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SCREENING MODELS OF ANTI-PARKINSONIAN AGENTS

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ABSTRACT: Parkinson's disease (PD), identified as the second most common neurodegenerative disorder, is a progressive disease that impairs the ability to control voluntary movements mainly. According to the findings of the pathogenesis of PD, lesions in dopaminergic innervation of the basal ganglia cause degeneration of neurons in the substantia nigra and thus leads to loss of striatal dopamine level. Hence, enhancement of dopaminergic transmission restores partial motor functions. In the current review, the possible *in-vivo* animal models have been described in detail, which mostly resembles the Parkinsonian state in humans and has helped research involving pathogenesis, treatment, and evaluating drugs having Anti-Parkinson activity. This study aims to set down all the possible models to screen agents that have a therapeutic or symptomatic role in treating Parkinson's disease. Every model has its advantage and disadvantage, which must be carefully considered when choosing the model to be used.

INTRODUCTION: Parkinson's disease is a progressive neurodegenerative illness characterized by tremors, muscular rigidity, bradykinesia, and postural imbalance that worsens over time [1]. It affects approximately 1% of the population over the age of 65 years [2] and is regarded as the second most common neurodegenerative disease [3]. Clinical diagnosis relies on the presence of such features, it is also associated with many non-motor symptoms that add to overall disability [4]. Also, the imbalance between dopamine and acetylcholine in the Substantia nigra (SN) is a cause of PD. Although characterized as a neuromuscular disorder, dementia, sialorrhoea [5], soft speech and difficulty in swallowing due to uncoordinated movements of mouth and throat also occurs at a much greater rate in PD patients over the normal-aged population.

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It is also reported as the decline in caudate-putamen dopamine content which led to the introduction of dopamine replacement therapy. The etiology of PD remains unknown; several factors appear to play a role, including the aging process, environmental chemicals, oxidative stress, and genetic aspects. A lesion in PD is a marked deficiency in the dopaminergic innervation of the basal ganglia [6]. The discovery that subjects exposed to pyridine derivative, 1 - methyl - 4 - phenyl - 1, 2, 3, 6 - tetrahydropyridine (MPTP), a by-product of synthetic heroin, developed a profound parkinsonian state that led to intense study of the pathogenesis of PD. The primary motor control-related symptoms have shown to be a result of dysregulation of the motor cortex via the nigrostriatal pathway.

This dysregulation is caused by the depletion of DA-producing neurons within the pars compacta region of the substantia nigra that project to the striatum. This is often accompanied by Lewy bodies, which are abnormal aggregates of protein that develop inside nerve cells, formed mainly by α-synuclein and ubiquitin [7].

Nevertheless, PD symptoms are not seen until about 80% of the dopaminergic neurons in the striatum have been destroyed. Thus, significant disease progression must occur before an observable reduction of motor movement and control [8].

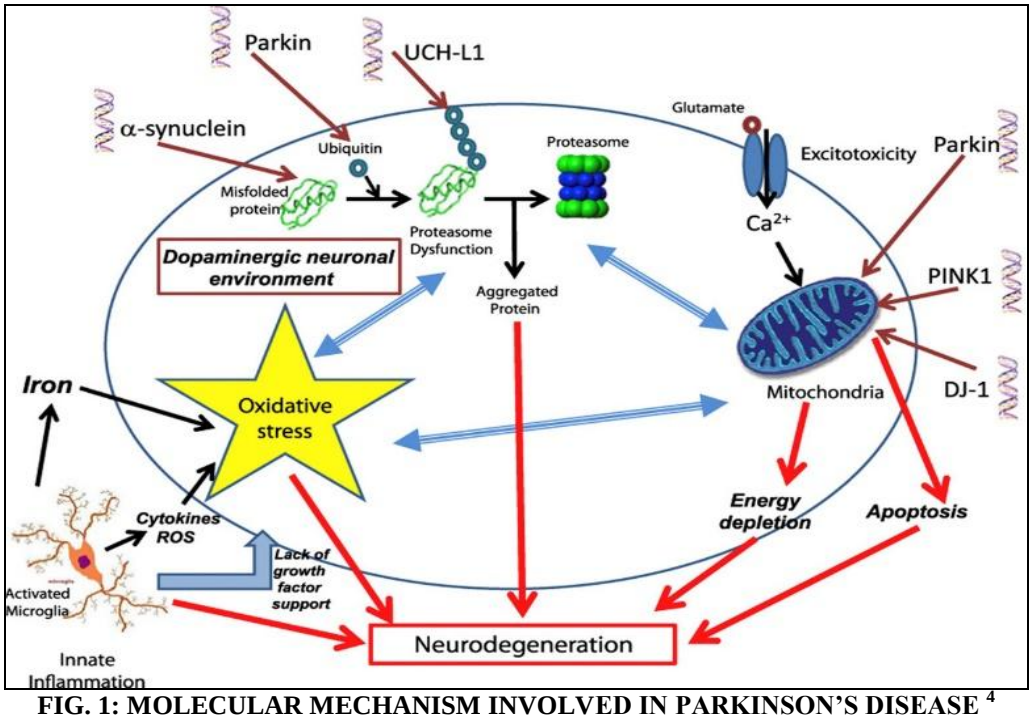

FIG. 1: MOLECULAR MECHANISM INVOLVED IN PARKINSON'S DISEASE [4]

TABLE 1: ANIMAL MODELS OF PD [7]

Risk Factor	Model	Species used
Aging	Intact Aged Animals	Monkey
Genetic	Transgenic animals α-syn	Mice
Neurotoxic	6- OHDA intranigral	Rat
	6- OHDA intraventricular	Rat
	6-OHDA intrastriatal	Rat
	MPTP systemic "acute"	Mice
	MPTP systemic "chronic"	Mice
	Rotenone systemic	Rat
	Lactacystin	Rat
Epigenetic	Prenatal exposure to bacteriotoxin	Rat
Mixed	Prenatal bacteriotoxin + 6-OHDA	Rat
	α – synoverexpression + systemic MPTP	Mice
	Aged + systemic MPTP	Mice

***In-vivo* Methods:**

1. Pharmacological Models: As the name suggests, pharmacological agents induce a parkinsonian state in animals. A drawback of this model is that these are not effective for drugs used for repeated administration.

a) Tremorine and Oxotremorine Antagonism: These agents are muscarine agonists and induce parkinsonism-like symptoms such as tremor, ataxia, spasticity, salivation, lacrimation, and hypothermia. Oxotremorine induces oxidative stress and is implicated as a common pathway in the development of Parkinson's symptoms [9]. They increase reflex and spontaneous activity. An increase in reflex activity is accompanied by rigidity whereas spontaneous activity consists of rhythmic bursts of discharges [10]. Muscarine antagonists are used for reversing the effects.

Methodology: Three groups of six male NMRI mice are used. Group A serves as a control. Group B is dosed orally with test compound, whereas group C is given standard (5mg/kg Benzatropine mesylate) 1 h before administration of 0.5mg/kg oxotremorine. Body temperature is noted before administration of test compound (basal value) and after administration at an interval of 1 h for 3 h. This model measures only central anticholinergic activity.

Observation and Evaluation:

Hypothermia: The difference in body temperature between intervals is observed and compared with standard and control groups.

Tremor: Tremor is scored after oxotremorine injection in a 10s observation period every 15min for 1 h.

Tremor	Score
Absent	0
Slight	1
Medium	2
Severe	3

Salivation and Lacrimation: These parameters are scored 15-20min after oxotremorine injection.

Absent	0
Slight	1
Medium	2
Severe	3

b) Reserpine Antagonism: The reserpine-treated rodent was one of the earliest animal models employed in PD research [11]. Reserpine binds to storage vesicles of catecholamines and also blocks VAMT-2 (Vesicular monoamine transporter-2). Thus, it temporarily depletes the storage of dopamine in their respective neurons in the brain. In addition, there is a gradual loss of vesicle stored dopamine as it is used up by release so that the storage vesicles eventually become dysfunctional. The reductant is the depletion of dopamine in neuron [8]. Thus, reserpine is used to induce Parkinson-like symptoms. Reserpine produces ~85% loss of dopamine in the Substantia nigra and >95% dopamine depletion in the striatum. This is regarded as the fastest method of inducing PD.

Methodology: Male NMRI mice are used. They are injected with 5mg/kg *i.p.* reserpine. After 24 h animals are tested. The test compound is injected 30 min before observation. The animals are placed onto the floor of a Perspex container and observed.

FIG. 2: PERSPEX CONTAINER

Observation and Evaluation: Horizontal movements are recorded for 10min. Also rearing, grooming episodes are observed. They are scored according to the severity and are compared using ANOVA [6].

c) Haloperidol Induced Catalepsy: Haloperidol causes the dysfunction of many neurotransmitters such as acetylcholine, GABA, and serotonin. Primarily used as an anti-psychotic drug, it blocks dopamine receptors in the brain. It functions by disrupting receptors of dopamine D1 and D2 in medium spiny neurons, which include motor circuit indirect and direct pathways. This leads to blockage of striatal dopamine transmission, which causes abnormal downstream firing in the basal ganglia as symptoms of muscle stiffness, locomotive activity, and catalepsy [12]. It induces catalepsy by increasing oxidative stress. It also induces a state in mice/rats in which the animal cannot correct the externally imposed posture (catalepsy) [5].

Methodology: Rats are divided into five groups. Each group contains 6 rats. Catalepsy is induced by injecting 1mg/kg i.p. haloperidol. Group 1 serves as vehicle control. Group 2 serves as standard (levodopa 6mg/kg p.o.). Group 3-5 serves as the test group in which the test compound is injected.

The standard bar test is used to measure catalepsy in animals.

Observation and Evaluation: The observations are noted every 30min for 210min. The duration for which the rats retain the four paws extended and resting on the elevated bar was considered as a cataleptic score [5].

d) Circling Behavior in Nigrostriatal Lesioned Rats: 6-hydroxydopamine (6-OHDA) is a neurotoxin that is used to induce lesions in rats. They act by several mechanisms: formation of free radicals, inhibition of mitochondrial respiratory chain complexes 1 and 4, inhibition of respiratory enzymes [13]. The rats show a typical behavior of rotating in the direction of lesion *i.e.,* ipsilateral when an indirect-acting compound (amphetamine) is administered and contralateral when a direct-acting compound is administered. A disadvantage of this method is low sensitivity to small changes in striatal dopamine [7]. This test measures central dopamine function and evaluates the mode of action of novel drugs on dopaminergic neurons.

Methodology: Male Wistar rats are used. The animals are anesthetized with sodium pentobarbital (60mg/kg i.p.). A sagittal cut is made to the skin of the skull. A 2mm wide hole is drilled.

Then a 30G Stainless steel cannula connected to a Hamilton syringe is aimed at the anterior zona compacta of the substantia nigra. Then 8mg of 6-OHDA in saline is injected. The wound is closed and allowed to recover. During the recovery time, the development of lesions occurs. The test compound is administered to the animals, and the circling behavior is noted.

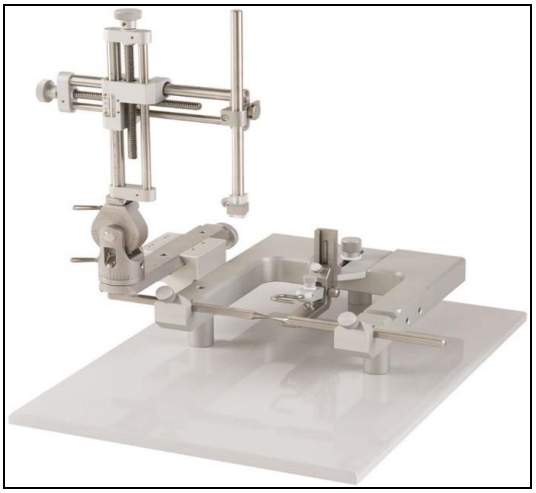

FIG. 3: STEREOTAXIC INSTRUMENT USED TO INJECT NEUROTOXIN TO INDUCE LESION

Observation and Evaluation: The number of full turns, ipsilateral or contralateral to the lesions, is recorded every 15 min for 2 h. ED_{50} values are calculated.

e) Elevated Body Swing test: Borlongan and Sanberg in 1995 proposed this test to measure asymmetrical motor behavior. Here, 6-OHDA is used to induce lesions.

Methodology: 8 week old male Sprague-Dawley rats are used. They are anesthetized with sodium pentobarbital 60mg/kg *i.p.* and mounted on Kopf stereotaxic frame **Fig. 3**. 8mg 6-OHDA in 4ml saline containing 0.02% ascorbic acid is injected in the left substantia nigra. After 7 days behavioral test is performed. The animal is allowed to attain a neutral position resting its four paws on the ground. The animal is then lifted 2.5cm above the tail. A swing is recorded when the animal moves its head to either side of the central axis.

Observation and Evaluation: Swings are recorded at the interval of 15 sec for 60 sec. The percentage of left and right swings are determined. Two-way ANOVA is used to analyze swing behavior across data.

f) Skilled Paw Reaching in Rats: 6-OHDA is used to induce impairment of paw reaching on both sides. These effects can be reversed by anti-parkinsonian drugs or by transplantation of nigral cell suspension.

Methodology: The apparatus used in this test is a narrow Perplex chamber with a lid **Fig. 2**. A double staircase is placed at the end of the box. Each staircase contains a well, and inside each well 45 mg, saccharin-flavored pellets are kept. Two groups, each containing 6 rats, are used. Group A is kept deprived of food a week before starting the test, whereas group B receives a regular nutrition diet. During this period, they are made familiar with appetitive saccharin-flavored pellets. The animals are placed in the box once per day for 10-15 min for 4 weeks. The animals are observed and noted.

Lesions are induced by injecting 4mg/ml 6-OHDA in 0.9% saline containing 0.01% ascorbic acid. The animals are treated with test compound or saline 30min before 6-OHDA induced lesions.

Observations and Evaluation: The number of pellets eaten during the test period is noted. This indicates the rat's success in grasping and retrieving the pellets. The number of steps from which pellets have been removed is noted. This indicates attempts to reach food and how far the rat can reach. The number of missed pellets is noted. This shows a lack of sensorimotor coordination in grasping and retrieving the pellets. Also, which forepaw is used first by the rat to reach the pellet is noted. All the above parameters are subjected to two-way ANOVA.

g) Stepping test in Rats: This test was introduced by Schallert *et al.* in 1992 as a relevant model for Parkinsonian akinesia. Lesions were induced by 6-OHDA, which caused impairment in the initiation of stepping movements. In such tests, training and testing are involved, which is time-consuming.

Methodology: Female Sprague Dawley rats are used. They receive two 6-OHDA 3.6mg/ml injections in 0.2mg/ml ascorbate-saline into the right ascending mesostriatal pathway. The experimental setup consists of a smooth-surfaced wooden ramp of 1m in length connected to the rat's

cage. The observations are made and the test is performed for three consecutive days.

The test is repeated weekly to determine baseline after 6-OHDA lesions. The test drug is administered only once.

Observations and Evaluation: The wooden ramp's initiation time, stepping time, and step length are observed. The data obtained is subjected to ANOVA and Fisher post hoc test [6].

2. Toxin-induced Rodent Models: Toxin-induced destruction of the nigrostriatal pathway has proved highly effective in detecting novel dopaminergic approaches to treatment and avoiding or reversing motor fluctuations and motor complications that occur during therapy as a result of disease progression. These models represent the classic and oldest experimental models of Parkinson's disease [10]. They aim to mimic the disease in humans by the use of certain neurotoxins, which primarily cause lesions in the brain. Neurotoxins such as MPTP and 6-OHDA are commonly used. These models have been established and validated as useful models for the development of therapeutic strategies aimed to treat motor symptoms and to study alterations of the basal ganglia that occur in this disease.

a) MPTP Model in Monkey: The discovery that MPTP produces PD in humans led to the discovery of the MPTP-induced PD model in primates. It is a commonly used toxin for inducing both rodent and primate models of PD based on its ability to induce persistent Parkinsonism in man. MPTP (N-Methyl-4 - phenyl - 1, 2, 3, 6 - tetrahydropyridine) is a mitochondrial complex-1 inhibitor used to induce symptoms of Parkinson's disease. It induces neurotoxicity in the dopaminergic neurons in mice, rats, cats, dogs, monkeys, and other higher mammals. It causes partial destruction of basal ganglia, which is a major cause of developing PD [14]. They selectively target dopaminergic neurons. MPTP is metabolized by the enzyme MAO-B to 1-methyl-4-phenyl-2,3-dihydropyridine (MPDP+), generating the respective Pyridium species MPP+ and dopaminergic neurons are selectively vulnerable to MPP+ which thus leads to the development of PD. MPTP-treated mice do not systematically show hypokinesia (paucity of movements); unilateral models can have increased activity due to spontaneous rotations. MPTP injected into rats causes only transient Parkinsonian-like symptoms since rats are exceptionally resistant to MPTP [15].

An advantage of this model is that long-term spontaneous compensatory dopaminergic striatal sprouting can be observed. MPTP can be administered acutely or chronically by different routes. Chronic MPTP-induced monkey models of PD also show dopaminergic cell loss, α-synuclein aggregation, α-synuclein upregulation and neuritic α-synuclein pathology [4]. It is found that nicotine protects dopaminergic neurons in the MPTP mouse model, and it also has protective effects in the primate MPTP model and the 6-OHDA-, rotenone-, and paraquat-induced animal models of PD [10]. MPTP easily crosses the blood-brain barrier because it is a lipophilic molecule where it is metabolized to MPP− by non-neural cells [3]. It is an effective model for repeated drug evaluation.

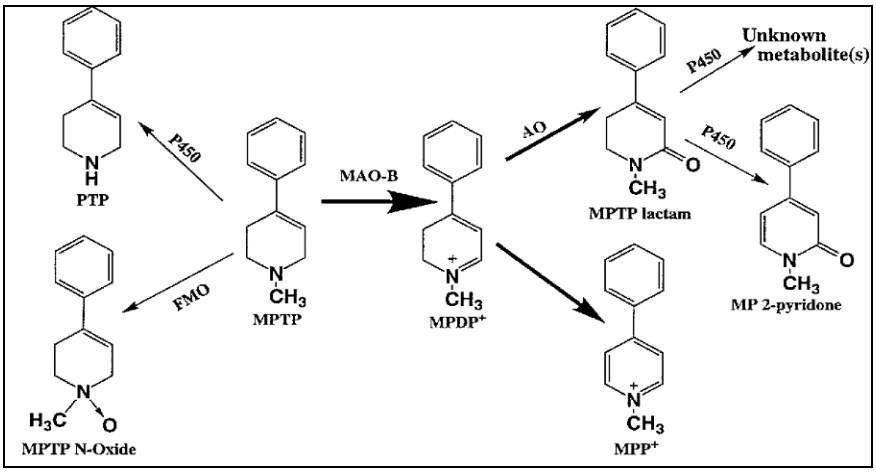

FIG. 4: METABOLIC PATHWAY OF MPTP

Methodology: Eight adult rhesus monkeys are injected with a cumulative dose of N-MPTP up to 10-18mg/kg i.v. for a period of 5-8days.
They show parkinsonism-like symptoms. The test compound is administered and observed for reversal of symptoms.

Observations and Evaluation: The symptoms are scored based on their severity.

1. To access movements:

0	Normal
1	Reduced
2	Sleepy

2. To check movements:

0	Present
1	Reduced
2	Absent

3. To check attention and blinking:

0	Normal
1	Abnormal

4. To check posture:

0	Normal
1	Abnormal trunk
2	Abnormal trunk and tail
3	Abnormal trunk, tail, and limbs
4	Flexed posture

5. To check balance and co-ordination:

0	Normal
1	Impaired
2	Unstable
3	falls

6. To check reactions:

0	Normal
1	Reduced
2	Slow
3	Absent

7. To check vocalizations:

0	Normal
1	Reduced
2	Absent

b) 6-hydroxydopamine Lesioned Rat Model: 6-hydroxydopamine (6-OHDA) is an analogue of dopamine, and norepinephrine is also being increasingly used with genetically modified mice. It is the first chemical to be found which induces PD in rodents. This is a prototypical model involving the use of 6-OHDA, which when injected locally (Intracerebral) produces neurotoxicity. 6-OHDA does not penetrate the blood brain barrier; hence, direct administration into the brain parenchyma is required. It is accounted to be the first animal model ever generated, as 6-OHDA was the first compound discovered to induce selective catecholaminergic cell death [10]. They act by several mechanisms: formation of free radicals, inhibition of mitochondrial respiratory chain complexes 1 and 4, inhibition of respiratory enzymes. Stereotaxic injection of 6-OHDA into the Substantia nigra pars compacta or the striatum induces neuronal cell death of the tyrosine hydroxylase (TH)-containing neurons in rat and mouse brain, which decreases the dopamine levels in the TH-positive terminals of the striatum. This procedure grants the highest amount of dopamine depletion within 2-3 days. This model is also widely used to evaluate the integrity of the nigrostriatal system and post-synaptic supersensitivity. 6-OHDA has been found in the urine of L-DOPA-treated patients with PD, suggesting that 6-OHDA may play a role in the pathogenesis of PD as an endogenous hydroxylated metabolite of dopamine [4].

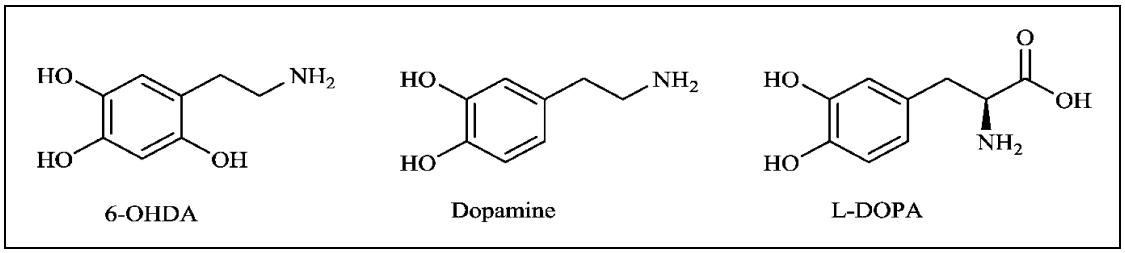

FIG. 5: STRUCTURAL SIMILARITY BETWEEN 6-OHDA, DOPAMINE, AND L-DOPA

Methodology: Rats are divided into six groups, and each group contains six animals. They are anesthetized with an i.p injection of 50mg/kg sodium pentobarbital and fixed in a stereotaxic apparatus. Stainless steel needle is inserted unilaterally in the substantia nigra, and an injection of 6-OHDA is made over 5min and the needle was left in place for a further 5min. The wound area is covered, and the animal is allowed to recover. The treatment of animals is started after 48 h of 6-OHDA induction once a day for 55 days. Group 1 receives normal saline 10ml/kg p.o. Group 2 serves

as 6-OHDA control and receives normal saline 10ml/kg p.o. Group 3 serves as standard (levodopa 6mg/kg p.o.). Group 4-6 receives test compound.

Observation and Evaluation: Behavioral assessment of all the six groups is performed. Locomotor activity is evaluated by using a digital actophotometer.

c) Lipopolysaccharide-induced Model: Recent advances in the pathogenesis of PD states that neuroinflammation is a major mediator in the initiation of the disease. This led to various animal model studies that use bacterial endotoxin lipopolysaccharide (LPS) to induce PD. It causes intense tissue inflammation and is directly infused into the nigrostriatal pathway of rats. When injected *via* the Intranigral route, it results in activation of microglia and degeneration of the dopaminergic system.

Lipopolysaccharide is itself not a neurotoxin, but the cytotoxins secreted by them possess the potential for developing the disease. In addition, LPS causes the accumulation of α-synuclein and ubiquitin in neurons. Also, they cause marked rotational behavior, ipsilateral to the lesioned side in response to systemic administration of amphetamine [16].

Methodology: Rats/mice of either sex are used. The animals are divided into three groups. Group A serves as a control. Group B receives a standard drug (Levodopa). Group C receives test compounds to be screened. Intrastriatal administration of 30 µg of LPS causes a reduction in the number of TH-positive cells in SN. The observations are made up to 4 weeks of LPS administration. The rotarod test is performed every week for four weeks, and a reduction in time spent on rotarod is noted. It is compared to standard and control groups.

3. Pesticide-induced Model: Various pesticides are used as neurotoxins for inducing PD into animals. Both rotenone and paraquat act as inhibitors of the complex I component of the mitochondrial respiratory chain.

a) Rotenone Induced Model: Rotenone is a naturally occurring compound that impairs oxidative phosphorylation in the mitochondria by inhibiting reduced nicotinamide adenine dinucleotide (NADH)-ubiquinone reductase activity. Similar to MPTP, the insecticide rotenone is highly lipophilic, so it readily crosses the blood brain barrier and diffuses into neurons where, like MPTP, it accumulates within mitochondria and inhibits complex I. The production of Reactive Oxygen Species (ROS) is thought to induce oxidative stress [11]. The Rotenone model shows various symptoms of PD, such as complex I blockade of mitochondrial function, behavioral dysfunctions, inflammation, synuclein aggregation, Lewy body-like formations, and oxidative stress. However, because of the difficulties associated with using rotenone to generate a model of PD, limited data have been reported [11]. It is both a herbicide and insecticide from Leguminosaplants, having a half-life of 3-5 days.

Methodology: The animals are divided into four groups, each containing 6 animals. Group 1 serves as a control. Group 2 is administered rotenone for 35 days. Group 3 and 4 receive test drugs for 35 days.

Observation and Evaluation: After 24 h of the last dose, behavioural studies are performed. Histological, biochemical analyses are done. Neurochemical studies are also done.

b) Paraquat Induced Model: It is known that paraquat exerts its deleterious effects through oxidative stress and its toxicity through cellular redox cycling. Paraquat, chemically known as 1,1′-dimethyl-4,4′-bipyridinium is used widely as an herbicide, and it exhibits a structural resemblance to MPP+.

However, unlike MPP+, paraquat exerts deleterious effects on dopaminergic neurons through oxidative stress-mediated damage of lipids, proteins, DNA, and RNA by generating reactive oxidative species, superoxide radicals, hydrogen peroxide, and hydroxyl radicals in mice. Paraquat enters the brain *via* neutral amino acid transporter. Once inside the brain, it induces both indirect neuronal toxicity as well as direct inhibition of mitochondrial complex 1. The latter effect usually occurs at high doses. Paraquat reduces motor activity and induces a dose-dependent loss of striatal tyrosine hydroxylase (TH)-positive neurons of mice. In addition, paraquat induces an increase in α-synuclein and the

formation of Lewy-like bodies in dopaminergic Substantia nigra pars compacta neurons. However, in several cases of paraquat-induced PD models, there is no patent decrease in the striatal dopaminergic innervation. There is only a transient decrease in the striatal TH levels [11].

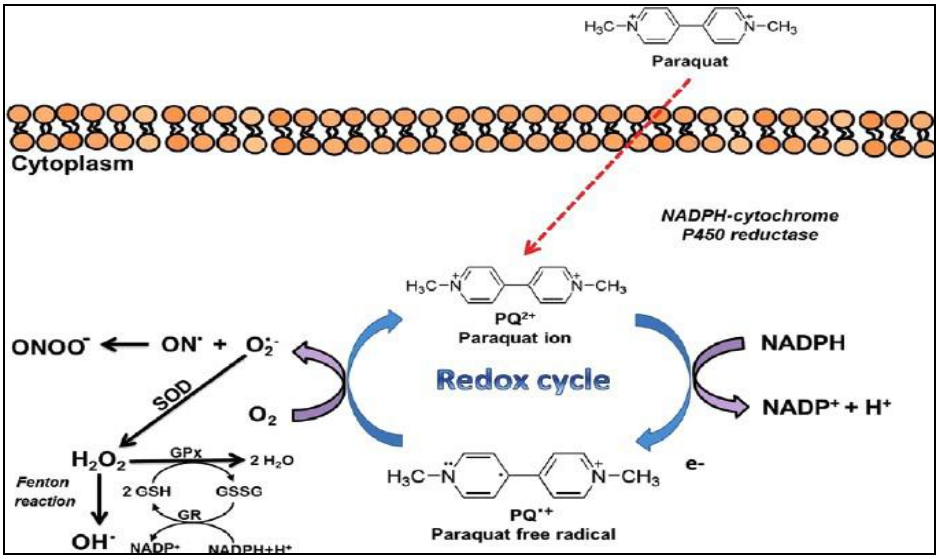

FIG. 6: MOLECULAR MECHANISM OF PARAQUAT TOXICITY

Methodology: Rats/mice of either sex are used. The animals are divided into three groups. Group A serves as a control. Group B receives a standard drug (Levodopa). Group C receives test compounds to be screened. Intrastriatal administration of paraquat leads to NADPH consumption and ROS generation, mainly hydrogen peroxide (H_20_2) and hydroxyl radical (HO), with consequent cellular deleterious effects. The animals are observed for reversal of effects. It is compared to standard and control groups.

c) Maneb Induced Model: Maneb is a fungicide manganese ethylene-bis-dithiocarbamate that has been associated with an increased incidence of PD [11]. Attempts have been made to model PD using this agent. Maneb enters the brain and causes inhibition of complex III of the mitochondrial respiratory chain. When combined with paraquat, it is shown to produce enhanced toxicity.

d) Methamphetamine Induced Models: Methamphetamine has neurotoxic effects on the nervous system that cause functional deficits and structural alterations. However, although selective dopaminergic or serotonergic neuronal cell loss occurs in rodents following the administration of high doses of methamphetamine, this model is not very reliable; the results only produce a long-term loss of TH enzyme but are not examined in the PD-dependent behavioral tests [4].

4. Transgenic Animal Models of Parkinson's disease: These models are made by manipulating genes [3], which have few motor deficits, and little neurodegeneration.

a) α-synuclein: SNCA was the first gene to be linked to familial PD, and together with the finding that the encoded protein α-synuclein is aggregated in Lewy bodies, this led to a breakthrough in PD research [16].

The most prominent models are related to α-synuclein. α-Synuclein is a presynaptic neuronal protein that is responsible for rare forms of PD. It contributes to PD pathogenesis because of the presence of soluble oligomeric conformations, termed protofibrils. These are toxic species that mediate the disruption of cellular homeostasis and neuronal death. It also contributes to disease progression [17]. The first transgenic mice that expressed α-synuclein was developed by Masliah *et al.* (2000). These mice showed progressive accumulation of α-synuclein in neurons of the neocortex, hippocampus, and substantia nigra. This led to the loss of dopaminergic terminals in the basal ganglia with motor impairments. Also, mutations in α-synuclein models were developed, which causes a rare form of autosomal dominant PD. Hence, targeting the toxic function of α-synuclein protein when it is dysregulated can be used as a novel therapeutic strategy in PD.

Various α-synuclein transgenic mice have been developed, but no significant nigrostriatal degeneration has been found [10]. Recent researches suggest that the mechanism of the phosphorylation process of α-synuclein in the brain might be a sensitive and effective biomarker candidate for PD [4]. α-synuclein may exert deleterious effects on neighbouring cells, including seeding of aggregation, thus possibly contributing to disease propagation. Other transgenic models used in screening anti-parkinsonian agents are weaver mutant mice [6], mutations in Leucine-rich repeat serine/threonine kinase 2 (LRRK2), and PTEN-induced putative kinase 1 (PINK1), Parkin, DJ-1.

b) Multiple Transgenic Mice: Multiple transgenic mouse lines have been developed by the crossing of α-synuclein transgenic mice with parkin or DJ-1 Knock-Out (KO) mice or silencing of PINK-1, DJ-1, parkin. None of them produced nigrostriatal degeneration.

c) MitoPark Mouse Model: This mouse is produced by the elimination of the nuclear genome encoded mitochondrial Tfam gene. This gene is responsible for mitochondrial DNA replication and transcription.

Therefore, mitochondrial function is selectively disrupted in dopaminergic neurons. Such animals survive to adulthood and progressively show PD-like symptoms. It has been found that L-DOPA normalizes motor deficits of MitoPark mice. Hence, this genetic model may represent a valuable tool for developing and screening new therapeutic strategies in PD [10]. In such mice, L-DOPA efficacy weakens with age.

d) Rat Genetic Models: Transgenic rat models producing α-synuclein mutations have recently been developed. They are of much advantage than transgenic mice because the rat's neuronal circuitry more closely resembles that of humans, and they are less prone to anxiety, which represents a major advantage for behavioral evaluation. But these show no signs of dopaminergic cell loss.

Alternative Models: Due to the lack of nigrostriatal degeneration in transgenic models and disappointing results thus obtained, alternative models have been developed with reduced genomic complexity and greater ease of manipulation. These models allow the possibility of conducting high-throughput experiments. Such models are in the earlier stage of development, and research on such models should be encouraged in drug discovery for PD.

1. Drosophila Model: Of the available models, the Drosophila model has received much attention because a large no. of human genes such as parkin, UCH-L1, PINK1, DJ-1, and LRRK2, have highly conserved homologues in Drosophila. The fruit fly Drosophila melanogaster has emerged to be a suitable model for screening anti-parkinsonian agents and studying the mechanisms of PD-related neurodegeneration. Drosophila is much similar to humans in terms of dopaminergic neurons and metabolic pathways for DA synthesis. The first PD-like Drosophila model was generated by neuronal overexpression of human α-synuclein for which no-fly homologous exists. These flies showed age-dependent and selective loss of dopaminergic neurons, the formation of fibrillary inclusions containing α-synuclein, and progressive loss of climbing activity, which is an indication of motor deficit. These effects were found to be counteracted by L-DOPA or DA agonists [10].

FIG. 7: THE FRUIT FLY DROSOPHILA MELANOGASTER

Methodology: Flies are subjected to rotenone chronically. Male adult flies 7-8days old are used. They are divided into 6 groups. Group 1 serves as a control. Group 2 serves as standard (Levodopa). Groups 3 to 6 receive test compounds.

Observation and Evaluation: Flies are placed in a flask, tapped at the bottom for 60sec; it is possible to study climbing activity, *i.e.*, locomotor activity.

2. *C. elegans* Model: The multiple offspring (~350) for rapid production of models and high-throughput screening makes *C. elegans* another

ideal platform for genetic studies. The nematode *Caenorhabditis elegans* (*C. elegans*) is an optimal model because it is a multicellular organism that is simple to be studied and has a well-characterized genome [10]. It is one of the simplest organisms with a nervous system and it is easy to disrupt the functions of specific genes. The organism is transparent, and hence it is easy to observe dopaminergic neurons with the help of green fluorescent proteins. It is homologous to PD-related proteins, including parkin, LRRK2, PINK1, DJ-1 but not α-synuclein. Overexpression of human α-synuclein induces a loss of dopaminergic neurons, which can then be further used as a model for studying the anti-parkinsonian effect. It has also been used for large-scale screening of potential modifiers of α-synuclein. Also used for discovering novel proteins and pathways, especially related to the α-synuclein pathway.

FIG. 8: *CAENORHABDITIS ELEGANS*

3. Zebrafish Model: The utilization of zebrafish for drug discovery increased at the beginning of the twenty-first century. It is a 3–4 cm long vertebrate that has been used for many years to study development and gene function as a potential model of PD amenable to high throughput *in-vivo* drug screening [11]. Zebrafish (*Danio rerio*) is a popular aquarium fish and has features that make it a simple model for evaluating pathological mechanisms in PD [10].

Fish larvae can be bred in 96-well plates and thus perform high-throughput screening of innovative therapeutic agents. There exist extensive similarities to the mammalian nervous system. The dopaminergic neurons in zebrafish are sensitive to PD-inducing toxins. Like Drosophila and C.elegans, extensive genomic data are available on zebrafish. Also, proteins such as parkin, DJ-1, PINK1, and LRKK2 are detected in zebrafish, but no homologue to α-synuclein has been observed. Overexpression of parkin protein protects the fish from cellular stress.

Therefore, parkin KO causes moderate loss of dopaminergic neurons, reduced mitochondrial complex I activity, and increased susceptibility to toxins. Similarly, PINK-1 KO alters dopamine projections and induces locomotor defects. Zebrafish can readily absorb hydrophilic compounds when added to their tank water. Therefore, the model represents a potential tool for high throughput screening of novel molecules that may counteract pathological mechanisms in PD [10].

FIG. 9: ZEBRAFISH

Behavioral Parameters Studied in Fish:

Latency to Travel from One Point to Another: PD induced in zebrafish makes them difficult to move. Reversal of such effects can be observed by administration of test compounds and compared with standard results.

Complete Cataleptic Time: locomotive activity can be evaluated.

Time Spent Near the Bottom of the Tank: PD-induced fish makes it difficult for them to move; hence such activity can be noted.

Monoaminergic System in Zebrafish: The role of the monoaminergic system is in the adjustment of movement, which is highly preserved in vertebrates. TH plays a major role in this system, and zebrafish contains two enzymes th1 and th2, which are similar to the mammalian system. The dopamine transporter is also detected in this system. So, the model is very similar to humans, with a slight difference in the dopaminergic neurons absent in the midbrain. Zebrafish as a model for screening anti-parkinsonian agents was first performed in 2004. Various chemical agents, toxins, pesticides, and herbicides are used to induce PD in zebrafish as follows:

Chemicals used: MPTP, 6-OHDA, Paraquat, Rotenone, Cytotoxic metabolite of metronidazole Titanium dioxide nanoparticles, Zeram.

Genetic Manipulations: β/γ-1 synucleins knockdown, γ-1 synuclein overexpression, Human α-1 synuclein overexpression, PINK-1 knockdown, Parkin knockdown, DJ-1 knockdown [8].

Ex-vivo:

Cell Transplantation into Lesioned Animals: In Parkinson's disease, embryonic stem-cell-derived dopaminergic neurons may replace the degenerated neurons in the brain [6]. To achieve this, numerous animal experiments were performed. Many authors used 6-OHDA to induce lesions, whereas MPTP was also used. Due to the complexity of such experiments, clinical trials with cell transplantation for Parkinson's disease have had disappointing results.

CONCLUSION: This review has discussed models for screening novel anti-parkinsonian agents based on symptomatic relief of mainly motor symptoms and the complications arising from the treatment. We are still unable to devise models that truly reflect the widespread and progressive pathology of the illness. Rats and mice are widely used as models for PD. Also, non-human primates serve as useful PD models. The availability of a "perfect" model for screening anti-parkinsonian agents is still a crucial need, yet there are models that portray the basic characteristics needed. Although many models have been developed, each model has its specifications and limitations. Hence, many factors need to be considered while choosing a particular model depending on the type of agent to be screened or the purpose of selecting a particular model. The model thus selected should have a majority of similarities with the human system. Testing in such models provides useful information to understand mechanisms of action and differences in treatment success. At present various toxic and transgenic models are developed. The major limitation of transgenic models is that neuronal degeneration is not prominent in most of the models. In contrast, neuronal degeneration occurs in the toxic model and good replication of PD motor symptoms. Also, suppose the role of selective proteins involved in PD pathogenesis is investigated. In that case, a specific transgenic model can be developed, but there are also many limitations to the development and use of transgenic animals.

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