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ACROSS ANIMAL AND DROSOPHILA MODELS**

NEUROPROTECTIVE POTENTIAL OF PROANTHOCYANIDINS AND ANTHOCYANIDINS AGAINST ROTENONE-INDUCED NEUROTOXICITY: A REVIEW ACROSS ANIMAL AND DROSOPHILA MODELS

Atere TG¹, Osuntokun OS²

1. Department of Medical Biochemistry, College of Health Sciences, Osun State University, Osogbo, Nigeria.
2. Department of Health and Sport Science, Regent College London, University of Bolton, England

Correspondences to: Atere TG, Department of Medical Biochemistry, College of Health Sciences, Osun State University, Osogbo, Nigeria. **E-mail:** tope.ateret@uniosun.edu.ng

ABSTRACT

Introduction: Rotenone, a natural pesticide and neurotoxin, is commonly used to replicate key features of Parkinson's disease in experimental models due to its ability to disrupt mitochondrial function and trigger oxidative stress, inflammation, and neuronal damage. As interest grows in plant-based therapies, compounds like proanthocyanidins and anthocyanidins found in berries, grape seeds, and colourful fruits, have shown beneficial effects due to their antioxidant and neuroprotective effects. These phytochemicals may counteract the harmful effects of rotenone by preserving mitochondrial health and reducing inflammation. This review aims to synthesize existing evidence on the protective roles of proanthocyanidins and anthocyanidins in animal and Drosophila models exposed to rotenone.

Methods: This review draws on a broad literature search across PubMed, Scopus, and Web of Science to summarize current evidence on how proanthocyanidins and anthocyanidins protect against rotenone-induced toxicity.

Results: Key findings indicate that these compounds effectively reduce oxidative stress markers, improve mitochondrial integrity, and alleviate motor deficits in both models. Comparative analysis reveals

similarities in mechanisms of action across species while highlighting differences in experimental approaches and outcomes.

Conclusion: Despite the promising preclinical results, significant gaps remain in translating these findings into human clinical applications. Standardized dosing regimens, deeper mechanistic studies, and clinical trials are essential to harness their full therapeutic potential. This review emphasizes the need for continued exploration of proanthocyanidins and anthocyanidins as adjunctive therapies in neurodegenerative diseases, particularly Parkinson's disease, offering a pathway toward novel, plant-derived interventions for human health.

Keywords: Rotenone toxicity, Neurodegenerative diseases, Proanthocyanidins, Anthocyanidins, Oxidative stress

INTRODUCTION

Rotenone, a naturally occurring compound derived from the roots of plants in the Fabaceae family, has long been used as an insecticide and piscicide.^{1, 2} Its neurotoxicity arises from its primary mechanism of action: the inhibition of mitochondrial complex I in the electron transport chain³. This inhibition disrupts ATP production, leading to the overproduction of reactive oxygen species

(ROS), which subsequently drive oxidative stress and trigger lipid peroxidation, protein damage, mitochondrial dysfunction, and neuronal apoptosis.³ These pathological effects render rotenone a valuable agent for modeling neurodegenerative diseases, particularly Parkinson's disease (PD). The rotenone-induced PD model replicates hallmark features of the disease, including selective loss of dopaminergic neurons and α -synuclein aggregation, providing a robust platform for studying the pathophysiology of neurodegeneration and evaluating therapeutic interventions.⁴ As the global burden of neurodegenerative diseases like PD continues to rise, the search for effective interventions to counteract rotenone-induced neurotoxicity becomes increasingly critical.

Among the emerging therapeutic candidates are plant-derived bioactive compounds, particularly proanthocyanidins and anthocyanidins, which are gaining attention for their ability to address oxidative stress and inflammation which are key drivers of rotenone-induced toxicity and neurodegenerative diseases.⁵ Proanthocyanidins are flavonoids composed of oligomeric or polymeric flavan-3-ols and are abundantly found in fruits such as grapes, apples, and berries, as well as in cocoa.⁶ Their complex chemical structure underpins their potent antioxidant activity, enabling them to effectively scavenge free radicals and bolster cellular defense mechanisms.⁷ Similarly, anthocyanidins, the water-soluble pigments responsible for the vibrant red, blue, and purple hues in many fruits and vegetables, exhibit a unique flavylium cation structure that contributes to their antioxidant, anti-inflammatory, and neuroprotective properties.⁸ These compounds are not only capable of reducing oxidative stress but also play a vital role in preserving mitochondrial integrity, modulating inflammatory signaling pathways, and preventing neuronal apoptosis—all of which are critical in mitigating the detrimental effects of rotenone toxicity.⁹

The therapeutic relevance of these phytochemicals lies in their multifaceted mechanisms of action. Proanthocyanidins and anthocyanidins have demonstrated remarkable potential in attenuating the oxidative and inflammatory cascades initiated by rotenone. By scavenging ROS, enhancing endogenous antioxidant defenses, and regulating inflammatory mediators, these compounds address the core pathological processes underlying neurodegenerative diseases. Moreover, their ability to protect mitochondrial function and modulate neuroprotective signaling pathways positions them as promising candidates for advancing neuroprotection in experimental models of rotenone-induced toxicity.^{5,10}

This review aims to comprehensively synthesize evidence on the protective roles of proanthocyanidins and anthocyanidins against rotenone-induced neurotoxicity, with a focus on both animal and *Drosophila* models. Through an analysis of existing studies, this work seeks to elucidate the mechanisms through which these phytochemicals exert their protective effects and assess their potential for translational application in treating neurodegenerative diseases. Additionally, the review highlights gaps in current research and identify key directions for future exploration, emphasizing the need for standardized protocols, rigorous studies on mechanisms, and clinical trials to optimize the use of these compounds as therapeutic agents.

METHODS

The present review was conducted using a narrative approach to synthesize existing evidence on the protective effects of proanthocyanidins and anthocyanidins against rotenone-induced toxicity. The methodology involved a systematic search of peer-reviewed literature from established scientific databases, including PubMed,

Scopus, and Web of Science, to ensure comprehensive coverage of relevant studies.

Sources of Literature

Searches focused on articles published in peer-reviewed journals and gray literature that explored rotenone toxicity, mechanisms of action, and the therapeutic potential of proanthocyanidins and anthocyanidins. The selection included studies performed in animal models, *Drosophila melanogaster*, and in vitro systems that mimicked rotenone-induced oxidative stress and neurodegeneration.

Inclusion and Exclusion Criteria

Studies were included if they (1) reported the use of rotenone as a model for neurotoxicity or neurodegenerative diseases, (2) investigated the therapeutic effects of proanthocyanidins and anthocyanidins, or (3) provided mechanistic insights into oxidative stress and inflammation mitigation by these compounds. Studies that lacked experimental rigor, involved unrelated compounds, or were focused on non-toxicological aspects of rotenone were excluded.

Search Terms and Timeframe

Search terms included combinations of keywords such as "rotenone toxicity," "proanthocyanidins," "anthocyanidins," "neuroprotection," "oxidative stress," and "neurodegeneration." Boolean operators (AND, OR) were applied to refine the search. The review encompassed studies published between 2000 and 2024, aligning with the growing interest in phytochemical interventions for neurodegeneration in the 21st century.

MECHANISM OF ROTENONE TOXICITY/MECHANISM OF ACTION

Rotenone primarily exerts its toxic effects through the inhibition of mitochondrial complex I (Complex I, a core component of the mitochondrial respiratory chain, functions as a major energy transducer within the inner mitochondrial membrane), a key enzyme in

the electron transport chain (ETC) responsible for oxidative phosphorylation. By binding to the NADH dehydrogenase subunit of complex I, rotenone inhibits electron flow to coenzyme Q10, thereby disrupting ATP production.¹¹ This blockade leads to the leakage of electrons and the generation of reactive oxygen species (ROS), which cause oxidative damage to lipids, proteins, and DNA.¹²

The resultant oxidative stress triggers a cascade of deleterious cellular events, including mitochondrial dysfunction, endoplasmic reticulum stress, and activation of pro-apoptotic signaling pathways¹³. By generating ROS, rotenone is presumed to damage mitochondrial structures, including mtDNA, thereby initiating apoptosis.¹³ In neuronal cells, rotenone-induced oxidative damage disrupts calcium homeostasis, activates caspases, and promotes apoptosis, culminating in the selective degeneration of dopaminergic neurons in the substantia nigra, a hallmark of Parkinson's disease.¹⁴ Furthermore, rotenone promotes the aggregation of α -synuclein, a pathological feature linked to neurodegeneration.¹⁵

Models Used for Study

Relevance of Animal Models

Animal models, particularly rodents (e.g., mice, rats and guinea pigs), are extensively used in neurotoxicology studies to investigate rotenone-induced neurodegeneration and to evaluate therapeutic interventions. These models replicate key features of human neurodegenerative diseases, including oxidative stress, neuroinflammation, and motor deficits.⁴ Rodent studies provide valuable insights into the molecular and cellular mechanisms of toxicity and offer a robust platform for testing the neuroprotective effects of compounds like proanthocyanidins and anthocyanidins.

In addition to mimicking core pathological features of Parkinson's disease (PD), such as dopaminergic neuronal loss in the substantia

nigra and mitochondrial dysfunction, rotenone-based rodent models have been instrumental in elucidating the roles of α -synuclein aggregation and neuroinflammatory responses. Studies have shown that chronic systemic administration of low-dose rotenone in rats results in selective nigrostriatal degeneration and the formation of Lewy body-like inclusions, closely resembling human PD pathology¹⁴. These models are especially useful for exploring the interplay between environmental toxins and genetic susceptibility, helping to bridge gaps between basic research and clinical application.

However, while rodent models offer numerous advantages, they are not without limitations. Species differences in drug metabolism, lifespan, and immune response can affect the translational relevance of findings. Moreover, behavioral manifestations in animals, such as motor deficits or cognitive impairment, may not fully capture the complexity of human symptoms. To mitigate these issues, researchers often employ complementary models, such as non-human primates or genetically modified rodents, which can provide additional physiological and anatomical relevance. Despite these limitations, animal models remain indispensable tools in neurotoxicology and therapeutic research, offering ethical and practical alternatives for preclinical evaluation.

Importance of *Drosophila melanogaster*

The fruit fly (*Drosophila melanogaster*) has emerged as a powerful genetic model for studying rotenone toxicity due to its well-characterized genome, short lifespan, and ease of genetic manipulation. *Drosophila* models have been instrumental in elucidating the role of mitochondrial dysfunction and oxidative stress in rotenone-induced neurotoxicity.¹⁶ The use of *Drosophila* also facilitates high-throughput screening of neuroprotective compounds and enables the

study of conserved molecular pathways implicated in neurodegeneration. Its relevance as a model organism is underscored by its ability to replicate behavioral and physiological phenotypes associated with rotenone toxicity, such as impaired locomotion and reduced lifespan.¹⁶

While the *Drosophila melanogaster* genome comprises approximately 13,600 genes—fewer than those found in the human genome—this numerical difference does not diminish its value as a model organism in biomedical research.¹⁷ Comparative genomic studies between humans and *Drosophila* have highlighted notable differences, such as a higher microsatellite mutation rate in humans and greater nucleotide diversity in flies. Despite these genetic distinctions, *Drosophila* remains a powerful model for studying the molecular and cellular mechanisms underlying many human diseases.

Nonetheless, there are inherent limitations to using *Drosophila* in disease modeling. One key drawback is that certain pathogenic processes are specific to vertebrates and may not be adequately captured in an invertebrate model. For instance, complex immunological disorders like multiple sclerosis cannot be effectively replicated in flies due to fundamental differences in immune system architecture. Similarly, cerebrovascular conditions such as brain infarcts or hemorrhages are not amenable to study in *Drosophila*, as they lack a true vascular system and their blood cells are limited to primitive hemocytes¹⁷. *Drosophila* models successfully mirror crucial aspects of human pathologies, particularly in the fields of neurodegeneration, cancer, and metabolic disorders.

PROANTHOCYANIDINS AND ANTHOCYANIDINS: MECHANISMS OF ACTION

Overview of Pharmacological Properties

Proanthocyanidins and anthocyanidins are two classes of flavonoids, are known for their

potent pharmacological properties. Both compounds exhibit antioxidant activity, primarily through their ability to scavenge reactive oxygen species (ROS) and inhibit lipid peroxidation.¹⁸ These phytochemicals enhance cellular antioxidant defenses by upregulating endogenous enzymes like superoxide dismutase (SOD) and catalase (CAT), reducing oxidative damage to cellular components.¹⁹

Additionally, both compounds possess anti-inflammatory properties, mediated through the inhibition of pro-inflammatory mediators such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and cyclooxygenase-2 (COX-2).²⁰ Their neuroprotective effects have been linked to their ability to attenuate oxidative stress, reduce neuroinflammation, and prevent neuronal apoptosis, suggesting potential therapeutic applications for neurodegenerative diseases.²¹

Interaction with Rotenone-Induced Pathways

Proanthocyanidins and anthocyanidins exhibit therapeutic potential in mitigating rotenone-induced toxicity by targeting specific molecular pathways. Oxidative stress, a hallmark of rotenone toxicity, is alleviated by the ability of these compounds to neutralize ROS, repair damaged cellular components, and maintain mitochondrial integrity.²² Proanthocyanidins, in particular, have been shown to enhance mitochondrial function by preserving ATP synthesis and reducing mitochondrial membrane potential collapse.²³ Both compounds also modulate signaling pathways involved in apoptosis and cell survival. They inhibit the activation of pro-apoptotic proteins like Bax and caspases while promoting anti-apoptotic markers like Bcl-2.²⁴ Furthermore, they regulate neuroprotective signaling pathways, such as the Nrf2/ARE pathway, which enhances the expression of antioxidant enzymes, and the PI3K/Akt pathway, which promotes cell survival under stress conditions.²⁵ These mechanisms underscore their ability to

counteract rotenone-induced neuronal damage and dysfunction.

EVIDENCE FROM ANIMAL MODELS

Studies on Proanthocyanidins

Several studies have demonstrated the efficacy of proanthocyanidins in mitigating rotenone-induced toxicity in animal models. For example, Tabeshpour et al.²⁶ reported that treatment with grape seed proanthocyanidin extract (GSPE) significantly reduced oxidative stress in rats exposed to rotenone by enhancing SOD and glutathione peroxidase (GPx) activity while lowering malondialdehyde (MDA) levels. GSPE was administered at doses ranging from 50 to 200 mg/kg via oral gavage, resulting in improved motor function and reduced neuronal apoptosis (as depicted in the Figure 1).

In another study, proanthocyanidins were found to attenuate neuroinflammation in rotenone-treated mice by inhibiting the activation of microglia and astrocytes and reducing the production of pro-inflammatory cytokines.²⁷ These effects were associated with improved histopathological outcomes, including reduced dopaminergic neuronal loss in the substantia nigra.

Studies on Anthocyanidins

Anthocyanidins, particularly those derived from blueberries and blackberries, have also been shown to be effective in treating rotenone-induced neurotoxicity. A study by Marques-da-Silva et al.²⁸ demonstrated that cyanidin, an anthocyanidin found in berries, significantly attenuated rotenone-induced motor deficits and oxidative stress in rats.

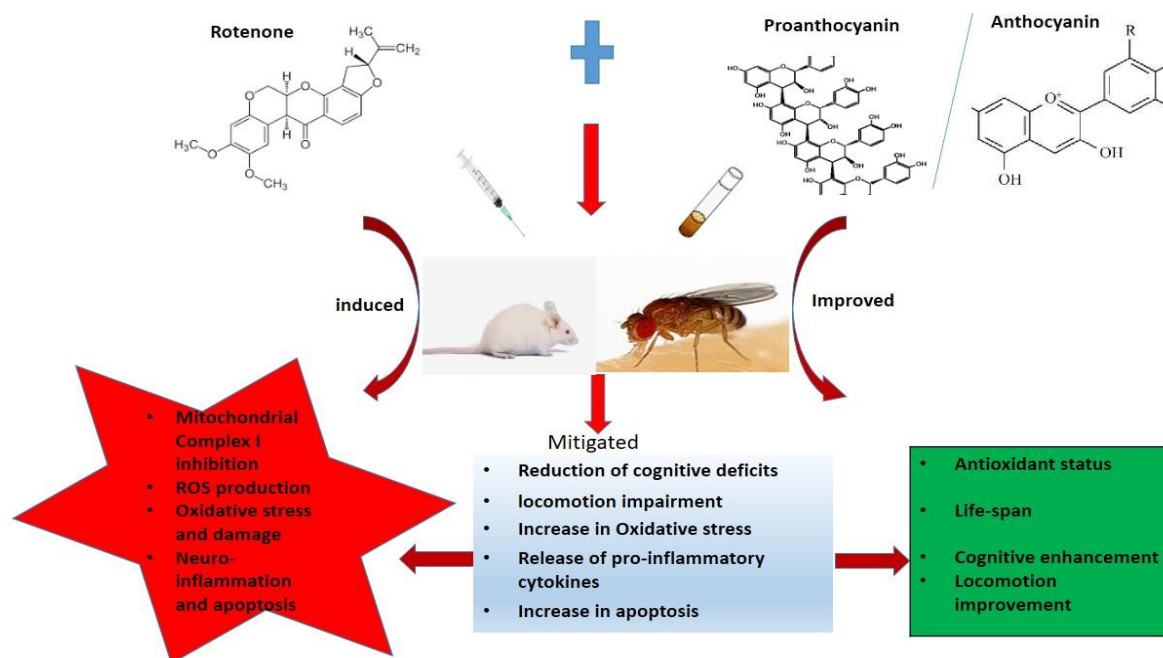


Figure 1: Schematic representation of Proanthocyanidins and Anthocyanin-Mediated Mitigation of Rotenone toxicity in Rats and *Drosophila melanogaster*

Cyanidin supplementation at 50 mg/kg improved mitochondrial function by restoring complex I activity and reducing ROS production.

Comparative studies have highlighted the superior antioxidant and neuroprotective effects of anthocyanidins over standard antioxidants like vitamin C and E.²⁹ Their ability to cross the blood-brain barrier and exert direct effects on the central nervous system is a key factor in their efficacy.

Moreover, anthocyanidins have been shown to modulate signaling pathways implicated in neurodegeneration. For instance, they activate the Nrf2/ARE pathway, a critical cellular defense mechanism against oxidative damage, while concurrently inhibiting pro-inflammatory cytokines such as TNF- α and IL-1 β .³⁰ These dual actions—antioxidant and anti-inflammatory—make anthocyanidins promising candidates for neurodegenerative disease therapy.

Emerging evidence also suggests that dietary anthocyanidins can improve cognitive performance and neuroplasticity. In

Drosophila models, cyanidin-3-glucoside was found to enhance locomotor activity and extend lifespan in flies challenged with rotenone, mirroring some effects seen in mammalian systems.³¹ This cross-species consistency underscores the translational potential of anthocyanidins in Parkinson's disease and related disorders. Importantly, their natural abundance in common fruits makes them accessible and relatively safe for long-term use.

Mechanistic Insights to action of Proanthocyanidins and Anthocyanidins Against Rotenone-Induced Neurotoxicity

Biochemical analyses from these studies revealed significant reductions in markers of oxidative stress (e.g., MDA, ROS) and improvements in antioxidant enzyme levels (e.g., SOD, CAT). Histopathological

evaluations showed reduced neuronal apoptosis and preservation of dopaminergic neurons. Behavioral assessments, such as improved motor function and reduced tremors, further validated the neuroprotective effects of these compounds.³²

EVIDENCE FROM DROSOPHILA MODELS

Relevance of Drosophila in Rotenone Toxicity Studies

The fruit fly (*Drosophila melanogaster*) is a valuable model organism for studying rotenone-induced toxicity due to its genetic simplicity, short life cycle, and translational relevance to human neurodegenerative diseases. *Drosophila* models replicate key pathological features of rotenone toxicity, including mitochondrial dysfunction, oxidative stress, and motor deficits.³³ Genetic tools available in *Drosophila* enable precise manipulation of genes and pathways involved in oxidative stress and neurodegeneration, making it an ideal platform for studying therapeutic interventions.

The fruit fly (*Drosophila melanogaster*) is a valuable model organism for studying rotenone. In particular, rotenone-exposed *Drosophila* exhibit dopaminergic neuronal loss and impaired climbing ability—hallmarks that parallel Parkinsonian phenotypes in humans. Ayajuddin et al.³⁴ demonstrated that chronic exposure to rotenone in flies led to significant neurodegeneration in the protocerebral posterior lateral cluster, a region enriched in dopaminergic neurons, coupled with age-dependent behavioral impairments. This similarity underscores the value of *Drosophila* not only in mechanistic studies but also in evaluating neuroprotective compounds.

Moreover, *Drosophila* offers an excellent platform to study gene-environment interactions in neurotoxicity. For example, mutations in PD-related genes such as *parkin*

and *PINK1* exacerbate rotenone-induced toxicity, while antioxidant interventions like overexpression of *superoxide dismutase* (SOD) or administration of polyphenolic compounds have been shown to alleviate rotenone-induced damage.³⁵ These findings provide a strong rationale for using *Drosophila* as a high-throughput, cost-effective model for screening neuroprotective strategies against environmental toxins like rotenone.

Studies on Proanthocyanidins and Anthocyanidins in Flies

Studies have demonstrated that proanthocyanidins and anthocyanidins confer significant protection against rotenone-induced neurotoxicity in *Drosophila*. Johri and Chandra showed that dietary supplementation with GSPE extended the lifespan of rotenone-exposed flies, improved climbing ability, and reduced markers of oxidative stress, such as hydrogen peroxide and lipid peroxidation.³⁶

Similarly, anthocyanidin-rich extracts from blueberries were found to restore mitochondrial function and reduce neurodegeneration in rotenone-treated *Drosophila* models. These effects were associated with increased expression of antioxidant genes, such as catalase and glutathione-S-transferase, and improved mitochondrial dynamics, including reduced fragmentation and enhanced fusion.³⁷

Potential pathways targeted by these compounds in *Drosophila* include the activation of the Nrf2 pathway, modulation of mitochondrial biogenesis through PGC-1 α , and inhibition of apoptotic signaling cascades.³⁸ These findings highlight the relevance of *Drosophila* as a model for screening neuroprotective phytochemicals and elucidating their mechanisms of action.

COMPARATIVE ANALYSIS OF ANIMAL AND FLY MODELS

Similarities in Mechanisms of Protection

Both animal and *Drosophila* models demonstrate consistent protective mechanisms of proanthocyanidins and anthocyanidins against rotenone-induced toxicity. In both models, these compounds mitigate oxidative stress by enhancing the activity of endogenous antioxidants like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). They also reduce markers of lipid peroxidation, such as malondialdehyde (MDA), and protect mitochondrial integrity by preserving mitochondrial membrane potential and ATP production.³⁹ Additionally, the compounds show anti-apoptotic effects by regulating the expression of pro-apoptotic and anti-apoptotic proteins (e.g., Bax and Bcl-2), along with suppressing caspase activation⁴⁰. (Table 1)

Differences in Experimental Setups, Doses, and Outcomes

Animal studies, particularly in rodents, often use oral gavage or intraperitoneal injections to deliver proanthocyanidins or anthocyanidins, with doses ranging from 50 to 200 mg/kg body weight.⁴¹ In contrast, *Drosophila* studies typically incorporate these compounds into the diet at lower concentrations, such as 5–10 mM, reflecting the metabolic and physiological differences between the two models.⁴²

The outcomes observed in animal models include improved motor coordination (tested through rotarod or grip strength assays), reduced neuroinflammation, and preservation of dopaminergic neurons in the substantia nigra.⁴³ In *Drosophila*, the benefits manifest as increased lifespan, enhanced climbing ability, and reduced neurodegeneration in dopaminergic clusters.⁴⁴ While both models show neuroprotective effects, the quantitative differences in outcomes may stem from variations in metabolism, drug bioavailability, and experimental setups (Table 1).

Table 1: Comparative Analysis of Animal and Fly Models in Rotenone-Induced Neurotoxicity Studies

| Category | Animal Models (Rodents) | <i>Drosophila</i> Models (Fruit Flies) |
|---------------------------------------|---|---|
| Mechanism of Protection | Enhance endogenous antioxidants: SOD, CAT, GPx Reduce MDA Protect mitochondrial membrane potential & ATP Regulate Bax/Bcl-2 Suppress caspase activation | Enhance endogenous antioxidants: SOD, CAT, GPx Reduce MDA Protect mitochondrial membrane potential & ATP Regulate Bax/Bcl-2 Suppress caspase activation |
| Compound Delivery | Oral gavage or intraperitoneal injection | Dietary incorporation |
| Typical Dose | 50–200 mg/kg body weight | 5–10 Mm |
| Measured Outcomes | - Improved motor coordination (e.g., rotarod, grip strength) - Reduced neuroinflammation - Preservation of dopaminergic neurons (e.g., substantia nigra) | - Increased lifespan - Enhanced climbing ability - Reduced dopaminergic neurodegeneration |
| Experimental Advantages | Closer to human metabolism allows complex behavioral and histological assessments | Rapid life cycle. Genetic tractability, Cost-effective, high-throughput screening |
| Limitations | Higher cost, longer experimental timeframes | Simpler nervous system, metabolic differences may limit direct translation |
| Reason for Outcome Differences | Variations in metabolism, drug bioavailability, and experimental protocols | Variations in metabolism, drug bioavailability, and experimental protocols |

Translational Value of Findings

The findings from animal and *Drosophila* models highlight the potential translational relevance of proanthocyanidins and anthocyanidins for human neurodegenerative diseases. Both models replicate critical features of rotenone-induced neurotoxicity, including mitochondrial dysfunction, oxidative stress, and motor deficits, providing insights into therapeutic mechanisms. However, differences in physiology and complexity between models and humans necessitate careful interpretation of these findings before clinical application.⁴⁵

Importantly, these models serve as foundational tools for screening natural compounds before moving into more complex preclinical and clinical settings. While rodents provide closer physiological relevance due to their mammalian systems, *Drosophila* models offer rapid, cost-effective screening of genetic and pharmacological interventions. This dual-model approach strengthens the reliability of observed neuroprotective effects.

Furthermore, proanthocyanidins and anthocyanidins are naturally occurring compounds already present in the human diet—found in berries, grapes, and dark-colored fruits—making them promising candidates for preventive or adjunctive strategies. The consistency of their protective effects across species supports the idea of incorporating these bioflavonoids into neurodegenerative disease research pipelines, especially in early-stage interventions or as dietary supplements. Nonetheless, rigorous human studies are essential to determine appropriate dosages, bioavailability, and long-term safety before any clinical translation can be confidently pursued.

THERAPEUTIC IMPLICATIONS

Potential Clinical Relevance

The neuroprotective effects of proanthocyanidins and anthocyanidins hold significant promise for treating

neurodegenerative diseases like Parkinson's disease (PD), where oxidative stress and mitochondrial dysfunction are central to pathogenesis. These compounds can potentially serve as adjunctive therapies to existing treatments like levodopa, targeting complementary pathways to slow disease progression.⁴⁶ Their ability to cross the blood-brain barrier and exert direct effects on neuronal cells enhances their clinical relevance.⁴⁷

Despite their promise, several challenges exist in translating these findings from preclinical models to humans. Key issues include variability in bioavailability, differences in metabolic processing, and the absence of standardized dosing regimens. Additionally, human studies need to account for the complexity of neurodegenerative diseases, including genetic and environmental factors that influence disease progression and treatment response.⁴⁸

Future Directions

Future research should aim to address these limitations through well-designed, multicenter studies that use standardized protocols. Clinical trials are essential to confirm the safety, efficacy, and optimal dosing of proanthocyanidins and anthocyanidins in humans. Furthermore, studies exploring the synergistic effects of these compounds with other therapeutic agents, such as coenzyme Q10, curcumin, or conventional neuroprotective drugs, may provide insights into combination therapies for neurodegenerative diseases.

Additional research should also focus on understanding the long-term effects of these compounds and their impact on disease progression. Advances in bioinformatics and systems biology could facilitate a deeper understanding of the molecular pathways targeted by these phytochemicals, aiding in the development of precision medicine approaches.⁴⁹

CONCLUSION

Proanthocyanidins and anthocyanidins demonstrate significant therapeutic potential in mitigating rotenone-induced toxicity by targeting oxidative stress, mitochondrial dysfunction, and apoptosis. Evidence from animal and *Drosophila* models highlights their ability to preserve neuronal integrity, improve motor function, and modulate neuroprotective signaling pathways.

However, translating these preclinical findings to clinical applications remains a challenge due to gaps in mechanistic understanding, variability in dosing, and limited human studies. Future research should focus on conducting rigorous clinical trials, exploring synergistic therapeutic combinations, and developing standardized protocols for these compounds. Given their antioxidant and anti-inflammatory properties, proanthocyanidins and anthocyanidins represent promising candidates for adjunctive therapies in neurodegenerative diseases like Parkinson's. Continued research will be crucial to unlocking their full potential and optimizing their clinical applicability for improving human health.

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