. Lezi, A.B. Crafter, R.H. Swerdlow, Regulation of neuron mitochondrial biogenesis and relevance to brain health, Biochim. Biophys. Acta 1802 (2010) 228–234.
[3] S.R. Subramaniam, M.F. Chesselet, Mitochondrial dysfunction and oxidative stress in Parkinson's disease, Prog. Neurobiol. 106-107 (2013) 17–32.

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[4] L.S. Sperling, J.I. Mechanick, I.J. Neeland, C.J. Herrick, J.P. Després, C.E. Ndumele, K. Vijayaraghavan, Y. Handelsman, G.A. Puckrein, M.R. Araneta, Q.K. Blum, K.K. Collins, S. Cook, N.V. Dhurandhar, D.L. Dixon, B.M. Egan, D.P. Ferdinand, L.M. Herman, S.E. Hessen, T.A. Jacobson, R.R. Pate, R.E. Ratner, E.A. Brinton, A.D. Forker, L.L. Ritzenthaler, S.M. Grundy, The ardioMetabolic Health Alliance: working toward a New care model for the metabolic syndrome, J. Am. Coll. Cardiol. 66 (2015) 1050–1067.
[5] L. Lee, R.A. Sanders, Metabolic syndrome, Pediatr. Rev. 33 (2012) 459–466.
[6] P. Holvoet, Stress in obesity and associated metabolic and cardiovascular disorders, Scientifica (Cairo) (2012) 205027.
[7] C.K. Roberts, A.L. Hevener, R.J. Barnard, Metabolic syndrome and insulin resistance: underlying causes and modification by exercise training, Compr. Physiol. 3 (2013) 1–58.
[8] H.K. Lee, Y.M. Cho, S.H. Kwak, S. Lim, K.S. Park, E.B. Shim, Mitochondrial dysfunction and metabolic syndrome-looking for environmental factors, Biochim. Biophys. Acta 3 (2010) 282–289.
[9] H.K. Lee, E.B. Shim, Extension of the mitochondria dysfunction hypothesis of metabolic syndrome to atherosclerosis with emphasis on the endocrine-disrupting chemicals and biophysical laws, J. Diabetes Investig. 4 (2013) 19–33.
[10] S. Tangvarasittichai, Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus, World J. Diabetes 6 (2015) 456–480.
[11] J.W. Eriksson, Metabolic stress in insulin's target cells leads to ROS accumulation - a hypothetical common pathway causing insulin resistance, FEBS Lett. 581 (2007) 3734–3742.
[12] M.R. Hayden, S.C. Tyagi, Intimal redox stress: accelerated atherosclerosis in metabolic syndrome and type 2 diabetes mellitus, Atheroscleropathy, Cardiovasc. Diabetol. 27 (2002) (1:3).
[13] E. Gammella, S. Recalcati, I. Rybinska, P. Buratti, G. Cairo, Iron-induced damage in cardiomyopathy: oxidative-dependent and independent mechanisms, Oxidative Med. Cell. Longev. (2015) 230182.
[14] U.J. Jung, M.S. Choi, Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease, Int. J. Mol. Sci. 15 (2014) 6184–6223.
[15] J.C. Cruz, A.F. Flôr, M.S. França-Silva, C.M. Balarini, V.A. Braga, Reactive oxygen species in the paraventricular nucleus of the hypothalamus alter sympathetic activity during metabolic syndrome, Front. Physiol. 6 (2015) 384.
[16] A. Guleria, S. Chandna, ATM kinase: much more than a DNA damage responsive protein, DNA Repair (Amst) 39 (2016) 1–20.
[17] L. Zhao, X. Guo, O. Wang, H. Zhang, Y. Wang, F. Zhou, J. Liu, B. Ji, Fructose and glucose combined with free fatty acids induce metabolic disorders in HepG2 cell: a new model to study the impacts of high-fructose/sucrose and high-fat diets in vitro, Mol. Nutr. Food Res. 60 (2016) 909–921.
[18] C.J. Andersen, K.E. Murphy, M.L. Fernandez, Impact of obesity and metabolic syndrome on immunity, Adv. Nutr. 7 (2016) 66–75.
[19] H.A. Hadi, J.A. Suwaidi, Endothelial dysfunction in diabetes mellitus, Vasc. Health Risk Manag. 3 (2007) 853–876.
[20] B. Uttara, A.V. Singh, P. Zamboni, R.T. Mahajan, Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options, Curr. Neuropharmacol. 1 (2009) 65–74.
[21] R.P. Patel, J.M.c. Andrew, H. Sellak, C.R. White, H. Jo, B.A. Freeman, V.M. Darley-Usmar, Biological aspects of reactive nitrogen species, Biochim. Biophys. Acta 1411 (1999) 385–400.
[22] A.Y. Seo, A.M. Joseph, D. Dutta, J.C. Hwang, J.P. Aris, C. Leeuwenburgh, New insights into the role of mitochondria in aging: mitochondrial dynamics and more, J. Cell Sci. 123 (2010) 2533–2542.
[23] P. Kumar, N.K. Jha, S.K. Jha, K. Ramani, R.K. Ambasta, Tau phosphorylation, molecular chaperones, and ubiquitin E3 ligase: clinical relevance in Alzheimer's disease, J. Alzheimers Dis. 43 (2015) 341–361.
[24] N.K. Jha, S.K. Jha, D. Kumar, N. Kejriwal, R. Sharma, R.K. Ambasta, P. Kumar, Impact of insulin degrading enzyme and neprilysin in Alzheimer's disease biology: characterization of putative cognates for therapeutic applications, J. Alzheimers Dis. 48 (2015) 891–917.
[25] P.I. Moreira, C. Carvalho, X. Zhu, M.A. Smith, G. Perry, Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology, Biochim. Biophys. Acta 1802 (2010) 2–10.
[26] G. Sweeney, J. Song, The association between PGC-1α and Alzheimer's disease, Anat. Cell Biol. 49 (2016) 1–6.
[27] K. Dolma, G.J. Iacobucci, K. Hong Zheng, J. Shandilya, E. Toska, J.A. White 2nd, E. Spina, S. Gunawardena, Presenilin influences glycogen synthase kinase-3 β (GSK-3β) for kinesin-1 and dynein function during axonal transport, Hum. Mol. Genet. 23 (2014) 1121–1133.
[28] L. Pagani, A. Eckert, Amyloid-Beta interaction with mitochondria, Int. J. Alzheimers Dis. (2011) 925050.
[29] S. Sekar, J. McDonald, L. Cuyugan, J. Aldrich, A. Kurdoglu, J. Adkins, G. Serrano, T.G. Beach, D.W. Craig, J. Valla, E.M. Reiman, W.S. Liang, Alzheimer's disease is associated with altered expression of genes involved in immune response and mitochondrial processes in astrocytes, Neurobiol. Aging 36 (2015) 583–591.
[30] S.M. Khan, D.S. Cassarino, N.N. Abramova, P.M. Keeney, M.K. Borland, P.A. Trimmer, C.T. Krebs, J.C. Bennett, J.K. Parks, R.H. Swerdlow, W.D. Parker Jr., J.P. Bennett Jr., Alzheimer's disease cybrids replicate beta-amyloid abnormalities through cell death pathways, Ann. Neurol. 48 (2000) 148–155.
[31] K. Hirai, G. Aliev, A. Nunomura, H. Fujioka, R.L. Russell, C.S. Atwood, A.B. Johnson, Y. Kress, H.V. Vinters, M. Tabaton, S. Shimohama, A.D. Cash, S.L. Siedlak, P.L. Harris, P.K. Jones, R.B. Petersen, G. Perry, M.A. Smith, Mitochondrial abnormalities in Alzheimer's disease, J. Neurosci. 9 (2001) 3017–3023.
[32] D. Fang, Z. Zhang, H. Li, Q. Yu, J.T. Douglas, A. Bratasz, P. Kuppusamy, S.S. Yan, Increased electron paramagnetic resonance signal correlates with mitochondrial dysfunction and oxidative stress in an Alzheimer's disease mouse brain, J. Alzheimers Dis. 51 (2016) 80.
[33] P. Mao, P.H. Reddy, Aging and amyloid beta-induced oxidative DNA damage and mitochondrial dysfunction in Alzheimer's disease: implications for early intervention and therapeutics, Biochim. Biophys. Acta 1812 (2011) 1359–1370.
[34] H. Prentice, J.P. Modi, J.Y. Wu, Mechanisms of neuronal protection against excitotoxicity, endoplasmic reticulum stress, and mitochondrial dysfunction in stroke and neurodegenerative diseases, Oxidative Med. Cell. Longev. (2015) 964518.
[35] L.P. Klosinski, J. Yao, F. Yin, A.N. Fonteh, M.G. Harrington, T.A. Christensen, E. Trushina, R.D. Brinton, White matter lipids as a ketogenic fuel supply in aging female brain: implications for Alzheimer's disease, EBioMedicine 2 (2015) 1888–1904.
[36] M.G. Chooneman, F.M. Vaz, S.M. Houten, M.R. Soeters, Acylcarnitines: reflecting or inflicting insulin resistance? Diabetes 62 (2013) 1–8.
[37] P.H. Reddy, Is the mitochondrial outermembrane protein VDAC1 therapeutic target for Alzheimer's disease? Biochim. Biophys. Acta 1832 (2013) 67–75.
[38] P. Kumar, D. Kumar, S.K. Jha, N.K. Jha, R.K. Ambasta, Ion channels in neurological disorders, Adv. Protein Chem. Struct. Biol. 103 (2016) 97–136.
[39] A.M. Pickrell, M. Pinto, A. Hida, C.T. Moraes, Striatal dysfunctions associated with mitochondrial DNA damage in dopaminergic neurons in a mouse model of Parkinson's disease, J. Neurosci. 31 (2011) 17649–17658.
[40] A. Migdalska-Richards, A.H. Schapira, The relationship between glucocerebrosidase mutations and Parkinson disease, J. Neurochem. (2016).
[41] S. Jana, M. Sinha, D. Chanda, T. Roy, K. Banerjee, S. Munshi, B.S. Patro, S. Chakrabarti, Mitochondrial dysfunction mediated by quinone oxidation products of dopamine: implications in dopamine cytotoxicity and pathogenesis of Parkinson's disease, Biochim. Biophys. Acta 1812 (2011) 663–673.
[42] G.E. Gibson, A. Starkov, J.P. Blass, R.R. Ratan, M.F. Beal, Cause and consequence: mitochondrial dysfunction initiates and propagates neuronal dysfunction, neuronal death and behavioral abnormalities in age-associated neurodegenerative diseases, Biochim. Biophys. Acta 1802 (2010) 122–134.
[43] Y. Cabeza-Arvelaiz, R.H. Schiestl, Transcriptome analysis of a rotenone model of parkinsonism reveals complex I-tied and -untied toxicity mechanisms common to neurodegenerative diseases, PLoS ONE 7 (2012) e44700.
[44] M. Vairetti, A. Ferrigno, V. Rizzo, G. Ambrosi, A. Bianchi, P. Richelmi, F. Blandini, M.T. Armentero, Impaired hepatic function and central dopaminergic denervation in a rodent model of Parkinson's disease: a self-perpetuating crosstalk? Biochim. Biophys. Acta 1822 (2012) 176–184.
[45] K.J. Thomas, M.R. Cookson, The role of PTEN-induced kinase 1 in mitochondrial dysfunction and dynamics, Int. J. Biochem. Cell Biol. 41 (2009) 2025–2035.
[46] V.S. Van Laar, S.B. Berman, The interplay of neuronal mitochondrial dynamics and bioenergetics: implications for Parkinson's disease, Neurobiol. Dis. 51 (2013) 43–55.
[47] C. Pacelli, D. De Rasmo, A. Signorile, I. Grattagliano, G. di Tullio, A. D'Orazio, B. Nico, G.P. Comi, D. Ronchi, E. Ferranini, D. Pirolo, P. Seibel, S. Schubert, A. Gaballo, G. Villani, T. Cocco, Mitochondrial defect and PGC-1α dysfunction in parkin-associated familial Parkinson's disease, Biochim. Biophys. Acta 1812 (2011) 1041–1053.
[48] Y. Han, P. Gao, S. Qiu, L. Zhang, L. Yang, J. Zuo, C. Zhong, S. Zhu, W. Liu, MTERF2 contributes to MPP(+)-induced mitochondrial dysfunction and cell damage, Biochem. Biophys. Res. Commun. 471 (2016) 177–183.
[49] X. Ye, Y. Han, L. Zhang, W. Liu, J. Zuo, MTERF4 regulates the mitochondrial dysfunction induced by MPP(+) in SH-SY5Y cells, Biochem. Biophys. Res. Commun. 464 (2015) 214–220.
[50] A.C. Guerra de Souza, R.D. Prediger, H. Cimarosti, Mitochondrial dysfunction and SUMOylation targets in Parkinson's disease, J. Neurochem. 13599 (2016).
[51] V. Dias, E. Junn, M.M. Mouradian, The role of oxidative stress in Parkinson's disease, J. Park. Dis. 3 (2013) 461–491.
[52] J. Labbadia, R.I. Morimoto, Huntington's disease: underlying molecular mechanisms and emerging concepts, Trends Biochem. Sci. 38 (2013) 378–385.
[53] P.H. Reddy, P. Mao, M. Manczak, Mitochondrial structural and functional dynamics in Huntington's disease, Brain Res. Rev. 61 (2009) 33–48.
[54] N.N. Naseri, H. Xu, J. Bonica, J.P. Vonsattel, E.P. Cortes, L.C. Park, J. Arjomand, G.E. Gibson, Abnormalities in the tricarboxylic acid cycle in Huntington disease and in a Huntington disease mouse model, J. Neuropathol. Exp. Neurol. 74 (2015) 527–537.
[55] M. Corrado, L. Scorrano, S. Campello, Mitochondrial dynamics in cancer and neurodegenerative and neuroinflammatory diseases, Int. J. Cell Biol. (2012) 729290.
[56] V. Leoni, C. Caccia, The impairment of cholesterol metabolism in Huntington disease, Biochim. Biophys. Acta 1851 (2015) 1095–1105.
[57] I.P. De Castro, L.M. Martins, R. Tufi, Mitochondrial quality control and neurological disease: an emerging connection, Expert Rev. Mol. Med. 12 (2010) e12.
[58] P.H. Reddy, U.P. Shirendeb, Mutant huntingtin, abnormal mitochondrial dynamics, defective axonal transport of mitochondria, and selective synaptic degeneration in Huntington's disease, Biochim. Biophys. Acta 1822 (2012) 101–110.
[59] H. Yano, S.V. Baranov, O.V. Baranova, J. Kim, Y. Pan, S. Yablonska, D.L. Carlisle, R.J. Ferrante, A.H. Kim, R.M. Friedlander, Inhibition of mitochondrial protein import by mutant huntingtin, Nat. Neurosci. 17 (2014) 822–831.
[60] S. Hwang, M.H. Disatnik, D. Mochly-Rosen, Impaired GAPDH-induced mitophagy contributes to the pathology of Huntington's disease, EMBO Mol. Med. 7 (2015) 1307–1326.
[61] J.H. Lee, L. Tecedor, Y.H. Chen, A.M. Monteys, M.J. Sowada, L.M. Thompson, B.L. Davidson, Reinstating aberrant mTORC1 activity in Huntington's disease mice improves disease phenotypes, Neuron 85 (2015) 303–315.
[62] A. Mehrotra, A. Kanwal, S.K. Banerjee, R. Sandhir, Mitochondrial modulators in experimental Huntington's disease: reversal of mitochondrial dysfunctions and cognitive deficits, Neurobiol. Aging 36 (2015) 2186–2200.
[63] I.L. Ferreira, M.V. Nascimento, M. Ribeiro, S. Almeida, S.M. Cardoso, M. Grazina, J. Pratas, M.J. Santos, C. Januário, C.R. Oliveira, A.C. Rego, Mitochondrial-dependent apoptosis in Huntington's disease human cybrids, Exp. Neurol. 222 (2010) 243–255.
[64] M. Orsini, A.B. Oliveira, O.J. Nascimento, C.H. Reis, M.A. Leite, J.A. De Souza, C. Pupe, O.G. De Souza, V.H. Bastos, M.R. De Freitas, S. Teixeira, C. Bruno, E. Davidovich, B.

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Smidt, Amyotrophic lateral sclerosis: new perpectives and update, Neurol. Int. 7 (2015) 5885.
[65] H. Muyderman, T. Chen, Mitochondrial dysfunction in amyotrophic lateral sclerosis - a valid pharmacological target? Br. J. Pharmacol. 171 (2014) 2191–2205.
[66] M. Mattiazzi, M. D'Aurelio, C.D. Gajewski, K. Martushova, M. Kiaei, M.F. Beal, G. Manfredi, Mutated human SOD1 causes dysfunction of oxidative phosphorylation in mitochondria of transgenic mice, J. Biol. Chem. 277 (2002) 29626–29633.
[67] L.J. Martin, Mitochondrial pathobiology in ALS, J. Bioenerg. Biomembr. 43 (2011) 569–579.
[68] C.M. Higgins, C. Jung, Z. Xu, ALS-associated mutant SOD1G93A causes mitochondrial vacuolation by expansion of the intermembrane space and by involvement of SOD1 aggregation and peroxisomes, BMC Neurosci. 4 (2003) 16.
[69] P.C. Wong, C.A. Pardo, D.R. Borchelt, M.K. Lee, N.G. Copeland, N.A. Jenkins, S.S. Sisodia, D.W. Cleveland, D.L. Price, An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria, Neuron 14 (1995) 1105–1116.
[70] S. Sasaki, M. Iwata, Dendritic synapses of anterior horn neurons in amyotrophic lateral sclerosis: an ultrastructural study, Acta Neuropathol. 91 (1996) 278–283.
[71] F. Tafuri, D. Ronchi, F. Magri, G.P. Comi, S. Corti, SOD1 misplacing and mitochondrial dysfunction in amyotrophic lateral sclerosis pathogenesis, Front. Cell. Neurosci. 9 (2015) 336.
[72] M. Zhu, A. Li, J. Chen, S. Zhang, J. Wu, Effects of optineurin mutants on SH-SY5Y cell survival, Mol. Cell. Neurosci. 74 (2016) 18–24.
[73] Z. Jiang, W. Wang, G. Perry, X. Zhu, X. Wang, Mitochondrial dynamic abnormalities in amyotrophic lateral sclerosis, Transl. Neurodegener. 4 (2015) 14.
[74] E. Mendoza, J.A. Miranda-Barrientos, R.A. Vázquez-Roque, E. Morales-Herrera, A. Ruelas, G. De la Rosa, G. Flores, E. Hernández-Echeagaray, In vivo mitochondrial inhibition alters corticostriatal synaptic function and the modulatory effects of neurotrophins, Neuroscience 280 (2014) 156–170.
[75] K. Fukunaga, Y. Shinoda, H. Tagashira, The role of SIGMAR1 gene mutation and mitochondrial dysfunction in amyotrophic lateral sclerosis, J. Pharmacol. Sci. 127 (2015) 36–41.
[76] H. Suzuki, M. Matsuoka, Overexpression of nuclear FUS induces neuronal cell death, Neuroscience 287 (2015) 113–124.
[77] A. Sharma, A.M. Varghese, K. Vijaylakshmi, R. Sumitha, V.K. Prasanna, S. Shruthi, B.K. Chandrasekhar Sagar, K.K. Datta, H. Gowda, A. Nalini, P.A. Alladi, R. Christopher, T.N. Sathyaprabha, T.R. Raju, M.M. Srinivas Bharath, Cerebrospinal fluid from sporadic amyotrophic lateral sclerosis patients induces mitochondrial and lysosomal dysfunction, Neurochem. Res. 41 (2016) 965–984.
[78] E. Grünblatt, J. Bartl, P. Riederer, The link between iron, metabolic syndrome,and Alzheimer's disease, J. Neural Transm. (Vienna) 118 (2011) 371–379.
[79] S.M. de la Monte, M. Tong, Brain metabolic dysfunction at the core of Alzheimer's disease, Biochem. Pharmacol. 88 (2014) 548–559.
[80] R. Sandhir, S. Gupta, Molecular and biochemical trajectories from diabetes to Alzheimer's disease: a critical appraisal, World J. Diabetes 6 (2015) 1223–1242.
[81] M.W. Akhtar, S. Sanz-Blasco, N. Dolatabadi, J. Parker, K. Chon, M.S. Lee, W. Soussou, S.R. McKercher, R. Ambasudhan, T. Nakamura, S.A. Lipton, Elevated glucose and oligomeric β-amyloid disrupt synapses via a common pathway of aberrant protein S-nitrosylation, Nat. Commun. 7 (2016) 10242.
[82] K. Maiese, Z.Z. Chong, S. Wang, Y.C. Shang, Oxidant stress and signal transduction in the nervous system with the PI 3-K, Akt, and mTOR cascade, Int. J. Mol. Sci. 13 (2012) 13830–13866.
[83] M. Wong, Mammalian target of rapamycin (mTOR) pathways in neurological diseases, Biomed. J. 36 (2013) 40–50.
[84] G. Razay, A. Vreugdenhil, G. Wilcock, The metabolic syndrome and Alzheimer disease, Arch. Neurol. 64 (2007) 93–96.
[85] G. Zuliani, M. Cavalieri, M. Galvani, S. Volpato, A. Cherubini, S. Bandinelli, A.M. Corsi, F. Lauretani, J.M. Guralnik, R. Fellin, L. Ferrucci, Relationship between low levels of high-density lipoprotein cholesterol and dementia in the elderly, the InChianti study, J. Gerontol. A Biol. Sci. Med. Sci. 65 (2010) 559–564.
[86] A.D. De Kloet, E.G. Krause, S.C. Woods, The renin angiotensin system and the metabolic syndrome, Physiol. Behav. 100 (2010) 525–534.
[87] R. Tsuruta, M. Fujita, T. Ono, Y. Koda, Y. Koga, T. Yamamoto, M. Nanba, M. Shitara, S. Kasaoka, I. Maruyama, M. Yuasa, T. Maekawa, Hyperglycemia enhances excessive superoxide anion radical generation, oxidative stress, early inflammation, and endothelial injury in forebrain ischemia/reperfusion rats, Brain Res. 1309 (2010) 155–163.
[88] M. Hassan, S.A. Sehgal, S. Rashid, Regulatory cascade of neuronal loss and glucose metabolism, CNS Neurol. Disord. Drug Targets 13 (2014) 1232–1245.
[89] A. Rozycka, P.P. Jagodzinski, W. Kozubski, M. Lianeri, J. Dorszewska, Homocysteine Level and Mechanisms of Injury in Parkinson's disease as related to MTHFR, MTR, and MTHFD1 genes polymorphisms and L-Dopa treatment, Curr. Genomics 14 (2013) 534–542.
[90] M.E. Lull, M.L. Block, Microglial activation and chronic neurodegeneration, Neurotherapeutics 7 (2010) 354–365.
[91] N. Chaudhari, P. Talwar, A. Parimisetty, C. Lefebvre d'Hellencourt, P. Ravanan, A molecular web: endoplasmic reticulum stress, inflammation, and oxidative stress, Front. Cell. Neurosci. 8 (2014) 213.
[92] W. Dauer, S. Przedborski, Parkinson's disease: mechanisms and models, Neuron 39 (2003) 889–909.
[93] P. Zhang, B. Tian, Metabolic syndrome: an important risk factor for Parkinson's disease, Oxidative Med. Cell. Longev. 729194 (2014).
[94] Y.L. Wang, Y.T. Wang, J.F. Li, Y.Z. Zhang, H.L. Yin, B. Han, Body mass index and risk of Parkinson's disease: a dose-response meta-analysis of prospective studies, PLoS ONE 10 (2015) e0131778.
[95] J.E. Vance, Dysregulation of cholesterol balance in the brain: contribution to neurodegenerative diseases, Dis. Model. Mech. 5 (2012) 746–755.
[96] J.M. Karasinska, M.R. Hayden, Cholesterol metabolism in Huntington disease, Nat. Rev. Neurol. 7 (2011) 561–572.
[97] R.C. Block, E.R. Dorsey, C.A. Beck, J.T. Brenna, I. Shoulson, Altered cholesterol and fatty acid metabolism in Huntington disease, J. Clin. Lipidol. 4 (2010) 17–23.
[98] P.B. Verghese, J.M. Castellano, D.M. Holtzman, Apolipoprotein E in Alzheimer's disease and other neurological disorders, Lancet Neurol. 10 (2011) 241–252.
[99] S.T. Ngo, F.J. Steyn, The interplay between metabolic homeostasis and neurodegeneration: insights into the neurometabolic nature of amyotrophic lateral sclerosis, Cell Regen. (Lond.) 4 (2015) 5.
[100] W. Robberecht, T. Philips, The changing scene of amyotrophic lateral sclerosis, Nature Reviews Neuroscience 14 (2013) 248–264.
[101] J.C. Dodge, C.M. Treleaven, J.A. Fidler, T.J. Tamsett, C. Bao, M. Searles, T.V. Taksir, K. Misra, R.L. Sidman, S.H. Cheng, L.S. Shihabuddin, Metabolic signatures of amyotrophic lateral sclerosis reveal insights into disease pathogenesis, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 10812–10817.
[102] S.T. Ngo, F.J. Steyn, L. Huang, S. Mantovani, C.M. Pfluger, T.M. Woodruff, J.D. O'Sullivan, R.D. Henderson, P.A. McCombe, Altered expression of metabolic proteins and adipokines in patients with amyotrophic lateral sclerosis, J. Neurol. Sci. 357 (2015) 22–27.
[103] P. Mergenthaler, U. Lindauer, G.A. Dienel, A. Meisel, Sugar for the brain: the role of glucose in physiological and pathological brain function, Trends Neurosci. 36 (2013) 587–597.
[104] P.H. Reddy, Inhibitors of mitochondrial fission as a therapeutic strategy for diseases with oxidative stress and mitochondrial dysfunction, J. Alzheimers Dis. 40 (2014) 245–256.
[105] S. Tangvarasittichai, S. Pongthaisong, S. Meemark, O. Tangvarasittichai, Abdominal Obesity Associated with Elevated Serum Butyrylcholinesterase Activity, Insulin Resistance and Reduced High Density Lipoprotein-Cholesterol Levels, Indian J Clin Biochem. 30 (2015) 275–280.
[106] M.A. Alam, M.M. Rahman, Mitochondrial dysfunction in obesity: potential benefit and mechanism of Co-enzyme Q10 supplementation in metabolic syndrome, J. Diabetes Metab. Disord. 13 (2014) 60.
[107] W.I. Sivitz, M.A. Yorek, Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities, Antioxid. Redox Signal. (2010) 537–577.
[108] C. Stockburger, D. Miano, M. Baeumlisberger, T. Pallas, T.N. Arrey, M. Karas, K. Friedland, W.E. Müller, A mitochondrial role of SV2a protein in aging and Alzheimer's disease: studies with levetiracetam, J. Alzheimers Dis. 50 (2015) 201–215.
[109] Y.J. Chen, H.Y. Zheng, X.X. Huang, S.X. Han, D.S. Zhang, J.Z. Ni, X.Y. He, Neuroprotective effects of icariin on brain metabolism, mitochondrial functions, and cognition in triple-transgenic Alzheimer's disease mice, CNS Neurosci. Ther. 22 (2016) 63–73.
[110] B. Thrash-Williams, S.S. Karuppagounder, D. Bhattacharya, M. Ahuja, V. Suppiramaniam, M. Dhanasekaran, Methamphetamine-induced dopaminergic toxicity prevented owing to the neuroprotective effects of salicylic acid, Life Sci. (2016) 30122–30129.
[111] D. Colle, D.B. Santos, J.M. Hartwig, M. Godoi, D.F. Engel, A.F. De Bem, A.L. Braga, M. Farina Succinobucol, A lipid-lowering drug, protects against 3-nitropropionic acid-induced mitochondrial dysfunction and oxidative stress in SH-SY5Y cells via upregulation of glutathione levels and glutamate cysteine ligase activity, Mol. Neurobiol. 53 (2016) 1280–1295.
[112] A.C. Sirianni, J. Jiang, J. Zeng, L.L. Mao, S. Zhou, P. Sugarbaker, X. Zhang, W. Li, R.M. Friedlander, X. Wang, *N*-acetyl-l-tryptophan, but not *N*-acetyl-*d*-tryptophan, rescues neuronal cell death in models of amyotrophic lateral sclerosis, J. Neurochem. 134 (2015) 956–968.
[113] B. Kim, J. Park, K.T. Chang, D.S. Lee, Peroxiredoxin 5 prevents amyloid-beta oligomer-induced neuronal cell death by inhibiting ERK-Drp1-mediated mitochondrial fragmentation, Free Radic. Biol. Med. 90 (2016) 184–194.
[114] S. Fossati, P. Giannoni, M.E. Solesio, S.L. Cocklin, E. Cabrera, J. Ghiso, A. Rostagno, The carbonic anhydrase inhibitor methazolamide prevents amyloid beta-induced mitochondrial dysfunction and caspase activation protecting neuronal and glial cells in vitro and in the mouse brain, Neurobiol. Dis. 86 (2016) 29–40.
[115] E.A. Rudnitskaya, N.A. Muraleva, K.Y. Maksimova, E. Kiseleva, N.G. Kolosova, N.A. Stefanova, Melatonin attenuates memory impairment, amyloid-β accumulation, and neurodegeneration in a rat model of sporadic Alzheimer's disease, J. Alzheimers Dis. 47 (2015) 103–116.
[116] H.G. Kim, G. Park, S. Lim, H. Park, J.G. Choi, H.U. Jeong, M.S. Kang, M.K. Lee, M.S. Oh, Mori Fructus improves cognitive and neuronal dysfunction induced by beta-amyloid toxicity through the GSK-3β pathway in vitro and in vivo, J. Ethnopharmacol. 171 (2015) 196–204.
[117] Y.P. Li, G.J. Yang, L. Jin, H.M. Yang, J. Chen, G.S. Chai, L. Wang, Erythropoietin attenuates Alzheimer-like memory impairments and pathological changes induced by amyloid β42 in mice, Brain Res. 1618 (2015) 159–167.
[118] E. Kornelius, C.L. Lin, H.H. Chang, H.H. Li, W.N. Huang, Y.S. Yang, Y.L. Lu, C.H. Peng, C.N. Huang, DPP-4 inhibitor linagliptin attenuates Aβ-induced cytotoxicity through activation of AMPK in neuronal cells, CNS Neurosci. Ther. 21 (2015) 549–557.
[119] S. Sarkar, S. Jun, J.W. Simpkins, Estrogen amelioration of Aβ-induced defects in mitochondria is mediated by mitochondrial signaling pathway involving ERβ, AKAP and Drp1, Brain Res. 1616 (2015) 101–111.
[120] E. Kim, M. Park, J. Jeong, H. Kim, S.K. Lee, E. Lee, B.H. Oh, K. Namkoong, Cholinesterase inhibitor donepezil increases mitochondrial biogenesis through AMP-activated protein kinase in the hippocampus, Neuropsychobiology 73 (2016) 81–91.
[121] M. Leirós, E. Alonso, M.E. Rateb, W.E. Houssen, R. Ebel, M. Jaspars, A. Alfonso, L.M. Botana, Gracilins: spongionella-derived promising compounds for Alzheimer disease, Neuropharmacology 93 (2015) 285–293.
[122] L. Yu, S. Wang, X. Chen, H. Yang, X. Li, Y. Xu, X. Zhu, Orientin alleviates cognitive deficits and oxidative stress in Aβ1-42-induced mouse model of Alzheimer's disease, Life Sci. 121 (2015) 104–109.
[123] D. Pinkaew, C. Changtam, C. Tocharus, S. Thummayot, A. Suksamrarn, J. Tocharus, Di-O-demethylcurcumin protects SK-*N*-SH cells against mitochondrial and endoplasmic reticulum-mediated apoptotic cell death induced by Aβ25-35, Neurochem. Int. 80 (2015) 110–119.
[124] A.K. Sachdeva, A. Kuhad, K. Chopra, Naringin ameliorates memory deficits in experimental paradigm of Alzheimer's disease by attenuating mitochondrial dysfunction, Pharmacol. Biochem. Behav. 127 (2014) 101–110.

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[125] G. Kulasekaran, S. Ganapasam, Neuroprotective efficacy of naringin on 3-nitropropionic acid-induced mitochondrial dysfunction through the modulation of Nrf2 signaling pathway in PC12 cells, Mol. Cell. Biochem. 409 (2015) 199–211.
[126] J. Li, X. Ji, J. Zhang, G. Shi, X. Zhu, K. Wang, Paeoniflorin attenuates Aβ25-35-induced neurotoxicity in PC12 cells by preventing mitochondrial dysfunction, Folia Neuropathol. 52 (2014) 285–290.
[127] X.F. Ji, T.Y. Chi, Q. Xu, X.L. He, X.Y. Zhou, R. Zhang, L.B. Zou, Xanthoceraside ameliorates mitochondrial dysfunction contributing to the improvement of learning and memory impairment in mice with intracerebroventricular injection of aβ1-42, Evid. Based Complement. Alternat. Med. (2014) 969342.
[128] H. Lei, C.Y. Zhao, D.M. Liu, Y. Zhang, L. Li, X.L. Wang, Y. Peng, l-3-*n*-Butylphthalide attenuates β-amyloid-induced toxicity in neuroblastoma SH-SY5Y cells through regulating mitochondrion-mediated apoptosis and MAPK signaling, J. Asian Nat. Prod. Res. 16 (2014) 854–864.
[129] D. Wang, L. Liu, X. Zhu, W. Wu, Y. Wang, Hesperidin alleviates cognitive impairment, mitochondrial dysfunction and oxidative stress in a mouse model of Alzheimer's disease, Cell. Mol. Neurobiol. 34 (2014) 1209–1221.
[130] E.T. Bayrakdar, G. Armagan, Y. Uyanikgil, L. Kanit, E. Koylu, A. Yalcin, Ex vivo protective effects of nicotinamide and 3-aminobenzamide on rat synaptosomes treated with Abeta(1–42), Cell Biochem. Funct. 32 (2014) 557–564.
[131] A. Prakash, A. Kumar, Implicating the role of lycopene in restoration of mitochondrial enzymes and BDNF levels in β-amyloid induced Alzheimer's disease, Eur. J. Pharmacol. 741 (2014) 104–111.
[132] C. Lv, X. Liu, H. Liu, T. Chen, W. Zhang, Geniposide attenuates mitochondrial dysfunction and memory deficits in APP/PS1 transgenic mice, Curr. Alzheimer Res. 11 (2014) 580–587.
[133] I.G. Onyango, J. Dennis, S.M. Khan, Mitochondrial Dysfunction in Alzheimer's Disease and the Rationale for Bioenergetics Based Therapies, Aging Dis. 15 (2016) 201–214.
[134] M. Lagouge, C. Argmann, Z. Gerhart-Hines, H. Meziane, C. Lerin, F. Daussin, N. Messadeq, J. Milne, P. Lambert, P. Elliott, B. Geny, M. Laakso, P. Puigserver, J. Auwerx, Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha, Cell 127 (2006) 1109–1122.
[135] D.M. Wang, S.Q. Li, W.L. Wu, X.Y. Zhu, Y. Wang, H.Y. Yuan, Effects of long-term treatment with quercetin on cognition and mitochondrial function in a mouse model of Alzheimer's disease, Neurochem. Res. 39 (2014) 1533–1543.
[136] D.L. Chen, P. Zhang, L. Lin, H.M. Zhang, S.D. Deng, Z.Q. Wu, S. Ou, S.H. Liu, J.Y. Wang, Protective effects of bajijiasu in a rat model of $A\beta_{25-35}$-induced neurotoxicity, J. Ethnopharmacol. 154 (2014) 206–217.
[137] Q. Sun, H. Hu, W. Wang, H. Jin, G. Feng, N. Jia, Taurine attenuates amyloid β 1-42-induced mitochondrial dysfunction by activating of SIRT1 in SK-*N*-SH cells, Biochem. Biophys. Res. Commun. 447 (2014) 485–489.
[138] Y.L. Jia, S.J. Sun, J.H. Chen, Q. Jia, T.T. Huo, L.F. Chu, J.T. Bai, Y.J. Yu, X.X. Yan, J.H. Wang, SS31, a small molecule antioxidant peptide, attenuates β-amyloid elevation, mitochondrial/synaptic deterioration and cognitive deficit in SAMP8 mice, Curr. Alzheimer Res. 13 (2016) 297–306.
[139] N.E. Gray, J.A. Zweig, C. Kawamoto, J.F. Quinn, P.F. Copenhaver, STX a novel membrane estrogen receptor ligand, protects against amyloid-β toxicity, J. Alzheimers Dis. 51 (2016) 391–403.
[140] M.J. McManus, M.P. Murphy, J.L. Franklin, The mitochondria-targeted antioxidant MitoQ prevents loss of spatial memory retention and early neuropathology in a transgenic mouse model of Alzheimer's disease, J. Neurosci. 31 (2011) 15703–15715.
[141] C. Feillet-Coudray, G. Fouret, R. Ebabe Elle, J. Rieusset, B. Bonafos, B. Chabi, D. Crouzier, K. Zarkovic, N. Zarkovic, J. Ramos, E. Badia, M.P. Murphy, J.P. Cristol, C. Coudray, The mitochondrial-targeted antioxidant MitoQ ameliorates metabolic syndrome features in obesogenic diet-fed rats better than Apocynin or Allopurinol, Free Radic. Res. 48 (2014) 1232–1246.
[142] S. Hagl, R. Grewal, I. Ciobanu, A. Helal, M.T. Khayyal, W.E. Muller, G.P. Eckert, Rice bran extract compensates mitochondrial dysfunction in a cellular model of early Alzheimer's disease, J. Alzheimers Dis. 43 (2015) 927–938.
[143] Q. Sun, N. Jia, W. Wang, H. Jin, J. Xu, H. Hu, Protective effects of astragaloside IV against amyloid beta1-42 neurotoxicity by inhibiting the mitochondrial permeability transition pore opening, PLoS ONE 9 (2014) e98866.
[144] J. Nataraj, T. Manivasagam, A. Justin Thenmozhi, M.M. Essa, Neuroprotective effect of asiatic acid on rotenone-induced mitochondrial dysfunction and oxidative stress-mediated apoptosis in differentiated SH-SYS5Y cells, Nutr. Neurosci. (2016).
[145] J.A. Bayliss, Z.B. Andrews, Ghrelin is neuroprotective in Parkinson's disease: molecular mechanisms of metabolic neuroprotection, Ther. Adv. Endocrinol. Metab. 4 (2013) 25–36.
[146] H. Mortiboys, R. Furmston, G. Bronstad, J. Aasly, C. Elliott, O. Bandmann, UDCA exerts beneficial effect on mitochondrial dysfunction in LRRK2(G2019S) carriers and in vivo, Neurology 85 (2015) 846–852.
[147] T. Chen, Y. Deng, X. Liao, J. Zhao, G. Wen, G. Weng, F. Ma, Y. Zheng, Effect of curcumin on oligomer formation and mitochondrial ATP-sensitive potassium channels induced by overexpression or mutation of α-synuclein, Zhonghua Yi Xue Yi Chuan Xue Za Zhi 32 (2015) 462–467.
[148] G.F. Zhang, Y. Zhang, G. Zhao, Crocin protects PC12 cells against MPP(+)-induced injury through inhibition of mitochondrial dysfunction and ER stress, Neurochem. Int. 89 (2015) 101–110.
[149] H. Liu, P. Mao, J. Wang, T. Wang, C.H. Xie, Allicin protects PC12 cells against 6-OHDA-induced oxidative stress and mitochondrial dysfunction via regulating mitochondrial dynamics, Cell. Physiol. Biochem. 36 (2015) 966–979.
[150] N.P. Mena, O. García-Beltrán, F. Lourido, P.J. Urrutia, R. Mena, V. Castro-Castillo, B.K. Cassels, M.T. Núñez, The novel mitochondrial iron chelator 5-((methylamino)methyl)-8-hydroxyquinoline protects against mitochondrial-induced oxidative damage and neuronal death, Biochem. Biophys. Res. Commun. 463 (2015) 787–792.
[151] Z. Tan, H. Xu, X. Shen, H. Jiang, Nesfatin-1 antagonized rotenone-induced neurotoxicity in MES23.5 dopaminergic cells, Peptides 69 (2015) 109–114.
[152] S. Srivastav, S.K. Singh, A.K. Yadav, S. Srikrishna, Folic acid supplementation rescues anomalies associated with knockdown of parkin in dopaminergic and serotonergic neurons in Drosophila model of Parkinson's disease, Biochem. Biophys. Res. Commun. 460 (2015) 780–785.
[153] Y. Binawade, A. Jagtap, Neuroprotective effect of lutein against 3-nitropropionic acid-induced Huntington's disease-like symptoms: possible behavioral, biochemical, and cellular alterations, J. Med. Food 16 (2013) 934–943.
[154] J. Nataraj, T. Manivasagam, A.J. Thenmozhi, M.M. Essa, Lutein protects dopaminergic neurons against MPTP-induced apoptotic death and motor dysfunction by ameliorating mitochondrial disruption and oxidative stress, Nutr. Neurosci. (2015).
[155] Y. Lee, H.R. Park, H.J. Chun, J. Lee, Silibinin prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease via mitochondrial stabilization, J. Neurosci. Res. 93 (2015) 755–765.
[156] Z. Cai, W. Zeng, K. Tao, F. Lu, G. Gao, Q. Yang, Myricitrin alleviates MPP^+-induced mitochondrial dysfunction in a DJ-1-dependent manner in SN4741 cells, Biochem. Biophys. Res. Commun. 458 (2015) 227–233.
[157] W.N. Cong, W. Chadwick, R. Wang, C.M. Daimon, H. Cai, J. Amma, W.H. Wood III, K.G. Becker, B. Martin, S. Maudsley, et al., J. Biol. Chem. 290 (2015) 2728–2743.
[158] S. Gupta, B. Sharma, Pharmacological benefits of agomelatine and vanillin in experimental model of Huntington's disease, Pharmacol. Biochem. Behav. 122 (2014) 122–135.
[159] M. Damiano, L. Galvan, N. Déglon, E. Brouillet, Mitochondria in Huntington's disease, Biochim. Biophys. Acta 1802 (2010) 52–61.
[160] S. Shetty, A. Hariharan, T. Shirole, A.G. Jagtap, Neuroprotective potential of escitalopram against behavioral, mitochondrial and oxidative dysfunction induced by 3-nitropropionic acid, Ann. Neurosci. 22 (2015) 11–18.
[161] J. Mishra, A. Kumar, Improvement of mitochondrial function by paliperidone attenuates quinolinic acid-induced behavioural and neurochemical alterations in rats: implications in Huntington's disease, Neurotox. Res. 26 (2014) 363–381.
[162] J. Mishra, T. Chaudhary, A. Kumar, Rosiglitazone synergizes the neuroprotective effects of valproic acid against quinolinic acid-induced neurotoxicity in rats: targeting PPARγ and HDAC pathways, Neurotox. Res. 26 (2014) 130–151.
[163] S. Gupta, B. Sharma, Protective effects of phosphodiesterase-1 (PDE1) and ATP sensitive potassium (KATP) channel modulators against 3-nitropropionic acid induced behavioral and biochemical toxicities in experimental Huntington's disease, Eur. J. Pharmacol. 732 (2014) 111–122.
[164] J. Mishra, A. Kumar, Improvement of mitochondrial NAD(+)/FAD(+)-linked state-3 respiration by caffeine attenuates quinolinic acid induced motor impairment in rats: implications in Huntington's disease, Pharmacol. Rep. 66 (2014) 1148–1155.
[165] D.J. Wright, T. Renoir, Z.M. Smith, A.E. Frazier, P.S. Francis, D.R. Thorburn, S.L. McGee, A.J. Hannan, L.J. Gray, *N*-acetylcysteine improves mitochondrial function and ameliorates behavioral deficits in the R6/1 mouse model of Huntington's disease, Transl. Psychiatry 5 (2015) e492.
[166] V. Srinivasan, D.W. Spence, S.R. Pandi-Perumal, G.M. Brown, D.P. Cardinali, Melatonin in mitochondrial dysfunction and related disorders, Int. J. Alzheimers Dis. (2011) 326320.
[167] L. Palamiuc, A. Schlagowski, S.T. Ngo, A. Vernay, S. Dirrig-Grosch, A. Henriques, A.L. Boutillier, J. Zoll, A. Echaniz-Laguna, J.P. Loeffler, F. René, A metabolic switch toward lipid use in glycolytic muscle is an early pathologic event in a mouse model of amyotrophic lateral sclerosis, EMBO Mol. Med. 7 (2015) 526–546.
[168] R.S. Pandya, H. Zhu, W. Li, R. Bowser, R.M. Friedlander, X. Wang, Therapeutic neuroprotective agents for amyotrophic lateral sclerosis, Cell. Mol. Life Sci. 70 (2013) 4729–4745.
[169] V. Veereshwarayya, P. Kumar, K.M. Rosen, R. Mestril, H.W. Querfurth, Differential effects of mitochondrial heat shock protein 60 and related molecular chaperones to prevent intracellular beta-amyloid-induced inhibition of complex IV and limit apoptosis, J. Biol. Chem. 281 (2006) 29468–29478.
[170] G.R. Luo, S. Chen, W.D. Le, Are heat shock proteins therapeutic target for Parkinson's disease? Int. J. Biol. Sci. 3 (2006) 20–26.
[171] R.K. Leak, Heat shock proteins in neurodegenerative disorders and aging, J. Cell Commun. Signal. 8 (2014) 293–310.
[172] E.K. Butler, A. Voigt, A.K. Lutz, J.P. Toegel, E. Gerhardt, P. Karsten, B. Reinartz, A. Falkenburger, K.F. Winklhofer, J.B. Schulz, The mitochondrial chaperone protein TRAP1 mitigates α-Synuclein toxicity, PLoS Genet. 8 (2012) e1002488.
[173] A. Wyttenbach, O. Sauvageot, J. Carmichael, C. Diaz-Latoud, A.P. Arrigo, D.C. Rubinsztein, Heat shock protein 27 prevents cellular polyglutamine toxicity and suppresses the increase of reactive oxygen species caused by huntingtin, Hum. Mol. Genet. 11 (2002) 1137–1151.
[174] J. Abubaker, A. Tiss, M. Abu-Farha, F. Al-Ghimlas, I. Al-Khairi, E. Baturcam, P. Cherian, N. Elkum, M. Hammad, J. John, S. Kavalakatt, A. Khadir, S. Warsame, S. Dermime, K. Behbehani, M. Dehbi, DNAJB3/HSP-40 cochaperone is downregulated in obese humans and is restored by physical exercise, PLoS ONE 8 (2013) e69217.
[175] A.L. Orr, S. Huang, M.A. Roberts, J.C. Reed, S. Li, X.J. Li, Sex-dependent effect of BAG1 in ameliorating motor deficits of Huntington disease transgenic mice, J. Biol. Chem. 283 (2008) 16027–16036.
[176] A. Israelson, D. Ditsworth, S. Sun, S. Song, J. Liang, M. Hruska-Plochan, M. McAlonis-Downes, S. Abu-Hamad, G. Zoltsman, T. Shani, M. Maldonado, A. Bui, M. Navarro, H. Zhou, M. Marsala, B.K. Kaspar, S. Da Cruz, D.W. Cleveland, Macrophage migration inhibitory factor as a chaperone inhibiting accumulation of misfolded SOD1, Neuron 89 (2015) 218–232.
[177] J. Deng, M. Yang, Y. Chen, X. Chen, J. Liu, S. Sun, H. Cheng, Y. Li, E.H. Bigio, M. Mesulam, Q. Xu, S. Du, K. Fushimi, L. Zhu, J.Y. Wu, FUS Interacts with HSP60 to promote mitochondrial damage, PLoS Genet. 11 (2015) e1005357.
[178] I. Padmalayam, The heat shock response: its role in pathogenesis of type 2 diabetes and its complications, and implications for therapeutic intervention, Discov. Med. 18 (2014) 29–39.
[179] C.W. Zhang, H.B. Adeline, B.H. Chai, E.T. Hong, C.H. Ng, K.L. Lim, Pharmacological or genetic activation of Hsp70 protects against loss of Parkin function, Neurodegener. Dis. (2016).
[180] L.F. Burbulla, C. Schelling, H. Kato, D. Rapaport, D. Woitalla, C. Schiesling, C. Schulte, M. Sharma, T. Illig, P. Bauer, S. Jung, A. Nordheim, L. Schöls, O. Riess, R. Krüger,

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Dissecting the role of the mitochondrial chaperone mortalin in Parkinson's disease: functional impact of disease-related variants on mitochondrial homeostasis, Hum. Mol. Genet. 19 (2010) 4437–4452.
[181] A.C. Costa, S.H. Loh, L.M. Martins, Drosophila Trap1 protects against mitochondrial dysfunction in a PINK1/parkin model of Parkinson's disease, Cell Death Dis. 4 (2013) e467.
[182] H.Q. Wang, Y.X. Xu, X.Y. Zhao, H. Zhao, J. Yan, X.B. Sun, J.C. Guo, C.Q. Zhu, Overexpression of F(0)F(1)-ATP synthase alpha suppresses mutant huntingtin aggregation and toxicity in vitro, Biochem. Biophys. Res. Commun. 390 (2009) 1294–1298.
[183] O.V. Forlenza, V.J. De-Paula, B.S. Diniz, Neuroprotective effects of lithium: implications for the treatment of Alzheimer's disease and related neurodegenerative disorders, ACS Chem. Neurosci. 5 (2014) 443–450.
[184] L. Luo, G.J. Sun, Y.J. Du, Effect of "kidney-reinforcing and govenor vessel-regulating" of acupuncture plus moxibustion on mitochondrial dynamics-related proteins in hippocampal neurons of rats with Alzheimer's disease, Zhen Ci Yan Jiu 40 (2015) 270–274.
[185] M. Itakura, H. Nakajima, T. Kubo, Y. Semi, S. Kume, S. Higashida, A. Kaneshige, M. Kuwamura, N. Harada, A. Kita, Y.T. Azuma, R. Yamaji, T. Inui, T. Takeuchi, Glyceraldehyde-3-phosphate dehydrogenase aggregates accelerate Amyloid-β amyloidogenesis in Alzheimer disease, J. Biol. Chem. 290 (2015) 26072–26087.
[186] J.L. Jin, A.K. Liou, Y. Shi, K.L. Yin, L. Chen, L.L. Li, X.L. Zhu, L. Qian, R. Yang, J. Chen, Y. Xu, CART treatment improves memory and synaptic structure in APP/PS1 mice, Sci. Rep. 5 (2015) 10224.
[187] A.D. Chaudhuri, D.C. Choi, S. Kabaria, A. Tran, E. Junn, MicroRNA-7 regulates the function of mitochondrial permeability transition pore by targeting VDAC1 expression, J. Biol. Chem. 291 (2016) 6483–6493.
[188] L. Hang, J. Thundyil, K.L. Lim, Mitochondrial dysfunction and Parkinson disease: a Parkin-AMPK alliance in neuroprotection, Ann. N. Y. Acad. Sci. 1350 (2015) 37–47.
[189] A. Nanou, A. Higginbottom, C.F. Valori, M. Wyles, K. Ning, P. Shaw, M. Azzouz, Viral delivery of antioxidant genes as a therapeutic strategy in experimental models of amyotrophic lateral sclerosis, Mol. Ther. 21 (2013) 1486–1496.
[190] Z. Mao, S. Zhang, H. Chen, Stem cell therapy for amyotrophic lateral sclerosis, Cell Regen. (Lond.) 4 (2015) 11.
[191] S.R. Colberg, R.J. Sigal, B. Fernhall, J.G. Regensteiner, B.J. Blissmer, R.R. Rubin, L. Chasan-Taber, A.L. Albright, B. Braun, Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement, Diabetes Care 33 (2010) e147–e167.
[192] E.A. Richter, N.B. Ruderman, AMPK and the biochemistry of exercise: implications for human health and disease, Biochem. J. 418 (2009) 261–275.
[193] S.M. de la Monte, Intranasal insulin therapy for cognitive impairment and neurodegeneration: current state of the art, Expert Opin. Drug Deliv. 10 (2013) 1699–1709.
[194] A.S. Paintlia, S. Mohan, I. Singh, Combinatorial Effect of Metformin and Lovastatin Impedes T-cell autoimmunity and neurodegeneration in experimental autoimmune encephalomyelitis, J. Clin. Cell Immunol. 30 (2013) 4.
[195] I. Ullah, N. Ullah, M.I. Naseer, H.Y. Lee, M.O. Kim, Neuroprotection with metformin and thymoquinone against ethanol-induced apoptotic neurodegeneration in prenatal rat cortical neurons, BMC Neurosci. 19 (2012) (13:11).
[196] C. Davis, J. Mudd, M. Hawkins, Neuroprotective effects of leptin in the context of obesity and metabolic disorders, Neurobiol. Dis. 72 (2014) 61–71.
[197] A.P. Signore, F. Zhang, Z. Weng, Y. Gao, J. Chen, Leptin neuroprotection in the CNS: mechanisms and therapeutic potentials, J. Neurochem. 106 (2008) 1977–1990.
[198] J. Chen, S. Li, W. Sun, J. Li, Anti-diabetes drug pioglitazone ameliorates synaptic defects in AD transgenic mice by inhibiting cyclin-dependent kinase5 activity, PLoS ONE 10 (2015) e0123864.

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Review

Re-expression of cell cycle markers in aged neurons and muscles: Whether cells should divide or die?

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ABSTRACT

Emerging evidence revealed that abrogated cell cycle entry into highly differentiated mature neurons and muscles is having detrimental consequences in response to cell cycle checkpoints disruption, altered signaling cascades, pathophysiological and external stimuli, for instance, Aβ, Parkin, p-tau, α-synuclein, impairment in TRK, Akt/GSK3β, MAPK/Hsp90, and oxidative stress. These factors, reinitiate undesired cell division by triggering new DNA synthesis, replication, and thus exquisitely forced mature cell to enter into a disturbed and vulnerable state that often leads to death as reported in many neuro- and myodegenerative disorders. A pertinent question arises how to reverse this unwanted pathophysiological phenomenon is attributed to the usage of cell cycle inhibitors to prevent the degradation of crucial cell cycle arresting proteins, cyclin inhibitors, chaperones and E3 ligases. Herein, we identified the major culprits behind the forceful cell cycle re-entry, elucidated the cyclin re-expression based on disturbed signaling mechanisms in neuromuscular degeneration together with plausible therapeutic strategies.

1. Introduction

The etiology of neuromuscular degeneration is characterized by the canonical deposition of non-functional/toxic proteins such as amyloid β, tau, parkin, α-synuclein, mutant huntingtin, and chronic inflammations in neuromuscular disorders (NMDs) including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Inclusion body myositis (IBM) and Poly myositis (PM). The deposition of pathological proteins or inclusions creates a physiological burden on cell that triggers multiple signaling cascades including mitogen-activated protein kinase (MAPK), c-Jun N-Terminal Kinases (JNK), Phosphatidylinositol 3-kinase/serine threonine kinase Akt/Glycogen synthase kinase 3 (PI3K/Akt/GSK3β), Notch and apoptotic signaling pathways. The consequent overexpression of cell cycle markers has been observed with the symptoms of cell cycle re-entry (CCR) [1]. Moreover, the co-localization of cell cycle markers with pathological proteins has reinforced the role of cell cycle machinery as a trigger to degeneration [2]. Further evidence suggests that re-expression of cell cycle markers occurs at prodromal stages before the appearance of pathological hallmarks in these neuromuscular disorders. A plethora of studies have demonstrated the involvement of oxidative stress, aging, mutation, mitochondrial dysfunction; ubiquitin proteasome system (UPS) shut down and loss of function of several protective proteins in the pathogenesis of these NMDs. For instance, prolonged activation or under the influence of acute insults such as DNA damage, oxidative stress, neurotrophic factor deprivation and altered microRNA (miR) function; cell cycle machinery turns detrimental and drives the cascade of DNA synthesis and consequent neuronal death culminating into neurodegeneration [3]. Interestingly, numerous studies have established cell cycle re-entry as a true phenomenon in neurodegeneration with actual DNA synthesis and cannot be viewed as an epiphenomenon of other processes. Moreover, cell cycle markers' re-expression in terminally differentiated neurons is also reported to fulfill the physiological demands pertaining to synaptic plasticity, neuronal migration and maturation [4]. Importantly, current studies advocate that aberrant cell cycle re-entry is not a consequence, but rather a cause of neuromuscular degeneration, depicting the importance of targeting cell cycle re-entry as a therapeutic window for treating neuromuscular disorders. These days several compounds/biomolecules have been implicated for targeting cell cycle re-entry in neurons and muscles. Moreover, potential role of ubiquitin E3 ligases and heat shock proteins have also been demonstrated to revert the damaging effect caused by cell cycle re-entry in neuromuscular disorders.

2. Relevance of cell cycle markers (cyclins, CDKs, CDKIs) in maintaining cell cycle homeostasis

The eukaryotic cell cycle is orchestrated machinery that accomplishes fundamental roles in cell replenishment such as DNA replication,

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Review

Tau Phosphorylation, Molecular Chaperones, and Ubiquitin E3 Ligase: Clinical Relevance in Alzheimer's Disease

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Abstract. Alzheimer's disease (AD) is characterized by dementia, cognitive disabilities, and tauopathy. Tau is a microtubule associated protein that helps maintain the neuronal network. While phosphorylation of tau protein causes disruption of the microtubular network, dephosphorylation allows reconstitution of the microtubule network. Several kinases, e.g., MARK, MAPK, and protein kinase C, are known to hyperphosphorylate tau, leading to disruption of the microtubular network and formation of neurofibrillary tangles (NFTs), which are further glycosylated, glycated, and have lipid peroxide adducts that impair the neuronal transport system and affect memory formation and retention. Moreover, intracerebral administration of amyloid-β oligomers causes hyperphosphorylation of tau, but whether it is involved in the formation of NFTs is still unclear. Further, amyloid burden activates AMP-activated protein kinase that increases phosphorylation of tau at position Ser262/Ser356 and Ser396. Several phosphatases are present at low levels in AD brains indicating that their down regulation results in abnormal hyperphosphorylation of tau. However, evidence strengthens a possible link between tau phosphorylation and molecular chaperone mediated tau metabolism for the clearance of toxic tau accumulation and has a crucial role in tauopathy. Furthermore, accumulation of phosphorylated tau protein and the possibility of removing the toxic phosphorylated tau protein from the milieu indicates that the chaperone interacts with phosphorylated tau and promotes its degradation. For instance, Hsp90 and cdc37 regulate tau stability and phosphorylation dynamics whereas Hsp27 is able to modulate neuronal plasticity, while 14-3-3 is involved in the interaction of tau with small HSPs. Hsp70 ATPase acts as a modulator in AD therapeutics while Hsc70 rapidly engages tau after microtubular destabilization. Herein, we highlight the various causes of tauopathy and HSP-E3 ligase mediated therapeutics in AD.

Keywords: Heat shock proteins, tau phosphorylation, tauopathy, ubiquitin E3 ligase

INTRODUCTION

The cytoskeletal structure of neurons plays an important role in maintaining cell structure, shape, and function. The cytoskeleton is primarily composed of microtubules. These microtubules are maintained and stabilized by a major type of microtubule associated

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Ion Channels in Neurological Disorders

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Contents

Abstract

The convergent endeavors of the neuroscientist to establish a link between clinical neurology, genetics, loss of function of an important protein, and channelopathies behind neurological disorders are quite intriguing. Growing evidence reveals the impact of ion channels dysfunctioning in neurodegenerative disorders (NDDs). Many neurological/neuromuscular disorders, viz, Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, amyotrophic lateral sclerosis, and age-related disorders are caused due to altered function or mutation in ion channels. To maintain cell homeostasis, ion channels are playing a crucial role which is a large transmembrane protein. Further, these channels are important as it determines the membrane potential and playing

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Role of Oxidative Stress, ER Stress and Ubiquitin Proteasome System in Neurodegeneration

Abstract

Neurodegenerative disorders (NDDs) are progressive and chronic disorders characterized by destruction of neurons in sensory, motor and cognitive systems. Free radical's accumulation, oxidative stress, ER stress and a dysfunctional ubiquitin proteasome system can regulate prognosis in NDDs. Oxidative trauma in the brain can result from high rate of oxidative metabolism in contrast to the diminished functional levels of the antioxidant enzymes responsible for detoxification. Endoplasmic Reticulum (ER) advocates a degree of control on cellular parameters such as proper protein folding, posttranslational modification and subsequent protein trafficking in order to maintain normal cellular homeostasis. However, an abnormal ER functioning can lead to loss of integrity of the ER thus resulting in ER stress. In addition to this impairment in the ubiquitin proteasome system (UPS) machinery results in the accumulation of toxic proteins in the brain thus resulting in severe neuronal trauma and subsequent damage. This review explores the disease critical interactions and roles of three critical NDD determinants viz. oxidative stress, ER stress and UPS dysfunction in neurodegenerative conditions.

Keywords

NDD; Free radicals; ROS; Oxidative stress; ER stress; UPS; E3-ligases; Mitochondrial dysfunction

Review Article

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Abbreviations

NDD: Neurodegenerative Disorders; ER: Endoplasmic Reticulum; UPS: Ubiquitin Proteasome System; ROS: Reactive Oxygen Species; AD: Alzheimer's Disease; PD: Parkinson's Disease; MS: Multiple Sclerosis; HD: Huntington's Disease; ALS: Amyotrophic Lateral Sclerosis; PS: Presenilin; APP: Amyloid beta (A4) Precursor Protein; MARK1: Microtubule Affinity-Regulating Kinase 1; SOD-1: Superoxide Dismutase 1; VAMP : Vesicle-Associated Membrane Protein; ALS2: Amyotrophic Lateral Sclerosis 2; DCTN1: Dynactin 1; FUS : FUS RNA binding protein; TDP- 43: TAR DNA binding Protein; PERK: Phosphorylation of the ER stress Kinases; IRE 1: Inositol-Requiring Enzyme 1; SCA: Spinocerebellar Ataxia; GSK: Glycogen Synthase Kinase; ASK1: Apoptosis Signal-Regulating Kinase 1; JNK: c-Jun NH2-terminal kinase; Keap1: Kelch-like ECH-associated protein 1; MGRN1: Mahogunin Ring finger 1, E3 ubiquitin protein ligase; MYCBP2: MYC Binding Protein 2, E3 ubiquitin protein ligase; UHRF2: Ubiquitin-like with PHD and Ring Finger domains 2; ZNRF1: Zinc and Ring Finger 1, E3 ubiquitin protein ligase; NEDD4: Neural precursor cell Expressed, Developmentally Down-regulated 4, E3 ubiquitin protein ligase; NEDD4L: Neural precursor cell Expressed, Developmentally Down-regulated 4-like, E3 ubiquitin protein Ligase; HECTD2: HECT domain containing E3 ubiquitin protein ligase 2; PJA2: Praja ring finger 2, E3 ubiquitin protein ligase; RNF19: Ring finger protein 19A, RBR E3 ubiquitin protein ligase; HECTD1: HECT domain containing E3 ubiquitin protein ligase 1; MULAN: Mitochondrial E3 Ubiquitin protein Ligase 1; HACE1: HECT domain and ankyrin repeat containing E3 ubiquitin protein ligase 1; TRIM13: Tripartite Motif containing 13; AIMP2: Aminoacyl tRNA synthetase complex-Interacting Multifunctional Protein 2; NRF2: Nuclear Factor, erythroid 2-like 2; DVL1: Dishevelled Segment polarity protein 1; MYC: v-MYC avian Myelocytomatosis viral oncogene homolog; TSC2: Tuberous Sclerosis 2; FBXO45: F-Box protein 45; PCNP: PEST Proteolytic Signal Containing Nuclear Protein; SMAD2: SMAD family member 2

Introduction

Neurodegenerative disorders (NDDs) are characterized by the gradual and progressive loss of neurons and neuronal death that ultimately leads to deficient nervous system functioning. It can result due to diverse factors such as oxidative stress, ER stress, mitochondrial dysfunction, impaired ubiquitin proteasomal system and several other determinants such as endocrine conditions, gender, poor education, inflammation, stroke, smoking, hypertension, diabetes, infection, head trauma, depression, tumors, vitamin deficiencies, immune and metabolic conditions, chemical exposure, accumulation of reactive oxygen species (ROS), loss of mitochondrial membrane potential, and ATP depletion. The two hit hypothesis of neurodegeneration states that neuronal cells that have been subjected to a severely stress once, becomes more vulnerable to the negative impact of a second hit and the effect of the toxicity of both the hits of severe stress may be synergistic in nature. Most common neurodegenerative diseases include Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Schizophrenia, Amyotrophic lateral sclerosis (ALS) and Multiple Sclerosis (MS) [1-5].

Oxidative stress plays a critical role in the progression of several age related brain disorders. Severe oxidative trauma to the neurons can result in neuronal dysfunction and death.

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Review

Impact of Insulin Degrading Enzyme and Neprilysin in Alzheimer's Disease Biology: Characterization of Putative Cognates for Therapeutic Applications

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Abstract. Alzheimer's disease (AD) is a neurodegenerative process primarily characterized by amyloid-β (Aβ) agglomeration, neuroinflammation, and cognitive dysfunction. The prominent cause for dementia is the deposition of Aβ plaques and tau-neurofibrillary tangles that hamper the neuronal organization and function. Aβ pathology further affects numerous signaling cascades that disturb the neuronal homeostasis. For instance, Aβ deposition is responsible for altered expression of insulin encoding genes that lead to insulin resistance, and thereby affecting insulin signaling pathway and glucose metabolism in the brain. As a result, the common pathology of insulin resistance between Type-2 diabetes mellitus and AD has led AD to be proposed as a form of diabetes and termed 'Type-3 diabetes'. Since accumulation of Aβ is the prominent cause of neuronal toxicity in AD, its clearance is the prime requisite for therapeutic prospects. This purpose is expertly fulfilled by the potential role of Aβ degrading enzymes such as insulin degrading enzyme (IDE) and Neprilysin (NEP). Therefore, their molecular study is important to uncover the proteolytic and regulatory mechanism of Aβ degradation. Herein, (i) *In silico* sequential and structural analysis of IDE and NEP has been performed to identify the molecular entities for proteolytic degradation of Aβ in the AD brain, (ii) to analyze their catalytic site to demonstrate the enzymatic action played by IDE and NEP, (iii) to identify their structural homologues that could behave as putative partners of IDE and NEP with similar catalytic action and (iv) to illustrate various IDE- and NEP-mediated therapeutic approaches and factors for clearing Aβ in AD.

Keywords: Alzheimer's disease, amyloid-β, insulin degrading enzyme, Neprilysin, therapeutics

INTRODUCTION

Alzheimer's disease (AD) is a neurological disorder that is characterized by neuronal death, which is caused by the abnormal burden of amyloid-β (Aβ) in the brain resulting in memory loss and cognitive decline [1–3]. The cognitive collapse in AD occurs due to neuronal dysfunction that is attributable to the extracellular Aβ

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Review Article

Molecular Chaperones and Ubiquitin Proteasome System in Tumor Biogenesis: An Overview

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Abstract

The hallmark feature in cancer is uncontrolled cell-division and altered protein expression. Currently, cancer is one of the most detrimental diseases encountered by a large population across the globe. However, an absolute treatment strategy has still not been achieved by the researchers. Further, molecular mechanism and therapeutic to combat this lethal disease is a baffling issue. Molecular chaperones and ubiquitin proteasome system is mainly responsible for the maintenance of protein homeostasis and thus playing a crucial role in the cancer pathophysiology. Molecular chaperones are a superfamily of proteins which expressions are triggered under physiological, pharmacological and environmental insults and playing a protective role for cell survival. However, beyond a threshold of protection, molecular chaperones are unable to provide proper shape of non functional proteins that accumulate unwanted protein the cellular milieu. In order to get rid off these accumulated proteins ubiquitin proteasome system comes into action where an E3 ligase, specific enzymes of ubiquitination system, play a decisive role in the turnover of many essential regulatory proteins involved in cancer. It also mediates numerous functions, for instance, cell death, cell growth and DNA repair. Since, both molecular chaperones and E3 ligases have been involved in the progression of cancers it is necessary to understand and implement the role of these two molecules to use as diagnostic markers to treat cancer. Herein, we have comprehensively discussed the functional role of molecular chaperones, their differential protein expressions and a possible correction mechanism in cancer. Furthermore, comprehensive information has been documented regarding E3 ligases and their associated role in cancer that may be used as potential diagnostic biomarker for the treatment of various cancers.

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Keywords: Cancer; E3 ligases; Heat shock proteins; Molecular chaperones; Ubiquitin proteasome pathway

Introduction

The progression of cancer is so silent that it is hard to diagnose at the earlier stages, and it is an equally challenging task for the clinicians to treat this disease at early stage due to many unknown mechanisms with sites and tissues specificity issues. Cancer cells are immensely proliferatives, incursive, and metastatic in nature. The pathological hallmark in cancer is uncontrolled cellular proliferation and altered protein expression where hindrance in chaperoning machinery, and UPS is one of the major reasons. Molecular chaperones and ubiquitin E3 ligases are ubiquitous class of proteins that play an essential role in the conformational quality control of the proteins through interacting with various misfolded proteins, stabilizing and remodeling a wide range of non-native polypeptides [1,2]. Even though constitutively expressed under balanced growth conditions, many chaperones are upregulated upon exposure of heat shock or other insults that constitutively increases cellular protein level and for that reason, it is also known as stress or Heat Shock Proteins (HSPs) [3]. Molecular chaperones act together on unfolded or partially folded protein subunits, for instance, nascent chains emerging from the ribosome and with extended chains being translocated across sub-cellular membranes [4]. In addition, some chaperones are non-specific, and they easily interact with a broad range of protein subunits, while others are restricted to specific targets. They often require ATP binding/hydrolysis for their proper functions [4]. Moreover, they also stabilize the non-native conformation for correct folding of protein subunits [5] and not associate with native proteins nor do they form part of the final folded structures. HSPs were first identified as a group of proteins, which are brought about by heat shock and other kind of sources, including physical and chemical stressors in a broad range of species [6]. The HSPs have been afterwards described as molecular chaperones, which share common property of altering the structures and interactions of other proteins [7].

Importantly, molecular chaperone directs that the HSPs frequently interact in a stoichiometric manner with their substrates and also require high intracellular levels of proteins [6]. This is the reason why HSP28, HSP40, HSP70 and HSP110 genes have developed a highly effective mechanism for their synthesis during stress with transcriptional activation, efficient messenger RNA (mRNA) stabilization and their preferential translation [8] and boosts to become dominantly expressed proteins under such stress scenario [9]. Heat shock factor (hsf) family is the main regulatory machineries for HSP gene transcription, which ensure proper transcriptional activation and equally steep switch-off mechanism after recovery [10]. The hsf gene family comprises heat shock transcription factor 1 (hsf1), acts as central co-ordinator of heat shock stress response. Besides this, heat shock transcription factor 2 (hsf2) and heat shock transcription factor 4 (hsf4) are also involved in stress response but less active with respect to hsf1 [11]. HSP transcription needs activated hsf1, which is itself over-expressed in cancer and play a crucial role in invasion and metastasis [12]. Further, the molecular

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RESEARCH Open Access

Comparative study of anti-angiogenic activities of luteolin, lectin and lupeol biomolecules

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Abstract

Background: Angiogenesis is a hallmark feature in the initiation, progression and growth of tumour. There are various factors for promotion of angiogenesis on one hand and on the other hand, biomolecules have been reported to inhibit cancer through anti-angiogenesis mechanism. Biomolecules, for instance, luteolin, lectin and lupeol are known to suppress cancer. This study aims to compare and evaluate the biomolecule(s) like luteolin, lupeol and lectin on CAM assay and HT-29 cell culture to understand the efficacy of these drugs.

Method: The biomolecules have been administered on CAM assay, HT-29 cell culture, cell migration assay. Furthermore, bioinformatics analysis of the identified targets of these biomolecules have been performed.

Result: Luteolin has been found to be better in inhibiting angiogenesis on CAM assay in comparison to lupeol and lectin. In line with this study when biomolecules was administered on cell migration assay via scratch assay method. We provided evidence that Luteolin was again found to be better in inhibiting HT-29 cell migration. In order to identify the target sites of luteolin for inhibition, we used software analysis for identifying the best molecular targets of luteolin. Using software analysis best target protein molecule of these biomolecules have been identified. VEGF was found to be one of the target of luteolin. Studies have found several critical point mutation in VEGF A, B and C. Hence docking analysis of all biomolecules with VEGFR have been performed. Multiple allignment result have shown that the receptors are conserved at the docking site.

Conclusion: Therefore, it can be concluded that luteolin is not only comparatively better in inhibiting blood vessel in CAM assay, HT-29 cell proliferation and cell migration assay rather the domain of VEGFR is conserved to be targeted by luteolin, lupeol and lectin.

Keywords: CAM assay, Flavonoids, HT-29 cell, Anti-angiogenesis, Luteolin, Lupeol, Lectin

Background

Angiogenesis process is regulated by several factors that have a critical role in governing the initiation and progression of tumour. Angiogenic factors such as bFGF, HGF, VEGF, hyluronatelyase, collagenase, MMP supports the formation of new blood vessels. In addition, cell cycle markers, for instance, cyclin A2, Cyclin Dependent Kinase-2, 6 and MAPK1, 14, 10 promote the tumour progression whereas caspase 3 inhibits the tumour progression. Mounting evidence is suggesting the critical role of cyclin inhibitors, and inducers of apoptotic markers in cancer therapy. Furthermore, several biomolecules elicit the anti-cancerous property such as, luteolin, lectin and lupeol but comparative studies in terms of anti-angiogenic activity remain unsettled.

Luteolin is a flavonoid; lupeol is a triterpene and lectin is a protein possessing carbohydrate. Flavonoids are polyphenols that play an important role in defending plant cells against microorganisms, insects, and UV

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