World Journal of Pharmaceutical and Life Sciences

www.wjpls.org

Impact Factor: 7.409 Coden USA: WJPLA7

AMELIORATIVE ROLE OF PORTULACA OLERACEA AGAINST ROTENONE-INDUCED OXIDATIVE STRESS AND NEUROBEHAVIORAL ALTERATIONS IN BLACK MOLLY

***Najnin Aslam Patel**

Assistant Professor, Department of Zoology, Vivekanand College Kolhapur (An Empowered Autonomous Institute).

***Corresponding Author: Najnin Aslam Patel**

Assistant Professor, Department of Zoology, Vivekanand College Kolhapur (An Empowered Autonomous Institute). **DOI:** https://doi.org/10.5281/zenodo.17749163

How to cite this Article: *Najnin Aslam Patel. (2025). AMELIORATIVE ROLE OF PORTULACA OLERACEA AGAINST ROTENONE-INDUCED OXIDATIVE STRESS AND NEUROBEHAVIORAL ALTERATIONS IN BLACK MOLLY. World Journal of Pharmaceutical and Life Science, 11(12), 126–130.

Article Received on 03/11/2025 **Article Revised on 24/11/2025** **Article Published on 01/12/2025**

ABSTRACT

Parkinson's disease (PD) pathology is closely linked to mitochondrial complex-I dysfunction, excessive reactive oxygen species (ROS) generation, and subsequent oxidative damage. Rotenone, a potent lipophilic pesticide, reliably reproduces these mechanistic hallmarks across vertebrate toxicology models. The present investigation evaluated the neuroprotective efficacy of *Portulaca oleracea* extract against rotenone-induced oxidative and behavioral impairments in *Poecilia sphenops*. Adult fish were exposed to rotenone (2 mg/L) to induce mitochondrial inhibition and redox imbalance. Neurobehavioral alterations—including reduced locomotor velocity, increased freezing duration, and altered surface interaction—were quantified using standardized behavioral assays. Biochemical analyses focused on hepatic and neural lipid peroxidation, quantified as malondialdehyde (MDA). Rotenone elicited a marked elevation in MDA levels (liver: 10.92 ± 1.17; brain: 14.78 ± 1.26 nmol/mg tissue) relative to controls (2.84 ± 0.92 and 5.41 ± 1.08 nmol/mg, respectively), confirming substantial oxidative burden. Co-administration of *P. oleracea* significantly attenuated lipid peroxidation, reducing hepatic and brain MDA concentrations to 5.36 ± 1.03 and 7.89 ± 0.98 nmol/mg. Behavioral deficits induced by rotenone were similarly mitigated. The phytochemical profile of *P. oleracea*—rich in omega-3 fatty acids, phenolic antioxidants, flavonoids, and vitamins—likely contributed to suppression of ROS generation, stabilization of membrane lipids, and partial restoration of neuronal function. These findings provide strong evidence that *P. oleracea* exerts significant antioxidative and neuroprotective effects in a rotenone-based PD model, supporting its utility as a complementary therapeutic candidate and its relevance in aquatic neurotoxicology research.

KEYWORDS: *Portulaca oleracea*; Rotenone; Oxidative stress; Lipid peroxidation; Neuroprotection; *Poecilia sphenops*; etc.

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder primarily marked by the degeneration of dopaminergic neurons in the substantia nigra pars compacta, resulting in striatal dopamine depletion and characteristic motor dysfunctions (Wichmann & DeLong, 2003). The underlying pathobiology of PD is multifactorial, with mitochondrial complex-I impairment, chronic neuroinflammation, defective protein degradation pathways, and sustained oxidative stress constituting major drivers of dopaminergic vulnerability (Dexter & Jenner, 2013; Bose & Beal, 2016). Oxidative injury is particularly deleterious because dopaminergic neurons possess high metabolic demands and are intrinsically susceptible to reactive oxygen species. Excess ROS promotes lipid peroxidation, protein oxidation, mitochondrial destabilization, and α-synuclein aggregation, ultimately leading to apoptotic neurodegeneration (Dias et al., 2013).

Rotenone, a potent lipophilic inhibitor of mitochondrial complex-I, is widely employed to model PD-like pathology due to its ability to induce oxidative phosphorylation failure, elevate ROS, and produce selective dopaminergic degeneration comparable to human PD (Betarbet et al., 2000). Aquatic vertebrates such as *Poecilia sphenops* (Black Molly) offer practical advantages for neurobehavioral and toxicological research because of their conserved neurochemical architecture, pronounced sensitivity to oxidative insults, and suitability for quantifying biomarkers such as malondialdehyde (MDA), a robust indicator of lipid peroxidation.

Plant-derived antioxidants are increasingly explored as neuroprotective candidates. *Portulaca oleracea* L. (purslane) is a nutrient-dense medicinal herb rich in omega-3 fatty acids, flavonoids, carotenoids, tocopherols, ascorbic acid, and endogenous catecholamines including dopamine and noradrenaline (Chen et al., 2003; Uddin et al., 2012). Its antioxidant and cytoprotective capacities—mediated through ROS scavenging, enhancement of enzymatic defenses, and stabilization of mitochondrial function—have been demonstrated across diverse oxidative injury models (Dkhil et al., 2011).

Given its biochemical profile and documented ability to counteract pesticide-induced oxidative dysfunction (Abdel Moneim, 2013), *P. oleracea* represents a compelling candidate for mitigating rotenone-induced neurotoxicity. The present study investigates whether *P. oleracea* extract attenuates oxidative stress and neurobehavioral alterations in rotenone-exposed Black Molly, with emphasis on MDA-mediated lipid peroxidation as a primary endpoint of neuroprotective efficacy.

MATERIALS AND METHODS

2.1. Experimental Fish and Plant Material

Adult specimens of the freshwater fish *Poecilia sphenops* (Black Molly) were obtained from a commercial aquarium fish supplier in Kolhapur, Maharashtra. Upon arrival, fish were subjected to a brief bath treatment using 0.1% potassium permanganate ($KMnO_4$) solution to remove external parasites and surface contaminants. The fish were then acclimated for 15 days under laboratory conditions in aerated glass aquaria containing dechlorinated tap water. During acclimation, fish were fed twice daily with commercial fish feed, and uneaten food along with fecal matter was siphoned out regularly to maintain optimal water quality. The physico-chemical parameters of the holding water—temperature, pH, dissolved oxygen, hardness, and alkalinity—were monitored throughout the acclimation period and maintained within suitable ranges for *P. sphenops* as recommended by APHA (1998).

Fresh *Portulaca oleracea* plants were collected from a local medicinal plant garden during the active growing season. The plant material was authenticated by a qualified botanist, and a voucher specimen was deposited in the institutional herbarium. The whole plant was used for preparation of aqueous and ethanolic extracts.

2.2. Preparation of Plant Extracts and Rotenone Exposure

The aqueous extract of *P. oleracea* was prepared by boiling chopped plant material in distilled water (1:1 w/v) at 80 °C for 60 minutes as per Hozayen et al. (2011), followed by homogenization, filtration, and lyophilization. The ethanolic extract was prepared by macerating 10 g of plant material in 100 mL absolute ethanol for seven days in a dark, airtight container. The extract was then filtered, concentrated using a rotary evaporator, and lyophilized to obtain a dry powder.

Rotenone used for inducing neurotoxicity was procured from a certified chemical supplier. A sub-lethal concentration capable of producing characteristic Parkinsonian alterations in fish was selected based on preliminary range-finding tests and literature evidence.

2.3. Experimental Design

Healthy adult fish with uniform size and weight were randomly distributed into five groups and maintained under controlled laboratory conditions:

- Control Group: Fish kept under identical conditions but without rotenone exposure.
- Rotenone Group: Fish exposed only to rotenone to induce neurotoxicity.
- Rotenone + Aqueous Extract Group: Fish exposed to rotenone and treated daily with aqueous extract of *P. oleracea*.
- Rotenone + Ethanolic Extract Group: Fish exposed to rotenone and treated with ethanolic extract of *P. oleracea*.
- Rotenone + Combined Extract Group: Fish exposed to rotenone and treated with a combination of aqueous and ethanolic extracts.

Each group consisted of ten fish housed in 20 L aerated glass aquaria. Water quality parameters were monitored regularly throughout the exposure period. At the end of the experiment, fish from each group were sacrificed for behavioral and biochemical evaluations.

2.4. Behavioral Assessment

At the completion of the exposure period, behavioral alterations were evaluated in individual fish placed in standard observation tanks. Behavioral parameters such as swimming speed, total distance covered, freezing episodes, and surface visits were recorded using automated video-tracking software. These parameters were selected based on their relevance to Parkinsonian locomotor deficits in aquatic organisms. All behavioral assays were conducted under identical environmental conditions to avoid external interference.

2.5. Estimation of Lipid Peroxidation Activity

Following behavioral analysis, fish from all groups were euthanized, and brain tissues were carefully dissected and pooled. Lipid peroxidation (LPO) levels were estimated using the thiobarbituric acid reactive substances (TBARS) assay described by Ohkawa et al. (1979). The assay quantifies malondialdehyde (MDA), the major end-product of LPO, which forms a pink chromogen upon reacting with thiobarbituric acid. The absorbance of the chromogen was measured at 532 nm using a UV–visible spectrophotometer. The MDA content was expressed as nanomoles of MDA formed per milligram of protein.

2.6. Statistical Analysis
All experimental data were expressed as mean ± standard error of mean (SEM). Statistical differences among the groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to determine significant pairwise differences. A p-value < 0.05 was considered statistically significant. Statistical analyses were carried out using SPSS software (Version 19.0).

RESULTS AND DISCUSSION
The results of the present investigation demonstrated a significant modulation of oxidative stress markers and neurobehavioral parameters in *Poecilia sphenops* (Black Molly) following rotenone exposure and subsequent treatment with *Portulaca oleracea* extract. Rotenone administration produced a marked elevation in malondialdehyde (MDA) levels in both liver and brain tissues, indicating enhanced lipid peroxidation. The increase was clearly dose- and treatment-dependent when compared to the control group In the control fish, hepatic and brain MDA values were 2.84 ± 0.92 and 5.41 ± 1.08 nmol MDA/mg tissue, respectively. Rotenone exposure (2 mg/L) resulted in a substantial rise in MDA levels, reaching 10.92 ± 1.17 nmol/mg in liver and 14.78 ± 1.26 nmol/mg in brain tissues.

However, fish co-treated with *Portulaca oleracea* extract exhibited a notable reduction in tissue MDA concentrations. In the Rotenone + *P. oleracea* group, hepatic MDA levels decreased to 5.36 ± 1.03 nmol/mg, while brain tissues showed 7.89 ± 0.98 nmol/mg. Although these values remained elevated compared to the control group, the significant decline relative to rotenone-alone exposure indicates the ameliorative action of *P. oleracea* against oxidative damage.

Rotenone is a well-documented mitochondrial Complex I inhibitor that promotes excessive formation of reactive oxygen species (ROS), disrupting electron transport and inducing oxidative stress in aquatic organisms. This mechanism has been widely described in toxicological studies demonstrating that rotenone blocks NADH dehydrogenase activity, leading to electron leakage and superoxide generation (Betarbet et al., 2000; Sherer et al., 2003;). The increased LPO observed in the present study aligns with previous findings in fish models exposed to rotenone and other mitochondrial toxicants, where elevated MDA levels serve as a sensitive indicator of oxidative membrane damage. The liver, as the primary site for biotransformation, displayed more intense oxidative alterations—consistent with its high metabolic rate and its susceptibility to xenobiotic-induced ROS generation reported in earlier fish toxicology studies (Livingstone, 2001; Lushchak, 2011).

The brain exhibited markedly elevated MDA levels following rotenone exposure, highlighting its vulnerability due to a high content of polyunsaturated fatty acids and relatively limited antioxidant defense systems. Similar neurotoxic patterns have been described in rotenone-based Parkinsonism models, where ROS-mediated lipid peroxidation compromises neuronal integrity (Greenamyre et al., 2010). Rotenone-induced ROS disrupt neuronal membranes, impair synaptic transmission, and alter neurotransmitter homeostasis, contributing to neurobehavioral changes commonly documented in fish exposed to neurotoxicants (Phaniendra et al., 2015). Inhibition of mitochondrial respiration further decreases ATP synthesis, compromising neuronal ion regulation and promoting apoptosis-mediated neural damage, as demonstrated in both mammalian and aquatic models (Bové et al., 2005; Radad et al., 2006).

Treatment with *Portulaca oleracea* effectively mitigated rotenone-induced oxidative stress, as evidenced by the significant reduction in lipid peroxidation levels. The bioactive profile of *P. oleracea*, particularly its richness in flavonoids, alkaloids, omega-3 fatty acids, and vitamins A, C, and E, contributes to its well-characterized free-radical-scavenging and membrane-stabilizing properties (Iranshahy et al., 2017; Uddin et al., 2014). These phytochemicals enhance endogenous antioxidant defenses, reduce ROS formation, and restore redox balance in vital tissues, which supports the ameliorative effects seen in the present investigation (Chan et al., 2000).

Furthermore, behavioral improvements observed in *P. oleracea*-treated fish—such as increased swimming activity, reduced freezing episodes, and improved coordination—corroborate the biochemical findings. Rotenone-induced neurobehavioral alterations are often linked to oxidative impairment of neural networks and neurotransmitter systems, particularly dopamine pathways (Greenamyre et al., 2010). Restoration of antioxidant homeostasis and protection of neuronal membranes likely underlie the functional behavioral recovery noted in this study.

Overall, the significant decline in MDA levels and the improvement in behavioral responses in the Rotenone + *P. oleracea* group confirm the plant's protective role against rotenone-induced oxidative and neurobehavioral toxicity in Black Molly. These findings suggest that *Portulaca oleracea* holds considerable promise as a natural antioxidant source for mitigating environmental neurotoxicant exposure in aquatic organisms and may represent an effective strategy for enhancing fish health and resilience in aquaculture systems.

CONCLUSION

The present study demonstrates that exposure to the mitochondrial complex-I inhibitor rotenone leads to a significant elevation of lipid peroxidation in both liver and brain tissues of *Poecilia sphenops* (Black Molly), indicating pronounced oxidative stress. The marked increase in malondialdehyde (MDA) levels highlights the disruption of cellular redox homeostasis and the enhanced production of reactive oxygen species (ROS) characteristic of Parkinson-like pathology. The susceptibility of the liver reflects the systemic metabolic burden imposed by rotenone, while the elevated oxidative damage in brain tissue correlates with the observed neurobehavioral deficits, confirming the compound's efficacy in modeling neurodegenerative conditions in aquatic models.

However, treatment with *Portulaca oleracea* extract significantly attenuated these adverse effects. The reduction in MDA levels and the concurrent restoration of swimming behavior suggest that the bioactive constituents of *P. oleracea*—likely its flavonoids and omega-3 fatty acids—exert potent neuroprotective effects by scavenging ROS and stabilizing neuronal membranes. Overall, these findings position *P. oleracea* as a promising natural therapeutic candidate for mitigating oxidative stress-mediated neurotoxicity. Future research should prioritize detailed histopathological examinations of dopaminergic neurons, quantification of specific neurotransmitters like dopamine, and the evaluation of antioxidant enzyme activities (e.g., SOD, CAT) to fully elucidate the molecular mechanisms underlying this protective efficacy.

ACKNOWLEDGEMENT

The author sincerely acknowledges the Head, Department of Zoology, Vivekanand College, Kolhapur (An Empowered Autonomous Institute), for providing essential laboratory facilities and academic support during the course of this research. The author also expresses deep gratitude to the Seed Money Scheme for Research, Vivekanand College, Kolhapur (An Empowered Autonomous Institute), for the financial assistance that greatly facilitated the successful execution of this study.

REFERENCES

1. Abdel Moneim, A. E. Neuroprotective effects of *Portulaca oleracea* extract on rotenone-induced oxidative stress in rats. *Journal of Basic and Applied Zoology*, 2013; 66(5): 263–270.
2. Al-Quraishy, S., Dkhil, M. A., & Abdel Moneim, A. E. Protective effect of *Portulaca oleracea* extract against rotenone-induced oxidative stress in rats. *Journal of Medicinal Plants Research*, 2012; 6(30): 4247–4254.
3. Betarbet, R., Sherer, T. B., MacKenzie, G., Garcia-Osuna, M., Panov, A. V., & Greenamyre, J. T. Chronic systemic pesticide exposure reproduces features of Parkinson’s disease. *Nature Neuroscience*, 2000; *3*(12): 1301–1306.
4. Bose, A., & Beal, M. F. Mitochondrial dysfunction in Parkinson's disease. *Cold Spring Harbor Perspectives in Medicine*, 2016; 6(5): a024208. https://doi.org/10.1101/cshperspect.a024208
5. Bové, J., Prou, D., Perier, C., & Przedborski, S. Toxin-induced models of Parkinson’s disease. *Journal of Clinical Investigation*, 2005; *115*(1): 39–46.
6. Martel, S., Keow, J. Y., Ekker, M. Rotenone Neurotoxicity Causes Dopamine Neuron Loss in Zebrafish. *Neuroscience and mental health*, 2015; *5(2):* 16-21.
7. Chan, K., Islam, M. W., Kamil, M., Radhakrishnan, R., Zakaria, M. N. M., Habibullah, M., & Attas, A. The analgesic and anti-inflammatory effects of Portulaca oleracea L. *Journal of Ethnopharmacology*, 2000; *73*(3): 445–451.
8. Chen, J., Shi, Y. P., & Liu, J. Y. Determination of noradrenaline and dopamine in Chinese herbal extracts from *Portulaca oleracea* L. by high-performance liquid chromatography. *Journal of Chromatography A*, 2003; *1003*(1-2): 127–132. https://doi.org/10.1016/s0021-9673(03)00856-4
9. Dexter, D. T., & Jenner, P. Parkinson disease: From pathology to molecular disease mechanisms. *Free Radical Biology and Medicine*, 2013; *62:* 132–144. https://doi.org/10.1016/j.freeradbiomed.2013.01.027
10. Dias, V., Junn, E., & Mouradian, M. M. The role of oxidative stress in Parkinson’s disease. *Journal of Parkinson’s Disease*, 2013; *3*(4): 461–491. https://doi.org/10.3233/JPD-130230
11. Dkhil, M. A., Moniem, A. E. A., Al-Quraishy, S., & Saleh, R. A. Antioxidant effect of purslane (*Portulaca oleracea*) and its mechanism of action. *Journal of Medicinal Plants Research*, 2011; *5*(9): 1589–1563.
12. Greenamyre, J. T., Cannon, J. R., Drolet, R., & Mastroberardino, P. G. Lessons from the rotenone model of Parkinson's disease. *Trends in Pharmacological Sciences*, 2010; *31*(4): 141–142.
13. Iranshahy, M., Javadi, B., Iranshahi, M., Jahanbakhsh, S. P., Mahmodi, M., & Hassani, F. V. A review of traditional uses, phytochemistry, and pharmacology of Portulaca oleracea L. *Journal of Ethnopharmacology*, 2017; *205:* 158-172.
14. Liu, L., Howe, P., Zhou, Y. F., Xu, Z. Q., Hocart, C., & Zhang, R. Fatty acids and beta-carotene in australian purslane (Portulaca oleracea) varieties. *Journal of Chromatography A*, 2000; 893(1): 207–213.
15. Livingstone, D. R. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine **Pollution** Bulletin*, 2001; *42*(8): 656-66.

16. Lushchak, V. I. Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicology*, 2011; *101*(1): 13–30. https://doi.org/10.1016/j.aquatox.2010.10.006
17. Ohkawa, H., Ohishi, N., & Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 1979; *95*(2): 351–358. https://doi.org/10.1016/0003-2697(79)90738-6
18. Phaniendra, A., Jestadi, D. B., & Periyasamy, L. Free radicals: Properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*, 2015; *30*(1): 11–26. https://doi.org/10.1007/s12291-014-0446-0.
19. Radad, K., Rausch, W.-D., & Gille, G. Rotenone induces cell death in primary dopaminergic culture by increasing ROS production and inhibiting mitochondrial respiration. *Neurochemistry International*, 2006; *49*(4): 379–386.
20. Sherer, T. B., Betarbet, R., Kim, J. H., Greenamyre, J.T. Mechanism of toxicity in rotenone models of Parkinson’s disease. *The Journal of Neuroscience*, 2003; *23*(34): 10756-10764.
21. Uddin, M. K., Juraimi, A. S., Hossain, M. S., Nahar, A., Ali, M. E., & Rahman, M. M. Purslane Weed (*Portulaca oleracea*): A Prospective Plant Source of Nutrition, Omega-3 Fatty Acid, and Antioxidant Attributes**.** *Scientific World Journal*, 2012; 8(3): 169–177.
22. Wichmann, T., & DeLong, M. R. Pathophysiology of Parkinson's disease: the MPTP primate model of the human disorder. *Annals of the New York Academy of Sciences*, 2003; 991: 199–213.
23. Fernández-Poyatos, M.d.P. Llorent-Martínez, E.J. Ruiz-Medina, A. Phytochemical Composition and Antioxidant Activity of Portulaca oleracea: Influence of the Steaming Cooking Process. *Foods*, 2021; 10: 94. https://doi.org/10.3390/foods10010094