Evaluation of Neuroprotective Effect of a Novel Compound in a Zebrafish Model of Parkinson's Disease – A Review Article

Mrs. Roshini K.V[1], Ms. Fidha Fathima M M[2], Ms. Gouri Vijayan[3],
Ms. Nafeesathul Fidha[4], Ms. Rameesa T S[5]
[1]*Associate professor, Department of Pharmacology, Chemists College of Pharmaceutical Sciences and Research, Varikoli, Ernakulum, affiliated to Kerala University of Health Sciences, Thrissur, Kerala-680596, India*
[2,3,4,5]*Students, Chemists College of Pharmaceutical Sciences and Research, Varikoli, Ernakulum, affiliated to Kerala University of Health Sciences, Thrissur, Kerala-680596, India*

***Abstract*—Parkinson's disease (PD) is a progressive, chronic neurodegenerative illness defined by the early death of a large number of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and a widespread accumulation of alpha synuclein (aSyn), an intracellular protein. A reduction in dopamine levels in the basal ganglia is responsible for producing both motor and non-motor symptoms. Given their similarities with human brain physiology and anatomy, zebrafish have emerged as an important animal model system for studying Parkinson's disease. Like the mammalian brain, the zebrafish brain contains all three primary brain divisions (forebrain, midbrain, hindbrain), along with a diencephalon and telencephalon; furthermore, the primary neurotransmitter functions of the brain are largely equivalent to those found in mammals. This review outlines the various zebrafish-based models of Parkinson's disease, including those generated via the administration of neurotoxins such as MPTP, 6-OHDA, rotenone, and paraquat. Different behavioral assays like locomotor tracking, the novel tank diving test, and startle response are employed to assess both motor impairment and neurobehavioral modifications, while molecular analyses (e.g., RT-qPCR) are performed for the purpose of investigating changes in gene expression associated with neurodegeneration, oxidative stress, and apoptosis. Zebrafish models provide an inexpensive and efficient means of screening neuroprotective agents and understanding the mechanisms of the development of pathology within the disease process. The present review outlines the value of employing zebrafish in neuropharmacological research and their potential application in developing new treatment approaches for Parkinson's disease.**

***Index Terms*—Parkinson's disease, Zebrafish, α-synuclein, Neurotoxin-induced models, MPTP, 6-OHDA, Paraquat, Rotenone, RT-qPCR.**

I. INTRODUCTION

Parkinson's disease is also called Idiopathic or primary parkinsonism and hypokinetic rigid syndrome. It ranks as the second most common neurodegenerative disorder which progresses over time. This condition affects about 2% to 3% of individuals older than 65 years of age. The disease involves a loss of dopaminergic neurons in the substantia nigra along with the build-up of alpha-synuclein inside cells, known as Lewy bodies. This is the main neuropathological feature of Parkinson's [1]. Clinical presentation usually starts with uneven motor features and includes bradykinesia, resting tremor, stiffness and balance issues. Nonmotor symptoms like constipation, sleep issues, depression, cognitive decline and autonomic dysfunction often come before or alongside motor symptoms, heavily affecting morbidity. Regardless of causes such as environmental and genetic and other risks, α-synuclein misfolding and clumping, mitochondrial dysfunction, issues with protein cleanup (involving major ubiquitin-proteasome and autophagy-lysosomal systems), nerve inflammation and oxidative stress may also contribute to Parkinson's disease [2].

The zebrafish, or Danio rerio, has drawn interest as a potent animal model for a variety of brain disorders in humans. The transparent quality of zebrafish larvae

facilitates the examination of vertebrate neural development [3]. When compared to human brains, certain parts of the zebrafish brain may be linked to and are often very well-preserved. For example, the zebrafish ventral telencephalon is found to be homologous to the human striatum. Additionally, the behaviors and phenotypes exhibited by zebrafish are analogous to human behaviors. For instance, zebrafish exposed to neurotoxins demonstrate movement deficits, including reduced swimming speed and atypical swimming patterns, analogous to bradykinesia-like symptoms observed in Parkinson's disease patients. Genome sequencing analysis showed that the zebrafish genome is 70% similar to the human genome, also that approximately 80% of gene similarity with significant conserved synteny [4].

Also, zebrafish are great for high-throughput drug screening because they grow quickly, lay a lot of eggs, and are cheap to take care of. These traits make it possible to test hundreds of compounds at once, which speeds up the search for possible drugs and lets scientists test their effectiveness and safety early on [5].

II. NEUROTOXIN-INDUCED PARKINSON'S DISEASE MODELS

There are neurotoxins like MPTP, 6-OHDA, paraquat and rotenone that are used for a long time in zebrafish studies. These neurotoxins are used to produce symptoms that are similar to Parkinson's disease.

2.1 MPTP Induced Models

MPTP is 1 methyl 4 phenyl 1, 2, 3, 6 tetrahydropyridine causes serious motor problems, such as slower swimming speed and more freezing behavior. These problems are similar to those seen in people with Parkinson's disease [6]. MPTP is a type of meperidine analog that is produced when 1 methyl 4 phenyl-4-propionoxypiperidine (MPP) is synthesized. After crossing the Blood Brain Barrier Monoamine oxidase quickly turns MPTP into the toxic compound 1-methyl-4-phenylpyridinium iodide (MPP^+) [7]. MPP^+ is then transported by Dopamine transporter (DAT) to dopaminergic neurons. After being moved into cells by the DAT, MPP+ builds up in the mitochondria, binds to NADH dehydrogenase, and stops the flow of electrons from NADH to CoA. MPP+ selectively harms dopaminergic neurons because it inhibits complex, I function, which lowers ATP production and causes reactive oxygen species to build up [8].

2.2 6-OHDA Induced Models

6-hydroxydopamine (6-OHDA) is another neurotoxin commonly used to induce Parkinson's-like symptoms in animal models. Unlike MPTP, this neurotoxin cannot cross the blood-brain barrier and, therefore, must be injected directly into the target region of the brain. This neurotoxin is a highly oxidizable analogue of dopamine. It enters dopaminergic neurons via the dopamine transporter. Once inside the cells, monoamine oxidase oxidizes this neurotoxin to a Quinone, which then forms by-products such as hydrogen peroxide and other free reactive radicals. These free reactive radicals are responsible for oxidative stress-induced toxicity and microglia-induced neuroinflammation in the target region of the brain. In addition to oxidative stress-induced neurotoxicity, this neurotoxin has also been known to directly inhibit complex I of the mitochondria, leading to mitochondrial dysfunction [4]. Diminished dopaminergic function is commonly associated with behavioral deficits, such as decreased locomotor activity and altered motor coordination, which are characteristic of PD-like phenotypes [9].

2.3 Paraquat Induced Models

Paraquat is a polar herbicide that protects plants from invasive species. Because it can be harmful, it needs to be handled carefully and used only in certain ways. Paraquat exposure induces oxidative damage in dopaminergic neurons, resulting in behavioral deficits in vivo. Hence it can be used for producing PD like symptoms in Zebrafish [10].

Paraquat has a low affinity for mitochondrial complex I, which means that it doesn't stop complex I from working. This means that complex I doesn't play a big role in the neurotoxicity of paraquat. Paraquat commandeers the pentose phosphate pathway to augment NADPH reducing equivalents and promote paraquat redox cycling. Paraquat causes oxidative stress and activates AMP-activated protein kinase. Paraquat can only kill dopaminergic mesencephalic neurons when microglia are present. A single paraquat injection is enough to activate microglia and make dopaminergic neurons more likely to die with the next injections [11].

2.4 Rotenone Induced Models

Rotenone is a naturally occurring plant-based toxin that is used as a pesticide in fishing and farming. Rotenone is a specific inhibitor of mitochondrial complex I that causes microglial NADPH oxidase to make too many reactive oxygen species (ROS), lowers the levels of antioxidant enzymes like glutathione, raises lipid peroxidation, and causes mitochondrial membrane depolarization, which leads to the death of dopaminergic neurons. This is especially toxic in PD pathology [11]. Rotenone could easily cross the BBB and biomembrane, which does not depend on DAT. In dopaminergic neurons, rotenone stops mitochondrial complex I from working, which leads to the production of ROS and results in abnormal mitochondrial function [12]. Several studies show that after Rotenone treatment Zebrafish shows anxiety and depression along with olfactory dysfunction [13].

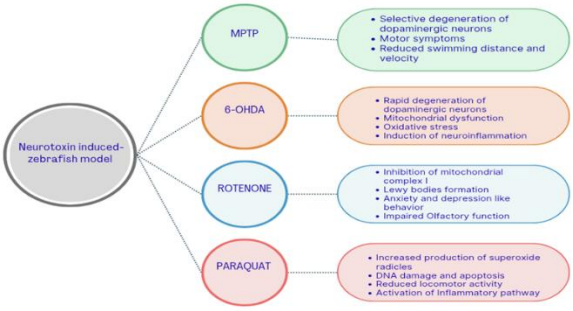

Figure 1: Neurotoxin-induced Parkinson's disease model in zebrafish

III. BEHAVIORAL ASSAYS IN ZEBRAFISH MODEL OF PARKINSONS DISEASE

Behavioral assays in zebrafish Parkinson's disease models, often induced by MPTP, 6-OHDA, paraquat, rotenone, detect crucial motor impairments like reduced locomotor activity (reduced speed, distance) and heightened freezing behavior [14].

Different behavioral assay includes,

- Locomotor Activity
- Novel Tank Test
- Startle Response Test (C-bend response).
- Light – Dark Preference Test

3.1 Locomotor Activity

Earlier research has shown that a decrease in dopaminergic neurons in the ventral diencephalon correlates with notable changes in zebrafish behavior. Evaluations of locomotor activity indicated that larvae exposed to MPTP experienced significant impairments, such as a dramatic reduction in both the total distance covered and swimming speed.

Moreover, various zebrafish models of Parkinson's disease induced by neurotoxins, including MPTP, MPP^+, 6-OHDA, and rotenone, consistently exhibit reduced locomotor activity. These treated zebrafish show diminished movement, slower swimming speeds, and shorter distances travelled, effectively replicating the bradykinesia typical of Parkinsonian conditions [8].

3.2 Novel Tank Test

The novel tank test (NTT) is commonly employed in zebrafish to evaluate anxiolytic or anxiogenic effects of compounds, or as a behavioral bioassay to elucidate the neurobiology of defensive (anxiety-like) behavior. The test takes advantage of zebrafish's natural tendency to prefer the bottom parts of a new tank, which is due to their biotic and abiotic environment in the wild. The NTT is easy to set up as a quick test, and it can also pick up on both anxiolytic and anxiogenic compounds. The main goal is to see how long the animal stays in the bottom third of the tank. Other goals include determining swimming abnormalities, freezing behavior, and swimming speed [15]. The outcomes are affected by a range of internal and external elements. Differences in variables such as the tank's height, the observation period, and the pre-stress levels experienced by the fish across various studies contribute to significant variability in NTT results [16].

3.3 Startle Response Test

The C-bend response in adult zebrafish, marked by the swift curvature of the body into a "C" shape followed by propulsion to evade a stimulus, was evaluated to assess muscular rigidity, a principal symptom of Parkinson's Disease. In this test, studies are conducted by exposing zebrafish to a number of vibrational stimuli, which elicit a characteristic 'C' shaped body bend response. The number of C bend response is recorded and used as a measure of behavioral activity. The frequency of the C-bend is directly related to how stiff the muscles are and how quickly they respond to movement [7].

3.4 Light- Dark Preference Test

Light/dark preference test is a behavioral test used to assess anxiety in zebrafish after exposure to neuro-toxins. This test usually uses a tank that is split into white and black sections. It uses the fish's natural preference for darkness to check for neurobehavioral problems. Anxiety is an emotion linked to risk assessment behaviors in response to potential threats, often triggered by exposure to a novel environment or a possible adverse stimulus. The light/dark preference in zebrafish varies according to the stimuli presented. When zebrafish are given the choice between a black and a white chamber, they consistently prefer the black chamber. Similar to rodent models, it has been proposed that the level of preference could serve as an indicator of anxiety [17].

IV. MOLECULAR ANALYSIS USING RT-QPCR

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) is a sensitive method of measuring the expression of messenger RNA (mRNA). The method involves the conversion of RNA into complementary DNA (cDNA) by the enzyme reverse transcriptase, followed by the amplification of the target DNA by specific primers. The accumulation of the amplified DNA product is monitored in real time by fluorescence, proportional to the original RNA target [18]. Molecular and gene expression techniques, RT-qPCR are commonly employed as a validation tool for neurotoxin-induced (MPTP, 6-OHDA, Rotenone, Paraquat) or genetic zebrafish models of Parkinson's Disease (PD). RT-qPCR targets important markers of dopaminergic neuronal degeneration, oxidative stress, mitochondrial damage, and inflammation in the posterior tuberculum of the zebrafish brain, which is analogous to the substantia nigra of the human brain [19].

Dopaminergic indicators, such as tyrosine hydroxylase (TH), are frequently examined to assess the functionality of dopaminergic neurons, which are predominantly impacted in Parkinson's disease. Furthermore, apoptotic pathways are explored by analyzing the expression of crucial genes, including those from the caspase family like caspase-3 and caspase-9, which are vital in facilitating neuronal cell death. Assessing these molecular markers sheds light on the processes of neurodegeneration and the level of neuronal damage [20,21].

Consequently, RT-qPCR is a vital tool for linking molecular changes with behavioral deficits, allowing for a thorough understanding of disease progression and potential neuroprotective approaches.

V. CONCLUSION

This review highlights the significance of using the zebrafish model to study Parkinson's disease. Models induced by neurotoxins successfully replicate dopaminergic neurodegeneration, while behavioral tests offer clear insights into motor dysfunction. Molecular techniques like RT-qPCR further deepen understanding by uncovering alterations in dopaminergic markers and apoptotic genes, such as caspases. Collectively, these methods provide a comprehensive framework for examining disease mechanisms and exploring potential therapeutic strategies. Overall, zebrafish models remain vital in advancing neurodegenerative research and hold considerable promise for identifying new therapeutic strategies and neuroprotective agents.

REFERENCE

[1] S. Zafar, F. Lui, and S. S. Yaddanapudi, "Parkinson disease," in StatPearls [Internet]. Treasure Island, FL, USA: StatPearls Publishing, Jan. 2026.

[2] J. Jankovic and E. K. Tan, "Parkinson's disease: Etiopathogenesis and treatment," Journal of Neurology, Neurosurgery & Psychiatry, vol. 91, pp. 795–808, 2020.

[3] L. M. Barnhill, H. Murata, and J. M. Bronstein, "Studying the pathophysiology of Parkinson's disease using zebrafish," Biomedicines, vol. 8, no. 7, p. 197, 2020.

[4] K. Razali, N. Othman, M. H. Mohd Nasir, et al., "The promise of the zebrafish model for Parkinson's disease: Today's science and tomorrow's treatment," Frontiers in Genetics, vol. 12, p. 655550, 2021.

[5] J. M. Doyle and R. P. Croll, "A critical review of zebrafish models of Parkinson's disease," Frontiers in Pharmacology, vol. 13, p. 835827, 2022.

[6] M. Bangeppagari, A. Manjunath, A. Srinivasa, et al., "Tiny fish, big hope: Zebrafish unlocking

secrets to fight Parkinson's disease," Biology, vol. 14, p. 1397, 2025.

[7] C. Ashok, N. K. Rajasekaran, S. Jeyabalan, et al., "Comparative evaluation of MPTP and rotenone as inducing agents for Parkinson's disease in adult zebrafish: Behavioural and histopathological insights," Toxicology Reports, vol. 15, p. 102084, 2025.

[8] M. Kalyn, K. Hua, S. Mohd Noor, et al., "Comprehensive analysis of neurotoxin-induced ablation of dopaminergic neurons in zebrafish larvae," Biomedicines, vol. 8, no. 1, p. 1, 2020.

[9] P. Carneiro, P. Pais, I. V. Oliveira, et al., "Amelioration of 6-OHDA-induced Parkinson's symptoms in zebrafish larvae by an almond skin acetonic extract," International Journal of Molecular Sciences, vol. 27, p. 2590, 2026.

[10] S. S. Kim, K. S. Hwang, H. Kan, et al., "Neurotoxicological profiling of paraquat in zebrafish model," Neurochemical Research, vol. 47, no. 8, pp. 2294–2306, 2022.

[11] T. Ranasinghe, Y. Seo, H. C. Park, et al., "Rotenone exposure causes features of Parkinson's disease pathology linked with muscle atrophy in developing zebrafish embryo," Journal of Hazardous Materials, vol. 480, p. 136215, 2024.

[12] X. S. Zeng, W. S. Geng, and J. J. Jia, "Neurotoxin-induced animal models of Parkinson disease: Pathogenic mechanism and assessment," ASN Neuro, vol. 10, no. 1, 2018.

[13] Y. Wang, W. Liu, J. Yang, et al., "Parkinson's disease-like motor and non-motor symptoms in rotenone-treated zebrafish," Neurotoxicology, vol. 58, pp. 103–109, 2017.

[14] C. Christensen, H. Þorsteinsson, V. H. Maier, et al., "Multi-parameter behavioral phenotyping of the MPP+ model of Parkinson's disease in zebrafish," Frontiers in Behavioral Neuroscience, vol. 14, p. 623924, 2020.

[15] I. Barra, D. C. Bruna, M. L. Maximino, and C. Maximino, "Novel tank test in zebrafish," protocols.io, 2023.

[16] T. Shinkai, M. Nakai, U. Takeshita, et al., "Refinement of the novel tank diving test: Toward standardized and robust analysis of anxiety-like behavior in zebrafish," Frontiers in Behavioral Neuroscience, vol. 19, p. 1624277, 2025.

[17] L. Magno, A. Fontes-Pereira, and B. Gonçalves, "Pharmacological study of the light/dark preference test in zebrafish (Danio rerio)," Pharmacology Biochemistry and Behavior, vol. 135, 2015.

[18] D. Bong, J. Sohn, and S. J. V. Lee, "Brief guide to RT-qPCR," Molecules and Cells, vol. 47, no. 12, p. 100141, 2024.

[19] A. Romero, A. Sanchez, J. D. Jones, et al., "Optimization of zebrafish larvae 6-OHDA exposure for neurotoxin induced dopaminergic marker reduction," Zebrafish, vol. 21, no. 4, pp. 287–293, 2024.

[20] H. Ye, D. Li, L. Zhang, et al., "Epicoccin A ameliorates PD-like symptoms in zebrafish," Marine Drugs, vol. 23, no. 4, p. 175, 2025.

[21] Y. H. Nies, W. L. Lim, N. Abd Karim, et al., "Metallothionein II treatment mitigates rotenone-induced neurodegeneration in zebrafish models of Parkinson's disease," Frontiers in Pharmacology, vol. 16, p. 1478013, 2025.