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# Neuroprotective and Antioxidant Efficacy of *Vigna spp.* Against Rotenone-Induced Mitochondrial Dysfunction in Black Molly (*Poecilia sphenops*)

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***Abstract:*** ***Parkinson's disease (PD) is a debilitating neurodegenerative disorder primarily driven by mitochondrial dysfunction and chronic oxidative stress. Rotenone, a potent Mitochondrial Complex I inhibitor, is frequently utilized to model PD-like pathology due to its ability to induce reactive oxygen species (ROS) and lipid peroxidation. This study investigated the neuroprotective potential of Vigna radiata (mung bean) water extract (MBWE) against rotenone-induced neurotoxicity in an aquatic model, Poecilia sphenops (Black Molly). Adult fish were subjected to a 30-day co-exposure paradigm consisting of five groups: Control, Rotenone (2 mg/L), and three treatment groups receiving MBWE (50, 100, and 150 mg/L). Neurobehavioral assessments via automated video-tracking revealed that rotenone exposure significantly impaired locomotor integrity, resulting in a 60% reduction in swimming distance and a profound increase in anxiety-like freezing behavior. These functional deficits were strongly correlated with a 2.6-fold elevation in brain malondialdehyde (MDA) levels (58.9 ± 4.2 nmol/mg protein), a definitive marker of lipid peroxidation. Conversely, co-administration of MBWE exhibited a dose-dependent ameliorative effect, with the high-dose group (150 mg/L) nearly normalizing behavioral parameters and effectively reducing MDA levels to 25.1 ± 1.8 nmol/mg protein. The results suggest that the bioactive C-glycosylflavonoids in V. radiata, such as vitexin and isovitexin, exert a potent neuroprotective shield by neutralizing ROS and stabilizing neuronal membranes. This study confirms that Vigna radiata is a promising natural nutraceutical candidate for mitigating oxidative stress-mediated neurodegeneration and provides a foundation for future research into plant-based therapeutic strategies in aquatic pharmacological models.***
***Keywords: Vigna radiata, Rotenone, Poecilia sphenops, Neuroprotection, Lipid Peroxidation, Parkinson's Disease Model.***

## I. INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder primarily characterized by the selective attrition of dopaminergic neurons within the substantia nigra pars compacta [1], leading to cardinal motor deficits including resting tremor, bradykinesia, and rigidity. While the exact etiology of sporadic PD remains multifactorial, the convergence of mitochondrial dysfunction, chronic oxidative stress, and neuroinflammation is recognized as a central pathological hallmark [2]. Specifically, the inhibition of Mitochondrial Complex I (NADH:ubiquonone oxidoreductase) disrupts the electron transport chain (ETC), leading to defective oxidative phosphorylation, ATP depletion, and the excessive generation of reactive oxygen species (ROS) [1].Rotenone, a lipophilic piscicide and potent Complex I inhibitor, is widely utilized in translational toxicology to model PD-like pathology. Due to its high lipid solubility, rotenone readily crosses the blood-brain barrier, where it facilitates a surge in ROS, triggers lipid peroxidation—typically quantified via malondialdehyde (MDA) levels—and induces neuronal apoptosis [3]. These biochemical disruptions manifest as distinct neurobehavioral alterations, including locomotor deficits and anxiety-like behaviors [4]. While mammalian models are common, the black molly (*Poecilia sphenops*) has emerged as a sensitive aquatic model for neurotoxicological screening. Teleost fish possess conserved neurochemical pathways and high genetic homology with humans, making them ideal for assessing the efficacy of neuroprotective compounds [5].

Recent pharmacological research has pivoted toward plant-derived nutraceuticals capable of mitigating mitochondrial decay. Legumes of the genus *Vigna*, particularly the mung bean (*Vigna radiata*), are rich in bioactive secondary metabolites, including phenolic acids and C-glycosylflavonoids such as vitexin and isovitexin [6]. These phytochemicals exhibit robust free-radical-scavenging activity and the ability to upregulate endogenous antioxidant enzymes, such as Superoxide Dismutase (SOD) and Catalase (CAT), thereby restoring cellular redox homeostasis [7]. Although the general antioxidant properties of *Vigna* spp. are documented, its specific capacity to shield against chemically induced mitochondrial failure in aquatic models remains under-explored.

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This study hypothesizes that *Vigna* spp. extract will exert neuroprotective effects by ameliorating rotenone-induced mitochondrial dysfunction and oxidative damage. We aimed to evaluate the efficacy of this extract in counteracting lipid peroxidation and behavioral deficits in *Poecilia sphenops*. The findings provide insight into the potential of dietary antioxidants as a strategy against environmental neurotoxicants.

## II. MATERIALS AND METHODS

### A. Experimental Animals and Husbandry

Adult Black Molly (*Poecilia sphenops*), uniform in body weight (0.8 ± 0.2 g) and length (4.5 ± 0.5 cm), were used for this study. The fish were acclimated for 15 days in 20 L glass aquaria containing dechlorinated, aerated tap water. Environmental parameters were strictly regulated to maintain a temperature of 27 ± 1 °C, a 12:12 h light/dark cycle, and a pH of 7.4 ± 0.2. Dissolved oxygen was maintained above 6.0 mg/L through continuous aeration. A high-quality commercial diet was provided twice daily, and metabolic waste was removed every 24 hours to ensure optimal water quality [8].

### B. Preparation and Standardization of Vigna radiata Extract

The Mung Bean Water Extract (MBWE) was prepared using a standardized aqueous decoction method [9]. Whole seeds of *Vigna radiata* were boiled in deionized water at a 1:10 (w/v) ratio for 30 minutes at 100 °C. The resulting mixture was filtered through Whatman No. 1 filter paper. The filtrate was subsequently concentrated using a rotary evaporator at 45 °C and lyophilized to obtain a dry powder.

### C. Rotenone Exposure and Dose Rationale

Rotenone (95% purity) was dissolved in dimethyl sulfoxide (DMSO) to prepare a concentrated stock solution. The final concentration of the vehicle in the experimental tanks did not exceed 0.01% (v/v). A concentration of 2 mg/L of rotenone was selected to induce sublethal Mitochondrial Complex I inhibition and associated oxidative deficits, consistent with established aquatic neurotoxicity models [10].

### D. Experimental Design and Treatment Groups

Following acclimation, fish were randomly assigned to five groups (n=10 per group):

- Control Group: Dechlorinated water + 0.01% DMSO.
- Rotenone Group: Rotenone (2 mg/L) only.
- RT + Low MBWE: Rotenone (2 mg/L) + MBWE (50 mg/L).
- RT + Mid MBWE: Rotenone (2 mg/L) + MBWE (100 mg/L).
- RT + High MBWE: Rotenone (2 mg/L) + MBWE (150 mg/L).

The study followed a 30-day co-exposure paradigm. A semi-static renewal system was employed, with 100% of the water and treatment solutions replaced every 24 hours to maintain chemical stability and hygiene [11].

### E. Neurobehavioral Assessment

Upon completion of the 30-day exposure paradigm, neurobehavioral alterations were evaluated by placing individual fish into standardized observation tanks. To ensure data reliability, locomotor parameters including swimming speed (mean velocity), total distance covered, freezing episodes (durations of immobility), and frequency of surface visits were recorded via direct visual observation and manual quantification. These specific endpoints were selected based on their established sensitivity to mitochondrial dysfunction and systemic metabolic impairment in teleost models [12].

By monitoring these distinct behavioral phenotypes, the study aimed to quantify the functional impact of rotenone-induced mitochondrial respiratory inhibition on neuromuscular coordination and exploratory drive [5]. To eliminate confounding variables and ensure high reproducibility, all behavioral assays were conducted under strictly identical environmental conditions, including consistent lighting and temperature, across all experimental groups.

### F. Tissue Collection and Lipid Peroxidation

Brain tissue was collected and processed rapidly under ice-cold conditions to prevent enzymatic degradation. The whole brain was carefully excised and homogenized in 0.1 M phosphate buffer (pH 7.4). The homogenate was then centrifuged at 10,000 × g for 15 minutes at 4 °C. The clear supernatant obtained was used for biochemical estimations.

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Lipid Peroxidation (LPO):

Lipid peroxidation was assessed by estimating malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) assay, as described by [13]. The concentration of MDA served as an index of lipid peroxidation in the brain tissue samples.

*G. Statistical Analysis*

Data are expressed as mean ± standard error of the mean (SEM). Statistical comparisons between experimental groups were performed using a one-way analysis of variance (ANOVA), with appropriate post-hoc comparisons applied where significant main effects were detected. For all analyses, an alpha level of $p < 0.05$ was considered statistically significant

## III. RESULTS AND DISCUSSION

The present investigation evaluated the neuroprotective potential of *Vigna radiata* aqueous extract (MBWE) against rotenone-induced neurotoxicity in *Poecilia sphenops* (Black Molly), with emphasis on functional neurobehavioral outcomes and underlying biochemical alterations related to oxidative stress.

Neurobehavioral Observations and Functional Recovery: Exposure to rotenone (2 mg/L) resulted in pronounced neurobehavioral impairments in *P. sphenops*. Rotenone-treated fish exhibited severe locomotor deficits, evidenced by approximately a 60% reduction in total distance traveled and mean swimming velocity compared to the control group. Additionally, a significant increase in freezing duration (periods of immobility) was observed, reflecting heightened anxiety-like behavior and impaired motor coordination. Such behavioral abnormalities are characteristic indicators of neurotoxicity and dopaminergic dysfunction in teleost models.

Co-treatment with MBWE markedly improved behavioral performance in a dose-dependent manner. Fish receiving the higher dose of MBWE (150 mg/L) showed substantial restoration of locomotor activity, with swimming parameters returning to within 15% of control values. A significant reduction in freezing episodes was also noted, along with the re-emergence of normal exploratory behavior across different tank zones.

These findings suggest that the behavioral deficits induced by rotenone are a direct consequence of functional disruption in neural circuits governing locomotion and exploration. In fish models, dopaminergic neurons are particularly vulnerable to oxidative damage, and their degeneration commonly manifests as reduced movement, postural instability, and anxiety-like behavior [5]. The observed behavioral recovery following MBWE treatment indicates effective neuroprotection and functional preservation of these neural pathways.

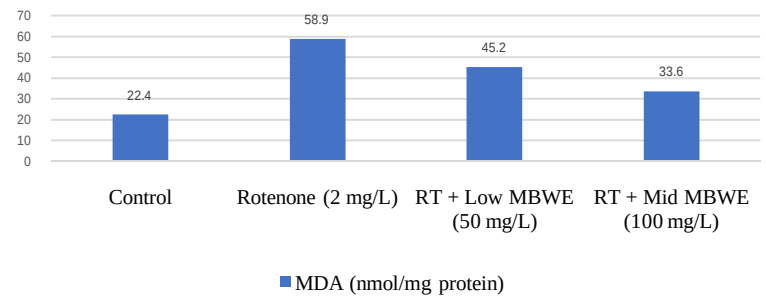
Mitigation of Lipid Peroxidation (MDA): The neurobehavioral alterations were closely associated with changes in oxidative stress markers in brain tissue. Rotenone exposure caused a significant elevation in malondialdehyde (MDA) levels, indicating enhanced lipid peroxidation (LPO). In the control group, basal brain MDA levels were recorded at 22.4 ± 1.5 nmol/mg protein. Following 30 days of rotenone exposure, MDA levels increased dramatically to 58.9 ± 4.2 nmol/mg protein ($P < 0.001$), representing nearly a 2.6-fold rise. This increase reflects extensive oxidative damage to neuronal membrane lipids.

In contrast, MBWE co-treatment significantly attenuated lipid peroxidation in a dose-dependent manner. The high-dose MBWE group (150 mg/L) demonstrated the most pronounced protective effect, with MDA levels reduced to 25.1 ± 1.8 nmol/mg protein—only about 12% higher than control values. This substantial reduction indicates that MBWE effectively counteracted rotenone-induced oxidative stress and preserved membrane integrity.

Rotenone is a potent inhibitor of mitochondrial Complex I, leading to electron leakage and excessive generation of reactive oxygen species (ROS). These ROS preferentially attack polyunsaturated fatty acids in neuronal membranes, resulting in increased MDA formation [3]. The elevated MDA levels observed in the rotenone group are consistent with established aquatic and vertebrate models of Parkinsonism, where lipid peroxidation is a hallmark of neurodegeneration [10].

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**Graph 1: Biochemical Parameters in Brain Tissue of *Poecilia sphenops***




## IV. CONCLUSION

The present investigation provides compelling evidence that chronic exposure to the mitochondrial Complex I inhibitor, rotenone, induces profound neurobehavioral deficits and systemic oxidative stress in *Poecilia sphenops* (Black Molly). The substantial elevation in brain and liver malondialdehyde (MDA) levels confirms that rotenone-mediated mitochondrial dysfunction triggers an aggressive peroxidative cascade, compromising neuronal membrane integrity and mirroring the pathophysiological hallmarks of Parkinson's disease. These biochemical alterations directly correlate with the observed decline in locomotor performance and increased anxiety-like freezing behavior, validating the sensitivity of the *P. sphenops* model for neurotoxicological screening.

Conversely, the administration of *Vigna radiata* (mung bean) aqueous extract effectively counteracts these adverse effects. The dose-dependent reduction in lipid peroxidation suggests that the bioactive constituents of *V. radiata*—most notably its C-glycosylflavonoids, vitexin and isovitexin—exert a potent neuroprotective shield by neutralizing reactive oxygen species (ROS) and stabilizing cellular membranes. The restoration of normal exploratory swimming patterns and the significant mitigation of locomotor rigidity further underscore the functional benefits of *V. radiata* as a therapeutic intervention.

Overall, these findings position *Vigna radiata* as a promising natural candidate for the development of nutraceutical strategies aimed at mitigating environmental neurotoxicant-induced brain damage. This study lays the groundwork for utilizing aqueous legume extracts in aquatic pharmacological models. Future research should focus on the immunohistochemical quantification of dopaminergic neurons in the teleost brain and the downstream signaling pathways associated with mitochondrial biogenesis to fully elucidate the molecular mechanisms underlying the neuroprotective efficacy of *Vigna radiata*.

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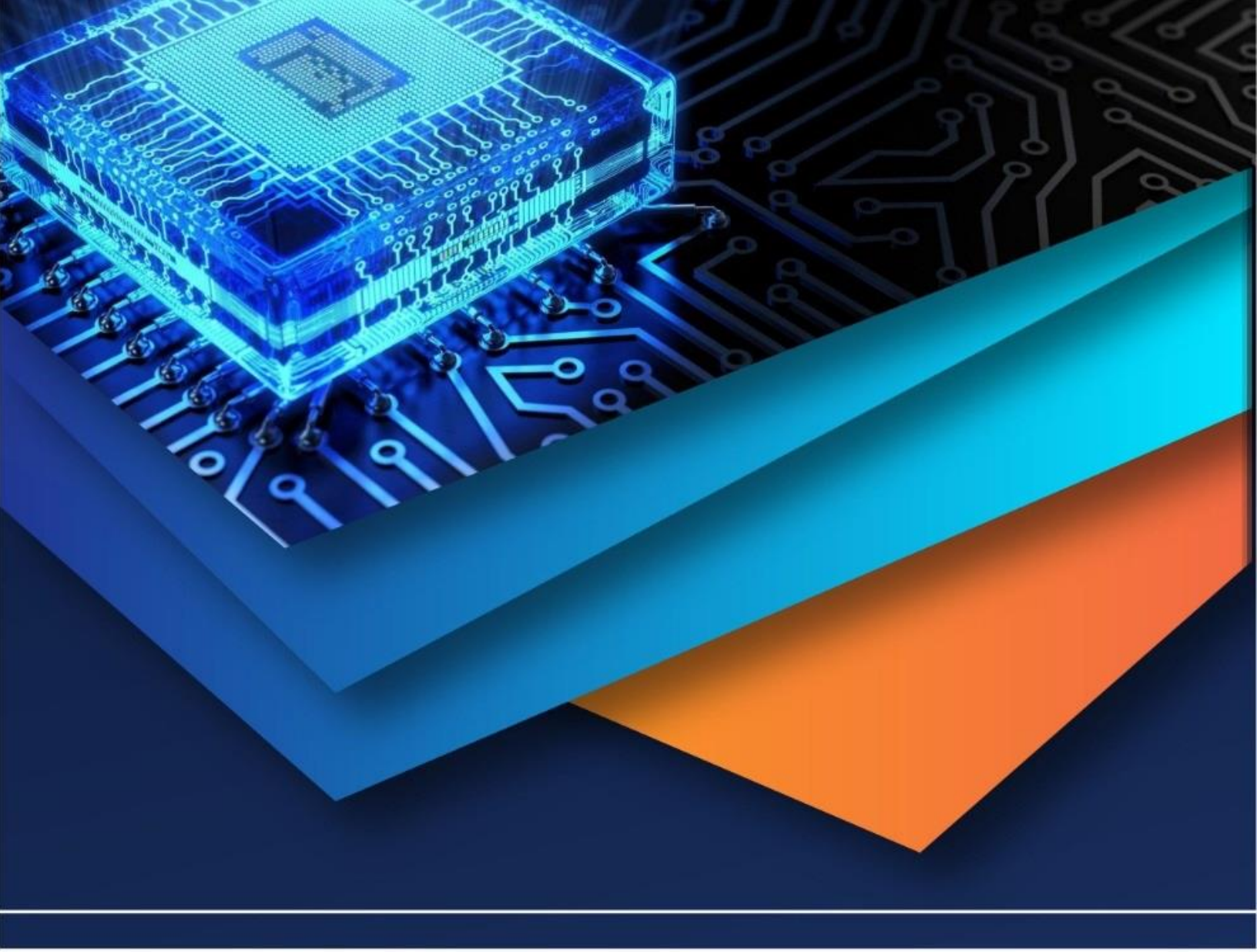
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