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Evaluation of the Synergistic Neuroprotective Effects of *Withania somnifera* and *Amomum subulatum* in Parkinson's disease via Nrf2 signalling pathway

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Abstract

Background: Parkinson's disease (PD) is a progressive neurodegenerative disorder defined by the selective loss of dopaminergic neurons in the substantia nigra pars compacta. Although oxidative stress and neuroinflammation are widely accepted as core pathological drivers, no disease-modifying pharmacotherapy is currently available. The Nrf2/Keap1/ARE signaling axis represents a pivotal endogenous cytoprotective mechanism and an attractive therapeutic target. *Withania somnifera* (Ashwagandha) and *Amomum subulatum* (Large Cardamom) individually exhibit neuroprotective properties; however, their combined potential through Nrf2 modulation has not been systematically explored.

Objective: To evaluate the synergistic neuroprotective effects of *Withania somnifera* and *Amomum subulatum*, individually and in combination, in a reserpine-induced rat model of Parkinson's disease, with mechanistic focus on Nrf2 pathway involvement.

Methods: Forty-two Wistar albino rats were divided into seven groups (n = 6): normal control, disease control (Reserpine 5 mg/kg, i.p.), standard drug (Selegiline 10 mg/kg), *Withania somnifera* ethanolic extract (100 mg/kg) (WS 100), po, *Amomum subulatum* methanolic extract 100 mg/kg po (AS 100), po, combination therapy *Withania somnifera* ethanolic extract (50 mg/kg), *Amomum subulatum* methanolic extract (50 mg/kg) (WS 50 + AS 50), po, and mechanistic group (Trigonelline 30 mg/kg, *Withania somnifera* ethanolic extract (50 mg/kg) and *Amomum subulatum methanolic* extract (50 mg/kg) (TG 30 + WS 50 + AS 50), po. Motor behavior, brain antioxidant enzyme activities (SOD, CAT, GSH, GPx), and substantia nigra histopathology were assessed after 21 days.

Results: Combination therapy produced the most pronounced improvements across all parameters near-normalization of akinesia (2.94 ± 0.21 s), locomotor counts (296.4 ± 11.2), rotarod retention (176.8 ± 6.2 s), and antioxidant enzymes exceeding individual plant treatments and approaching selegiline. Trigonelline-mediated Nrf2 inhibition significantly attenuated these effects, confirming pathway dependence.

Conclusion: The combination of *Withania somnifera* and *Amomum subulatum* exerts synergistic neuroprotection primarily through Nrf2/Keap1/ARE pathway activation, supporting its further development as a complementary strategy in PD management.

Keywords: *Parkinson's disease; Withania somnifera; Amomum subulatum; Nrf2/Keap1/ARE; neuroprotection; oxidative stress; reserpine model; synergism; dopaminergic neurons; antioxidant enzymes*

1. Introduction

Parkinson's disease (PD) is the second most prevalent progressive neurodegenerative disorder globally, affecting an estimated 10 million people, with prevalence projected to double by 2040 owing to demographic ageing[1]. The clinical picture of PD encompasses both motor disturbances resting tremor, bradykinesia, muscular rigidity, and postural instability and a broad spectrum of non-motor features including cognitive

impairment, autonomic dysfunction, sleep disorders, and neuropsychiatric symptoms[2], [3]. At the neuropathological level, PD is defined by the selective degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and by intraneuronal deposition of misfolded α-synuclein in the form of Lewy bodies[4]. Motor symptoms typically emerge only after approximately 70–80% of these neurons have been lost, highlighting the importance of identifying disease-modifying strategies capable of acting before this irreversible threshold is reached[5]. The pathogenesis of PD is multifactorial, involving mitochondrial respiratory chain dysfunction, excessive reactive oxygen species (ROS) generation, chronic neuroinflammation, impaired protein clearance pathways, and apoptotic cascades[6]. Among these, oxidative stress occupies a central position: the SNpc is intrinsically susceptible to oxidative injury due to pro-oxidant dopamine metabolism, elevated mitochondrial activity, and limited endogenous antioxidant reserves[7]. Neuroinflammatory amplification through microglial activation and pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) further accelerates ROS production and neuronal loss[8]. Current pharmacotherapy is centered on dopaminergic replacement principally levodopa, dopamine agonists, and monoamine oxidase-B (MAO-B) inhibitors which address symptoms but do not modify the underlying neurodegenerative process[9]. Long-term levodopa therapy is complicated by motor fluctuations and dyskinesias, while no disease-modifying therapy has yet achieved regulatory approval.

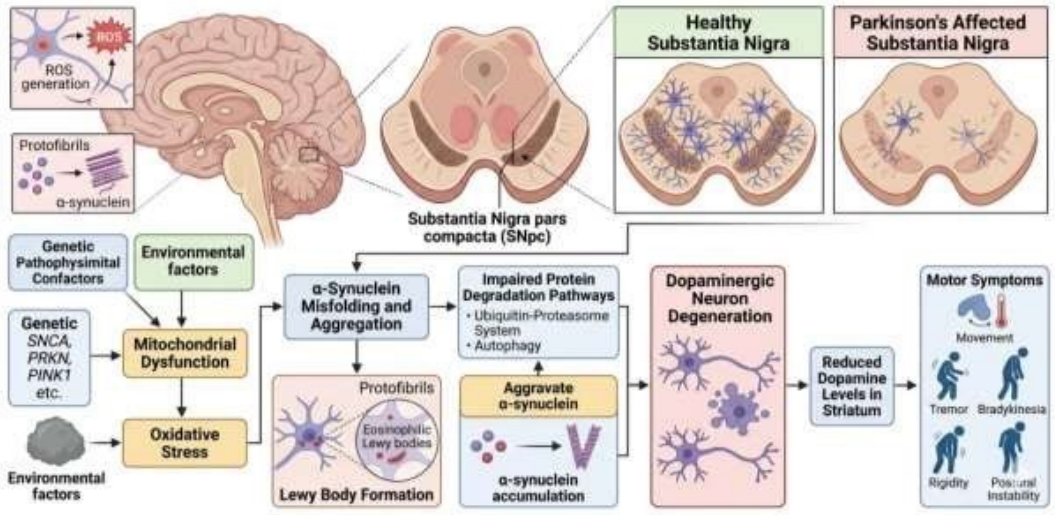

Figure 1.1: The Pathophysiological Cascade of Parkinson's disease (PD). This figure illustrates the multi-factorial neurodegenerative process within the Substantia Nigra pars compacta (SNpc) that leads to the clinical manifestation of Parkinson's Disease.

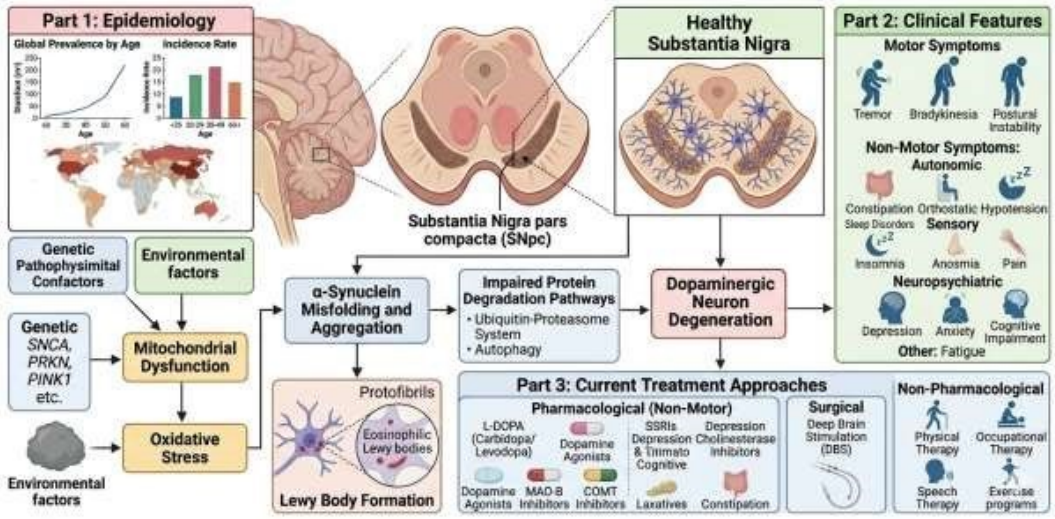

Figure 1.2: Comprehensive Overview of Parkinson's disease.This figure provides a multidimensional summary of Parkinson's Disease (PD), spanning from global population trends to cellular pathology and clinical management.

This unmet need has directed growing research attention toward pathways that can augment intrinsic cytoprotective mechanisms[10].The Nuclear factor erythroid 2-related factor 2 (Nrf2)/Kelch-like ECH-associated protein 1 (Keap1)/antioxidant response element (ARE) axis is an established master regulator of cellular redox homeostasis[11] .Under basal conditions Nrf2 is retained in the cytoplasm by Keap1 and undergoes proteasomal degradation; electrophilic or oxidative stress modifies critical Keap1 cysteine residues, liberating Nrf2 to translocate to the nucleus and drive ARE-dependent transcription of cytoprotective genes including HO-1, NQO1, SOD, catalase, and glutathione biosynthesis enzymes[12]. .Pharmacological Nrf2 activation has consistently attenuated dopaminergic neurodegeneration and improved behavioral outcomes in experimental PD models[13]Within this context, *Withania somnifera* Dunal (Ashwagandha), a foundational Rasayana herb in Ayurvedic medicine, has emerged as a well-characterized neuroprotective agent.[14] Its principal withanolide constituents withaferin A and withanolide A activate Nrf2 through direct modification of Keap1 cysteine residues, suppress α-synuclein aggregation, and protect dopaminergic neurons in multiple PD models[15]. *Amomum subulatum* Roxb (Large Cardamom; family Zingiberaceae), indigenous to the Eastern Himalayan foothills, is rich in 1,8-cineole, α-terpineol, limonene, and diverse phenolics and flavonoids[16].Emerging evidence indicates that 1,8-cineole inhibits NF-κB-mediated neuroinflammation and reduces oxidative stress markers in neurological disease models.[17]. The rationale for combining these two plants rests on pharmacodynamic complementarity: withanolides from *Withania somnifera* address Nrf2-Keap1 interaction and α-synuclein homeostasis, while terpenoid and phenolic constituents of *Amomum subulatum* suppress upstream NF-κB-mediated neuroinflammation and directly scavenge ROS.[18]. Despite individual evidence for each plant, systematic investigation of their combined efficacy and Nrf2 pathway mechanistics remains absent. The present study addresses this gap using a 21-day reserpine-induced Parkinsonian rat model with comprehensive behavioral, biochemical, and histopathological evaluation.

2. Material and Methods

2.1 Ethical Approval and Animals

Forty-two male Wistar albino rats (200–250gm) were procured from an approved institutional animal facility. All procedures were conducted in accordance with the CCSEA guidelines under prior Institutional Animal Ethics Committee approval. Animals were housed under standard conditions (25 ± 2°C, 12-h light/dark cycle) with standard diet and water ad libitum. A seven-day acclimatization period preceded all experimental procedures. Animals will be maintained under standard laboratory conditions according to CCSEA guidelines such as Drinking Water, Feeding , Bedding of Animals etc. The study Experimental protocol approved by the Institutional Animal Ethics Committee (IAEC), MVU University Protocol No. 015/IAEC/MVN/2025 .

2.2 Plant Material Authentication and Extract Preparation

The root powder of *Withania somnifera* and fruit powder of *Amomum subulatum* were procured from an authenticated herbal supplier, Ambe Naturals, and used for the preparation of plant extracts and dried roots of *Withania Somnifera* and fruits of *Amomum subulatum* were separately and subjected to extraction Soxhlet apparatus, The *Withania somnifera* root powder was extracted using ethanol, whereas the *Amomum subulatum* fruit powder was extracted using methanol for 48–72 hours. Extracts were filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator, and stored at 4°C until use.

2.3 Experimental Groups and Treatment Protocol

Animals were randomly allocated to seven groups of six animals each (Table 1). Parkinsonism was induced by a single intraperitoneal injection of reserpine (5 mg/kg), a VMAT-2 inhibitor that produces irreversible monoamine depletion and oxidative dopaminergic neurodegeneration closely mirroring human PD features.Following induction, oral gavage treatments were administered once daily for 21 days. Selegiline served as the standard reference drug due to its selective MAO-B inhibitory and neuroprotective properties[19].[20]. Trigonelline, a naturally occurring Nrf2/ARE inhibitor, was co-administered in the mechanistic group to pharmacologically interrogate Nrf2 pathway dependence[21]

Table 1. Experimental Groups, Treatments, and Dosing Schedule

Group	Treatment	Drug and Dose	No. of Animals
I	Normal Control	Normal saline (daily),OD	6
II	Disease Control	Reserpine 5 mg/kg (Day 1-Day 5),i.p,OD	6
III	Standard Drug	Selegiline 10 mg/kg + Reserpine 5 mg/kg,p.o,OD	6
IV	Test Drug1 (*Withania somnifera* treated) (WSEE)	Withania somnifera 100 mg/kg + Reserpine 5 mg/kg,p.o,OD	6
V	Test Drug 2 (*Amomum subulatum* treated)(ASME)	Amomum subulatum 100 mg/kg + Reserpine 5 mg/kg,p.o,OD	6
VI	Test Drug 1 & 2 (Combination treatment) (WSEE +ASME)	WS 50 mg/kg + AS 50 mg/kg + Reserpine 5 mg/kg,p.o,OD	6
VII	Nrf2 Antagonist + Test Drug 1 & Test Drug 2)(WSEE +ASME)	Trigonelline 30 mg/kg + WS 50 + AS 50 mg/kg + Reserpine 5 mg/kg,p.o,OD	6

List of Abbreviations: -

NC	Normal Control
DC	Disease Control
STD	Standard Drug
OD	Once a Day
IP	Intraperitoneal
p.o	Orally
WSEE	Withania Somniferous Ethanolic Extracts
ASME	Amomum Subulatum Methanolic Extracts

2.4 Behavioral Assessments

Behavioral tests were performed at baseline and on Days 7, and 21. **Akinesia** was quantified by the stepping/bar test, recording latency to initiate voluntary forelimb movement.[22] **Spontaneous locomotor activity** was measured by digital actophotometer over 5 minutes. **Motor coordination and grip strength** were assessed using the rotarod test (constant speed: 20 rpm) and wire-hanging test, with retention time and hanging latency recorded, respectively[23].

2.5 Biochemical Estimation of Antioxidant Enzymes

At study termination (Day 22), animals were euthanized by CO_2 asphyxiation. Brain tissue homogenates were prepared in ice-cold phosphate buffer (0.1 M, pH 7.4). Superoxide dismutase (SOD) activity was measured by the NBT reduction assay (absorbance at 560 nm)[24]. Catalase (CAT) activity was determined by monitoring H_2O_2 decomposition at 240 nm. Reduced glutathione (GSH) was estimated using Ellman's DTNB reagent (412 nm)[25]. Glutathione peroxidase (GPx) activity was assessed by the coupled NADPH oxidation method (340 nm). Protein concentrations were determined by the Lowry method; all enzyme activities were expressed per milligram of protein.

2.6 Histopathological Evaluation

Brain tissues were fixed in 10% neutral buffered formalin (24–48 h), processed for paraffin embedding, and sectioned at 4–5 µm using a rotary microtome. Substantia nigra sections were stained with hematoxylin and eosin (H&E) and examined by light microscopy. Neuronal degeneration was scored semi-quantitatively on a 0–4 scale encompassing neuronal density, vacuolization, pyknosis, gliosis, and inflammatory infiltration.

2.7 Statistical Analysis

All data are expressed as Mean ± Standard Error of Mean (SEM), n = 6. Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test (GraphPad Prism). A p-value < 0.05 was considered statistically significant. Significance levels are denoted as: ###/*** $p < 0.001$; ##/** $p < 0.01$; #/* $p < 0.05$ (### vs. normal control; *** vs. disease control; # vs. combination group).

3. Results

All reserpine-treated animals developed reliable Parkinsonian behavioral and biochemical alterations by Day 1 post-induction, validating the experimental model. No mortality or severe adverse events were recorded in any group throughout the 21-day treatment period.

3.1 Effect on Akinesia

Reserpine administration caused a marked increase in movement initiation time relative to normal controls (9.84 ± 0.52 s vs. 2.15 ± 0.18 s; $p < 0.001$), reflecting severe dopamine-depletion–induced akinesia.[22] Selegiline substantially reduced akinesia latency (3.28 ± 0.24 s; $p < 0.001$ vs. disease control). Individual *Withania somnifera* and *Amomum subulatum* treatments reduced akinesia to 5.46 ± 0.31 s and 5.98 ± 0.36 s, respectively (both $p < 0.01$). Combination therapy achieved near-normalization (2.94 ± 0.21 s; $p < 0.001$), while trigonelline co-treatment significantly reversed this improvement to 6.75 ± 0.42 s ($p < 0.05$ vs. combination), implicating Nrf2 pathway involvement.

3.2 Effect on Locomotor Activity

Actophotometric assessment demonstrated a profound reduction in spontaneous locomotor counts in disease control animals (108.4 ± 8.5 vs. 312.5 ± 12.6; $p < 0.001$). Both individual extracts produced moderate restorations (*Withania somnifera*: 210.8 ± 9.6; *Amomum subulatum*: 198.5 ± 10.1; both $p < 0.01$). Combination therapy produced the highest locomotor recovery (296.4 ± 11.2; $p < 0.001$), approaching normal control values. Trigonelline administration reduced locomotor counts to 176.2 ± 8.8, significantly lower than the combination group ($p < 0.05$).

3.3 Effect on Rotarod Performance and Wire-Hanging Test

Disease control animals exhibited dramatically impaired rotarod retention (68.4 ± 5.2 s vs. 182.6 ± 6.4 s in normal controls; $p < 0.001$). Selegiline restored retention to 165.3 ± 5.8 s. Individual plant treatments improved retention to 128.4 ± 3.8 s and 118.6 ± 5.1 s, respectively. Combination therapy produced the greatest restoration (176.8 ± 6.2 s; $p < 0.001$ vs. disease control), statistically equivalent to selegiline. Wire-hanging test results followed an identical trend, with the combination group demonstrating superior muscular endurance recovery. Trigonelline co-treatment reduced rotarod retention to 103.5 ± 3.9 s.

Table 2. Effect of Treatments on Behavioral Parameters (Mean ± SEM, n = 6)

S No	Group	Akinesia Time(s)	Locomotor Counts(s)	Rotarod Retention(s)	Hanging Time(s)
1	Normal Control	2.15 ± 0.18	312.5 ± 12.6	182.6 ± 6.4	78.4 ± 3.2
2	Disease Control	9.84 ± 0.52 ###	108.4 ± 8.5 ###	68.4 ± 5.2 ###	24.6 ± 2.1 ###
3	Standard Drug	3.28 ± 0.24 ***	275.6 ± 10.4 ***	165.3 ± 5.8 ***	72.1 ± 2.8 ***
4	Test Drug1 (*Withania somnifera* treated)(WSEE)	5.46 ± 0.31 **	210.8 ± 9.6 **	128.4 ± 3.8 **	55.8 ± 2.4 **
5	Test Drug 2 (*Amomum subulatum* treated)(ASME)	5.98 ± 0.36 **	198.5 ± 10.1 **	118.6 ± 5.1 **	51.3 ± 2.6 **
6	Test Drug 1 & 2 (Combination treatment)(WSEE +ASME)	2.94 ± 0.21 ***	296.4 ± 11.2 ***	176.8 ± 6.2 ***	75.6 ± 3.0 ***
7	Nrf2 Antagonist + Test Drug 1 & Test Drug 2)(WSEE +ASME)	6.75 ± 0.42 #	176.2 ± 8.8 #	103.5 ± 3.9 #	42.8 ± 2.2 #

p<0.001 vs Normal Control; ** p<0.01, *** p<0.001 vs Disease Control; # p<0.05 vs Combination group

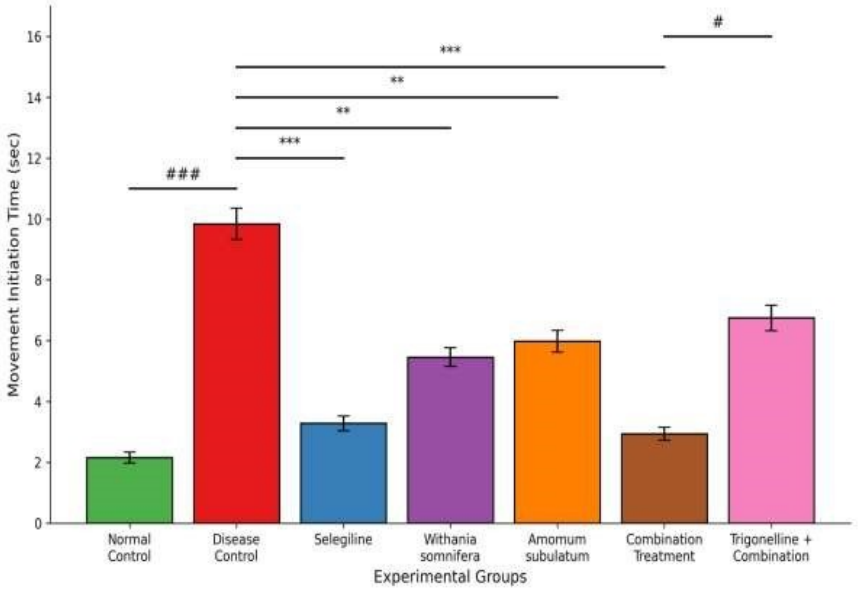

Figure: - 3 Effect of Treatments on Akinesia Test

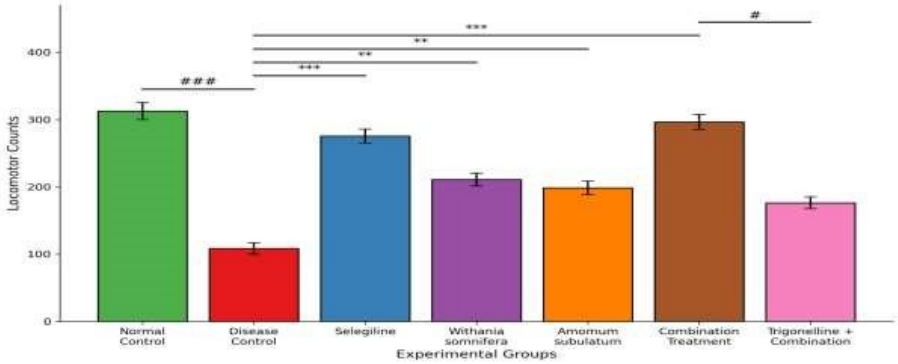

Figure: - 4 Effect of Treatments on Locomotor Activity.

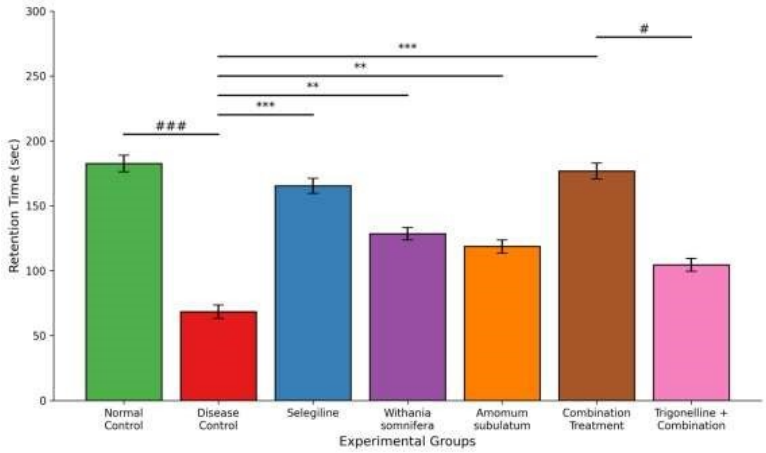

Figure: - 5 Effect of Treatments on Rotarod Performance.

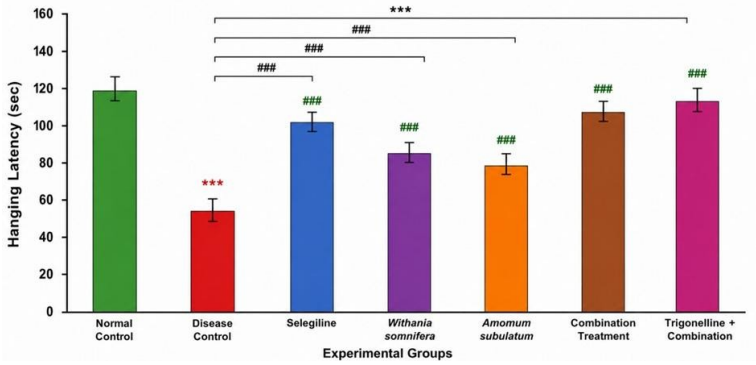

Figure: -6 Effects of Treatment on Hanging Test

3.4 Effect on Brain Antioxidant Enzyme Activities

Disease control animals displayed profound depletion of all four antioxidant parameters relative to normal controls (SOD: 3.15 ± 0.28 vs. 12.4 ± 0.62 U/mg protein; CAT: 16.2 ± 1.06 vs. 42.5 ± 1.84 µmol/min/mg protein; GSH: 2.48 ± 0.21 vs. 8.26 ± 0.42 µg/mg protein; GPx: 3.18 ± 0.24 vs. 9.82 ± 0.48 U/mg protein; all $p < 0.001$), confirming severe reserpine-induced oxidative stress.[26].[27].Combination therapy produced the most complete restoration across all enzymes, significantly exceeding individual plant treatments and approaching normal control levels. Trigonelline co-administration substantially attenuated this antioxidant restoration, supporting Nrf2 pathway dependence (Table 3).

Table 3. Effect of Treatments on Brain Antioxidant Enzyme Activities (Mean ± SEM, n = 6)

S No	Group	SOD(U/mg protein)	CAT (u/min/mg)	GSHx(ug/mg protein)	GPx(U/mg protein)
1	Normal Control	12.4 ± 0.62	42.5 ± 1.84	8.26 ± 0.42	9.82 ± 0.48
2	Disease Control	3.15 ± 0.28 ###	16.2 ± 1.06 ###	2.48 ± 0.21 ###	3.18 ± 0.24 ###
3	Standard Drug	10.85 ± 0.54 ***	38.6 ± 1.42 ***	7.14 ± 0.38 ***	8.64 ± 0.42 ***
4	Test Drug1 (Withania somnifera treated)(WSEE)	8.12 ± 0.41 **	30.4 ± 1.28 **	5.82 ± 0.31 **	6.58 ± 0.35 **
5	Test Drug 2 (Amomum subulatum treated)(ASME)	7.68 ± 0.39 **	28.8 ± 1.16 **	5.41 ± 0.28 **	6.12 ± 0.31 **
6	Test Drug 1 & 2 (Combination treatment)(WSEE +ASME)	11.72 ± 0.58 ***	40.5 ± 1.62 ***	7.92 ± 0.36 ***	9.18 ± 0.44 ***
7	Nrf2 Antagonist + Test Drug 1 & Test Drug 2)(WSEE +ASME)	6.42 ± 0.36 #	23.3 ± 1.18 #	3.38 ± 0.24 #	5.06 ± 0.28 #

p<0.001 vs Normal Control; ** p<0.01, *** p<0.001 vs Disease Control; # p<0.05 vs Combination group.

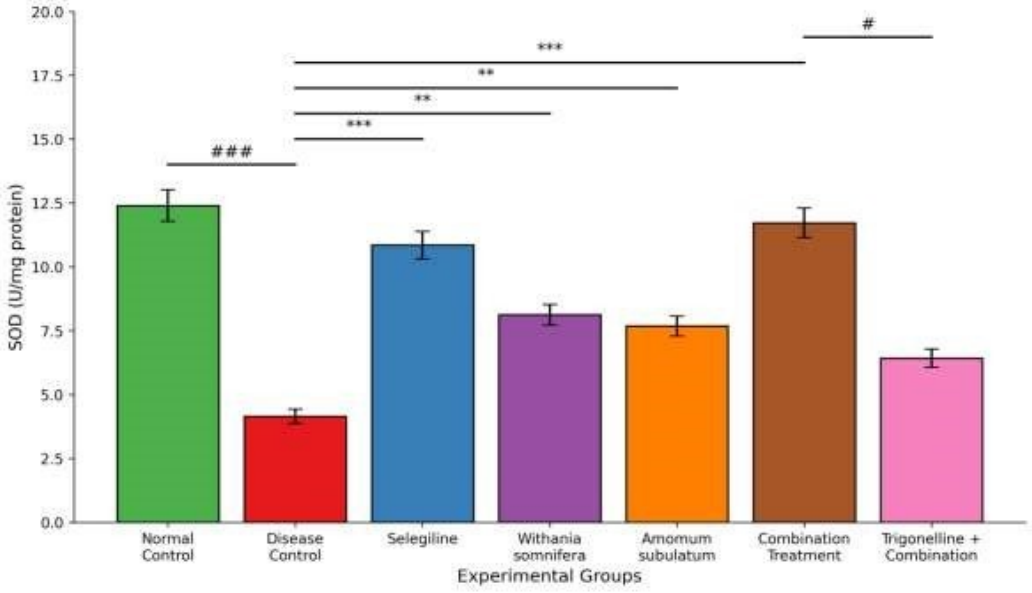

Figure:-7 Effects of Treatments of SOD Activity

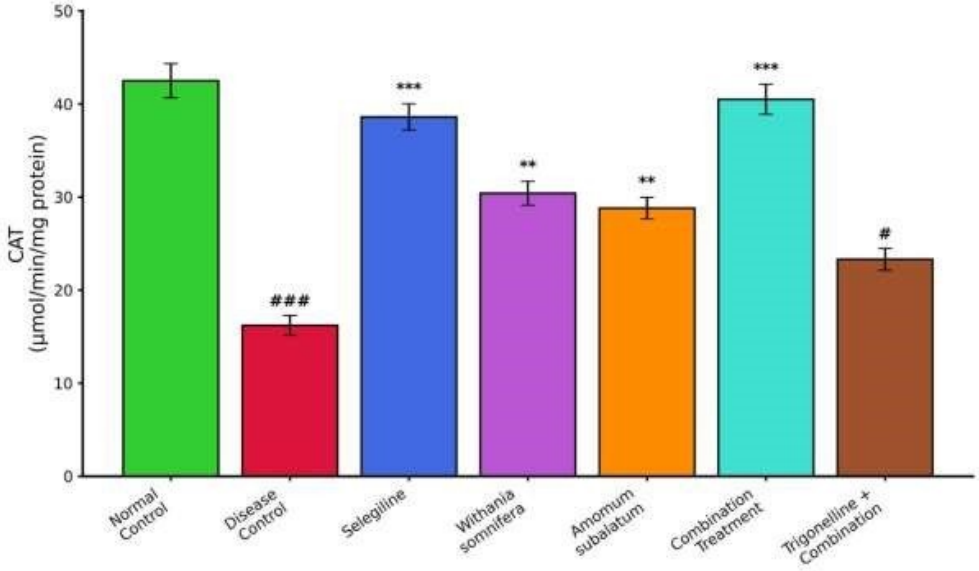

Figure:- 8 Effects of Treatments of CAT.

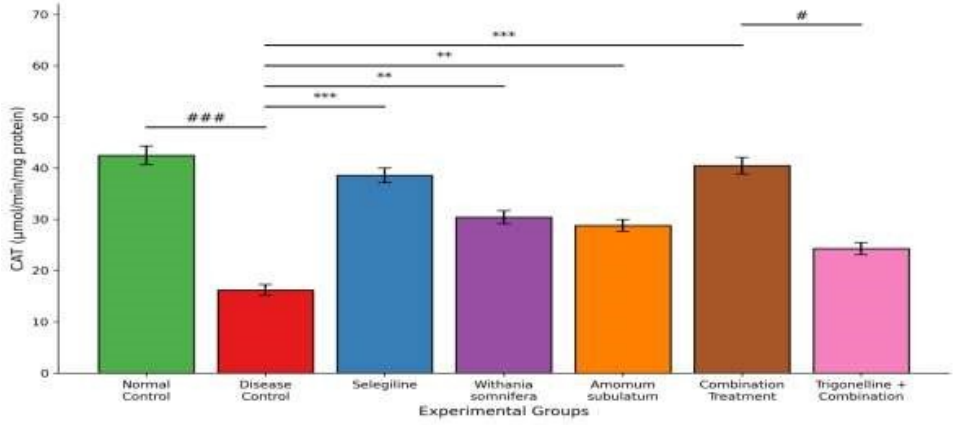

Figure:- 9 Effects of Treatment on GSH Level.

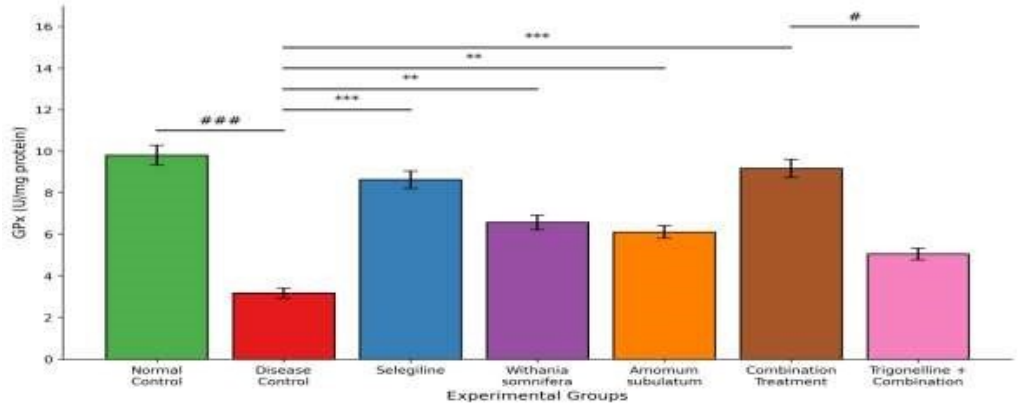

Figure:-10 Effects of Treatment Glutathione Peroxidase.

3.5 Histopathological Findings

Substantia nigra sections from normal control animals showed intact dopaminergic neuronal architecture with normal nuclear morphology and no inflammatory infiltration. Disease control animals demonstrated extensive neuronal degeneration, vacuolization, pyknotic nuclei, gliosis, and inflammatory cell infiltration (degeneration score: 3.00 ± 0.18)[28]. Selegiline treatment markedly preserved neuronal integrity (score: 0.84 ± 0.12). Individual plant treatments provided moderate neuroprotection (*Withania somnifera*: 1.62 ± 0.16; *Amomum subulatum*: 1.78 ± 0.14). Combination therapy produced the most significant neuronal preservation, with near-normal architecture and minimal gliosis (score: 0.48 ± 0.10). Trigonelline co-treatment partially reversed these benefits (score: 2.14 ± 0.18; $p < 0.05$ vs. combination), providing morphological corroboration of Nrf2 pathway involvement.

Table 4. Effect of Treatments on Brain Histopathological Studies (Mean ± SEM, n = 6)

S. No	Group Name	Neuronal Degeneration Score (Mean ± SEM)
1	Normal Control	0.25 ± 0.08
2	Disease Control	3.00 ± 0.18###
3	Standard Drug	0.84 ± 0.12***
4	Test Drug1 (*Withania somnifera* treated)(WSEE)	1.62 ± 0.16**
5	Test Drug 2 (*Amomum subulatum* treated)(ASME)	1.78 ± 0.14**
6	Test Drug 1 & 2 (Combination treatment)(WSEE +ASME)	0.48 ± 0.10***
7	Nrf2 Antagonist + Test Drug 1 & Test Drug 2)(WSEE +ASME)	2.14 ± 0.18#

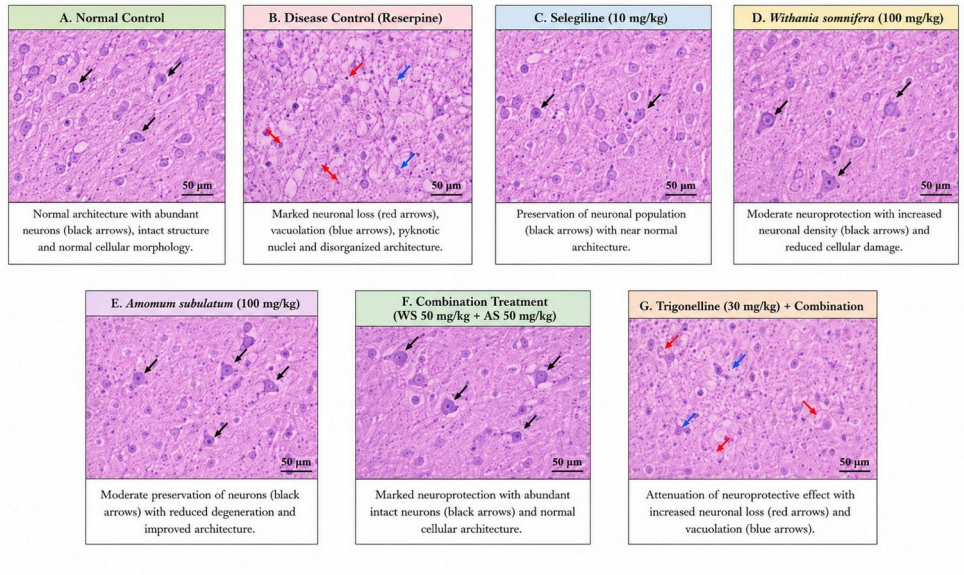

Figure No:-11 Effects of Treatment of Brain Histopathological Studies.

4. Discussion

To our knowledge, this is the first systematic preclinical investigation of the combined neuroprotective effects of *Withania somnifera* and *Amomum subulatum* in a reserpine-induced Parkinsonian model, with pharmacological interrogation of the Nrf2/Keap1/ARE pathway. The convergence of behavioral, biochemical, and histopathological evidence consistently favored the combination over individual plant treatments, and the mechanistic evidence from trigonelline-mediated Nrf2 inhibition confirms that this synergy is substantially pathway-dependent.Reserpine is a well-validated pharmacological tool for modeling key features of PD in rodents. Its irreversible blockade of VMAT-2 depletes vesicular dopamine stores, resulting in cytosolic accumulation of free dopamine that undergoes MAO-catalyzed oxidative metabolism generating superoxide radicals, hydrogen peroxide, and dopamine quinones[29]. This oxidative cascade overwhelms endogenous antioxidant defenses and drives dopaminergic neurodegeneration, faithfully recapitulating the motor deficits and biochemical oxidative burden characteristic of human PD.[23][30] The profound depletion of SOD, CAT, GSH, and GPx activities observed in the present study's disease control group is consistent with this well-established oxidative mechanism[31]. The anti-akinetic and pro-locomotor effects of *Withania somnifera* align with prior reports demonstrating that withanolides particularly withaferin A upregulate tyrosine hydroxylase expression, augment dopamine biosynthesis, reduce α-synuclein aggregation, and suppress NF-κB neuroinflammatory signaling in experimental PD models.[32],[33],[34]. The locomotor and motor coordination improvements with *Amomum subulatum* are consistent with reported MAO-B inhibitory activity of its 1,8-cineole and α-terpineol constituents and potent free radical scavenging through phenolic and flavonoid content.[35] . The superiority of combination therapy over individual plant treatments points to genuine pharmacodynamic synergy[36]. Withanolides from *Withania somnifera* directly target the Nrf2-Keap1 protein-protein interaction, while terpenoid and polyphenolic fractions from *Amomum subulatum* contribute upstream NF-κB suppression, direct ROS scavenging, and metal chelation. These distinct, converging mechanisms acting on shared cytoprotective nodes provide a pharmacological basis for the observed combination superiority[37]. The mechanistic group provided pivotal evidence: trigonelline, which competes with Nrf2 for ARE binding without direct cytotoxicity,[38]. significantly attenuated all combination-mediated improvements in a partial rather than complete manner. This partial reversal indicates that, while Nrf2/ARE activation is the primary mediator of the combination's neuroprotective efficacy, Nrf2-independent mechanisms — including direct free radical scavenging, MAO-B inhibition, and anti-neuroinflammatory activity also contribute meaningfully.[39] This multitarget pharmacological profile is particularly well-suited to the multifactorial pathogenesis of PD[40]. The comprehensive antioxidant enzyme restoration achieved by combination therapy is mechanistically consistent with known ARE-driven transcriptional induction of SOD, CAT, and glutathione biosynthesis enzymes by withanolide-mediated Nrf2 activation[41]. complemented by metal chelation and GSH-sparing effects of *Amomum subulatum* flavonoids and polyphenols[42],[43]. Histopathological near-normalization of substantia nigra architecture in the combination group provides definitive morphological confirmation of these functional findings[44]. Both plants carry well-established safety profiles with extensive histories of human use[45],[46]. Nonetheless, limitations include the reserpine model's inability to reproduce progressive neurodegeneration or Lewy body pathology, uncertainty regarding optimal combination dose ratios, and the absence of molecular Nrf2 target gene quantification and formal pharmacokinetic characterization.[47],[48]. Future studies employing transgenic α-synuclein models, [49],[50]. Western blot and immunohistochemical quantification of Nrf2/HO-1/NQO1, and full pharmacokinetic profiling will be essential for advancing this combination toward clinical evaluation[51].

5. Conclusion

The combination of *Withania somnifera* and *Amomum subulatum* exerts synergistic, multitarget neuroprotective effects in reserpine-induced experimental Parkinsonism. The combination achieved near-complete normalization of motor function, antioxidant enzyme activities, and dopaminergic neuronal architecture — outcomes comparable to the standard drug selegiline and significantly superior to either plant alone. Trigonelline-based Nrf2 inhibition confirmed that Nrf2/Keap1/ARE pathway activation is the principal mechanism, with secondary contributions from Nrf2-independent pathways. Given established safety profiles and traditional use in Ayurvedic medicine, this polyherbal combination represents a pharmacologically rational and evidence-based candidate for further development as a complementary neuroprotective strategy in Parkinson's disease management.

References

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