# Cellular and Animal Models of Parkinson's Disease: Rationale into Neuroprotective Effects of Naringenin

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## Abstract

Parkinson's disease (PD) is the most common movement condition, marked by motor and nonmotor characteristics. Because the pathophysiology has not been fully understood, the current therapeutic regimen for PD is primarily symptomatic. Several animal models have been developed to explore various facets of the disease to understand pathogenesis. The pathophysiology of PD remains complex, and existing PD therapies appear to be clinically inefficient. Detailed studies on developing novel pharmaceutical alternatives have shown that natural compounds, such as herbal remedies, extracts, and their secondary metabolites, offer immense potential as cytoprotective treatments in PD. Recent preclinical studies show that a variety of herbal medicines and their bioactive constituents can be turned into optimal pharmaceuticals for PD treatment. Naringenin (NAR) is a flavonoid showing neuroprotection in PD pathogenesis. Here, we focus on the neuroprotective ability of bioactive NAR in cellular and animal models of PD research.

**Keywords:** Naringenin, Neurodegeneration, Parkinson's disease, PD models, α-synuclein.

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## Cellular Models of PD

Selecting an appropriate model for the pathogenesis of PD of the best model system is very difficult. Numerous cell culture and animal models have been developed to understand better the underlying neurological mechanisms and the sequence of pathophysiological processes. Cell lines for PD research have benefited from relatively unrestricted access, enable sensitive detection for etiopathogenesis and therapeutic alternatives, and limit the number of clinical testing needed (Falkenburger, Schulz, *et al*. 2006). To the greatest extent attainable, immortalized cell lines demonstrate the advantages of cell culture models by presenting homogenous populations of continuously proliferating cells by displaying homogeneous groups of constantly increasing cells. This enables large-scale experimentation with high reproducibility across a wide range of tests. Chemical and physical procedures could effectively introduce genetic material into cells, preserving cells at -150$^0$C. This facilitates the construction of cell line libraries stably expressing numerous proteins of interest. Freezing aliquots of low passage cell stocks are essential for replicating results since even cell lines are reported to adapt their appearances and activity with increasing passages as epigenetic changes develop (Falkenburger, Schulz, *et al*. 2006). E13 mouse or rat embryos are commonly used to produce primary mesencephalic cultures. They include dopaminergic neurons from the midbrain grown in the context of their physiological neighbors. Like in similar primary neuronal cultures neurons rapidly differentiate and produce neurites and make connections, even though all these cultures are commonly referred to as "primary dopaminergic neurons" (Cruz-Hernandez, Agim, *et al*. 2018). Neurons are devoid of their natural environment, of afferent sensory connections, in all cell lines. This is a significant change since these connections are essential to a neuron's life in the brain. Even when cultured cells make cellular interconnections, these are only two-dimensional. The glass or plastic support typically one side of the cell, whereas the aqueous cell culture medium covers the other. This culture medium substitutes much of the "natural" environment. As a result, the specific culture conditions significantly affect the survival of particular types of cells. Primary cultures, for example, are almost entirely dopaminergic when mounted without serum. Serum increases glial proliferation, which improves neuron survival while complicating biochemistry and viability experiments unless cocultures of neurons and glia are designed to investigate their reciprocal interactions.

### Neurotoxins-induced PD Models

Toxins of many kinds are utilized to produce DA-ergic neurodegeneration. Despite their influence on mitochondria, most can potently suppress complex I or increase the production of reactive oxygen species (ROS) (Mailloux 2020). Some transporters preferentially uptake DA, enabling to target them specifically. Recent studies have focused on developing the model systems wherein exposure is

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prolonged and damage proceeds progressively to resemble human PD.

### 6-Hydroxydopamine (6-OHDA) induced Model of PD

The neurotoxic compound 6-OHDA is structurally identical to dopamine and norepinephrine (NE) and has a strong affinity for these catecholamines, plasma transport proteins (Cruz-Hernandez, Agim *et al*. 2018). A 6-Hydroxydopamine is often delivered unilaterally to the striatum, medial forebrain bundle (MFB), or SN. Most 6-OHDA concentrations damage the DA-ergic cells in SN within several hours of administration to the ventral midbrain, even before striatal terminals disappear. Because 6-OHDA does not penetrate through the blood-brain barrier, it should be administered intracerebrally (Chia, Tan, *et al*., 2020). It rapidly oxidizes inside the cell, generating superoxide radicals such as hydrogen peroxide, superoxide radicals, and hydroxyl radicals, which are extremely poisonous and cause mitochondrial dysfunction. Numerous investigations on 6-OHDA models have been conducted to investigate the preventative function of different compounds. For example, a combination of antioxidant and iron-chelating agents has indeed been shown to be effective in neutralizing 6-OHDA-induced neurodegeneration (Jing, Wei, *et al*., 2016). It is well established that the 6-OHDA model does not entirely match the etiology of PD, which lacks the development of Lewy Bodies (LB) (Haleagrahara, Siew, *et al*., 2013).

### 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced PD Model

MPTP is one of the most frequently used neurotoxins in PD animal models. It is a lipid-soluble molecule, enabling it to easily pass through the blood-brain barrier. After systemic injection, the monoamine oxidase B in astrocytes can metabolize MPTP into the potent dopamine neurotoxic 1-methyl-4-phenylpyridinium ion (MPP+). MPP+ is a harmful byproduct of that, due to the intrinsic similarity to dopamine, is rapidly absorbed by dopamine neurons via the dopamine transporter (DAT) (Martí, Matthaeus, *et al*., 2017). MPP+ subsequently induces gradual loss of DA-ergic neurons in the SN and a decrease in striatal levels of dopamine. Cell injury induced by MPP+ suppresses complex I in mitochondrial respiration. This causes a sudden drop in adenosine triphosphate (ATP) levels in the stria and SN, preceded by apoptosis of DA-ergic neurons and necrosis. MPTP is primarily utilized in nonhuman primates and rodents, but it's also been used in several other species, notably cats and dogs. MPTP can be administered in various ways, although systemic injection is the most common and reliable (subcutaneous, intravenous).

### Paraquat-induced PD Model

Paraquat is a frequently used herbicide implicated as a neurotoxicant because of its structural similarity to MPP+. The application of paraquat as a widespread insecticide has prompted general concerns because it may have a role in the pathogenesis of PD. According to epidemiological statistics, the use of insecticide enhances the likelihood of developing Parkinson's disease; however, in the case of PQ, there have only been 95 cases of PD linked to its neurotoxicity. PQ leads to oxidative stress, which is mediated by electron transfer, which produces ROS. The hydroxyl radical, peroxides, and superoxide anion, in particular, induce lipid, protein, DNA, and RNA damage (Blesa, Phani, *et al*., 2012).

Furthermore, systemic paraquat administration in the mice model resulted in dopaminergic neuron degeneration. According to reports, paraquat crosses the blood-brain barrier via a neutral protein transporter. Notwithstanding its structural similarity to MPP+, paraquat does not block mitochondria complexes. I like MPP+. Instead, paraquat inhibits glutathione and thioredoxin cycling (Blesa, Phani, *et al*., 2012). PQ's significance to Parkinson's investigators lies in its capacity to elevate α-synuclein content in individual Dopaminergic neurons in the Substantia nigra pars compacta and its ability to induce LB-like aggregates in Dopamine neurons in the SNpc (Manning-Bog, McCormack, *et al*. 2002). Its neurotoxicity can be ascribed to redox cycling and oxidative stress. PQ is carried into mitochondria through a carrier-mediated mechanism within the cells. Inside the mitochondria complex I, generating a PQ radical capable of oxidatively disrupting the mitochondria (Bastías-Candia, Zolezzi, *et al*. 2019).

### Rotenone-induced PD Model

Rotenone is a phytochemical found in plants and traditionally employed as an insecticide. It is lipid-soluble and can penetrate the blood-brain barrier to inhibit the mitochondrial complex like MPTP. On the other hand, Rotenone generates systemic inhibition, as opposed to MPTP, which targets catecholaminergic neurons (Bisbal and Sanchez 2019). Pure Rotenone is indeed an herbicide and a pesticide. It is the most toxic substance in the rotenoid family in nature in tropical plants. Chronically low rotenone administration disrupts the mitochondrial oxidative pathway in the rat brain. Rotenone has been delivered to animals through several means. Oral dosing appears to be toxic in a limited manner. The most popular method of delivery has been chronic systemic administration by osmotic pumps, especially in the Lewis rat, which may be more sensitive to Rotenone than other types of rats. Although mortality is high, intraperitoneal injections have elicited behavioral and neurochemical abnormalities. Intravenous injection can disrupt nigrostriatal DA-ergic neurons, triggering α-synuclein aggregation, Lewy-like body deposition, cytoplasmic inclusion, oxidative stress, and gastrointestinal issues. The obvious benefit of this model is that, like paraquat, it appears to recapitulate practically all of the symptoms of PD, including producing α-synuclein aggregation and the development of Lewy-like bodies (Cannon, Tapias, *et al*. 2009).

### Naringenin (NAR)

Naringenin (NAR,5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one), found in citrus fruits like grapes,

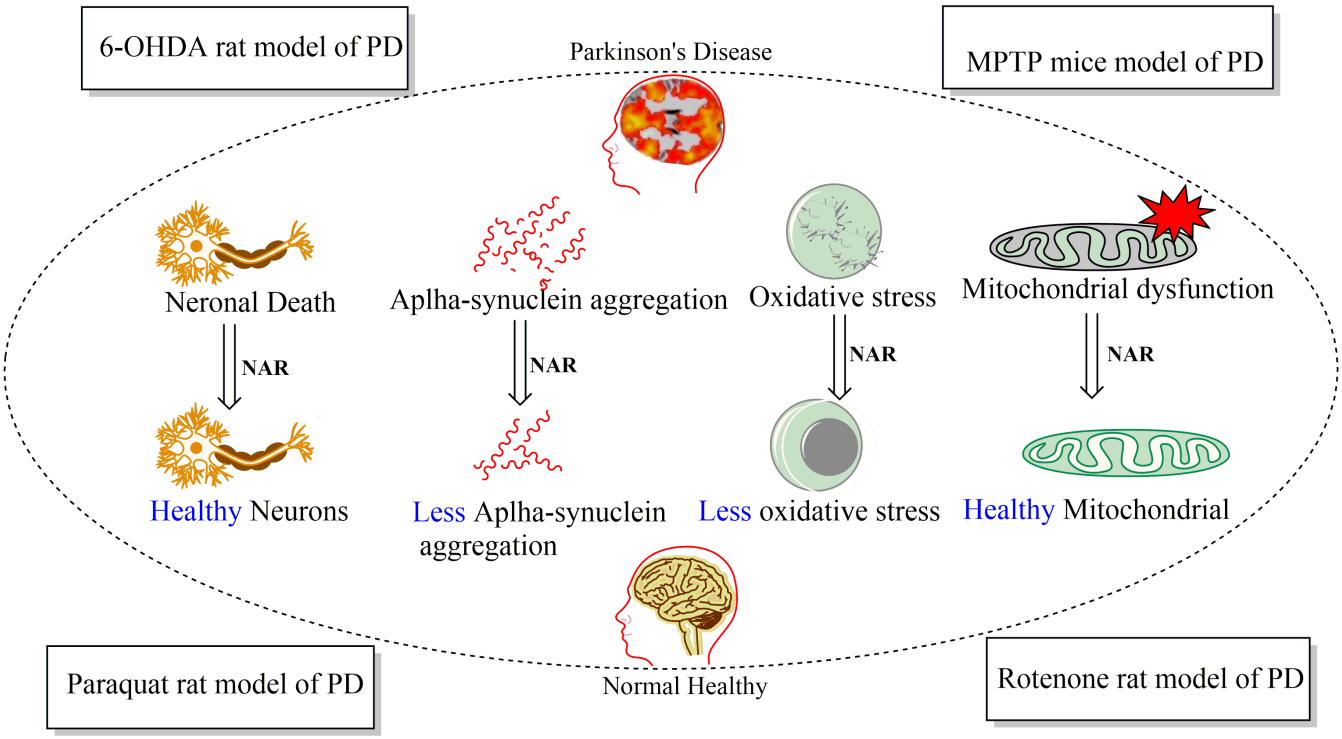


**Figure 1:** Diagrammatic representation of the possible mechanism of NAR's neuroprotective role in different PD models.

oranges, and lemons, possesses an antioxidant effect by reducing free radical like ROS and increasing the antioxidant activities in the neurodegenerative diseases, including PD (Zaidun, Thent, *et al*. 2018). NAR is a polyphenol that belongs to the flavanones class. It can be found in various citrus fruits, bergamot, tomatoes, and other fruits and in its glycoside form. Reports suggest that NAR has antioxidant, anticancer, antiviral, antibacterial, anti-inflammatory, antiadipogenic, and cardioprotective properties. Nonetheless, most of the evidence provided came from in vitro or in vivo investigations.

### Naringenin is used in Parkinson's Disease Therapy in Different Model Systems

NAR possesses an antioxidant effect by reducing free radicals like ROS and increasing SOD, GSH, and catalase's antioxidant activity in neurodegenerative diseases (de Oliveira, Brasil, *et al*., 2017). NAR modified the 6-OHDA lesions in the mouse model of PD (Bonito-Oliva, Masini, *et al*., 2014). In another 6-OHDA induced rat PD model, NAR exhibits neuroprotection in PD, which may be due to its antioxidant properties and ability to cross the blood-brain brain (Zbarsky, Datla *et al*. 2005). In yet another NAR protected against 6-OHDA-induced neurotoxicity in mice model via activation of the Nrf2/ARE signaling pathway (Lou, Jing, *et al*. 2014). A recent study found that NAR mitigates 6-OHDA-induced PD-like characteristics in SH-SY5Y cells and zebrafish models (Kesh, Kannan, *et al*., 2021). NAR treatment inhibited α-synuclein agglomeration, and its evaluation in MPTP induced PD mice model (Jayaraj, Elangovan, *et al*., 2014). NAR protected against MPTP-induced neuroinflammation and resulted in oxidative stress in SH-SY5Y cells (Mani, Sekar, *et al*., 2018). It ameliorated the toxicity effect in mitochondria isolated from rats in the paraquat-induced model of PD (Daneshgar, Rezaei, *et al*., 2016). NAR exhibited anti-inflammatory effects via a mechanism associated with the Nrf2/HO-1 axis in the paraquat-induced cellular model of PD (SH-SY5Y cells). Recently, our study showed that NAR alleviated paraquat-induced dopaminergic neuronal loss in SH-SY5Y cells and a rat model of PD (Ahmad, Fatima, *et al*., 2021). The possible neuroprotective role of NAR in different models of PD is given in Figure 1**.**

## Conclusion

These observations indicate that NAR provides neuroprotection in different cellular and animal PD models. The NAR-mediated neuroprotection in these PD models may be attributed to its radical scavenging property.

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