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Evaluation of Pharmacological Potential of Abietic Acid Against Rotenone Induced Parkinsonism in Caenorhabditis Elegans

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic neuronal loss, mitochondrial dysfunction, and oxidative stress. Natural compounds with antioxidant and anti-inflammatory activity have gained increasing interest as potential neuroprotective agents. Abietic acid, a diterpenoid resin acid derived from *Pinus* species, is known for its potent anti-inflammatory, antioxidant, and cytoprotective properties. Recent evidence from in vitro and in vivo studies—especially toxin-induced models such as rotenone and 6-OHDA—suggests its ability to modulate oxidative pathways, suppress neuroinflammation, and stabilize neuronal function. This review summarizes current knowledge on the biochemical profile of abietic acid, its pharmacological activities, mechanisms relevant to neuroprotection, and its potential therapeutic role in managing Parkinson's disease. The article also highlights gaps in research and proposes directions for future studies, including the need for mammalian model validation and molecular pathway characterization.

Keywords: Abietic acid, *Caenorhabditis elegans*, Parkinson's disease, Rotenone, Neuroprotection, Oxidative stress.

Introduction

Parkinson's Disease (PD) is a progressive neurodegenerative disorder primarily caused by loss of dopamine producing neurons in substantia nigra pars compacta of midbrain, resulting in reduced dopamine levels that disrupt normal motor function (Dauer et al., 2003). First described by British apothecary James Parkinson in his 1817 publication "*An Essay on the Shaking Palsy"*, disease was originally termed "paralysis agitans" or "shaking palsy." Though commonly associated with aging, PD can begin as early as age 30 (Parkinson J., 1817). It is second most prevalent neurodegenerative disorder and typically manifests clinically after more than 60% of dopaminergic neurons have been destroyed. Patients exhibit a range of motor symptoms such as resting tremors, muscle rigidity, bradykinesia and postural instability, along with difficulties in speech and writing. Non- motor symptoms include cognitive impairment, depression, anxiety, sleep disturbances, psychosis, constipation, urinary dysfunction, sexual dysfunction and difficulties in chewing and swallowing. These neuropsychiatric complications, often exacerbated by prolonged use of PD medications, have a profound impact on patient's quality of life, disease progression and mortality risk (Chaudhuri et al., 2006).

At pathological level, PD is characterized by presence of Lewy bodies, which are cytoplasmic inclusions composed of aggregated misfolded α- synuclein protein. These inclusions interfere with intracellular

transport, disrupt synaptic function and contribute to widespread neuronal dysfunction. Degeneration of extrapyramidal system and basal ganglia further contributes to classical motor symptoms of disease. PD belongs to broader category of Parkinsonian syndromes that includes multiple system atrophy, progressive supranuclear palsy, cortico- basal degeneration and dementia with Lewy bodies (Spillantini et al., 1997). Although exact cause of PD remains unknown, current evidence supports a multifactorial etiology involving genetic predispositions, environmental exposures and aging. Genetically, around 14% of cases have a familial history, with 10 to 12% resulting from monogenic mutations in genes such as *LRRK2*, *PARK1*, *PARK4* (linked to α- synuclein dysfunction), *UCHL1*, *PARKIN* and *PINK1* (involved in proteasome and autophagy pathways) and *ATP13A2* (associated with lysosomal function). These mutations impair protein degradation, mitochondrial integrity and cellular homeostasis, facilitating α-synuclein aggregation and neuronal death (Lesage et al., 2009).

Environmental risk factors include exposure to pesticides such as rotenone and paraquat, solvents and certain narcotic substances (Betarbet et al., 2000). However, aging remains most significant risk factor, as it leads to accumulated cellular damage, oxidative stress, impaired mitochondrial function and weakened compensatory mechanisms that accelerate neurodegeneration (Jenner et al., 2003). In addition to these, molecular processes such as proteasomal and lysosomal dysfunction, oxidative stress, mitochondrial failure and neuroinflammation - especially involving microglial and astrocytic activity further contribute to pathogenesis of PD. These interconnected pathways result in synaptic dysfunction, disrupted brain homeostasis and eventual cell death. Despite extensive research, initial molecular triggers that lead to PD onset remain poorly understood, highlighting need for continued investigation into its early pathophysiological mechanisms (Schapira et al., 2008).

In this study, we evaluated potential of Abietic acid (AA) to treat PD like phenotypes in wild- type *C. elegans* treated with rotenone. Rotenone model of PD using *C. elegans* is a valuable approach for studying neurodegeneration and testing potential therapeutic interventions (Nass et al., 2003). Rotenone, a plant-derived insecticide, acts as a high affinity inhibitor of mitochondrial complex I, leading to increased production of reactive oxygen species and mitochondrial dysfunction - key features observed in PD. When *C. elegans* are exposed to rotenone, worms exhibit reduced lifespan, impaired movement, degeneration of dopaminergic neurons and elevated oxidative stress, closely mirroring pathological and behavioral characteristics of human PD. This model is particularly beneficial because *C. elegans* is genetically tractable, has a simple and well mapped nervous system and reproduces rapidly, enabling high-throughput genetic and pharmacological screening. Transparency of worms allows for direct observation of neuronal changes, while the conservation of many diseases related pathways between *C. elegans* and humans enhances relevance of findings. Overall, rotenone model in *C. elegans* offers a robust and efficient platform for unraveling disease mechanisms and identifying new therapeutic strategies for PD (Katzenschlager et al., 2002). AA, a naturally occurring tricyclic diterpenoid resin acid ($C_{20}H_{30}O_2$; MW: 302.45 g/mol) primarily obtained from pine trees, has drawn increasing attention for its neuroprotective potential, particularly in addressing key pathological features of Parkinson's disease (PD), such as neuroinflammation and oxidative stress. Structurally, AA belongs to the abietane group and features a phenanthrene-like framework composed of three fused cyclohexane rings (designated A, B and C), a carboxylic acid group at C18 and an isopropyl substituent at C13, with double bonds commonly located at C7 - C8 and C13 - C14, rendering it susceptible to isomerization and oxidation (Peters R. J., 2010). In a *C. elegans* model of rotenone-induced parkinsonism, AA was selected due to its ability to modulate inflammatory pathways and enhance antioxidant defense mechanisms, potentially mitigating cellular

damage and restoring neuronal function, thereby offering a holistic approach to neuroprotection. It is chiral compound with multiple stereocenters but most often found in (1R, 4aR, 4bR, 10aR) configuration that appears as colorless crystals in pure form but typically exists as a yellowish glassy solid commercially due to oxidative byproducts and related acids. It melts between 85 - 175°C (varying with purity), is insoluble in water, but readily dissolves in organic solvents such as ethanol, acetone, diethyl ether, dimethyl sulfoxide (DMSO) and petroleum ether (Park et al., 2023). AA is largely sourced from coniferous species like *Pinus sylvestris* and *Pinus strobus*, where it comprises 40 to 70% of rosin, the solid residue formed after volatile turpentine is removed from pine oleoresin, a sticky substance secreted by trees as a defense mechanism against pests, pathogens and injury (Gonzalez et al., 2009). Industrial extraction of AA typically involves steam distillation of the oleoresin, followed by thermal isomerization of precursor acids (e.g., levopimaric acid) and crystallization using solvents such as ethanol to isolate purified AA (Li et al., 2019), while advanced techniques like response surface methodology (RSM) have significantly optimized yield (up to 58%) and purity (up to 99%) using reagents like HCl and ethanolamine (Nong et al., 2014). Altogether, these biochemical properties and processing advances support the growing interest in AA as a candidate for therapeutic intervention in neurodegenerative conditions like PD.

AA demonstrates a wide range of therapeutic properties across different physiological systems. Its strong anti- inflammatory and immune regulating capabilities are evident through its ability to reduce key inflammatory mediators, including nitric oxide (NO), prostaglandin E2 (PGE2), tumor necrosis factor- α (TNF- α), interleukin- 1 β (IL- 1β) and interleukin- 6 (IL- 6) in immune cells (Alves et al., 2022). These effects are largely due to inhibition of nuclear factor kappa light chain enhancer of activated B cells (NF- κB) signaling pathway, which prevents movement of this key regulator into cell nucleus, as well as suppression of mitogen- activated protein kinase (MAPK) signaling by decreasing activation of c- Jun N- terminal kinase (JNK), extracellular signal regulated kinase (ERK) and p38 mitogen activated protein kinase (p38). AA also helps restore immune system balance, as shown in psoriasis models where it adjusted Th17/Treg ratio and lowered levels of cytokines such as IL- 17A and IL- 23. Additionally, AA acts as a strong antioxidant by both neutralizing reactive oxygen species (ROS) and activating Nrf2/ARE pathway, which leads to increased production of body's own antioxidant enzymes. These antioxidant's effects help protect against diseases linked to oxidative stress, such as neurodegenerative disorders and cancer. In terms of anticancer activity, AA selectively targets cancer cells, causing cell cycle arrest, initiating apoptosis through both mitochondrial and external mechanisms and triggering ferroptosis. It also helps reduce metastasis by inhibiting matrix metalloproteinase- 9 (MMP-9) and vascular endothelial growth factor (VEGF) through suppression of NF- κB activity. Beyond cancer, AA has antimicrobial properties, damaging microbial cell membranes and enhancing effectiveness of antifungal treatments against drug-resistant Candida species. It also has antiemetic potential through interactions with serotonin (5HT3) and muscarinic receptors (González et al., 2015; Festa et al., 2015). Moreover, AA supports tissue repair by encouraging new blood vessel formation through ERK/p38 MAPK pathway activation in endothelial parts and influences gut microbiota composition, which aids its anti-inflammatory effects in conditions like psoriasis (Sargazifar et al., 2024). Overall, these diverse pharmacological actions highlight AA's potential as a multi- target therapeutic agent for managing inflammation, oxidative stress, infections, cancer and tissue regeneration

Material and methodology

Selection of phytoconstituent

AA was selected in this study due to its strong mechanistic resemblance to several plant derived compounds known to offer neuroprotection in preclinical PD models. Like rosmarinic acid, berberine and astragaloside IV, AA exhibits potent antioxidant and anti- inflammatory activities, crucial in mitigating the dopaminergic neuron loss, oxidative stress and microglial activation induced by rotenone, a mitochondrial complex- I inhibitor. It modulates key inflammatory pathways by inhibiting NF- κB, COX- 2 and iNOS, while also enhancing endogenous antioxidants like SOD and GSH.

Although direct studies in PD models are limited, AA's protective effects in renal and hepatic injury models, low toxicity profile and natural origin support its potential. These multifaceted actions make AA a promising candidate for neurodegenerative disease intervention, particularly in PD.

Collection, authentication and processing of phytoconstituent

Phytoconstituent AA was purchased from "Yucca Enterprises, Wadala (E), Mumbai" and stored in an air tight container at 15- 20°C. Authentication certificate was given by Yucca enterprises and comparing observations with those reported in literature.

Acquiring of worms

Wild type *C. elegans* during this study were procured from National Institute of Immunology (NII), New Delhi, India. Strain was cultivated and kept under conventional laboratory settings at 20°C on nematode growth medium (NGM) agar plates layered with a lawn of *E. coli* OP50 (Brenner et al., 1974). Synchronized populations were prepared by treating gravid hermaphrodites with sodium hypochlorite (Fabian & Johnson, 1994)

Preparation of Growth media

To formulate 1 liter of NGM agar, combine 3.0 g of sodium chloride (NaCl), 2.5 g of peptone and 17 g of agar in a 2 - liter Erlenmeyer flask. Add 975 ml of distilled water and loosely cover flask with aluminum foil. Sterilize mixture by autoclaving at 121°C for 30 minutes (Stiernagle et al., 2006).

After autoclaving, place flask in a 55°C water bath, allowed to cool for at least 1 hour, ensuring temperature stabilizes within range of 55 - 58°C. Once solution has cooled, supplement it with 25 ml of 1 M potassium phosphate buffer (pH 6.0) prepared from 108.3 g KH_2PO_4 and 35.6 g K_2HPO_4 in water to make 1 liter. Also add 1 ml each of 1 M calcium chloride ($CaCl_2$), 1 M magnesium sulfate ($MgSO_4$) and 5 mg/ml cholesterol solution (dissolved in ethanol). Mix gently after each addition to ensure uniform distribution and to minimize bubble formation.

Using a peristaltic pump, dispense medium into Petri dishes, filling each plate to roughly two- thirds capacity (approximately 10 ml for 60 mm plates). Leave plates open in a laminar flow hood for about 20 minutes to allow agar to solidify and excess moisture to evaporate.

Let plates sit at room temperature for 2 - 3 days to monitor for contamination and allow residual moisture to dissipate. For storage, keep unseeded plates in airtight containers at 4°C and use within four weeks. Seeded plates (those inoculated with *E. coli* OP50, typically 24 hours post- pouring) should be used within 7 - 10 days for optimal results.

Preparation of bacterial food source

Cultivation of uracil- deficient *e. coli* OP50 as a dietary source for *C. elegans* is a standard but critical procedure in nematode research laboratories (Avery et al., 1993). OP50 is strain of choice due to its restricted growth, which minimizes lawn overgrowth on NGM plates and facilitates clear observation of worm phenotypes (Brenner, 1974; Stiernagle, 2006). All steps involving bacterial handling are conducted within a laminar flow hood to minimize risk of contamination and ensure user safety. To begin, a single bacterial colony from a streaked plate is aseptically transferred into a nutrient- rich medium, such as Luria-Bertani (LB) broth or Lysogeny broth [composition: 10 g Bacto- tryptone, 5 g Bacto- yeast extract, 5 g NaCl, water to 1 liter; pH adjusted to 7.0 using 1 M NaOH]. Dispense 100 ml of this medium into 250 ml screw- cap flasks or bottles, then sterilize by autoclaving. se sterilized media containers can be stored at ambient temperature for several months (Byerly et al., 1976). Following inoculation, incubate culture at 37°C with gentle shaking overnight. bacterial culture should reach saturation (optical density at 600 nm between ~1.5 - 2.0), indicated by visible turbidity. For seeding, pipette 100 µL of saturated OP50 culture onto 60 mm NGM agar plates. Allow bacterial lawn to dry and establish at room temperature inside laminar airflow cabinet or incubate at 37°C for several hours to overnight before introducing worms (Stiernagle, 2006; Porta et al., 2012). It is advisable to use seeded plates within a few days to maintain bacterial viability and prevent contamination. Consistent use of sterile technique is vital throughout entire process to ensure well- being and experimental reproducibility of *C. elegans* populations (Sutphin & Kaeberlein, 2009).

Methodology and maintenance of *C. elegans* stocks

Worms cultured and maintained on NGM agar plates of varying diameters (60 mm and 90 mm) each seeded with a lawn of *E. coli* OP50. Plates with a 60 mm diameter were primarily used for experimental procedures, while both 90 mm plates and some 60 mm plates were utilized for routine cultivation and maintenance of worm populations. For transferring individual worms, standard worm- picking method was employed, commonly regarded as most effective technique for isolating single nematodes. worm pick consists of one inch segment of 32 gauge platinum wire affixed to a handle, typically a glass rod or occasionally a bacteriological loop holder. Since platinum have its own advantages as compared to nichrome or any other metal as primary advantages is its high resistance to corrosion and oxidation, allowing it to withstand repeated sterilization with a Bunsen burner without degrading, which is essential for maintaining aseptic conditions during worm handling. Its flexibility and strength make it easy to shape into a pick, while still being firm enough to manipulate worms effectively without harming them. Additionally, platinum's smooth surface minimizes risk of physically damaging worms or agar plates. It also heats and cools quickly, reducing chance of heat injury to animals during transfers. se characteristics make platinum wire a reliable and efficient tool for routine *C. elegans* maintenance and experimentation. To prevent contamination during transfer of worms between plates, platinum wire pick was sterilized using a flame prior to each use. Caution was exercised throughout process to avoid injuring worms or damaging agar surface. An alternative and efficient method for transferring worm cultures is known as **chunking**, wherein a sterile spatula is used to move a small section of agar containing worms from one plate to another. nematodes typically remain attached to agar piece and gradually migrate onto fresh NGM plate seeded with *E. coli* OP50, dispersing across bacterial lawn. Transfers were performed under a dissecting stereomicroscope by visually locating and picking *C. elegans*. Cultures can be maintained at temperatures ranging from 16°C to 25°C, with optimal growth occurring at 20°C (Hope, 1999).

Alkaline hypochlorite treatment (age synchronization)

This approach is commonly employed to generate age- synchronized populations of *C. elegans* for experimental use. Since focus of our study involved L4- stage young adult worms that typically develop 45 to 50 hours post- egg laying (progressing through L1, L2 and L3 larval stages). To isolate eggs, gravid adult worms were subjected to alkaline hypochlorite treatment. This method selectively lyses adult worm bodies and eliminates microbial contaminants, while leaving eggs intact. Initially, M9 buffer was used to rinse gravid worms from culture plates, which were then transferred into 2 ml microcentrifuge tubes. After allowing worms to settle for about 2 minutes, supernatant was carefully removed, leaving approximately 200 µL of M9 buffer containing worm pellet. A bleaching solution consisting of 4% sodium hypochlorite and 5 M NaOH (mixed in a 2:1 ratio) was added to tube and volume was adjusted to 2 ml with M9 buffer. Tube was gently vortexed every 2 minutes over a ten minutes period to facilitate digestion of worm tissue. Eggs were recovered by centrifugation at 1000 × g (approximately 4200 rpm for a centrifuge with a 4.9 cm radius). After spinning, supernatant was discarded and pellet resuspended in fresh M9 buffer. This washing step was repeated three to four times to ensure complete removal of bleach.

Finally, egg suspension was pipetted onto freshly seeded NGM plates containing *E. coli* OP50 and incubated at 20°C. This process destroys all larval and adult stages but preserves eggs, which hatch overnight into a synchronized population of L1 larvae (Fabian & Johnson, 1994).

Toxicity assessment of AA

AA (Yucca Enterprises, Wadala (E), Mumbai) 3.02 mg was dissolve in dimethyl sulfoxide (DMSO, CDH) to prepare 100 µM stock. For evaluation of toxic effect of AA, toxicity assay was performed with 25, 50, 75 and 100 µM. Up to 25 µM, AA was found non- toxic among all of other concentrations. Hence 6.25, 12.5 and 25 µM concentrations were taken as test concentrations and 0.05% DMSO was used as control. Animal plates were freshly prepared before use.

Curve representing toxicity data in which X-axis represents time course from Day 1 to Day 7, while y-axis indicated mortality percentage ranging from 0% to 60%. Six distinct treatment groups were tested, each corresponding to a specific AA concentration: 6.25 µM, 12.5 µM, 25.0 µM, 50.0 µM, 75.0 µM and 100.0 µM. Percentage mortality on day 1, 3, 5 and 7 was calculated.

Different colored lines represent six distinct doses of AA: 100µM (blue), 75µM (red), 50µM (green), 25µM (purple), 12.5 µM (orange) and 6.25 µM (black). It shows that at higher concentrations; mortality rates increased over time. Lower concentrations of 6.25 µM, 12.5 µM and 25.0 µM exhibited no mortality throughout study suggesting that these doses were non- toxic to *C. elegans* and could be considered safe for further use. However, at 50.0 µM, mortality began to emerge by Day 3 and gradually increased, reaching approximately 30% by Day 7. Group with 75.0 µM showed a steady increase in mortality with approximately 40% of population dead by Day 7. Most severe outcome was observed at 100.0 µM concentration, where mortality sharply increased over time and peaked at around 55% by end of exposure period. These findings confirmed that AA induced a concentration dependent toxic effect in *C. elegans*. While lower concentrations (up to 25.0 µM) were well tolerated, higher concentrations (50.0 µM and above) resulted in significant mortality over time. Steep mortality trend at 75.0 µM and 100.0 µM indicated that prolonged exposure to elevated doses of AA might overwhelm organism's physiological defenses, potentially through mechanisms such as oxidative stress, mitochondrial disruption or interference with cellular metabolism. In contrast, three concentration 25, 12.5 and 6.25µM are completely merged along0% response line on x- axis throughout all time period of study.

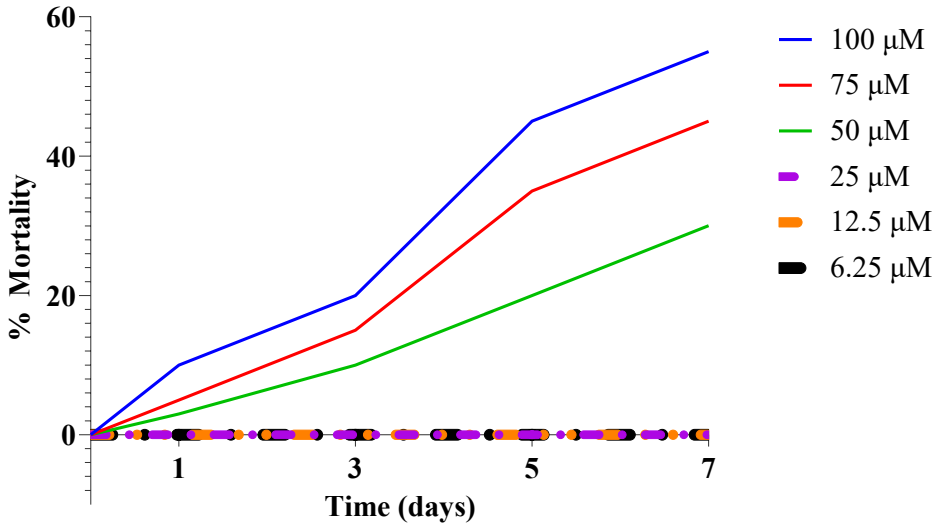

Fig. 1 Percentage mortality of *C. elegans* over a period of seven days following exposure to varying concentrations of AA

Administration of rotenone and treatment drug to *C. elegans*

Fresh stored 60 mm NGM plates were seeded with animal food i.e. *e. coli* op50 in incubation chamber at controlled temperature up to 20 °C and allowed *e. coli* to grow on NGM plate for 24 hours. Next day, by maintaining sterility *C. elegans* loaded chunk were transferred to op50 seeded plates and allowed *C. elegans* to grow and reproduce for three days. After completion of time period *C. elegans* were firmly washed from NGM plates and performed bleaching process to obtain eggs. Transferred eggs to fresh NGM plate and allowed to grow till required stage i.e. young adult stage is achieved. Transferred animals to different NGM plates according to requirement of assay without *e. coli* op50 and proper marking was given to plates. Three to five plates of each group were prepared and marked. To prepare 5 µM rotenone solution, 100 ml of 1 Mm of rotenone was prepared using microbalance. Separately, a 0.05% DMSO solution was prepared by measuring 0.05 mL (50 µL) of DMSO and adding it to approximately 80 mL of distilled water in a clean beaker. Weighed rotenone was transferred into DMSO- water mixture. Since rotenone is only slightly soluble in water but soluble in DMSO, mixture was stirred thoroughly and gentle warming was applied to aid dissolution. Once a uniform solution was obtained, mixture was transferred to a 100 mL volumetric flask. Beaker was rinsed with small volumes of DMSO solution and all rinsings were added to flask. Finally, volume was brought up to 100 mL with 0.05% DMSO solution. Solution was mixed thoroughly and labeled as 5 µM Rotenone. Then, 1 mM AA stock solution, approximately 30.25 mg of AA was accurately weighed and transferred into a clean 250 mL beaker. In a separate container, 0.05% DMSO was prepared by adding 50 µL of DMSO to about 80 mL of distilled water. This DMSO solution was then added to beaker containing AA. Mixture was stirred thoroughly and gently heated to assist in dissolving compound. Once a uniform solution was achieved, it was allowed to cool to room temperature. Solution was transferred quantitatively into a 100 mL volumetric flask. Volume was then brought up to 100 mL with same 0.05% DMSO solution and final stock was mixed thoroughly and labeled as a 1 mM AA stock. Using this stock, three working solutions were prepared. For 6.25 µM solution, 62.5 µL of stock was pipetted into a clean tube and diluted to 10 mL with 0.05% DMSO. For 12.5 µM solution, 125 µL of stock was similarly diluted to 10 ml. Lastly, 250 µL of stock was used to prepare 10 ml of 25 µM solution strength in same manner. Each solution was mixed well to ensure homogeneity and labeled accordingly. All solutions were protected from light and stored under appropriate conditions for further

use. After that, 100 ul of OP50 to each plate, add 50 ul of AA and 50 ul of rotenone to respective groups and 100uL DMSO to controlled group was transferred (Lee et al., 2019).

Experimental Design

L4-stage worms were divided into:

- Control
- Rotenone group
- Rotenone + Abietic Acid (various concentrations)

Rotenone exposure induced PD-like phenotypes, followed by abietic acid treatment.

Homogenate preparation

For preparation of homogenate, collected approximately 300 *C. elegans* worms into a tube containing PBS. To estimate density of worms, took 20 µL of suspension and placed it under a microscope. Counted number of worms in this 20 µL solution and found that average number of worms higher than target range (9 - 11 worms per 20 µL) so diluted sample by adding more PBS, count is lower than target, allow worms to settle for 10 minutes, then carefully remove some of PBS from top to concentrate sample. Once density adjusted, transferred 260 µL of worm suspension into a new 1.5 mL microcentrifuge tube. Mixed gently by pipetting up and down to resuspend worms evenly. Attached a clean pestle to a motor- driven tissue grinder. Rinsed pestle with ice- cold PBS and wipe it with absorbent paper. Placed tube containing worm pellet into an ice bucket to keep it cold during process. Pressed pestle against pellet firmly and grinded worms for 10 seconds, paused for 2 seconds and grinded again for another 10 seconds. Used 200 µL of ice- cold PBS to rinse any remaining material off pestle and back into same tube after that centrifuge tube at 5000 × g for 5 minutes at 4 °C. After centrifugation, carefully collected 200 µL of clear supernatant and divided it equally into four tubes, each containing 50 µL, for fur r used for biochemical analyses (Zhang et al., 2017).

Biochemical parameters

Superoxide dismutase (SOD) level measurement

SOD is protective enzyme that protects cells from oxidative damage by catalyzing dismutation of superoxide radicals (O_2^-) into oxygen (O_2) and hydrogen peroxide (H_2O_2). Measuring SOD activity can be a useful way to assess antioxidant capacity of a biological system, such as *C. elegans* exposed to neurotoxins. Here's a general method to measure SOD activity using a simple spectrophotometric approach (Kono et al., 1978).

Key reactions involved:

Step- I $\quad$ Xanthine + O_2 $\xrightarrow{\text{Xanthine oxidase}}$ Uric acid + Superoxide radical ($O_2\bullet^-$)

Step- II $\quad$ NBT + Superoxide radical ($O_2\bullet^-$) $\longrightarrow$ Formazan (measured at 660 nm)

Step - III $\quad$ Superoxide radical ($O_2\bullet^-$) + 2 H^+ $\xrightarrow{\text{SOD}}$ $O_2 + H_2O_2$

Procedure

In this experiment, 0.15 ml of hydroxylamine hydrochloride was added to reaction mixture that included 0.1 ml of supernatant, 1.9 ml distilled water, 75 µL of NBT and 0.15 mL of Triton- X 100. Absorbance was then measured at 570nm using UV- visible spectrophotometer to determine extent of NBT reduction inhibition brought on by superoxide dismutase present in supernatant. This measurement was contrasted

with blank to provide a baseline. This measurement was contrasted with a blank sample to provide a baseline. Lastly, units/mg of protein for SOD concentrations were calculated (Kono et al., 1978).

Glutathione (GSH) level measurement

Procedure

DTNB (5,5'- Dithiobis- (2- nitrobenzoic acid)), is commonly utilized to quantify GSH levels in biological samples. To precipitate protein, an equal volume of 10% trichloroacetic acid was added to supernatants and mixture was centrifuged at 2000 rpm for 10 minutes at 4°C. Then, 0.1 mL of cleared supernatant, 0.5 mL of DTNB reagent, 2 mL of phosphate buffer (pH 8.4) and 0.4 mL of distilled water were added to create reaction mixture. This was allowed to react with DTNB and produce a yellow product 5′- thio- 2- nitrobenzoic acid (TNB) by incubating it for 15 minutes. Absorbance was determined with spectrophotometer at 412 nm (Caito et al., 2015; Rahman et al., 2006). Calculations were based on standard curve of known GSH concentrations. Final data was normalized using the protein concentrations of each group.

Key reaction involved:

GSH + DTNB $\longrightarrow$ GS-TNB (mixed disulfide) + 2-nitro-5-thiobenzoate anion (TNB^{2-})

This TNB^{2-} is yellow colored compound measured at 412 nm spectrophotometrically.

Acetylcholinesterase (AChE) estimation

Procedure

Enzyme activity of AChE was measured using Ellman method. For this 0.4 mL of supernatant, 2.6 mL of sodium phosphate buffer (0.01 M; pH 8), 0.1 mL of DTNB and 0.02 mL of acetylthiocholine iodide (ATCI) solution were all added to reaction mixture. For 15 minutes, this combination went through incubation at room temperature. Thiocholine is created when AChE breaks down ATCI. Thiocholine combines with DTNB and generate yellow 5- thio- 2- nitrobenzoate ion. Absorbance at 412 nm helps to detect reaction (Prokić et al., 2017; Ellman et al., 1961).

Statistical analysis

All computations were performed using GraphPad Prism v 8.4.2 analytic program. To analyze data. Following significance levels were indicated: *P<0.05, **P<0.01 and ***P<0.001. Tukey's Honestly Significant Difference (HSD) post-hoc test was applied to perform pairwise comparisons between all groups. Tukey's test was chosen because it controls for familywise error rate and is well-suited for evaluating all possible group comparisons in a balanced design, ensuring that observed differences, particularly those between rotenone group and the other experimental conditions are statistically robust. Data presented as mean ± standard error of the mean (SEM), which reflects precision of sample mean and allows for easier comparison between groups. SEM is commonly used in experimental biology and pharmacology to illustrate how accurately sample mean estimates true population mean, especially when focus is on comparing central tendencies rather than spread of individual data points, which would be better represented by standard deviation (SD). Verifying equal variances is a critical prerequisite for applying parametric tests like one-way ANOVA, which was subsequently used to identify statistically significant differences among groups. Together, these tools provided a systematic and statistically sound approach to evaluating the effects of experimental treatments on *C. elegans*. (Brown et al., 1974).

Results

Effect of AA on basal slowing response

To test BSR, 20 animals were washed in M9 buffer and transfer to NGM plates with or without OP50 bacterial. frequency of body bends, known as basal slowing were recorded for 20 seconds for each worm and analyzed body bands in presence and absence of food.

Basal slowing assay was conducted in *C. elegans* to assess how presence and absence of food affect locomotion behavior under different treatment conditions. In this assay, five groups were prepared: Control, Vehicle, AA 6.25 µM, AA 12.5 µM and AA 25 µM). Each group consisted of 20 worms and locomotion was quantified by counting number of body bends per 20 seconds for each worm in both absence and presence of food in three plates simultaneously and body bend is defined as movement of worm's head (or neck) through midline in either direction during forward or backward movement. From these values, percentage basal slowing was calculated using formula below to reflect neuronal capacity to modulate their movement in response to food, a behavior reliant on intact sensory and dopaminergic function.

$$\text{Basal slowing (\%)} = \frac{\text{Body bends (food absence)} - \text{Body bends (food presence)}}{\text{Body bends (food absence)}} \times 100$$

Plate 1

Basal slowing response of C. elegans was evaluated to assess dopaminergic function. As shown in **Fig. 2.,** there is significantly reduced (***P< 0.001) response of vehicle group compared to control. AA treatment with 6.25, 12.5, and 25 µM improved the response in a dose-dependent manner. Significance level showed a modest increase at 6.25 µM, 12.5 µM (**P < 0.01) and 25 µM (***P <0.001).

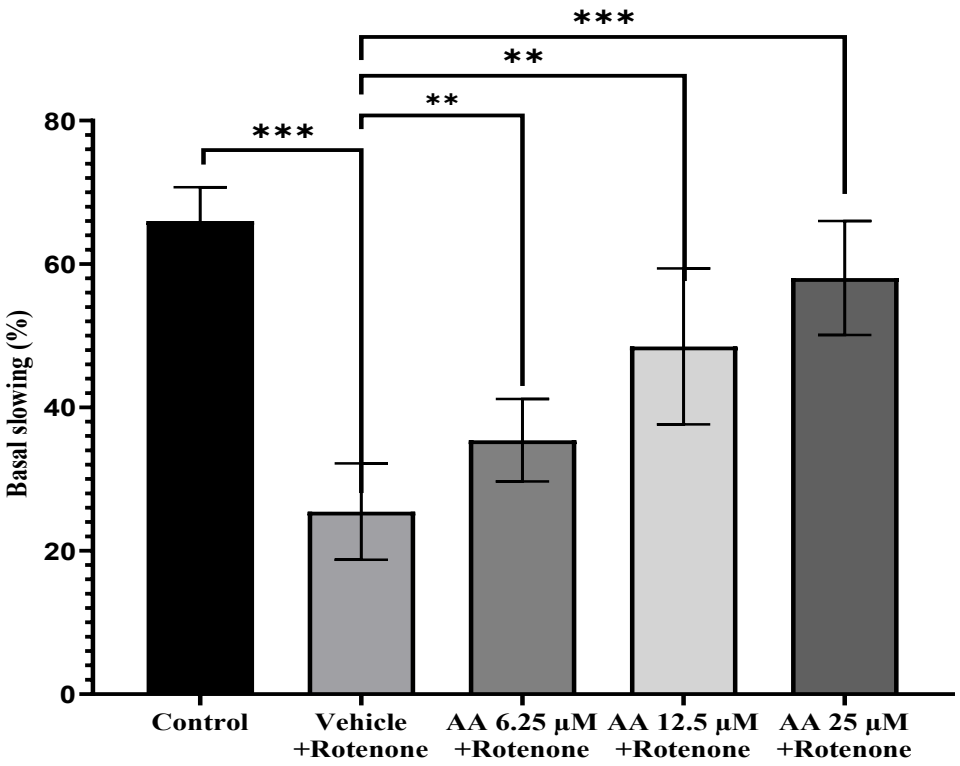

Fig. 2 Effect of AA on basal slowing response in plate 1

Values for body bends analyzed for 20 seconds for each worm with and without food and shown as mean ± SEM for n = 20. One way ANOVA with Tukey's analysis F (4, 95) = 95.79 were used to evaluate data. Followings were used to indicate significance levels: **P<0.01 and ***P<0.001.

Plate 2

Basal slowing response of C. elegans was evaluated to assess dopaminergic function. As shown in **Fig . 3,** there is significantly reduced (***p< 0.001) response of vehicle group compared to control. AA treatment with 6.25, 12.5, and 25 µM improved the response in a dose-dependent manner. Significance level showed a modest increase at 6.25 µM, 12.5 µM (**P < 0.01) and 25 µM (***P <0.001).

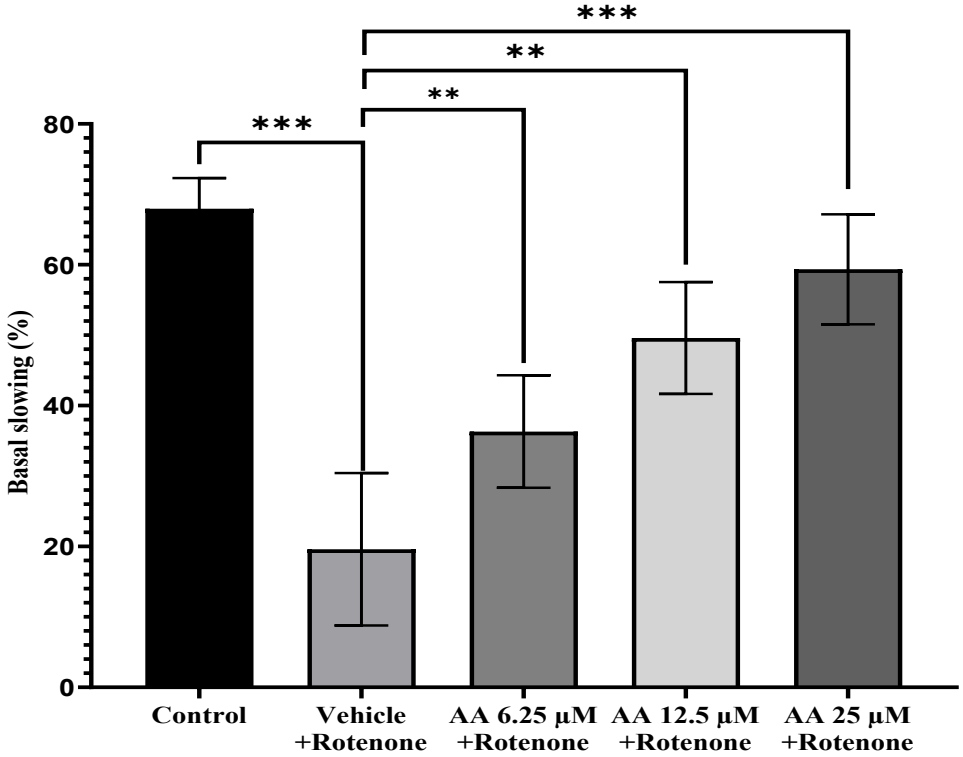

Fig. 3 Effect of AA on basal slowing response in plate 2

Values for body bends analyzed for 20 seconds for each worm with and without food and shown as mean ± SEM for n = 20. One way ANOVA with Tukey's analysis F (4, 95) = 113 were used to evaluate data. Followings were used to indicate significance levels: **P<0.01 and ***P<0.001.

Plate 3

Basal slowing response of C. elegans was evaluated to assess dopaminergic function. As shown in Fig. 4, there is significantly reduced (***P < 0.001) response of vehicle group compared to control. AA treatment with 6.25, 12.5, and 25 µM improved the response in a dose-dependent manner. Significance level showed a modest increase at 6.25 µM, 12.5 µM (**P < 0.01) and 25 µM (***P <0.001). As shown in Fig. 2 Fig. 3 and Fig. 4 control group in each carrying highest basal slowing response in plate 1, plate 2 and plate 3 respectively, indicating normal behavioral responsiveness to food. In contrast, vehicle treated group exhibited a significantly reduced basal slowing data, suggesting a disruption in neural signaling pathways responsible for food induced slowing. Consistent and statistically significant improvement across replicates suggests that AA has restorative effect on food dependent locomotion in *C. elegans* via modulation of neural pathways involved in sensory integration and motor output.

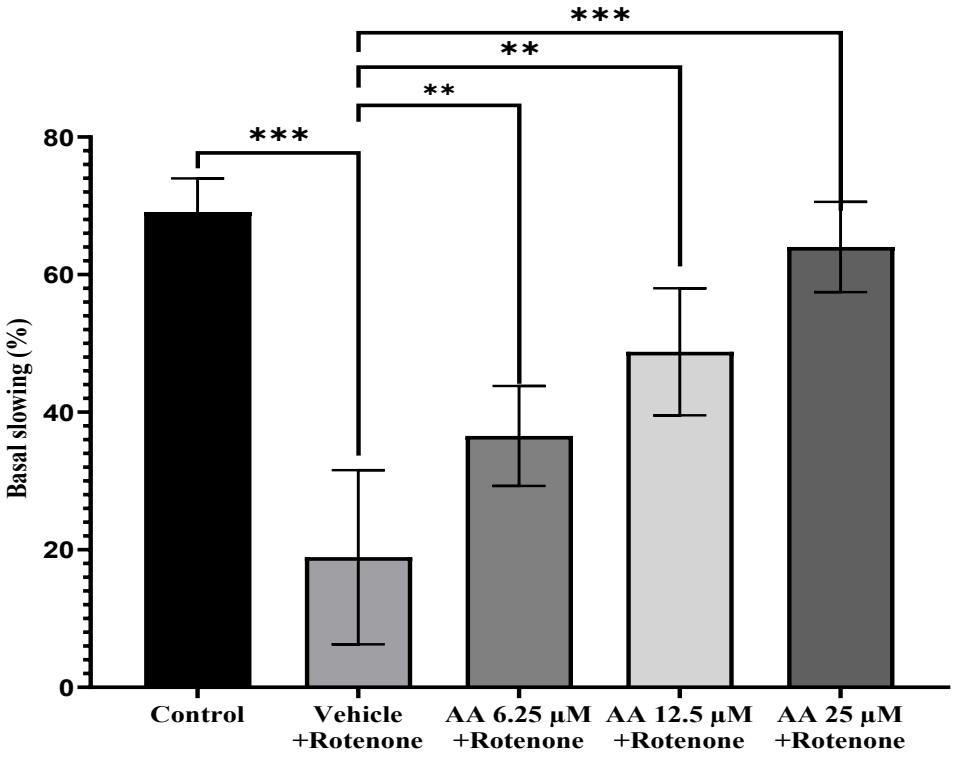

Fig. 4 Effect of AA on basal slowing response plate 3

Values for body bends analyzed for 20 seconds for each worm with and without food and shown as mean ± SEM for n = 20. One way ANOVA with Tukey's analysis F (4, 95) = 114.5 were used to evaluate data. Followings were used to indicate significance levels: **P <0.01 and ***P <0.001.

5.3 Effect of Abietic acid on chemotactic behaviour

Procedure

To assess ethanol preference as an indicator of dopaminergic function, animals were first exposed to ethanol and then transferred to assay plates divided into two equal sections (Fatima et al., 2014). Ethanol was applied to one of quadrants *and* worms were allowed to freely explore plate for 30 minutes, during which their location preference was monitored. For experimental setup, both AA- treated and untreated day- 3 adult *C. elegans* were washed with M9 buffer and placed at center of agar plates. Each plate had 10 µL of an attractant (*e. coli* op50) on one side and 10 µL repellent (either 1 M sodium acetate or ethanol). To immobilize worms upon reaching either side, 10 µL of 1 M sodium azide was added at both sides of plate. *C. elegans* with Parkinsonian- like dopamine deficits are attracted to ethanol because their defective dopamine system disrupts normal avoidance behavior, providing a quantifiable behavioral marker of dopaminergic dysfunction in PD models (Bargmann et al., 2006; Jee et al., 2022).

$$\textbf{Chemotaxis index (CI)} = \frac{N_A - N_C}{N_T}$$

Where:

N_A = number of worms at attractant spot

N_C = number of worms at control spot

N_T = total number of scored worms ($N_A + N_C + N_O$), where N_O are worms that went neither to attractant nor control)

Chemotaxis index was measured to evaluate sensory neuronal function under different treatment conditions. Chemotaxis index measured for three replicates i.e. plate 1, plate 2 and plate 3 respectively across five experimental gups, highlighting degree of sensory or behavioral response in each condition. Chemotaxis index is a quantitative measure of an organism's ability to detect and move toward a chemical stimulus, often used to assess neurological or sensory function, particularly relevant in *C. elegans* models.

$$\text{Chemotaxis index} = \frac{\text{No. of worms (Attractant)} - \text{No. of worms (Repellent)}}{\text{No. of worms (Attractant)} + \text{No. of worms (Repellent)}}$$

Plate 1

As shown in Fig. 5 chemotaxis index remained high in control group each plate as compared to vehicle treated with chemotaxis index values 0.7. Significant reduction ($P < 0.001$) in chemotaxis index observed between control and vehicle approximately value up to 1.08, indicates impaired chemotaxis suggesting neuronal damage, particularly to olfactory circuits. Lower concentration of AA group showed slight improvement with chemotaxis as compared to vehicle treated and further improvements at higher doses, indicates improvements in chemotactic behaviour in animals.

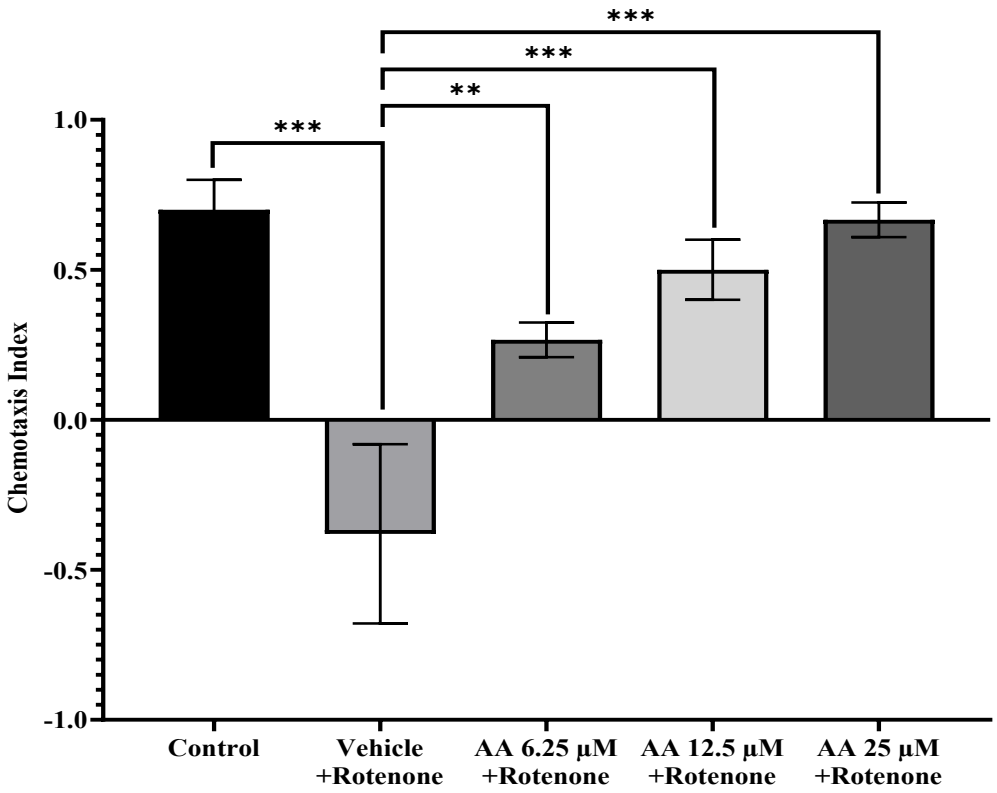

Fig. 5 Effect of different concentrations of AA on chemotaxis index in plate 1

Values for body bends analyzed for 20 seconds for each worm with and without food and shown as mean ± SEM for n = 20 (number of replicates). One way ANOVA with Tukey's analysis F (4, 95) = 95.79 were used to evaluate data. Followings were used to indicate significance levels: **P<0.01 and ***P<0.001.

Plate 2

As shown in Fig. 6 chemotaxis index remained high in control group each plate as compared to vehicle

treated animals. Significant reduction ($P < 0.001$) in chemotaxis index observed between control and vehicle indicates impaired chemotaxis suggesting neuronal damage, particularly to olfactory circuits. Lower concentration of AA group showed slight improvement with chemotaxis as compared to vehicle treated with P- value 0.01 and further improvements at higher doses with P- value 0.001, indicates improvements in chemotactic behaviour in animals.

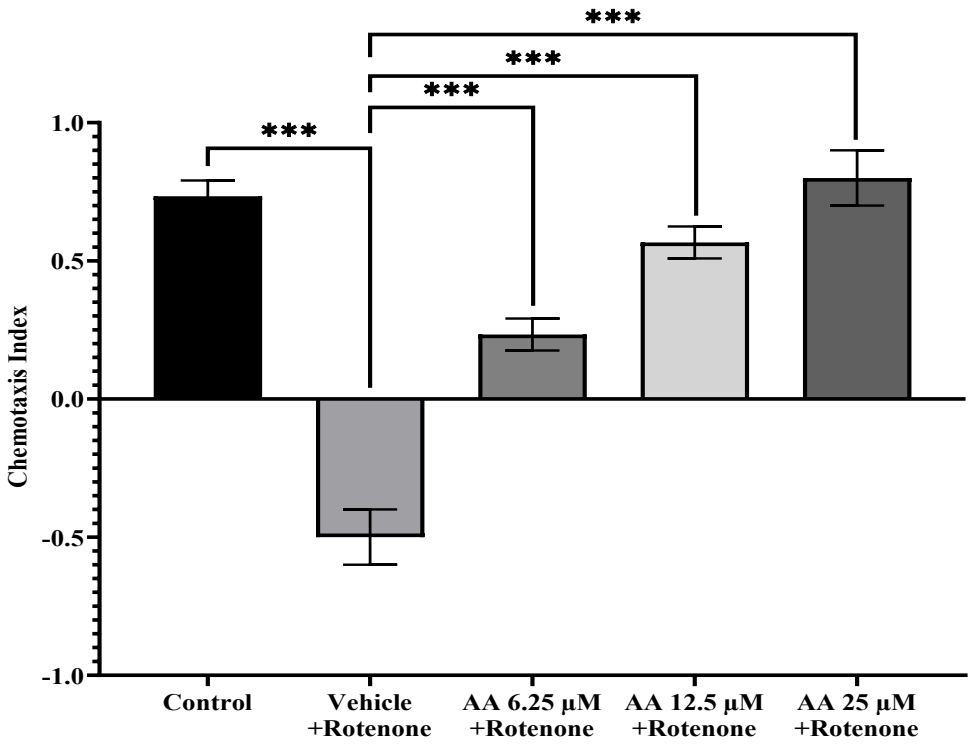

Fig. 6 Effect of different concentrations of AA on chemotaxis index in plate 2

Values for body bends analyzed for 20 seconds for each worm with and without food and shown as mean ± SEM for n = 20 (number of replicates). One way ANOVA with Tukey's analysis F (4, 95) = 141.4 were used to evaluate data. Followings were used to indicate significance levels: **P<0.01 and ***P<0.001.

Plate 3

As shown in **Fig. 7** chemotaxis index remained high in control group each plate as compared to vehicle treated animals. Significant reduction ($P < 0.001$) in chemotaxis index observed between control and vehicle indicates impaired chemotaxis suggesting neuronal damage, particularly to olfactory circuits. Lower and higher concentration of AA group showed nearly similar improvement in chemotactic behaviour as compared to vehicle treated with P-value 0.001, indicates improvements in chemotactic behaviour in animals.

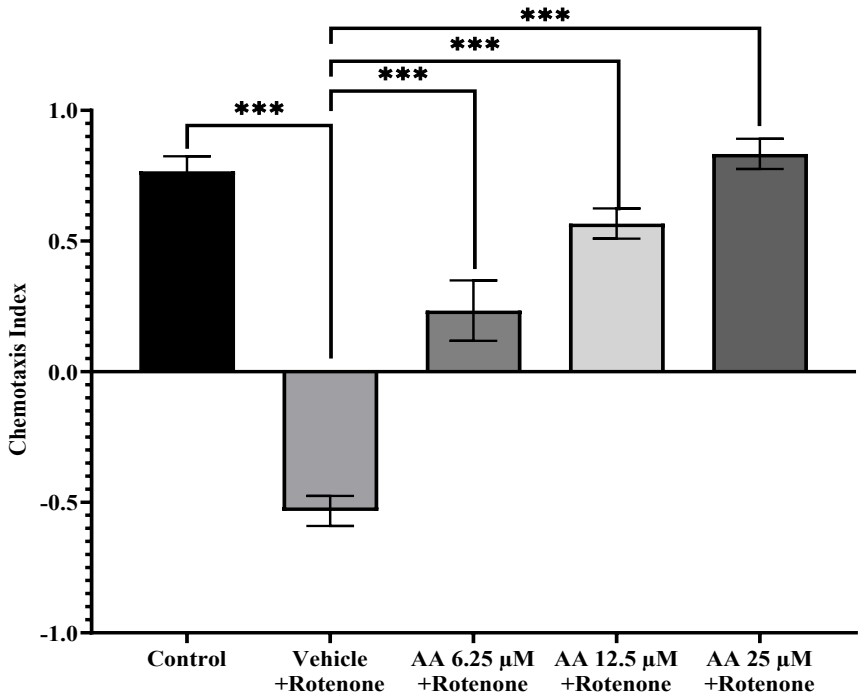

Fig. 7 Effect of different concentrations of AA on chemotaxis index in plate 3

Values were shown as mean ± SEM for n = 20. One-way ANOVA and Tukey's test with F (4, 95) = 175.1 were used to evaluate data. Followings were used to indicate significance levels: ***P<0.001.
As shown in Fig. 5, Fig. 6 and Fig. 7. control group in each carrying highest chemotaxis index in plate 1, plate 2 and plate 3 respectively, indicating normal behavioral responsiveness to food. In contrast, vehicle treated group exhibited a significantly reduced chemotactic behaviour, suggesting a disruption in neural signaling pathways. Consistent and statistically significant improvement across replicates suggests that AA has restorative effect on preference in *C. elegans* via modulation of neural pathways involved in sensory integration and motor output.

5.4. Effect of AA on total protein content

Standard curve of bovine serum albumin was used to find out total protein content in biological sample. Protein content was calculated across five experimental, expressed in **Fig. 8.** Results showing control group exhibited highest TPC, indicating baseline protein expression under normal or untreated conditions. Vehicle treated group showed lowest TPC to around, which was statistically significant compared to control, marked with *** ($P < 0.001$) Treatment with AA at 6.25 µM resulted in slightly increase in protein content to 159.9 µg, indicating a stronger therapeutic or protective effect as compared to vehicle treated, statistically marked with *($P < 0.05$). Increase in TPC was observed in group treated with AA at 12.5 µM, which reached about 216.5 µg, showing significant difference from control group, denoted by ** ($P < 0.01$). Treatment with AA at 25 µM showed substantial increase in TPC around 325.47 µg near to control group, suggesting a strong protective effect of AA against rotenone induced oxidative damage to protein content.

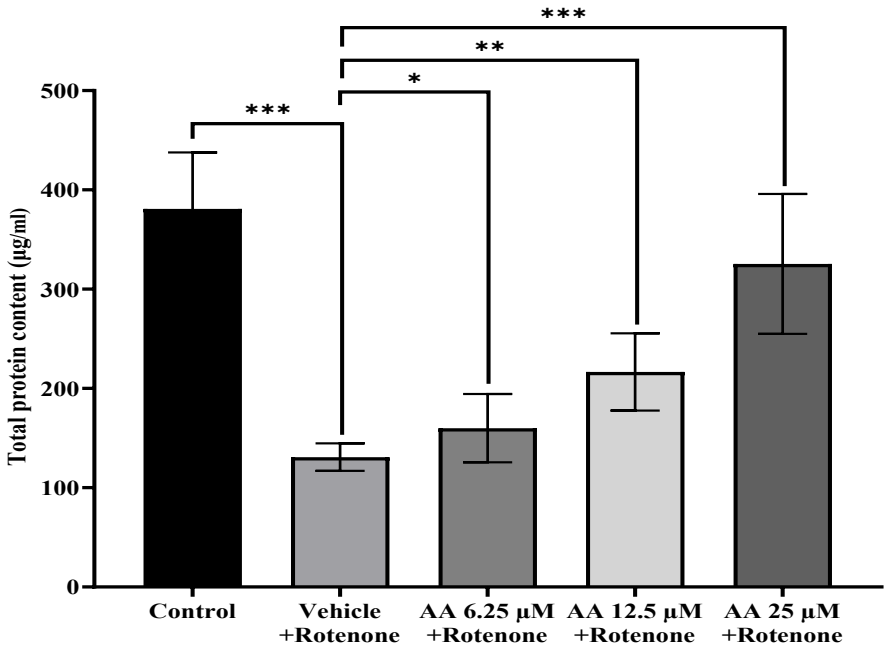

Fig. 8 Effect of different concentrations of AA on total protein concentration

Values were shown as mean ± SEM for n = 3. One-way ANOVA and Tukey's test with F (4, 10) = 15.53 were used to evaluate data. Followings were used to indicate significance levels: *P <0.05, **P <0.01 and ***P <0.001.

5.5. Superoxide Dismutase (SOD) Activity

SOD activity assay conducted in *C. elegans* to assess oxidative stress response under different treatment conditions. This assay involved five experimental groups: control group, vehicle treated and AA with concentrations 6.25 µM, 12.5 µM and 25 µM as shown in fig. 5.5. Enzymatic activity of SOD was measured in units per µg of protein, serving as a biomarker for organism's antioxidant defense capacity. In control group, SOD activity was at its highest, approximately 3.237 units/µg protein, reflecting normal physiological antioxidant status of worms. In contrast, vehicle group exhibited a dramatic and statistically significant reduction in SOD activity (about 2.47 units/µg protein), confirming detrimental effect of rotenone on oxidative balance ($P < 0.001$) as shown in fig. 5.6. This highlights rotenone's effectiveness in impairing antioxidant defense mechanisms, thus creating a model for oxidative stress. Upon treatment with compound AA, a dose- dependent restoration of SOD activity was observed. At concentration 6.25 µM, SOD activity increased significantly to 1.173 units/µg compared to vehicle treated group ($P < 0.05$), suggesting partial recovery. Concentration 12.5 µM led to a further significant enhancement in SOD levels to approximately 1.635 ($P < 0.01$) and 25 µM concentration brought SOD activity close to control levels approximately 2.87 units/µg, indicating near complete restoration of antioxidant function ($P < 0.001$) as compared to vehicle group. Recovery observed at 25 µM was substantial, suggesting a strong protective effect of AA against rotenone induced oxidative damage. Statistical analysis using Tukey's test and One-way ANOVA yielded a highly significant result $F (4, 10) = 118.7$, confirming that differences among groups were not due to random variation but were indeed statistically meaningful. SOD is an important antioxidant enzyme that helps protect cells from oxidative stress by catalyzing the dismutation of superoxide radicals into oxygen and hydrogen peroxide. Increase in SOD activity with higher doses of

treatment drugs underscores importance of dosage in the pharmacological management of oxidative stress-related conditions.

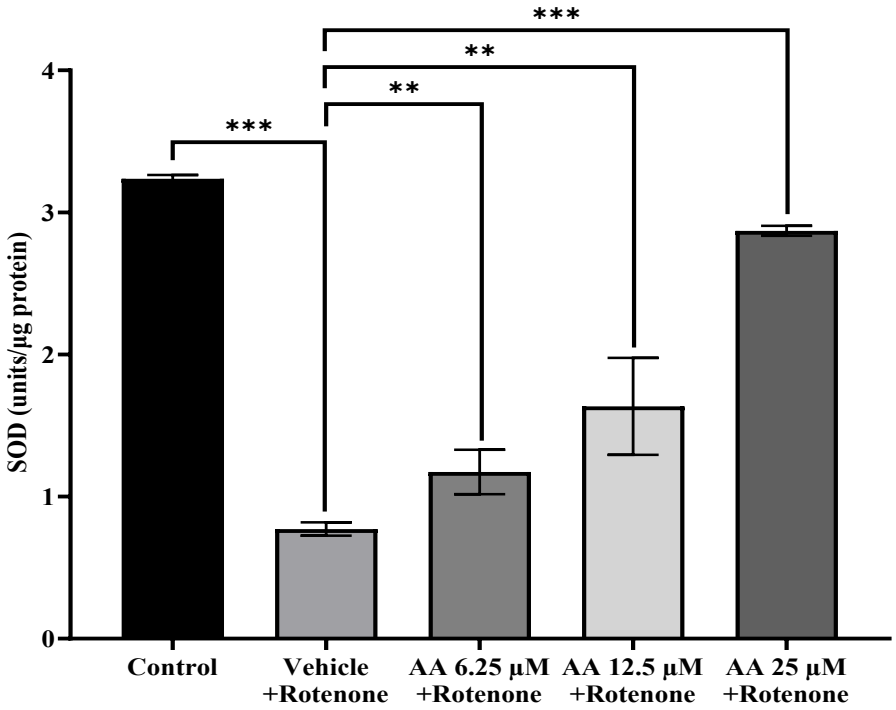

Fig. 9 Effect of different concentrations of AA on SOD level

Values were shown as mean ± SEM for n = 3. One-way ANOVA and Tukey's analysis with F (4, 10) = 118.7 were used to evaluate data. Followings were used to indicate significance levels: *P <0.05, **P <0.01 and ***P <0.001.

Effect of AA on reduced glutathione (GSH) level

GSH assay was performed to evaluate oxidative stress and antioxidant response under various treatment conditions. Study included five experimental groups: control group, vehicle group and AA with concentrations 6.25 µM, 12.5 µM and 25 µM as shown in fig. 5.6. In control group, GSH content was highest, approximately 13.23 nmol/µg protein, reflecting a normal antioxidant status in untreated worms. Rotenone treatment (Vehicle group) caused a dramatic and highly significant depletion in GSH levels (reduced to ~9.957 nmol/µg protein), confirming that rotenone induces severe oxidative stress by impairing glutathione- based antioxidant defense ($P < 0.001$) as shown in fig. 5.8. Treatment with AA resulted in a dose- dependent restoration of GSH levels. At 6.25 µM, GSH levels were significantly elevated to 7.473 nmol/µg compared to vehicle group ($P < 0.05$), suggesting partial mitigation of oxidative damage. AA 12.5 µM dose showed a greater restoration ($P < 0.01$) with GSH level 9.039 nmol/µg protein and 25 µM dose resulted in approximately 12.69 nmol/µg protein of GSH levels near complete recovery to control group ($P < 0.001$). Statistical evaluation using One-Way ANOVA with Tukey's test confirmed robustness of these findings, with a highly significant $F(4,10) = 482.6$, indicating that observed differences among groups were statistically significant.

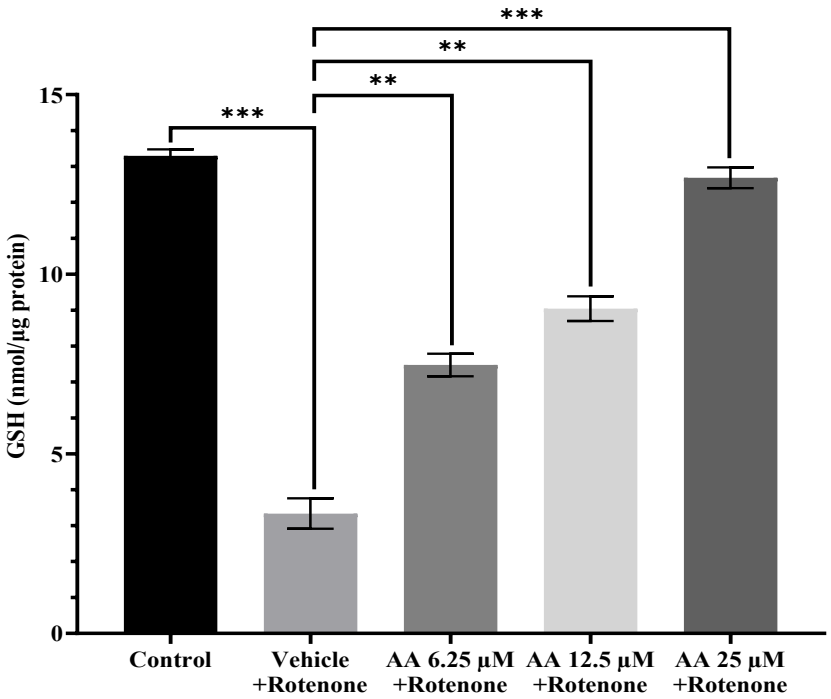

Fig. 10 Effect of different concentrations of AA on GSH level

Values were shown as mean ± SEM for n = 3. One way ANOVA and Tukey's analysis with F (4, 10) = 482.6 were used to evaluate data. Followings were used to indicate significance levels: *p<0.05, **p<0.01 and ***p<0.001.

Effect of AA on acetylcholinesterase (AChE)

AChE activity estimation was performed across five experimental groups: Control, Vehicle and three treatment groups with AA 6.25 µM, 12.5 µM and 25 µM shown in fig. 5.7. AChE activity was expressed in nmol/µg protein, indicating enzyme's catalytic function in hydrolyzing acetylcholine. In control group, AChE activity was highest (~49.37 nmol/µg protein), representing normal cholinergic function. Exposure to vehicle group resulted in a marked reduction in AChE activity to ~30.09 nmol/µg protein, a highly significant drop ($P < 0.001$) that reflects neurotoxic effects of rotenone, known to disrupt synaptic transmission and cholinergic signaling. Treatment with compound AA led to a dose- dependent restoration of AChE activity. At 6.25 µM of AA, AChE activity significantly increased ($P < 0.01$) with value 31.59 nmol/µg, indicating partial neuroprotection. AA at 12.5 µM concentration further elevated AChE activity to 41.23 nmol/µg, showing a significant difference ($P < 0.01$). AA at 25 µM concentration nearly restored AChE activity to control levels (~46.5 nmol/µg protein) with $P < 0.001$ significance, suggesting strong neuroprotective efficacy of AA at this concentration. Treatment with compound AA significantly and dose-dependently restores AChE function. Robust statistical validation by One- Way ANOVA and Tukey's test provided F (4,10) = 257.7, supports conclusion that AA offers potent neuroprotective effects. Estimation of acetylcholinesterase activity is a valuable tool in field of neurotoxicology. AChE is a crucial enzyme in nervous system that breaks down acetylcholine (ACh), a neurotransmitter involved in transmitting signals between neurons. Estimation of AChE levels and their changes can provide insight into cholinergic dysfunction associated with PD. Increased AChE activity typically correlates with cognitive decline, while a decrease indicates altered cholinergic activity.

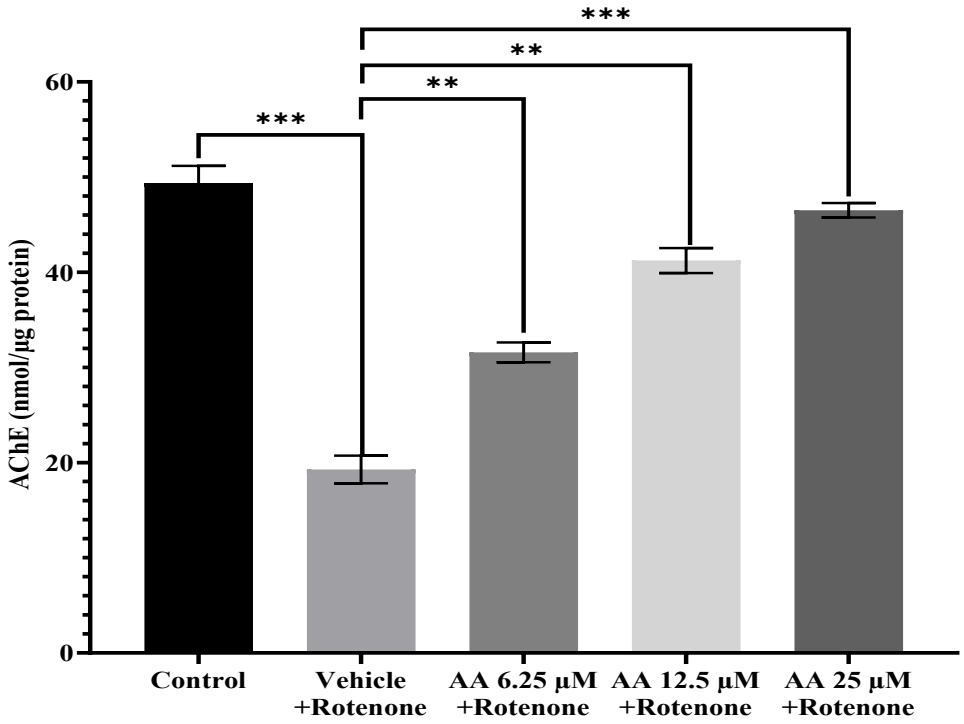

Fig. 11 Effect of different concentrations of AA on AChE level

In bar graph, values were shown as mean ± SEM for n = 3. One-way ANOVA and Tukey's analysis with F (4, 10) = 257.7 were used to evaluate data. Followings were used to indicate significance levels: *P <0.05, **P <0.01 and ***P <0.001.

Discussion

PD is a complex, age- associated neurodegenerative disorder that primarily results from progressive loss of dopaminergic neurons within substantia nigra pars compacta. Despite being second most prevalent neurodegenerative disorder globally, underlying causes of PD remain exclusive and its multifactorial pathogenesis poses challenges for development of effective disease- modifying treatments. Current therapeutic options, such as levodopa and dopamine agonists, primarily offer symptomatic relief but do not halt or reverse disease progression. Thus, identifying novel neuroprotective compounds that can intervene in key pathological processes including oxidative stress, mitochondrial dysfunction, neuroinflammation and neurotransmitter dysregulation is a critical priority in PD research. In this context, present study aimed to evaluate the therapeutic efficacy of AA, a naturally occurring diterpenoid resin acid against rotenone induced Parkinsonian pathology using *C. elegans* model.

C. elegans were chosen for its unique advantages in neurodegenerative disease modeling. Nematode possesses a simple and well- characterized nervous system composed of exactly 302 neurons in hermaphrodites, including eight dopaminergic neurons, many of which are homologous to mammalian systems. Transparency of worm allows for real time visualization of neuronal degeneration and behavior and its short lifespan, genetic manipulability and cost- effectiveness make it ideal for high- throughput screening. Furthermore, dopaminergic system in *C. elegans* is functionally conserved, allowing use of behavioral assays such as basal slowing response (BSR), chemotaxis and body bending frequency to evaluate dopaminergic integrity that were also found in (Zhang et al., 2018). In this study, exposure to rotenone an environmental neurotoxicant known to inhibit mitochondrial complex- I and induce oxidative

stress successfully replicated key features of Parkinsonism, including impaired motor behavior, altered neurotransmitter activity and oxidative imbalance that mimic those found in PD (Greenamyre et. al., 2010: Thirugnanam et. al., 2022; da Silva et. al., 2024). Administration of rotenone *C. elegans* displayed marked deficits in body bending movements and chemotaxis behaviour. Basal slowing response, a behavior regulated by dopaminergic signaling, was significantly reduced in rotenone treated group, indicating functional impairment of dopamine neurons. Similarly, chemotaxis is ability of worms to move toward a chemical attractant was compromised, reflecting both sensory neuron and dopaminergic dysfunction which also found in (Thirugnanam et. al., 2022: Gonzalez et. al., 2021). These behavioral impairments are consistent with previous reports utilizing rotenone in PD models and further validate effectiveness of *C. elegans* based experimental design. Moreover, biochemical assays showed significant reductions in antioxidant enzymes such as SOD and GSH alongside AChE activity. These findings collectively support induction of a PD like state in nematodes by rotenone, driven by mitochondrial damage, oxidative stress and neurotransmitter dysregulation. Treatment with AA significantly ameliorated these behavioral and biochemical impairments in a dose dependent manner. At concentrations of 6.25, 12.5 and 25 µM, AA restored BSR to levels approaching that of control group. This improvement in locomotor regulation indicates that AA exerts a protective effect on dopaminergic neurons. Restoration of chemotaxis behavior further suggests that AA not only preserves motor function but also protects sensory circuits and neuronal communication pathways. These behavioral outcomes are indicative of AA's capacity to counteract rotenone induced dopaminergic degeneration. In terms of biochemical modulation, AA demonstrated strong antioxidant properties. Rotenone exposure resulted in a marked depletion of SOD and GSH- two pivotal antioxidants that safeguard cellular structures against oxidative damage. AA treatment significantly reversed these effects, restoring antioxidant enzyme levels in a concentration- dependent manner. This finding is in alignment with existing literature that has reported antioxidant potential of AA in other disease models, including renal and hepatic oxidative injury. Upregulation of endogenous antioxidant defenses suggests that AA mitigates mitochondrial- derived ROS accumulation and preserves redox homeostasis. Given that oxidative stress is a central event in PD pathogenesis, this action represents a key mechanism of neuroprotection (B Mythri et al., 2012).

Present study also highlighted impact of AA on cholinergic signaling through assessment of acetylcholinesterase activity. AChE, which regulates acetylcholine levels in synaptic cleft, was significantly altered in rotenone exposed group, suggesting cholinergic dysfunction. AA treatment normalized AChE activity, indicating preservation of cholinergic transmission. This effect is particularly important given involvement of both dopaminergic and cholinergic systems in motor and cognitive symptoms of PD. By concurrently modulating both neurotransmitter pathways, AA presents a dual- action therapeutic profile. Since AA was found to be non- toxic up to 25 µM, as confirmed through toxicity assay. This is an important consideration, as many synthetic agents carry a risk of long- term toxicity or induce compensatory pathologies such as dyskinesia when administered chronically. Multifaceted mechanisms of action observed in AA treated groups align it with other plant derived neuroprotective agents such as rosmarinic acid, berberine, astragaloside IV and glycyrrhizic acid. These compounds are known for their antioxidant, anti- inflammatory and anti- apoptotic properties, have shown similar protective effects in PD models. Specifically, AA's ability to inhibit key pro- inflammatory mediators like NF- κB, COX- 2 and iNOS while simultaneously upregulating SOD and GSH, supports a multimodal action that targets both primary and secondary degenerative mechanisms (Nataraj et al., 2017). Studies have shown that AA stabilizes mitochondrial membrane potential, inhibits mitochondrial permeability transition and prevents

apoptosis in non- neuronal models. These attributes underscore therapeutic potential of AA in diseases where mitochondrial dysfunction plays a critical role. From a broader perspective, current findings support inclusion of AA in growing list of natural compounds with neuroprotective potential. *C. elegans* model served as an effective and efficient system for screening AA's therapeutic benefits and deciphering its mechanistic pathways. Neuroprotective effects observed in this model warrant further exploration in more complex organisms, such as 6- OHDA- induced rodent models or MPTP- treated primates. Moreover, studies on AA's effect on α- synuclein aggregation, synaptic integrity and neuroinflammation in mammalian systems would provide deeper insights into its applicability for human PD. In future studies, integrating molecular biology techniques such as transcriptomic and proteomic profiling will be essential to identify downstream effectors and signaling cascades regulated by AA. It would also be valuable to investigate potential synergistic effects of AA in combination with existing PD therapies, such as levodopa or MAO- B inhibitors to explore adjunctive treatment strategies as found in (Gupta et al., 2016). Moreover, understanding pharmacokinetics, blood- brain barrier permeability and bioavailability of AA in higher organisms will be critical steps toward clinical translation (Vidal-Gadea et al., 2011).

Collectively, results of this study establish AA as a promising neuroprotective compound against rotenone induced Parkinsonian pathology in *C. elegans*. Observed improvements in behavioral parameters, restoration of antioxidant defenses and normalization of neurotransmitter enzyme activity highlight AA's potential to target multiple pathogenic pathways in PD. Given its natural origin, safety profile and multifactorial mode of action, AA holds significant promise as a candidate for further preclinical development. This work not only contributes to understanding of plant- based neurotherapeutics but also reinforces relevance of simple animal models like *C. elegans* in advancing translational neuroscience research.

Conclusion

PD continues to be one of the most prevalent and debilitating neurodegenerative disorders, with a complex etiology encompassing oxidative stress, mitochondrial dysfunction, neuroinflammation and progressive dopaminergic neuronal loss. Despite extensive advancements in understanding PD pathophysiology, therapeutic strategies remain largely symptomatic, with no currently available treatment capable of altering disease progression. This underscores the urgent need to explore alternative therapeutic avenues, particularly those involving naturally derived compounds with multi-targeted neuroprotective mechanisms.

Present study aimed to evaluate potential of AA, a natural diterpenoid resin acid, as a neuroprotective agent in a rotenone induced model of PD using *C. elegans*. Rotenone exposure in *C. elegans* reliably replicated PD associated features, including behavioral impairments such as reduced basal slowing response, chemotaxis deficits and body bending irregularities, as well as biochemical abnormalities marked by depleted antioxidant enzymes (SOD and GSH) and altered acetylcholinesterase activity. These findings validated efficacy of rotenone model for studying PD like neurodegeneration in vivo. Treatment with AA produced significant neuroprotective effects in a concentration-dependent manner. Behavioral assays demonstrated notable improvement in locomotor and sensory behaviors, indicative of dopaminergic functional preservation. Concurrently, AA enhanced antioxidant defense systems by increasing GSH and SOD levels, thus countering rotenone induced oxidative damage. Moreover, AA stabilized cholinergic neurotransmission by normalizing AchE activity. Collectively, these results confirm that AA effectively ameliorates both functional and biochemical parameters associated with PD pathology.

Notably, AA exhibited a favorable safety profile, showing no toxicity at doses up to 25 µM. Its pleiotropic mode of action like spanning antioxidant, anti-inflammatory and neurotransmitter modulatory pathways supports its therapeutic promise in targeting multifactorial nature of PD. These findings of this study firmly establish AA as a compelling candidate for further exploration in PD research. *C. elegans* model proved to be a valuable in vivo system for initial screening of neuroprotective efficacy and mechanistic insight. Given AA's natural origin, safety profile and ability to target multiple facets of PD pathology, it holds significant potential for future preclinical trials in higher animal models and eventual translational application. Study opens new directions for development of plant- based neurotherapeutics and highlights value of integrative research approaches that combine classical pharmacology with modern neurogenetic tools. Future work should focus on validating AA's efficacy in mammalian models, elucidating its impact on α -synuclein aggregation and exploring its suitability for combinatorial therapies. Through such endeavors, AA may one day contribute meaningfully to development of disease modifying treatments for Parkinson's disease.

References

1. Aarsland, D., & Kramberger, M. G. (2015). Neuropsychiatric symptoms in Parkinson's disease. Journal of Parkinson's Disease, 5(3), 659-667.
2. Aarsland, D., Marsh, L., & Schrag, A. (2009). Neuropsychiatric symptoms in Parkinson's disease. *Movement disorders: official journal of the Movement Disorder Society*, *24*(15), 2175-2186.
3. Alexander, G. E. (2004). Biology of Parkinson's disease: pathogenesis and pathophysiology of a multisystem neurodegenerative disorder. *Dialogues in clinical neuroscience*, *6*(3), 259-280.
4. Alves, A. L. V., da Silva, L. S., Faleiros, C. A., Silva, V. A., & Reis, R. M. (2022). The role of ingenane diterpenes in cancer therapy: From bioactive secondary compounds to small molecules. *Natural Product Communications*, *17*(7), 1934578X221105691.
5. Avery, L. (1993). The genetics of feeding in Caenorhabditis elegans. *Genetics*, *133*(4), 897-917.
6. B Mythri, R., & M Srinivas Bharath, M. (2012). Curcumin: a potential neuroprotective agent in Parkinson's disease. *Current pharmaceutical design*, *18*(1), 91-99.
7. Balestri, W., Sharma, R., da Silva, V. A., Bobotis, B. C., Curle, A. J., Kothakota, V., ... & Reinwald, Y. (2024). Modeling the neuroimmune system in Alzheimer's and Parkinson's diseases. *Journal of neuroinflammation*, *21*(1), 32.
8. Bargmann, C. I. (1998). Neurobiology of the Caenorhabditis elegans genome. *Science*, *282*(5396), 2028-2033.
9. Bargmann, C. I. (2006). Chemosensation in C. elegans. *WormBook: The online review of C. elegans biology [Internet]*.
10. Barker, R. A., & Williams-Gray, C. H. (2016). The spectrum of clinical features seen with alpha synuclein pathology. *Neuropathology and applied neurobiology*, *42*(1), 6-19.
11. Betarbet, R., Sherer, T. B., MacKenzie, G., Garcia-Osuna, M., Panov, A. V., & Greenamyre, J. T. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nature neuroscience*, *3*(12), 1301-1306.
12. Bhushan, R., Goel, F., & Singh, S. (2025). Beyond dopamine: novel therapeutic pathways for Parkinson's disease through receptor signaling. *CNS & Neurological Disorders-Drug Targets*.
13. Blauwendraat, C., Nalls, M. A., & Singleton, A. B. (2020). The genetic architecture of Parkinson's disease. *The Lancet Neurology*, *19*(2), 170-178.

14. Blauwendraat, C., Reed, X., Krohn, L., Heilbron, K., Bandres-Ciga, S., Tan, M., ... & Singleton, A. B. (2020). Genetic modifiers of risk and age at onset in GBA associated Parkinson's disease and Lewy body dementia. *Brain*, *143*(1), 234-248.
15. Blesa, J., Trigo-Damas, I., Quiroga-Varela, A., & Jackson-Lewis, V. R. (2015). Oxidative stress and Parkinson's disease. *Frontiers in neuroanatomy*, *9*, 91.
16. Bloem, B. R., Okun, M. S., & Klein, C. (2021). Parkinson's disease. *The Lancet*, 397(10291), 2284 - 2303.
17. Borsche, M., König, I. R., Delcambre, S., Petrucci, S., Balck, A., Brüggemann, N., ... & Klein, C. (2020). Mitochondrial damage-associated inflammation highlights biomarkers in PRKN/PINK1 parkinsonism. *Brain*, *143*(10), 3041-3051.
18. Bose, A., & Beal, M. F. (2016). Mitochondrial dysfunction in Parkinson's disease. *Journal of neurochemistry*, *139*, 216-231.
19. Boulos, C., Yaghi, N., El Hayeck, R., Heraoui, G. N., & Fakhoury-Sayegh, N. (2019). Nutritional risk factors, microbiota and Parkinson's disease: what is the current evidence? *Nutrients*, *11*(8), 1896.
20. Bové, J., & Perier, C. (2012). Neurotoxin-based models of Parkinson's disease. *Neuroscience*, *211*, 51-76.
21. Braak, H., Del Tredici, K., Rüb, U., De Vos, R. A., Steur, E. N. J., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of aging*, *24*(2), 197-211.
22. Brenner, S. (1974). The genetics of Caenorhabditis elegans. *Genetics*, *77*(1), 71-94.
23. Brochard, V., Combadière, B., Prigent, A., Laouar, Y., Perrin, A., Beray-Berthat, V., ... & Hunot, S. (2008). Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *The Journal of clinical investigation*, *119*(1).
24. Brown, M. B., & Forsythe, A. B. (1974). Robust tests for the equality of variances. *Journal of the American statistical association*, *69*(346), 364-367.
25. Buck, S. A., & Sanders, L. H. (2025). LRRK2-mediated mitochondrial dysfunction in Parkinson's disease. *Biochemical Journal*, *482*(11), BCJ20253062.
26. Byerly, L., Cassada, R. C., & Russell, R. L. (1976). The life cycle of the nematode Caenorhabditis elegans: I. Wild-type growth and reproduction. *Developmental biology*, *51*(1), 23-33.
27. Caito, S., & Aschner, M. (2015). Neurotoxicity of metals. *Handbook of clinical neurology*, *131*, 169-189.
28. Cannon, J. R., & Greenamyre, J. T. (2011). The role of environmental exposures in neurodegeneration and neurodegenerative diseases. *Toxicological Sciences*, *124*(2), 225-250.
29. Cannon, J. R., Tapias, V., Na, H. M., Honick, A. S., Drolet, R. E., & Greenamyre, J. T. (2009). A highly reproducible rotenone model of Parkinson's disease. *Neurobiology of disease*, *34*(2), 279-290.
30. Cebrián, C., Loike, J. D., & Sulzer, D. (2014). Neuroinflammation in Parkinson's disease animal models: a cell stress response or a step in neurodegeneration? *Behavioral Neurobiology of Huntington's Disease and Parkinson's Disease*, 237-270.
31. Cenci, M. A., & Björklund, A. (2020). Animal models for preclinical Parkinson's research: an update and critical appraisal. *Progress in brain research*, *252*, 27-59.
32. Chagas, M. H. N., Zuardi, A. W., Tumas, V., Pena-Pereira, M. A., Sobreira, E. T., Bergamaschi, M. M., ... & Crippa, J. A. S. (2014). Effects of cannabidiol in the treatment of patients with Parkinson's disease: an exploratory double-blind trial. *Journal of Psychopharmacology*, *28*(11), 1088-1098.

33. Chaudhuri, K. R., Healy, D. G., & Schapira, A. H. (2006). Non-motor symptoms of Parkinson's disease: diagnosis and management. *The Lancet Neurology*, *5*(3), 235-245.
34. Chen, C., Zhang, D., Chen, F., Wu, D., Chen, W., Qin, H., & Hu, Y. (2024). A retrospective study of Parkinson's disease in Southwest China 2021–2024: An age-based approach. *Experimental Gerontology*, *195*, 112532.
35. Cheng, H. C., Ulane, C. M., & Burke, R. E. (2010). Clinical progression in Parkinson disease and the neurobiology of axons. *Annals of Neurology*, 67(6), 715 -725.
36. Choudhary, D., Bhattacharyya, S., & Bose, S. (2017). Efficacy and safety of Ashwagandha (Withania somnifera (L.) Dunal) root extract in improving memory and cognitive functions. *Journal of dietary supplements*, *14*(6), 599-612.
37. Cooper, A. J. (2018). Role of astrocytes in maintaining cerebral glutathione homeostasis and in protecting the brain against xenobiotics and oxidative stress. In *Glutathione in the nervous system* (pp. 91-115). CRC Press.
38. da Silva, L. P. D., da Cruz Guedes, E., Fernandes, I. C. O., Pedroza, L. A. L., da Silva Pereira, G. J., & Gubert, P. (2024). Exploring Caenorhabditis elegans as Parkinson's disease model: neurotoxins and genetic implications. *Neurotoxicity Research*, *42*(1), 11.
39. Damier, P., Hirsch, E. C., Agid, Y., & Graybiel, A. (1999). The substantia nigra of the human brain: II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. *Brain*, *122*(8), 1437-1448.
40. Dauer, W., & Przedborski, S. (2003). Parkinson's disease: mechanisms and models. *Neuron*, *39*(6), 889-909.
41. Deep, S. N., Baitharu, I., Sharma, A., Gurjar, A. K. S., Prasad, D., & Singh, S. B. (2016). Neuroprotective role of L-NG-nitroarginine methyl ester (L-NAME) against chronic hypobaric hypoxia with crowding stress (CHC) induced depression-like behaviour. *PLoS One*, *11*(4), e0153371.
42. Dehay, B., Bourdenx, M., Gorry, P., Przedborski, S., Vila, M., Hunot, S., ... & Meissner, W. G. (2015). Targeting α-synuclein for treatment of Parkinson's disease: mechanistic and therapeutic considerations. *The Lancet Neurology*, *14*(8), 855-866.
43. Del Tredici, K., & Braak, H. (2016). Sporadic Parkinson's disease: development and distribution of α-synuclein pathology. *Neuropathology and applied neurobiology*, *42*(1), 33-50.
44. DeLong, M. R., & Wichmann, T. (2015). Basal ganglia circuits as targets for neuromodulation in Parkinson disease. *JAMA Neurology*, 72(11), 1354-1360.
45. Deng, H., & Yuan, L. (2014). Genetic variants and animal models in SNCA and Parkinson disease. *Ageing research reviews*, *15*, 161-176.
46. Deng, X., Mehta, A., Xiao, B., Chaudhuri, K. R., Tan, E. K., & Tan, L. C. (2024). Parkinson's disease subtypes: Approaches and clinical implications. *Parkinsonism & Related Disorders*, 107208.
47. Dorsey, E. R., Elbaz, A., Nichols, E., Abbasi, N., Abd-Allah, F., Abdelalim, A., ... & Murray, C. J. (2018). Global, regional, and national burden of Parkinson's disease, 1990 -2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology*, *17*(11), 939-953.
48. EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Silano, V., Barat Baviera, J. M., Bolognesi, C., Chesson, A., Cocconcelli, P. S., ... & Lampi, E. (2020). Safety assessment of the process Somoplast-Riachi & Co, based on Starlinger de CON technology, used to recycle post-consumer PET into food contact materials. *EFSA Journal*, *18*(10), e06252.

49. El Ghazal, N., Nakanishi, H., Martinez-Nunez, A. E., Al Sabbakh, N. K., Segun-Omosehin, O. A., Bourdakos, N. E., ... & Matar, R. (2023). The effects of deep brain stimulation on mood and quality of life in Parkinson's disease: a systematic review and meta-analysis. *Cureus*, *15*(8).
50. Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology*, *7*(2), 88-95.
51. ElMosbah, D. E., Ibrahim, M., Khalil, H., El-Asssal, M., & El Miniawy, H. (2024). The improvement potential of camel milk whey as a natural remedy in comparison with Rivastigmine chitosan-loaded nanoparticles in aluminum chloride induced Alzheimer-like disease in rats. *Egyptian Journal of Veterinary Sciences*, *55*(5), 1435-1446.
52. Emmons, S. W. (2015). The beginning of connectomics: a commentary on White et al. (1986) 'The structure of the nervous system of the nematode Caenorhabditis elegans'. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1666), 20140309.
53. Exner, N., Lutz, A. K., Haass, C., & Winklhofer, K. F. (2012). Mitochondrial dysfunction in Parkinson's disease: molecular mechanisms and pathophysiological consequences. *The EMBO journal*, *31*(14), 3038-3062.
54. Fabian, T. J., & Johnson, T. E. (1994). Production of age-synchronous mass cultures of Caenorhabditis elegans. *Journal of gerontology*, *49*(4), B145-B156.
55. Festa, C., D'Auria, M. V., Sepe, V., Ilaš, J., Leick, A., N'Gom, S., & De Marino, S. (2015). Triterpenoid profile and bioactivity study of Oenothera maritima. *Phytochemistry Letters*, *13*, 324-329.
56. Fitzgerald, E., Murphy, S., & Martinson, H. A. (2019). Alpha-synuclein pathology and the role of the microbiota in Parkinson's disease. *Frontiers in neuroscience*, *13*, 369.
57. Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., ... & Salvioli, S. (2007). Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mechanisms of ageing and development*, *128*(1), 92-105.
58. Gauthier, S., & Schlaefke, S. (2014). Efficacy and tolerability of Ginkgo biloba extract EGb 761® in dementia: a systematic review and meta-analysis of randomized placebo-controlled trials. *Clinical interventions in aging*, 2065-2077.
59. Ghavami, S., Shojaei, S., Yeganeh, B., Ande, S. R., Jangamreddy, J. R., Mehrpour, M., ... & Łos, M. J. (2014). Autophagy and apoptosis dysfunction in neurodegenerative disorders. *Progress in neurobiology*, *112*, 24-49.
60. Ghosh, S., Kumar, V., Mukherjee, H., Saini, S., Gupta, S., Chauhan, S., ... & Roy, P. (2024). Assessment of the mechanistic role of an Indian traditionally used ayurvedic herb Bacopa monnieri (L.) Wettst. for ameliorating oxidative stress in neuronal cells. *Journal of ethnopharmacology*, *328*, 117899.
61. Goedert, M., Clavaguera, F., & Tolnay, M. (2010). The propagation of prion-like protein inclusions in neurodegenerative diseases. *Trends in neurosciences*, *33*(7), 317-325.
62. Goedert, M., Spillantini, M. G., Del Tredici, K., & Braak, H. (2013). 100 years of Lewy pathology. *Nature Reviews Neurology*, 9(1), 13 -24.
63. Goetz, C. G. (2011). The history of Parkinson's disease: early clinical descriptions and neurological therapies. *Cold Spring Harbor perspectives in medicine*, *1*(1), a008862.
64. Goldstein, D. S. (2012). Noradrenergic neurotransmission. In *Primer on the autonomic nervous system* (pp. 37-43). Academic Press.

65. Goldstein, D. S., Holmes, C., Sullivan, P., Mash, D. C., Sidransky, E., Stefani, A., ... & Sharabi, Y. (2015). Deficient vesicular storage: a common theme in catecholaminergic neurodegeneration. *Parkinsonism & related disorders*, *21*(9), 1013-1022.
66. González, M. A. (2015). Aromatic abietane diterpenoids: Their biological activity and synthesis. *Natural Product Reports*, *32*(5), 684-704.
67. Gonzalez-Hunt, C. P., Luz, A. L., Ryde, I. T., Turner, E. A., Ilkayeva, O. R., Bhatt, D. P., ... & Meyer, J. N. (2021). Multiple metabolic changes mediate the response of Caenorhabditis elegans to the complex I inhibitor rotenone. *Toxicology*, *447*, 152630.
68. Goronzy, J. J., & Weyand, C. M. (2013). Understanding immunosenescence to improve responses to vaccines. *Nature immunology*, *14*(5), 428-436.
69. Gratwicke, J., Jahanshahi, M., & Foltynie, T. (2015). Parkinson's disease dementia: a neural networks perspective. *Brain*, *138*(6), 1454-1476.
70. Greenamyre, J. T., Cannon, J. R., Drolet, R., & Mastroberardino, P. G. (2010). Lessons from the rotenone model of Parkinson's disease. *Trends in pharmacological sciences*, *31*(4), 141.
71. Greenamyre, J. T., Cannon, J. R., Drolet, R., & Mastroberardino, P. G. (2010). Lessons from the rotenone model of Parkinson's disease. *Trends in pharmacological sciences*, *31*(4), 141.
72. Guo, F., Liu, X., Cai, H., & Le, W. (2018). Autophagy in neurodegenerative diseases: pathogenesis and therapy. *Brain pathology*, *28*(1), 3-13.
73. Gupta, D. K., Hang, X., Liu, R., Hasan, A., & Feng, Z. (2016). Levodopa-induced motor and dopamine receptor changes in Caenorhabditis elegans overexpressing human alpha-synuclein. *Neurodegenerative Diseases*, *16*(3-4), 179-183.
74. Hanson, J. R. (2017). Diterpenoids of terrestrial origin. *Natural Product Reports*, *34*(10), 1233-1243.
75. Hartmann, A., Hunot, S., Michel, P. P., Muriel, M. P., Vyas, S., Faucheux, B. A., ... & Hirsch, E. C. (2000). Caspase-3: a vulnerability factor and final effector in apoptotic death of dopaminergic neurons in Parkinson's disease. *Proceedings of the National Academy of Sciences*, *97*(6), 2875-2880.
76. Henry, C. J., Huang, Y., Wynne, A. M., & Godbout, J. P. (2009). Peripheral lipopolysaccharide (LPS) challenge promotes microglial hyperactivity in aged mice that is associated with exaggerated induction of both pro-inflammatory IL-1β and anti-inflammatory IL-10 cytokines. *Brain, behavior, and immunity*, *23*(3), 309-317.
77. Hirsch, E. C., Breidert, T., Rousselet, E., Hunot, S., Hartmann, A., & Michel, P. P. (2003). The role of glial reaction and inflammation in Parkinson's disease. *Annals of the New York Academy of Sciences*, *991*(1), 214-228.
78. Hisahara, S., & Shimohama, S. (2011). Toxin-Induced and Genetic Animal Models of Parkinson′ s Disease. *Parkinson's disease*, *2011*(1), 951709.
79. Hope, I. A. (Ed.). (1999). *C. elegans: a practical approach* (Vol. 213). OUP Oxford.
80. Hu, Y. L., Xu, T. Q., Cheng, H. Y., Liu, Y., Zhang, X., Zhang, Y. B., & Zhou, G. X. (2022). Undescribed abietane-type diterpenoids and oleanane-type triterpenoids from the stem and branch of Tripterygium wilfordii. *Phytochemistry*, *201*, 113258.
81. Huang, S., Chen, Z., Fan, B., Chen, Y., Zhou, L., Jiang, B., ... & Li, Y. (2021). A selective NLRP3 inflammasome inhibitor attenuates behavioral deficits and neuroinflammation in a mouse model of Parkinson's disease. *Journal of Neuroimmunology*, *354*, 577543.
82. Jackson-Lewis, V., & Przedborski, S. (2007). Protocol for the MPTP mouse model of Parkinson's disease. *Nature protocols*, *2*(1), 141-151.

83. Jain, K. K., & PharmaBiotech, J. (2007). Drug delivery in central nervous system disorders. *Basel, Switzerland: Jain Pharma Biotech Publications*.
84. Jee, C., Batsaikhan, E., & Salim, C. (2022). Neurobiological Basis of Aversion-Resistant Ethanol Seeking in C. elegans. *Metabolites*, *13*(1), 62.
85. Jenner, P. (2003). Oxidative stress in Parkinson's disease. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, *53*(S3), S26-S38.
86. Johnson, M. E., & Bobrovskaya, L. (2015). An update on the rotenone models of Parkinson's disease: their ability to reproduce the features of clinical disease and model gene -environment interactions. *Neurotoxicology*, *46*, 101-116.
87. Kalia, L. V., & Lang, A. E. (2015). Parkinson's disease. *The Lancet*, 386(9996), 896 -912.
88. Katzenschlager, R., & Lees, A. J. (2002). Treatment of Parkinson's disease: levodopa as the first choice. *Journal of neurology*, *249*,
89. Katzenschlager, R., Evans, A., Manson, A., Patsalos, P. N., Ratnaraj, N., Watt, H., ... & Lees, A. J. (2004). Mucuna pruriens in Parkinson's disease: a double blind clinical and pharmacological study. *Journal of Neurology, Neurosurgery & Psychiatry*, *75*(12), 1672-1677.
90. Kaya, C., Murillo-Amador, B., & Ashraf, M. (2020). Involvement of L-cysteine desulfhydrase and hydrogen sulfide in glutathione-induced tolerance to salinity by accelerating ascorbate-glutathione cycle and glyoxalase system in capsicum. *Antioxidants*, *9*(7), 603.
91. Khan, E., Hasan, I., & Haque, M. E. (2023). Parkinson's disease: exploring different animal model systems. *International journal of molecular sciences*, *24*(10), 9088.
92. Kimura, H., Kurimura, M., Wada, M., Kawanami, T., Kurita, K., Suzuki, Y., ... & Kato, T. (2002). Female preponderance of Parkinson's disease in Japan. *Neuroepidemiology*, *21*(6), 292-296.
93. Klein, C., & Westenberger, A. (2012). Genetics of Parkinson's disease. *Cold Spring Harbor Perspectives in Medicine*, 2(1), a008888.
94. Kokoszka, J. E., Waymire, K. G., Levy, S. E., Sligh, J. E., Cai, J., Jones, D. P., ... & Wallace, D. C. (2004). The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature*, *427*(6973), 461-465.
95. Kono, Y. (1978). Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. *Archives of biochemistry and biophysics*, *186*(1), 189-195.
96. Kumar, P., Kale, R. K., Mukherjee, S., Prakash, K., McLean, P., & Baquer, N. Z. (2011). Antidiabetic effects of Trigonella foenum-graecum seed powder in a rat model. *Toxicological & Environmental Chemistry*, *93*(10), 2085-2097.
97. Lang, A. E., & Espay, A. J. (2018). Disease modification in Parkinson's disease: Current approaches, challenges, and future considerations. *Movement Disorders*, 33(5), 660 -677.
98. Lee, J., Jee, C., & McIntire, S. L. (2009). Ethanol preference in C. elegans. *Genes, Brain and Behavior*, *8*(6), 578-585.
99. Lee, Y., Lee, S., Chang, S. C., & Lee, J. (2019). Significant roles of neuroinflammation in Parkinson's disease: therapeutic targets for PD prevention. *Archives of pharmacal research*, *42*, 416-425.
100. Leelaja, B. C., & Rajini, P. S. (2012). Impact of phosphine exposure on development in Caenorhabditis elegans: Involvement of oxidative stress and the role of glutathione. *Pesticide biochemistry and physiology*, *104*(1), 38-43.
101. Lesage, S., & Brice, A. (2009). Parkinson's disease: from monogenic forms to genetic susceptibility factors. *Human molecular genetics*, *18*(R1), R48-R59.

102. Leung, A. W., & Halestrap, A. P. (2008). Recent progress in elucidating the molecular mechanism of the mitochondrial permeability transition pore. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1777*(7-8), 946-952.
103. Levites, Y., Amit, T., Mandel, S., & Youdim, M. B. (2003). Neuroprotection and neurorescue against Aβ toxicity and PKC-dependent release of non-amyloidogenic soluble precursor protein by green tea polyphenol (-)-epigallocatechin-3-gallate. *The FASEB Journal*, *17*(8), 1-23.
104. Li, Y., Xu, X., Niu, M., Chen, J., Wen, J., Bian, H., ... & Liu, X. (2019). Thermal stability of abietic acid and its oxidation products. *Energy & Fuels*, *33*(11), 11200-11209.
105. Lim, D. W., Lee, J. E., Lee, C., & Kim, Y. T. (2024). Natural products and their neuroprotective effects in degenerative brain diseases: a comprehensive review. *International journal of molecular sciences*, *25*(20), 11223.
106. Liu, T., Hao, Y., & Zhao, L. (2024). Pathogenic genes associated with Parkinson's disease: molecular mechanism overview. *Biocell*, *48*(5).
107. Lowry, O. H., & Rosebrough, N. J. (1951). Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J. biol. Chem*, *193*(1), 265-275.
108. Luo, Y., Qiao, L., Li, M., Wen, X., Zhang, W., & Li, X. (2025). Global, regional, national epidemiology and trends of Parkinson's disease from 1990 to 2021: findings from the Global Burden of Disease Study 2021. *Frontiers in Aging Neuroscience*, *16*, 1498756.
109. Lv, L., Zhang, H., Tan, X., Long, Z., Qin, L., Bai, R., ... & Wang, C. (2022). Associated factors and abnormal dorsal raphe nucleus connectivity patterns of freezing of gait in Parkinson's disease. *Journal of Neurology*, *269*(12), 6452-6466.
110. MacMahon Copas, A. N., McComish, S. F., Fletcher, J. M., & Caldwell, M. A. (2021). The pathogenesis of Parkinson's disease: a complex interplay between astrocytes, microglia, and T lymphocytes. *Frontiers in neurology*, *12*, 666737.
111. Martinez, T. N., & Greenamyre, J. T. (2012). Toxin models of mitochondrial dysfunction in Parkinson's disease. Antioxidants & redox signaling, 16(9), 920-934.
112. Mata, I. F., Shi, M., Agarwal, P., Chung, K. A., Edwards, K. L., Factor, S. A., ... & Zabetian, C. P. (2010). SNCA variant associated with Parkinson disease and plasma α-synuclein level. *Archives of neurology*, *67*(11), 1350-1356.
113. McCoy, M. K., & Cookson, M. R. (2012). Mitochondrial quality control and dynamics in Parkinson's disease. *Antioxidants & redox signaling*, *16*(9), 869-882.
114. McGeer, P. L., Itagaki, S., Boyes, B. E., & McGeer, E. G. (1988). Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology*, *38*(8), 1285-1285.
115. Meredith, G. E., & Rademacher, D. J. (2011). MPTP mouse models of Parkinson's disease: an update. *Journal of Parkinson's disease*, *1*(1), 19-33.
116. Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological chemistry*, *247*(10), 3170-3175.
117. Moon, H. E., & Paek, S. H. (2015). Mitochondrial dysfunction in Parkinson's disease. *Experimental neurobiology*, *24*(2), 103.
118. Moon, H., Kim, B., Kwon, I., & Oh, Y. (2023). Challenges involved in cell therapy for Parkinson's disease using human pluripotent stem cells. *Frontiers in Cell and Developmental Biology*, *11*, 1288168.

119. Nandipati, S., & Litvan, I. (2016). Environmental exposures and Parkinson's disease. *International journal of environmental research and public health*, *13*(9), 881.
120. Nascimento, M. H. M. D., & de Araújo, D. R. (2022). Exploring the pharmacological potential of glycyrrhizic acid: From therapeutic applications to trends in nanomedicine. *Future Pharmacology*, *2*(1), 1-15.
121. Nass, R., & Blakely, R. D. (2003). The Caenorhabditis elegans dopaminergic system: opportunities for insights into dopamine transport and neurodegeneration. *Annual review of pharmacology and toxicology*, *43*(1), 521-544.
122. Nataraj, J., Manivasagam, T., Justin Thenmozhi, A., & Essa, M. M. (2017). Neuroprotective effect of asiatic acid on rotenone-induced mitochondrial dysfunction and oxidative stress-mediated apoptosis in differentiated SH-SYS5Y cells. *Nutritional neuroscience*, *20*(6), 351-359.
123. Nawa, M., & Matsuoka, M. (2012). The method of the body bending assay using Caenorhabditis elegans. *Bio-protocol*, *2*(17), e253-e253.
124. Nawa, M., Kage-Nakadai, E., Aiso, S., Okamoto, K., Mitani, S., & Matsuoka, M. (2012). Reduced expression of BTBD10, an Akt activator, leads to motor neuron death. *Cell Death & Differentiation*, *19*(8), 1398-1407.
125. Nechushtai, L., Frenkel, D., & Pinkas-Kramarski, R. (2023). Autophagy in Parkinson's disease. *Biomolecules*, *13*(10), 1435.
126. Nong, W. J., Chen, X. P., Liang, J. Z., Wang, L. L., Tong, Z. F., Huang, K., ... & Li, K. X. (2014). Isolation and characterization of abietic acid. *Advanced Materials Research*, *887*, 551-556.
127. Orenstein, S. J., Kuo, S. H., Tasset, I., Arias, E., Koga, H., Fernandez-Carasa, I., ... & Cuervo, A. M. (2013). Interplay of LRRK2 with chaperone-mediated autophagy. *Nature neuroscience*, *16*(4), 394-406.
128. Pacelli, C., Giguère, N., Bourque, M. J., Lévesque, M., Slack, R. S., & Trudeau, L. É. (2015). Elevated mitochondrial bioenergetics and axonal arborization size are key contributors to the vulnerability of dopamine neurons. *Current Biology*, *25*(18), 2349-2360.
129. Papagiannakis, N., & Stefanis, L. (2021). 1Autophagy-lysosome pathway as a source of candidate biomarkers for Parkinson's disease. *Neuroimmunology and Neuroinflammation*, *8*(2), 101-110.
130. Park, J., Kim, J. E., Jin, Y. J., Roh, Y. J., Song, H. J., Seol, A., ... & Hwang, D. Y. (2023). Anti-atopic dermatitis effects of abietic acid isolated from rosin under condition optimized by response surface methodology in DNCB-spread BALB/c mice. *Pharmaceuticals*, *16*(3), 407.
131. Parkinson, J. (1817). *An Essay on the Shaking Palsy*. Sherwood, Neely, and Jones.
132. Parvez, S., Winkler-Stuck, K., Hertel, S., Schönfeld, P., & Siemen, D. (2010). The dopamine-D2-receptor agonist ropinirole dose-dependently blocks the Ca2+-triggered permeability transition of mitochondria. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1797(6-7), 1245-1250.
133. Perry, V. H. (2012). Innate inflammation in Parkinson's disease. *Cold Spring Harbor perspectives in medicine*, *2*(9), a009373.
134. Peters, R. J. (2010). Two rings in them all: the labdane-related diterpenoids. *Natural product reports*, *27*(11), 1521-1530.
135. Pickrell, A. M., & Youle, R. J. (2015). The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. *Neuron*, *85*(2), 257-273.

136. Pieperhoff, P., Südmeyer, M., Dinkelbach, L., Hartmann, C. J., Ferrea, S., Moldovan, A. S., ... & Amunts, K. (2022). Regional changes of brain structure during progression of idiopathic Parkinson's disease - A longitudinal study using deformation based morphometry. *Cortex*, *151*, 188-210.
137. Poewe, W., Seppi, K., Tanner, C. M., Halliday, G. M., Brundin, P., Volkmann, J., ... & Lang, A. E. (2017). Parkinson disease. *Nature reviews Disease primers*, *3*(1), 1-21.
138. Politis, M., Wu, K., Loane, C., Brooks, D. J., Kiferle, L., Turkheimer, F. E., ... & Piccini, P. (2014). Serotonergic mechanisms responsible for levodopa-induced dyskinesias in Parkinson's disease patients. *The Journal of clinical investigation*, *124*(3), 1340-1349.
139. Politis, M., Wu, K., Loane, C., Quinn, N. P., Brooks, D. J., Oertel, W. H., ... & Piccini, P. (2012). Serotonin neuron loss and nonmotor symptoms continue in Parkinson's patients treated with dopamine grafts. *Science translational medicine*, *4*(128), 128ra41-128ra41.
140. Porta-de-la-Riva, M., Fontrodona, L., Villanueva, A., & Cerón, J. (2012). Basic Caenorhabditis elegans methods: synchronization and observation. *Journal of visualized experiments: JoVE*, (64), 4019.
141. Postuma, R. B., & Berg, D. (2019). Prodromal Parkinson's disease: the decade past, the decade to come. *Movement disorders*, *34*(5), 665-675.
142. Postuma, R. B., Aarsland, D., Barone, P., Burn, D. J., Hawkes, C. H., Oertel, W., & Ziemssen, T. (2012). Identifying prodromal Parkinson's disease: pre-motor disorders in Parkinson's disease. *Movement Disorders*, *27*(5), 617-626.
143. Postuma, R. B., Berg, D., Stern, M., Poewe, W., Olanow, C. W., Oertel, W., ... & Deuschl, G. (2015). MDS clinical diagnostic criteria for Parkinson's disease. *Movement disorders*, *30*(12), 1591-1601.
144. Postuma, R. B., Iranzo, A., Hu, M., Högl, B., Boeve, B. F., Manni, R., ... & Pelletier, A. (2019). Risk and predictors of dementia and parkinsonism in idiopathic REM sleep behaviour disorder: a multicentre study. *Brain*, *142*(3), 744-759.
145. Prokić, M., Borković-Mitić, S., Krizmanić, I., Gavrić, J., Despotović, S., Gavrilović, B., ... & Saičić, Z. (2017). Comparative study of oxidative stress parameters and acetylcholinesterase activity in the liver of Pelophylax esculentus complex frogs. *Saudi Journal of Biological Sciences*, *24*(1), 51-58.
146. Rahman, I., Kode, A., & Biswas, S. K. (2006). Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nature protocols*, *1*(6), 3159-3165.
147. Raju, S., Shetty, K., Sahoo, L., Paramanandam, V., Iyer, J. M., Bowmick, S., ... & PRAI Consortium. (2025). Progressive Supranuclear Palsy in India: Past, Present, and Future. *Annals of Indian Academy of Neurology*, *28*(1), 17-25. Raju, S., Shetty, K., Sahoo, L., Paramanandam, V., Iyer, J. M., Bowmick, S., ... & PRAI Consortium. (2025). Progressive Supranuclear Palsy in India: Past, Present, and Future. *Annals of Indian Academy of Neurology*, *28*(1), 17-25.
148. Reynolds, A. D., Stone, D. K., Hutter, J. A., Benner, E. J., Mosley, R. L., & Gendelman, H. E. (2010). Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease. *The Journal of Immunology*, *184*(5), 2261-2271.
149. Rocha, E. M., De Miranda, B., & Sanders, L. H. (2018). Alpha-synuclein: Pathology, mitochondrial dysfunction and neuroinflammation in Parkinson's disease. *Neurobiology of disease*, *109*, 249-257.
150. Salimikia, I., Moridi Farimani, M., Monsef-Esfahani, H. R., & Gohari, A. R. (2016). A new rearranged tricyclic abietane diterpenoid from Salvia chloroleuca Rech. f. & Allen. *Natural Product Research*, *30*(1), 120-124.

151. Sargazifar, Z., Ghorbanian Charami, D., Esmaeilzadeh Kashi, M., Asili, J., & Shakeri, A. (2024). Abietane-Type Diterpenoids: Insights into Structural Diversity and Therapeutic Potential. *Chemistry & Biodiversity*, *21*(10), e202400808.
152. Sarrafchi, A., Bahmani, M., Shirzad, H., & Rafieian-Kopaei, M. (2016). Oxidative stress and Parkinson's disease: new hopes in treatment with herbal antioxidants. *Current pharmaceutical design*, *22*(2), 238-246.
153. Saunders, J. A. H., Estes, K. A., Kosloski, L. M., Allen, H. E., Dempsey, K. M., Torres-Russotto, D. R., ... & Gendelman, H. E. (2012). CD4+ regulatory and effector/memory T cell subsets profile motor dysfunction in Parkinson's disease. *Journal of Neuroimmune Pharmacology*, *7*, 927-938.
154. Sazanov, L. A. (2015). A giant molecular proton pump: structure and mechanism of respiratory complex I. *Nature reviews Molecular cell biology*, *16*(6), 375-388.
155. Schapira, A. H. (2008). Mitochondria in the aetiology and pathogenesis of Parkinson's disease. *The Lancet Neurology*, *7*(1), 97-109.
156. Schöndorf, D. (2018). *Mechanisms of GBA1 related PD in hiPSC derived neurons* (Doctoral dissertation, Universität Tübingen).
157. Schrag, A., Ben-Shlomo, Y., Brown, R., David Marsden, C., & Quinn, N. (1998). Young-onset Parkinson's disease revisited—clinical features, natural history, and mortality. *Movement disorders: official journal of the Movement Disorder Society*, *13*(6), 885-894.
158. Shakya, S., Prevett, J., Hu, X., & Xiao, R. (2022). Characterization of Parkinson's disease subtypes and related attributes. *Frontiers in neurology*, *13*, 810038.
159. Sherer, T. B., Betarbet, R., Testa, C. M., Seo, B. B., Richardson, J. R., Kim, J. H., ... & Greenamyre, J. T. (2003). Mechanism of toxicity in rotenone models of Parkinson's disease. *Journal of Neuroscience*, *23*(34), 10756-10764.
160. Singh, A., Zhi, L., & Zhang, H. (2019). LRRK2 and mitochondria: recent advances and current views. *Brain research*, *1702*, 96-104.
161. Singh, V., Singh, N., Verma, M., Kamal, R., Tiwari, R., Sanjay Chivate, M., ... & Mishra, V. (2022). Hexavalent-chromium-induced oxidative stress and the protective role of antioxidants against cellular toxicity. *Antioxidants*, *11*(12), 2375.
162. Spillantini, M. G., Schmidt, M. L., Lee, V. M. Y., Trojanowski, J. Q., Jakes, R., & Goedert, M. (1997). α-Synuclein in Lewy bodies. *Nature*, *388*(6645), 839-840.
163. Stamelou, M., & Bhatia, K. P. (2015). Atypical parkinsonism: diagnosis and treatment. *Neurologic clinics*, *33*(1), 39-56.
164. Stiernagle, T. (2006). Maintenance of C. elegans. *WormBook: The online review of C. elegans biology [Internet]*.
165. Su, D., Cui, Y., He, C., Yin, P., Bai, R., Zhu, J., ... & Feng, T. (2025). Projections for prevalence of Parkinson's disease and its driving factors in 195 countries and territories to 2050: modelling study of Global Burden of Disease Study 2021. *bmj*, *388*.
166. Su, Y., Shi, C., Wang, T., Liu, C., Yang, J., Zhang, S., ... & Xu, Y. (2022). Dysregulation of peripheral monocytes and pro-inflammation of alpha-synuclein in Parkinson's disease. *Journal of Neurology*, *269*(12), 6386-6394.
167. Surmeier, D. J., Guzman, J. N., & Sanchez-Padilla, J. (2010). Calcium, cellular aging, and selective neuronal vulnerability in Parkinson's disease. *Cell calcium*, *47*(2), 175-182.

168. Surmeier, D. J., Obeso, J. A., & Halliday, G. M. (2017). Selective neuronal vulnerability in Parkinson disease. *Nature Reviews Neuroscience*, *18*(2), 101-113.
169. Sutphin, G. L., & Kaeberlein, M. (2009). Measuring Caenorhabditis elegans life span on solid media. *Journal of visualized experiments: JoVE*, (27), 1152.
170. Tan, E. K., Chao, Y. X., West, A., Chan, L. L., Poewe, W., & Jankovic, J. (2020). Parkinson disease and the immune system—associations, mechanisms and therapeutics. *Nature Reviews Neurology*, *16*(6), 303-318.
171. Terrin, F., Tesoriere, A., Plotegher, N., & Dalla Valle, L. (2023). Sex and brain: the role of sex chromosomes and hormones in brain development and Parkinson's disease. *Cells*, *12*(11), 1486.
172. Thenganatt, M. A., & Jankovic, J. (2014). Parkinson disease subtypes. *JAMA neurology*, *71*(4), 499-504.
173. Thirugnanam, T., & Santhakumar, K. (2022). Chemically induced models of Parkinson's disease. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *252*, 109213.
174. Tieu, K. (2011). A guide to neurotoxic animal models of Parkinson's disease. *Cold Spring Harbor perspectives in medicine*, *1*(1), a009316.
175. Trinh, J., Hicks, A. A., Koenig, I. R., Delcambre, S., Lueth, T., Schaake, S., ... & Grünewald, A. (2022). Mitochondrial DNA heteroplasmy distinguishes disease manifestation in PINK1-and PRKN-linked Parkinson's disease 2022.05. 17.22275087. *22275087*.
176. Tysnes, O. B., & Storstein, A. (2017). Epidemiology of Parkinson's disease. *Journal of Neural Transmission*, 124(8), 901 -905.
177. Varghese, M., Pandey, M., Samanta, A., Gangopadhyay, P. K., & Mohanakumar, K. P. (2009). Reduced NADH coenzyme Q dehydrogenase activity in platelets of Parkinson's disease, but not Parkinson plus patients, from an Indian population. *Journal of the Neurological Sciences*, *279*(1-2), 39-42.
178. Vidal-Gadea, A., Topper, S., Young, L., Crisp, A., Kressin, L., Elbel, E., ... & Pierce-Shimomura, J. T. (2011). Caenorhabditis elegans selects distinct crawling and swimming gaits via dopamine and serotonin. *Proceedings of the National Academy of Sciences*, *108*(42), 17504-17509.
179. Vinothkumar, K. R., Zhu, J., & Hirst, J. (2014). Architecture of mammalian respiratory complex I. *Nature*, *515*(7525), 80-84.
180. White, J. G., Southgate, E., Thomson, J. N., & Brenner, S. (1986). The structure of the nervous system of the nematode Caenorhabditis elegans. *Philos Trans R Soc Lond B Biol Sci*, *314*(1165), 1-340.
181. Wirth, C., Brandt, U., Hunte, C., & Zickermann, V. (2016). Structure and function of mitochondrial complex I. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1857*(7), 902-914.
182. Xie, H. R., Hu, L. S., & Li, G. Y. (2010). SH-SY5Y human neuroblastoma cell line: in vitrocell model of dopaminergic neurons in Parkinson's disease. *Chinese medical journal*, *123*(8), 1086-1092.
183. Xie, Y., Zhang, A., Qi, M., Xiong, B., Zhang, S., Zhou, J., & Cao, Y. (2023). Pheochromocytoma crisis with refractory Acute Respiratory Distress Syndrome (ARDS), Takotsubo syndrome, emergency adrenalectomy, and need for Extracorporeal Membrane Oxygenation (ECMO) in a previously undiagnosed and asymptomatic patient, due to the use of metoclopramide. *BMC Endocrine Disorders*, *23*(1), 145.

184. Xie, Z., Yang, Q., Song, D., Quan, Z., & Qing, H. (2020). Optogenetic manipulation of astrocytes from synapses to neuronal networks: A potential therapeutic strategy for neurodegenerative diseases. Glia, 68(2), 215-226.
185. Xu, X., Fu, Z., & Le, W. (2019). Exercise and Parkinson's disease. *International review of neurobiology*, *147*, 45-74.
186. Xu, Z., Yang, D., Huang, X., & Huang, H. (2021). Astragaloside IV protects 6-hydroxydopamine-induced SH-SY5Y cell model of Parkinson's disease via activating the JAK2/STAT3 pathway. *Frontiers in neuroscience*, *15*, 631501.
187. Yadav, V. K., Dhanasekaran, S., Choudhary, N., Nathiya, D., Thakur, V., Gupta, R., ... & Patel, A. (2025). Recent advances in nanotechnology for Parkinson's disease: diagnosis, treatment, and future perspectives. *Frontiers in Medicine*, *12*, 1535682.
188. Yamada, T., McGeer, P. L., Baimbridge, K. G., & McGeer, E. G. (1990). Relative sparing in Parkinson's disease of substantia nigra dopamine neurons containing calbindin-D28K. *Brain research*, *526*(2), 303-307.
189. Yang, Z., Yu, Y., Sun, N., Zhou, L., Zhang, D., Chen, H., ... & Gao, Y. (2023). Ginsenosides Rc, as a novel SIRT6 activator, protects mice against high fat diet induced NAFLD. *Journal of Ginseng Research*, *47*(3), 376-384.
190. Yun, S. P., Kim, D., Kim, S., Kim, S., Karuppagounder, S. S., Kwon, S. H., ... & Ko, H. S. (2018). α-Synuclein accumulation and GBA deficiency due to L444P GBA mutation contributes to MPTP-induced parkinsonism. *Molecular neurodegeneration*, *13*, 1-19.
191. Zhang, J., Chen, R., Yu, Z., & Xue, L. (2017). Superoxide dismutase (SOD) and catalase (CAT) activity assay protocols for Caenorhabditis elegans. *Bio-protocol*, *7*(16), e2505-e2505.
192. Zhang, Z., Shen, Y., Luo, H., Zhang, F., Peng, D., Jing, L., ... & Jin, L. (2018). MANF protects dopamine neurons and locomotion defects from a human α-synuclein induced Parkinson's disease model in C. elegans by regulating ER stress and autophagy pathways. Experimental neurology, 308, 59-71.
193. Zorov, D. B., Juhaszova, M., & Sollott, S. J. (2014). Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiological reviews*, *94*(3), 909-950.