International Journal of Research in Pharmacology & Pharmacotherapeutics (IJRPP)

IJRPP |Vol.14 | Issue 3 | Jul - Sept -2025

www.ijrpp.com

ISSN: 2278-2648 DOI: https://doi.org/10.61096/ijrpp.v14.iss3.2025.342-355

Review

An Overview of Parkinson's Disease Screening Methods: Genetic, Clinical and Neurotoxin-Based Approaches

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Published on: 17 July 2025

Published by:
Futuristic Publications

Abstract

Parkinson's disease is a progressive neurodegenerative disorder marked by motor and non-motor symptoms due to dopaminergic neuron loss in the substantia nigra. Its complex etiology genetic, environmental, and age-related hinders development of disease-modifying therapies. To understand PD pathogenesis and evaluate potential therapeutics, various experimental models have been developed. Alterations in PD-associated genes have been used to develop animal and cell models. *In-vitro* models (Culture of Substantia Nigra and MTT^{+} Assay by Neuroblastoma SH-SY5y Cells (*In-vitro* approaches, including the use of SH-SY5Y cell lines and primary neuronal cultures, offer high-throughput platforms for mechanistic studies and drug screening) and *In-vivo* (Neurotoxin-induced, pharmacological, genetic models,). Neurotoxins such as MPTP, 6-OHDA, and rotenone are widely used to mimic dopaminergic neurodegeneration in animal models and in pharmacological model such as Haloperidol-Induced Model, Tremorine and Oxotremorine, Reserpine. Genetic models utilize mutations in PD-related genes (e.g., SNCA, PARK2, PINK1, LRRK2) to simulate familial PD. In these each model offers unique advantages and limitations, and a strategic combination of these methods enhances translational relevance in PD research.

Keywords: Parkinson's Disease

INTRODUCTION

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder after Alzheimer's disease. It is a chronic, progressive condition characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to a substantial decline in striatal dopamine levels. This dopaminergic loss manifests clinically as bradykinesia, rigidity, resting tremor, and postural instability, collectively known as the cardinal motor symptoms of PD [1,2]. In addition to motor impairments, patients often experience a range of non-motor symptoms, including cognitive dysfunction, sleep disturbances, mood diorders, and autonomic dysfunction, which significantly impact their quality of life [3,4]. Parkinson's disease (PD) becomes more prevalent with age, affecting about 1% of individuals over 60 and rising to 4% in those over 80. While the average age of onset is

around 60 years, about 10% of cases occur between the ages of 20 and 50, classified as young-onset PD, potentially representing a distinct subgroup. The disease is more common in men than women, with reported male-to-female ratios ranging from 1.1:1 to nearly 3:1. Oestrogen's protective effects may influence this gender difference in women [5]. The pathogenesis of PD is multifactorial and incompletely understood, involving complex interactions between genetic predispositions, environmental exposures, mitochondrial dysfunction, oxidative stress, and abnormal protein aggregation, notably of alpha-synuclein. Despite extensive research efforts, current therapies primarily offer symptomatic relief without altering disease progression, highlighting the urgent need for disease-modifying treatments [4,5].

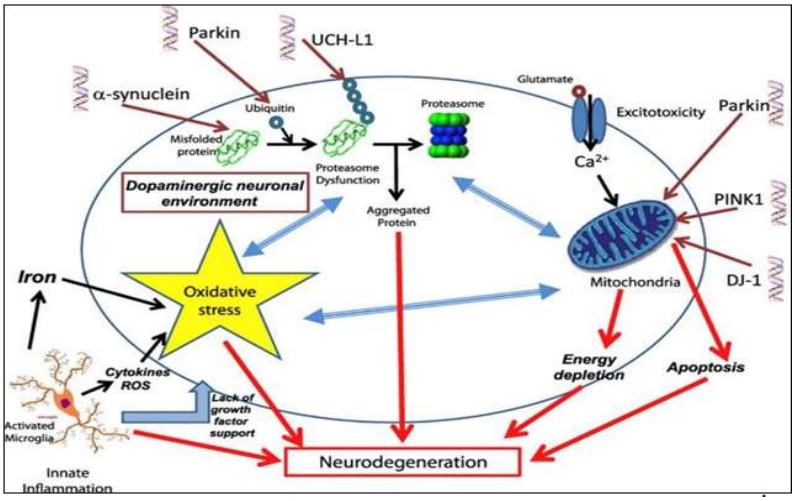


Fig.1: Molecular Mechanism Involved in Parkinson's Disease [4,7]

To advance the understanding of PD mechanisms and to evaluate novel therapeutics, a variety of experimental models have been developed. These include toxin-based and pharmacological animal models, genetic models, and *in-vitro* cellular systems. Each model recapitulates specific aspects of PD pathology and serves as a valuable tool in preclinical research. This review provides a comprehensive overview of the widely used screening methods in PD research, emphasizing their relevance, strengths, and limitations in modelling the disease and facilitating translational discoveries [7,8].

***In-vivo* models in parkinson's disease**

Neurotoxin-Induced Model

- 6-OHDA (6-hydroxydopamine)
- 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP)
- Rotenone
- Paraquat

Genetic Model`

- α-Synuclein Transgenic Mice
- LRRK2 Mutant Mice
- DJ-1, Parkin and PINK1 Knockout Mice

Pharmacological Model

- Haloperidol-Induced Model
- Tremorine and Oxotremorine
- Reserpine

Neurotoxin-Induced Model

Neurotoxin-induced models are among the most widely used experimental approaches to mimic Parkinson's disease pathology in animals. These models rely on selective toxins that target and degenerate dopaminergic neurons, particularly in the nigrostriatal pathway, thereby replicating key features of PD such as motor deficits and dopaminergic loss. The most common neurotoxins used include 6-OHDA, MPTP, rotenone, and paraquat. Each model varies in its mechanism of action, species specificity, and extent of pathology.

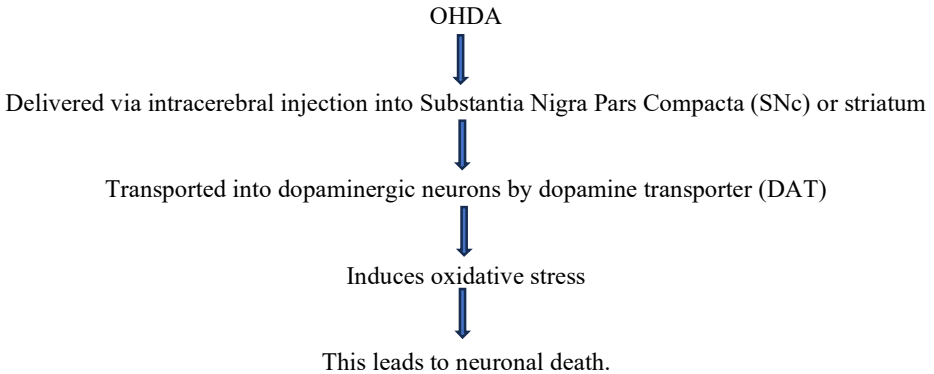
Table: 1 List of Various Parkinson's Disease Models with Species and Risk Factors

Model	Species used	Risk factor [7]
MPTP	Rhesus monkeys Mice (*Strains:* C57BL/6) Rats (less common)	Inside the neuron, MPP^+ disrupts mitochondrial complex I, leading to: • Oxidative stress • ATP depletion • Neuronal death
6-OHDA	Rats Mice Other species (Rarely used; 6-OHDA models are largely rodent-based)	• Generates reactive oxygen species (ROS). • Causes oxidative stress and mitochondrial dysfunction. • This leads to selective dopaminergic neuron degeneration.
Rotenone	Rats (particularly Lewis rats) Mice Drosophila melanogaster (fruit flies) Zebrafish C. elegans	Inhibits mitochondrial complex I → leads to: • ATP depletion • Excess reactive oxygen species (ROS) • Oxidative stress • α-synuclein aggregation
Paraquat	Mice (most commonly usedC57BL/6 strain) Rats Drosophila melanogaster (fruit flies) C. elegans	Redox cycling of paraquat generates large amounts of reactive oxygen species (ROS) • Oxidative stress • Lipid peroxidation • Mitochondrial dysfunction • Dopaminergic neuron death
Haloperidol	Rats Mice Primates (rarely)	• Dopaminergic modulation • Screening of anti-parkinsonian drugs • Drug-induced motor side effects

6-OHDA

6-OHDA is a hydroxylated analog of dopamine that selectively destroys catecholaminergic neurons by generating reactive oxygen species [ROS] and this inhibiting mitochondrial complex I. Due to its inability to cross the blood-brain barrier (BBB), 6-OHDA must be stereotactically injected directly into the substantia nigra, medial forebrain bundle, or striatum. The extent and site of injection influence the severity and progression of dopaminergic neuronal loss. This model is typically used in rats and results in unilateral lesions, allowing for behavioral assessment via rotational asymmetry tests (e.g., apomorphine-induced rotation). The 6-OHDA model is well-suited for studying motor symptoms and dopaminergic neurodegeneration, but this model does not replicate alpha-synuclein pathology or progressive disease features [7,8,9].

Mechanism of Action



This 6-OHDA model induced various alteration and its related symptoms are as follows

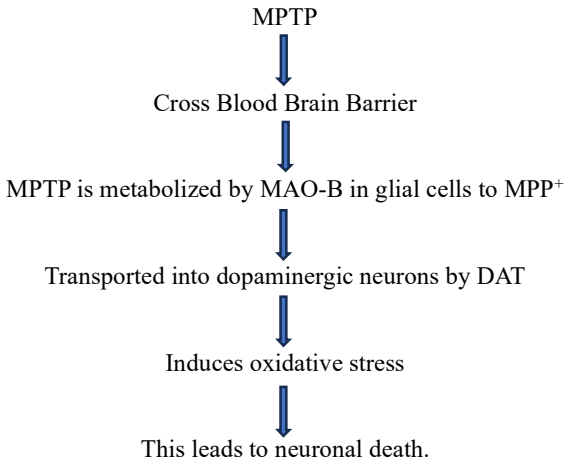
Table: 2 Behavioral, Motor, and Non-Motor alterations and its Symptoms in of 6-OHDA Model

Category	Symptoms/Features
Behavioral	Apathy or reduced motivation Impaired learning or memory in some paradigms Anxiety-like or depressive-like behaviors (depending on lesion site and extent)
Motor	Unilateral lesions: Asymmetrical motor deficits, rotational behavior (e.g., ipsilateral or contralateral turning after amphetamine or apomorphine challenge) Bradykinesia (slowness of movement) Rigidity (muscle stiffness) Postural instability Tremor (less consistently observed in rodents) Decreased spontaneous locomotion
Non-Motor	Cognitive deficits (working memory, executive function) Depression-like behavior (e.g., increased immobility in forced swim test) Anxiety-like behavior (e.g., reduced time in open arms of an elevated plus maze) Sleep disturbances Gastrointestinal dysfunction (slowed motility)

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

MPTP is a lipophilic neurotoxin that readily crosses the BBB and is converted by astrocytic monoamine oxidase-B into its active metabolite, MPP+. MPP+ selectively enters dopaminergic neurons via the dopamine transporter (DAT) and inhibits mitochondrial complex I, leading to oxidative stress and neuronal death. This model is predominantly used in mice and non-human primates. In mice, acute or subacute systemic MPTP administration induces rapid nigrostriatal degeneration and motor deficits. In primates, MPTP leads to a syndrome closely resembling human PD, including both motor and non-motor symptoms. However, similar to 6-OHDA, MPTP does not reproduce Lewy body pathology unless modified with additional factors (e.g., alpha-synuclein overexpression) [7,8,9].

Mechanism of Action



This MPTP model induced various alteration and its related symptoms are as follows:

Table: 3 Behavioral, Motor, and Non-Motor alterations and its Symptoms in of MPTP Model

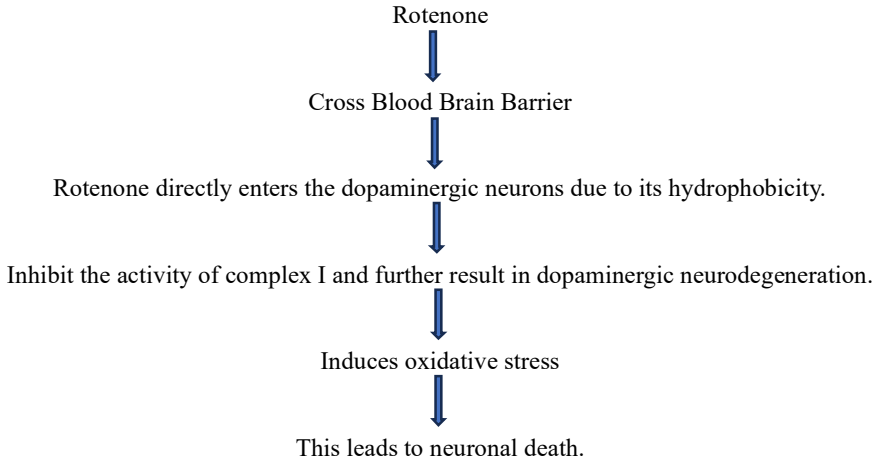
Category	Symptoms/Features
Behavioural	Reduced exploratory behavior Lethargy or hypokinesia Altered social interaction in primates Anhedonia or decreased motivation (in chronic models)
	Bradykinesia (slowness of movement) Rigidity (muscle stiffness)

Motor	Postural instability Tremor (especially in non-human primates) Impaired balance and coordination Decreased spontaneous movement or locomotor activity
Non-Motor	Cognitive impairments (attention, working memory, executive function) Mood disorders (anxiety, depression-like symptoms) Sleep disturbances Autonomic dysfunction (gastrointestinal issues, thermoregulation problems) Olfactory deficits (less studied but reported)

Rotenone

Rotenone is a natural mitochondrial complex I inhibitor that can induce systemic and selective dopaminergic neurodegeneration when administered chronically. Unlike MPTP or 6-OHDA, rotenone can replicate alpha-synuclein aggregation, making it useful for studying PD-related proteinopathy. It also induces oxidative stress, microglial activation, and gastrointestinal dysfunction, mimicking several aspects of idiopathic PD. However, rotenone's high variability in response and systemic toxicity, especially in rats, limit its reproducibility and practical utility. It is generally used to model both motor and non-motor symptoms of PD [7,8,9].

Mechanism of Action



This Rotenone model induced various alteration and its related symptoms are as follows:

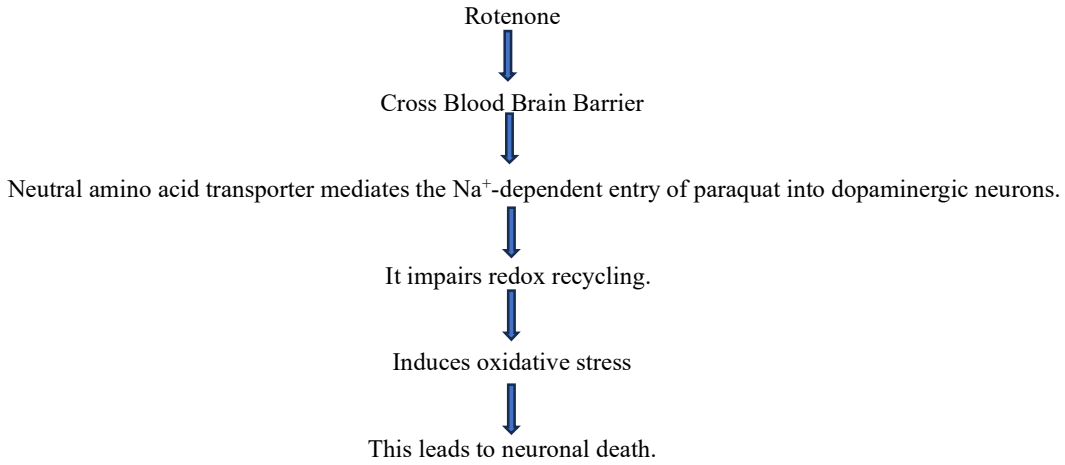
Table 4: Behavioral, Motor, and Non-Motor alterations and its Symptoms in of Rotenone Model

Category	Symptoms/Features
Behavioral	Reduced exploratory activity Decreased motivation (e.g., reduced sucrose preference) Social withdrawal (in rodents and flies)
Motor	Bradykinesia (slowed movement) Rigidity Postural instability Tremor-like activity (more apparent in primates) Impaired gait and coordination Decreased locomotion (open field test)
Non-Motor	Depression-like symptoms (e.g., increased immobility in forced swim test) Anxiety-like behavior (e.g., reduced time in open arms of the elevated plus maze) Cognitive impairments (memory, attention deficits) Olfactory deficits Gastrointestinal dysfunction (common due to enteric nervous system involvement) Sleep abnormalities (in longer-term exposure models)

Paraquat

Paraquat, a widely used herbicide, shares structural similarities with MPP+ and induces oxidative stress through redox cycling. It has been shown to cause selective dopaminergic cell death, especially when combined with maneb (a fungicide), to enhance neurotoxicity. Although paraquat exposure is epidemiologically linked to increased PD risk, its ability to replicate consistent nigrostriatal degeneration in animal models is debated. It is primarily used to study environmental toxin contributions to PD [7,8,9].

Mechanism of Action



This Paraquat model induced various alteration and its related symptoms are as follows:

Table: 5 Behavioral, Motor, and Non-Motor alterations and its Symptoms in of Paraquat Model

Category	Symptoms/Features
Behavioral	Reduced exploratory activity Decreased motivation (e.g., reduced sucrose preference) Social withdrawal (in rodents and flies)
Motor	Bradykinesia (slowed movement) Rigidity Postural instability Tremor-like activity (more apparent in primates) Impaired gait and coordination Decreased locomotion (open field test)
Non-Motor	Depression-like symptoms (e.g., increased immobility in forced swim test) Anxiety-like behavior (e.g., reduced time in open arms of the elevated plus maze) Cognitive impairments (memory, attention deficits) Olfactory deficits Gastrointestinal dysfunction (common due to enteric nervous system involvement) Sleep abnormalities (in longer-term exposure models)

Genetic Model

Genetic models of Parkinson's disease (PD) have been developed to recapitulate hereditary forms of the disease, which account for approximately 5–10% of all cases. These models involve the manipulation of genes known to be mutated in familial PD, including SNCA (α-synuclein), LRRK2 (leucine-rich repeat kinase 2), PARK2 (parkin), PINK1 (PTEN -induced kinase-1), DJ-1 (is also known as PARK7 stands for Parkinsonism associated deglycase 7), and GBA1 (Glucosylceramidase Beta 1). Genetic models are instrumental in dissecting the molecular and cellular mechanisms underlying PD, particularly those associated with protein aggregation, mitochondrial dysfunction, autophagy-lysosomal pathways, and neuroinflammation [20].

α-Synuclein Models

Mutations and multiplications of the SNCA gene, encoding α-synuclein, are linked to autosomal dominant PD. Transgenic animals overexpressing wild-type or mutant forms (e.g., A53T, A30P, E46K) of α-

synuclein develop intracellular protein aggregates resembling Lewy bodies, the pathological hallmark of PD. These models are commonly developed in mice, rats, and Drosophila, and exhibit progressive motor deficits, synaptic dysfunction, and selective dopaminergic neuron loss in some cases. However, the severity and onset of pathology depend on the promoter used and the genetic background. Although not all α-synuclein models display robust nigrostriatal degeneration, they are invaluable for studying protein misfolding, aggregation, and spread [10,12].

LRRK2 Models

Mutations in LRRK2, particularly G2019S, are the most frequent cause of familial and sporadic PD. LRRK2 is a multifunctional kinase implicated in vesicular trafficking, mitochondrial dynamics, and autophagy. Transgenic or knock-in mice harboring mutant LRRK2 display subtle motor deficits, mitochondrial abnormalities, impaired dopamine neurotransmission, and axonal pathology. However, they often lack overt dopaminergic neuron loss or α-synuclein aggregation. These models are essential for studying kinase-dependent neurodegeneration and for testing LRRK2 inhibitors [10,12].

Parkin, PINK1, and DJ-1 Models

PARK2 (parkin), PINK1, and DJ-1 mutations are associated with autosomal recessive early-onset PD. These genes are critical for mitochondrial quality control and oxidative stress regulation. Knockout models of these genes, especially in mice and flies, typically do not develop spontaneous nigrostriatal degeneration, but they exhibit mitochondrial dysfunction, increased susceptibility to oxidative stress, and deficits in motor coordination. Combining these models with environmental stressors (e.g., MPTP or rotenone) can unmask latent phenotypes and mimic gene-environment interactions [10,12].

GBA1 Models

Mutations in GBA1, which encodes glucocerebrosidase, are a major genetic risk factor for PD. GBA1-deficient models show α-synuclein accumulation, lysosomal dysfunction, and dopaminergic deficits. These models bridge the gap between lysosomal storage disorders and synucleinopathies, highlighting the role of impaired autophagy-lysosomal pathways in PD [10,12].

Pharmacological Model

Pharmacological models of PD involve the use of drugs that disrupt dopaminergic signalling to mimic Parkinsonian symptoms. These models are widely used for studying the mechanisms of motor dysfunction, evaluating dopaminergic pathways, and testing anti-Parkinson or neuroprotective drugs.

Haloperidol Model

Haloperidol (HP), a neuroleptic medication, is one of the main factors of drug-induced neurological disorders such as Parkinson's globally. The initial step of first-generation antipsychotic medication often prescribed to treat schizophrenia is haloperidol (HP). Haloperidol use reduces the drug's tendency to exhibit several extrapyramidal symptoms, including tardive dyskinesia and parkinsonism. Haloperidol-induced extrapyramidal symptoms' precise etiology is still undetermined [24].

Mechanism of Action

Haloperidol

⇓

blocking D_2 receptors in the nigrostriatal pathway

⇓

extrapyramidal symptoms such as bradykinesia, rigidity, tremors, and postural instability [28].

This 6-OHDA model induced various alteration and its related symptoms are as follows:

Table: 6 Behavioral, Motor, and Non-Motor Symptoms alterations and its Symptoms in of Haloperidol Model

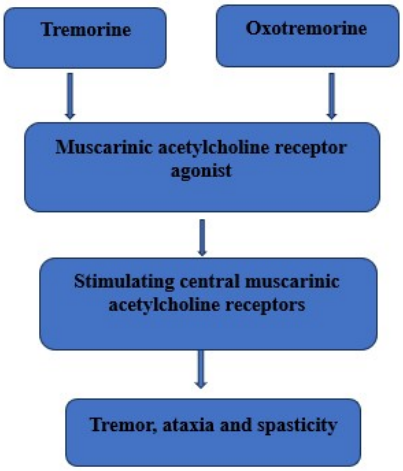
Category	Symptoms/Features
Behavioral	Reduced exploratory behavior Decreased motivation or reward-seeking (due to dopamine blockade) Sedation or lethargy Social withdrawal (in prolonged treatment)

Motor	Catalepsy (rigid, immobile posture held abnormally long hallmark feature in rodents) Bradykinesia (slow movement) Rigidity Tremor-like symptoms (occasionally in primates) Impaired locomotion and spontaneous activity Akinesia (loss of movement initiation)
Non-Motor	Anxiety-like behavior (e.g., elevated plus maze tests) Depressive-like symptoms (e.g., increased immobility in forced swim test) Cognitive deficits (attention and memory, especially with chronic use) Sleep disturbances (due to dopamine modulation) Anhedonia (loss of pleasure response)

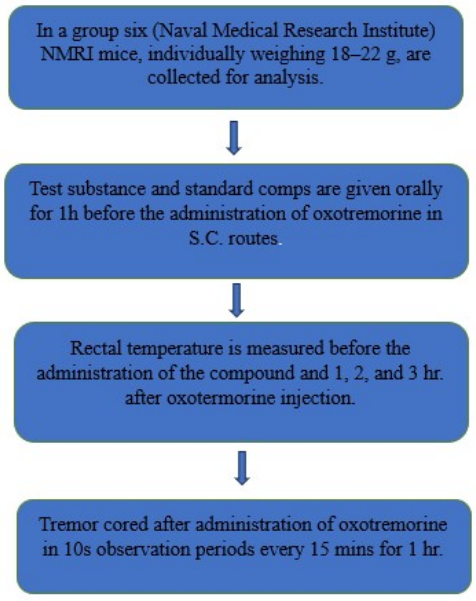
Tremorine and Oxotremorine

Muscarinic agonists Tremorine and oxotremorine evoke symptoms of Parkinson's disease. The indication of symptoms like hypothermia, ataxia, spasticity, tremors, lacrimation, and salivation. These symptoms are counteracted by anticholinergic medications.

Mechanism of Action



Procedure



Evaluation

Hypothermia: Differences in body temperature after 1, 2, 3hrs versus basal values are summarized in each group in control and test and their average values are compared.

Tremor: The scores for all animals in each group in three observational periods are noted to calculate the percentage protection in the test groups.

Salivation and Lacrimation: The scores for both symptoms for all animals in each group are summarized to express the protection of test compounds [22].

Scores for tremor, salivation, lacrimation

	Tremors Score	Salivation and Lacrimation Score
Absent	0	0
Slight	1	1
Medium	2	2
Severe	3	3

Reserpine

Reserpine-induced depletion of central catecholamine stores, these leads to decreased dopamine levels. The sedative effects might be produced in mice after the injection, followed by signs of eyelid ptosis, hypokinesia, rigidity, and immobility.

Procedure

Male NMRI mice of either sex weighing 20-25g

⇓

Intraperitoneal injection of reserpine 5 mg/kg and tested 24 hrs later

⇓

Test comps are injected 30 minutes before observation.

⇓

The animals are placed singly onto the floor of a Perspex container (30×26 cm high), situated on a panlab proximity sensor unit.

⇓

Horizontal movements are recorded for 10 min.

Evaluation

Locomotor activity and grooming scores of drugs treated and control groups are compared [22].

***In-vitro* models in parkinson's disease**

In-vitro models play a critical role in elucidating the molecular mechanisms of Parkinson's disease (PD) and in the early-stage screening of neuroprotective agents. These models offer high-throughput capabilities, cost-effectiveness, and mechanistic insights prior to *in-vivo* validation.

The most frequently used *in-vitro* methods are

Culture of Substantia Nigra

MTT^{+} Assay by Neuroblastoma SH-SY5y Cells

Other commonly used *in-vitro* models include

Immortalized cell lines

Primary neuronal cultures

Organotypic slices

Induced pluripotent stem cells (iPSCs)

Neurotoxin Model

Genetic Model

The most frequently used *in-vitro* methods are

Culture of Substantia Nigra

The culture of Substantia Nigra refers to the isolation and in vitro maintenance of neuronal and/or glial cells derived from the substantia nigra, a midbrain region rich in dopaminergic neurons, which are the primary

targets of Parkinson's disease [14,16].

Purpose

- To study dopaminergic neuron physiology, survival, and degeneration.
- To model Parkinson's disease mechanisms (e.g., oxidative stress, neurotoxicity).
- To screen neuroprotective compounds (like *Musa paradisiaca* peel extract).

Procedure

1. A suitable plane of anesthesia can be achieved by placing the animals on ice for two to three minutes.
2. Immediately after the animals are killed, 8–10 brains from P2–P5 C57BL/6 or SWR matings are taken out and inserted in a newly made dissociation medium (DM).
3. A portion of the midbrain, rostral to the cerebellum and caudal to the hippocampus, is isolated once the brains are positioned on their ventral surfaces. The whole midbrain or the ventrolateral midbrain, which contains the substantia nigra, is dissected and chopped into little pieces after this removed brain slab is laid flat in DM.
4. The tissue is triturated in 5 ml PM and the cell suspension is added to 2 ml (1 ml of BSA stock and 1 ml of ovalbumin stock). The minced substantia nigra or midbrain is then incubated in papain and DNase (Dissociation Kit, Worthington Biochemical Corp., Freehold, NJ, follow kit instructions) for 30 min at 37 8C. A second incubation with fresh papain solution (30 min, 37 8C) is then followed by three rinses in DM and one rinse in plating media (PM). Lastly, the MPTP in media is 50 nM.
5. The pellet is then resuspended in 1.0 ml of plating media containing 2% rat serum after the cell suspension has been spun for 8 minutes at 1400 rev/min.
6. Trypan blue (0.4%) is employed to count the cells until they have been resuspended to evaluate their vitality.
7. Cells are plated at 200,000 cells/cm in Lab-Tek 4-well Permanox chamber slides after being adjusted to 1.2310 cells/ml. The slides were previously coated with a 1:1 (v: v) solution of laminin (200 mg/ml, Collaborative Biomedical Products) and poly-D-lysine (200 mg/ml, Collaborative Biomedical Products), and rinsed once with deionized water. Two to three times a week, cells are fed with feeding media supplemented with 2% rat serum (RS) by exchanging roughly one-fifth of the media (100 Ml), and they are kept in an incubator at 37 8C with 5% CO [17,25,27].

MTT$^+$ Assay by Neuroblastoma SH-SY5y Cells

Purpose

The MTT assay is a colorimetric assay used to assess cell viability and cytotoxicity. It measures mitochondrial metabolic activity, which reflects the number of viable (living) cells.

Cell Line: SH-SY5Y

- Origin: Human neuroblastoma
- Properties: Can be differentiated into neuron-like cells (dopaminergic phenotype) using agents like retinoic acid.
- Relevance: Widely used in Parkinson's disease and neurodegeneration research.

Principle of the MTT Assay

- MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is reduced by mitochondrial dehydrogenases in viable cells to form insoluble purple formazan crystals.
- After solubilization, absorbance is measured spectrophotometrically (typically at 570 nm).
- Higher absorbance = more viable cells

SHSY-5Y Neuroblastoma cells treated with 1mM conc. Of haloperidol dissolved in 5% DMSO Soln and then treated with arbutin (5, 10, 15, and 20 µm) dissolved in distilled water

⇓

Incubate it for 48 hrs.

⇓

Neuroblastoma cells were cleaned with PBS and 100 µl MTT dye was loaded in all wells and retained for 3 hrs at 37^{o}C

⇓

After (Dimethyl Sulfoxide) DMSO was added well color from yellow to blue

⇓

Absorbance was assessed at 570nm.

Report: % protections of cell death of SH-SY5Y cells induced by haloperidol are measured [25].

Other commonly used *in-vitro* models include

Immortalized Cell Lines

Cell lines such as SH-SY5Y and (Lund human mesencephalic) LUHMES are widely used due to their ease of culture, reproducibility, and suitability for high-throughput drug screening. SH-SY5Y, a human neuroblastoma line, expresses low levels of dopaminergic markers but can acquire neuron-like features upon differentiation with agents like retinoic acid. LUHMES cells, derived from human fetal mesencephalon, must be differentiated to express dopaminergic characteristics such as (Tyrosine Hydroxylase) TH, (Dopamine Transporter) DAT, and (Volumetric Modulated Arc Therapy) VMAT. While SH-SY5Y cells are easier to maintain, LUHMES offer better physiological relevance due to their non-tumor origin. However, both lines are limited by differences from primary neurons in morphology and function [30].

Primary Dopaminergic Cultures

Primary neurons are typically isolated from embryonic rodent ventral midbrain and provide a more accurate dopaminergic phenotype, closely mimicking *in-vivo* neuronal morphology and function. These cultures express high levels of (Tyrosine hydroxylase) TH and (Dopamine Transporter) DAT and form functional synapses. However, they are heterogeneous, glia-rich, and technically challenging to prepare, limiting their use for large-scale screening. Moreover, their rodent origin poses translational limitations [30].

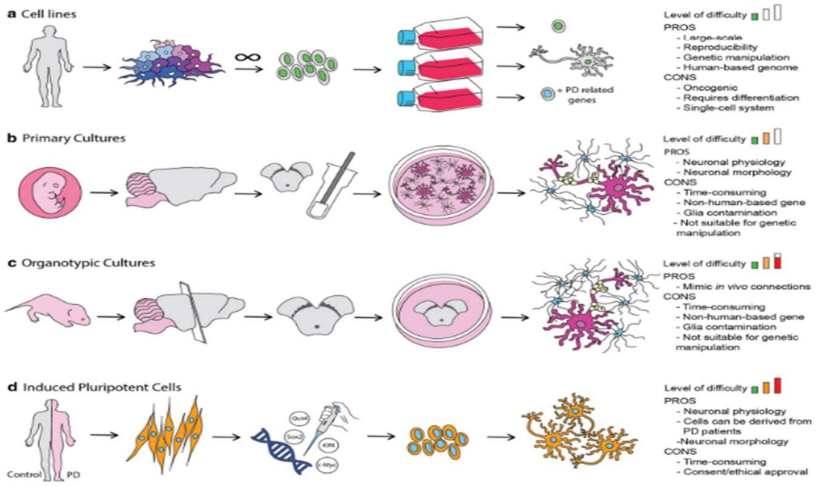


Fig.2: *In-vitro* models of Parkinson's disease vary in complexity, ranging from the simplest to the most technically demanding systems.

(a)*Cell line models* are derived from biopsied cells of multicellular organisms, which are clonally expanded and immortalized often through transformation with agents like large T antigen allowing continuous proliferation. These lines can be used in both undifferentiated and differentiated forms.
(b)*Primary neuronal cultures* are prepared by collecting intrauterine horns from pregnant rodents (E14 for rats, E13 for mice), followed by dissection of embryos to isolate the ventral midbrain area (VMA). Dopaminergic neurons are extracted using enzymatic and mechanical digestion and then cultured in optimized media.
(c)*Organotypic slice cultures* preserve the three-dimensional structure and natural cell interactions of brain tissue. In this method, brains from neonatal rodents are dissected to isolate the VMA, which is then sliced and cultured to maintain regional architecture.
(d)*Induced pluripotent stem cells (iPSCs)* are generated by reprogramming adult human somatic cells (commonly fibroblasts) using four transcription factors Oct4, Sox2, Klf4, and c-Myc. These cells can subsequently be directed to differentiate into dopaminergic neurons [30].

Organotypic Slice Cultures

Organotypic cultures maintain the three-dimensional architecture and cell–cell interactions of brain tissue. Obtained from neonatal rodent brain slices, these cultures preserve the nigrostriatal circuitry, making them

valuable for studying network-level neurodegeneration, neurogenesis, and electrophysiology. Toxins like 6-OHDA and MPP^+ are commonly used to induce PD pathology. Despite their physiological relevance, these models require skilled handling and have limited scalability [30].

Induced Pluripotent Stem Cells (iPSCs)

iPSC-derived dopaminergic neurons are emerging as the gold standard for in vitro PD modelling. They can be generated from patient-derived fibroblasts, enabling personalized disease modelling. Upon differentiation, these cells exhibit key dopaminergic markers and functional synaptic activity. Genetic PD models can be created directly from patients with mutations (e.g., SNCA, LRRK2) or through genome editing. However, iPSC cultures are time-consuming (4–10 weeks), technically demanding, and require ethical clearance. Furthermore, their 2D culture format may lack the structural complexity of the brain, although recent advances in 3D cerebral organoids show promise in overcoming these limitations [30].

Neurotoxin Model

Neurotoxin-induced models of Parkinson's disease use selective toxins like 6-OHDA, MPTP, rotenone, and paraquat to selectively damage dopaminergic neurons in the nigrostriatal pathway. These models effectively replicate key PD features such as motor dysfunction and dopamine loss, though they differ in mechanism, species sensitivity, and pathological outcomes as same as *in-vivo* models.

Genetic Model

Genetic models of Parkinson's disease mimic hereditary forms by manipulating genes like SNCA, LRRK2, PARK2, PINK1, DJ-1, and GBA1. They are crucial for studying PD-related mechanisms such as protein aggregation, mitochondrial dysfunction, impaired autophagy, and neuroinflammation as same as *in-vivo* models**.**

CONCLUSION

Parkinson's disease remains a complex neurodegenerative disorder with multifactorial origins, necessitating diverse approaches for its study and early detection. In this review article, we comprehensively discuss all major screening methods used for Parkinson's disease, including genetic, clinical, and neurotoxin-based approaches. Unlike previous studies that often focus on one or two techniques, this review offers a broad and integrated perspective. These will be helpful for further research work on Parkinson's disease. Neurotoxin-based *in-vivo* models MPTP, 6-OHDA, rotenone, and paraquat, have been invaluable for replicating Parkinson's disease pathology supporting preclinical testing of therapeutic candidates and investigation of underlying disease mechanisms. Genetic screening has revealed critical insights into familial and sporadic PD through the identification of mutations in genes such as *SNCA*, *LRRK2*, and *PINK1*, offering promising avenues for predictive diagnostics. Pharmacological assessments—including motor evaluations, imaging, and biomarker analysis—remain essential for diagnosis and monitoring, though they often capture the disease only after significant neuronal loss has occurred.

I*n-vitro* models are essential in Parkinson's disease research, providing efficient platforms for studying neurodegeneration and screening therapies. While cell lines like SH-SY5Y provide high-throughput utility, advanced models such as iPSC-derived neurons and organotypic slices offer greater physiological relevance. Although no single model fully replicates Parkinson's disease, their combined use particularly when integrated with in vivo studies enhances mechanistic insight and supports therapeutic discovery.

Future Aspects

Future screening strategies for Parkinson's disease (PD) are expected to increasingly integrate traditional experimental models such as neurotoxin-induced, genetic, and pharmacological systems with cutting-edge technologies like artificial intelligence (AI) and machine learning (ML). These computational tools are particularly adept at handling high-dimensional and heterogeneous data, making them ideal for identifying subtle early biomarkers, predicting disease onset and progression, and personalizing treatment strategies. AI-driven image analysis can enhance the sensitivity of detecting neurodegenerative changes in animal models, while behavioral tracking systems powered by ML can reveal nuanced motor and non-motor phenotypes. Furthermore, ML-based approaches can improve genotype-phenotype correlations, enabling deeper insights into the underlying molecular mechanisms of PD and paving the way for precision medicine. By combining biological relevance with computational power, this integrated approach holds significant promise for accelerating discovery, improving diagnostic accuracy, and ultimately advancing therapeutic development in Parkinson's disease research.

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