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"In Vitro Neuroprotective Evaluation of a Herbal Blend of Emblica Officinalis and WithaniaSomniferaagainst oxidative Stress-Induced Neurotoxicity in SH-SY5Y Cells"

Singh Sahahagal Surendra, Monika Pawar, Dr. Satish kumar Sarankar
Faculty of Pharmacy Mansarovar Global University, Sehore

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ABSTRACT

Background: Neurodegenerative disorders are progressive neurological conditions associated with neuronal loss, oxidative stress, neuroinflammation, mitochondrial dysfunction, apoptosis, and impaired neurotransmission. The limited ability of currently available pharmacological therapies to modify disease progression has encouraged the investigation of medicinal plants possessing antioxidant and neuroprotective properties. *Emblica officinalis* and *Withaniasomnifera* are traditionally used medicinal plants with diverse bioactive constituents that may provide complementary neuroprotective effects.

Objective: The present study was designed to evaluate the antioxidant, neuroprotective, and cholinesterase inhibitory potential of ethanolic extracts of *Emblica officinalis* fruits and *Withaniasomnifera* roots, individually and in combination as a 1:1 herbal blend.

Materials and Methods: Plant materials were extracted separately with 70% ethanol using a Soxhlet apparatus. Preliminary phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC), and HPTLC standardization were performed. Antioxidant activity was evaluated using DPPH, ABTS, and FRAP assays. Neuroprotective activity was assessed in H_2O_2 -induced oxidative stress in human SH-SY5Y neuroblastoma cells using MTT cell viability, DCFH-DA ROS estimation, morphological examination, and estimation of SOD, CAT, GSH, and MDA. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities were determined using Ellman's method. Donepezil was used as the reference drug. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

Results: The extraction yields of *E. officinalis* and *W. somnifera* were 18.74 ± 0.82% and 11.63 ± 0.54%, respectively. *E. officinalis* exhibited the highest TPC (182.64 ± 4.31 mg GAE/g) and TFC (104.83 ± 2.74 mg QE/g), while the herbal blend retained substantial phenolic and flavonoid contents. HPTLC analysis confirmed gallic acid and withanolide-A as characteristic marker compounds. The herbal blend demonstrated superior antioxidant activity, with DPPH and ABTS IC_{50} values of 18.36 ± 0.58 and 15.64 ± 0.49 µg/mL, respectively, and FRAP activity of 812.37 ± 16.48 µM Fe^{2+} equivalents/g extract. In SH-SY5Y cells, H_2O_2 reduced cell viability to 42.37 ± 1.96%, whereas treatment with the herbal blend restored viability to 84.73 ± 2.35%. The blend also markedly reduced intracellular ROS, restored SOD and CAT activities, increased GSH levels, and reduced MDA levels. AChE and BChE activities were also suppressed following treatment with the herbal formulation.

Conclusion: The findings demonstrate that the 1:1 herbal blend of *Emblica officinalis* and *Withaniasomnifera* possesses significant antioxidant, neuroprotective, and cholinesterase inhibitory activities. Its protective effects may be associated with the combined action of polyphenolic constituents and withanolides through modulation of oxidative stress and cholinergic pathways. The formulation therefore represents a promising natural therapeutic candidate for further investigation in oxidative stress-associated neurodegenerative disorders.

Keywords:*Emblica officinalis*; *Withaniasomnifera*; Neuroprotection; Oxidative stress; SH-SY5Y; Antioxidant; Cholinesterase; Herbal formulation.

I. Introduction

Neurodegenerative disorders are progressive diseases characterized by the gradual loss of neuronal structure and function, leading to cognitive, behavioral, and motor impairments. The increasing prevalence of conditions such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral

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sclerosis has become a major global health concern, particularly with the aging population. Although the exact causes of these disorders are multifactorial, oxidative stress, neuroinflammation, mitochondrial dysfunction, apoptosis, and impaired neurotransmission are recognized as key contributors to neuronal degeneration. Among these, oxidative stress and chronic inflammation play central roles in accelerating disease progression and cognitive decline.

Current pharmacological treatments mainly provide symptomatic relief and have limited ability to halt disease progression. Consequently, growing attention has been directed toward natural products with antioxidant, anti-inflammatory, and neuroprotective properties. Medicinal plants such as *Emblica officinalis* and *Withaniasomnifera* have shown promising potential in protecting neurons through multiple mechanisms, including the reduction of oxidative stress and preservation of cholinergic function. This chapter provides an overview of the nervous system, the molecular mechanisms underlying neurodegeneration, and the therapeutic potential of herbal interventions in the prevention and management of neurodegenerative disorders.

II. Materials and Methods

Plant Materials and Extraction

Fresh fruits of *Emblica officinalis* and dried roots of *Withaniasomnifera* were procured from authenticated herbal suppliers and botanically authenticated. The plant materials were shade-dried, powdered, and extracted separately with 70% ethanol using a Soxhlet apparatus. The extracts were concentrated under reduced pressure using a rotary evaporator, dried, and stored at 4°C until further use.

Preparation of Herbal Blend

The dried extracts of *E. officinalis* and *W. somnifera* were mixed in a 1:1 (w/w) ratio to prepare the herbal blend. Stock solutions were prepared in dimethyl sulfoxide (DMSO), and the final DMSO concentration in all experiments was maintained below 0.1%.

Chemicals and Reagents

Analytical-grade chemicals and cell culture reagents were obtained from Sigma-Aldrich, Merck, HiMedia, and Gibco. The human neuroblastoma SH-SY5Y cell line was obtained from the National Centre for Cell Science (NCCS), Pune, India. Donepezil hydrochloride was used as the reference standard.

Phytochemical Analysis and In Vitro Antioxidant Evaluation

Preliminary phytochemical screening of the ethanolic extracts of *Emblica officinalis* and *Withaniasomnifera* was performed using standard qualitative methods to identify major secondary metabolites, including alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, terpenoids, steroids, and carbohydrates.

The total phenolic content (TPC) and total flavonoid content (TFC) were determined by the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively. Gallic acid and quercetin were used as reference standards, and the results were expressed as mg gallic acid equivalents (GAE)/g extract and mg quercetin equivalents (QE)/g extract.

Phytochemical standardization of the extracts was carried out by high-performance thin-layer chromatography (HPTLC) using gallic acid and withanolide-A as marker compounds for *E. officinalis* and *W. somnifera*, respectively.

The antioxidant potential of the individual extracts and the herbal blend was evaluated using DPPH radical scavenging, ABTS radical cation decolorization, and ferric reducing antioxidant power (FRAP) assays. Ascorbic acid was used as the reference antioxidant, and antioxidant activity was expressed as percentage radical.

Cell Culture and Neuroprotective Evaluation

Human neuroblastoma SH-SY5Y cells (NCCS, Pune, India) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin, and 1% L-glutamine at 37°C in a humidified atmosphere containing 5% CO_2 . Cells were subcultured at 80–90% confluence using 0.25% trypsin–EDTA. Oxidative stress was induced with 200 µM hydrogen peroxide (H_2O_2) for 3 h. Cells were pretreated with *Emblica officinalis* extract, *Withaniasomnifera* extract, the herbal blend (1:1), or donepezil (5 µM) for 24 h before H_2O_2 exposure. Untreated and H_2O_2 -treated cells served as normal and toxic controls, respectively.

Assessment of Neuroprotective Activity

Cell viability was determined using the MTT assay, while intracellular reactive oxygen species (ROS) generation was measured using the DCFH-DA fluorescence assay. Morphological changes in SH-SY5Y cells were examined under an inverted phase-contrast microscope. Antioxidant status was evaluated by estimating superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA) levels in cell lysates using standard spectrophotometric methods.

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Cholinesterase Inhibition and Statistical Analysis
Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities were determined using Ellman's colorimetric method, with donepezil as the reference standard. Protein content was estimated by the Lowry method for normalization of enzyme activities. All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way ANOVA followed by Tukey's post hoc test in GraphPad Prism 10.0. A p value < 0.05 was considered statistically significant.
avenging and IC_{50} values where applicable.

Instruments, Reagents, and Statistical Analysis
All analytical and cell culture experiments were performed using standard laboratory instruments, including a UV–Visible spectrophotometer (Shimadzu UV-1800), HPTLC system (CAMAG, Switzerland), CO_2 incubator (Thermo Fisher Scientific), fluorescence and ELISA microplate readers (BioTek and Bio-Rad), inverted phase-contrast microscope (Olympus), and rotary vacuum evaporator (Heidolph, Germany). All reagents and buffer solutions, including phosphate-buffered saline (PBS), hydrogen peroxide (H_2O_2), MTT, DCFH-DA, and DTNB, were freshly prepared using analytical-grade chemicals according to standard protocols.
Calibration curves for gallic acid and quercetin (10–100 µg/mL) were used for the determination of total phenolic and total flavonoid contents, respectively. IC_{50} values for antioxidant and cholinesterase inhibition assays were calculated by nonlinear regression using GraphPad Prism software.
All experiments were performed in triplicate (n = 3), and the results were expressed as mean ± standard deviation (SD). Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using GraphPad Prism (Version 9.0/10.0). Differences were considered statistically significant at p< 0.05. Graphs were prepared using GraphPad Prism and OriginPro, and all experimental data were recorded and analyzed following standard laboratory and biosafety practices.

III. Results and Discussion

Extraction Yield and Phytochemical Characterization

Hydroethanolic Soxhlet extraction of *Emblica officinalis* fruits and *Withaniasomnifera* roots produced dark brown semisolid extracts with percentage yields of **18.74 ± 0.82%** and **11.63 ± 0.54%**, respectively. The higher yield obtained for *E. officinalis* may be attributed to its abundance of water-soluble phenolics and tannins. Both extracts showed good solubility in DMSO and were suitable for subsequent in vitro analyses.

Preliminary phytochemical screening revealed the presence of several bioactive constituents, including phenolics, flavonoids, glycosides, alkaloids, terpenoids, and saponins. *E. officinalis* was particularly rich in phenolics, flavonoids, and tannins, whereas *W. somnifera* contained higher levels of terpenoids, steroidal compounds, and alkaloids. These phytoconstituents are known for their antioxidant, anti-inflammatory, and neuroprotective activities, suggesting that the combined herbal formulation may exert synergistic protective effects against oxidative stress-induced neuronal injury.
The higher extraction yield and phytochemical richness observed in the present study are consistent with previous reports on hydroethanolic extracts of *E. officinalis* and *W. somnifera*, supporting their suitability as potential neuroprotective agents.

Table1OrganolepticCharacteristicsandPercentageYieldofHerbalExtracts

Parameter	***E.officinalis* Extract**	***W. somnifera*Extract**
Plantpartused	Fruit	Root
Extractionsolvent	70%Ethanol	70%Ethanol
Appearance	Darkbrownsemisolid	Brownsemisolid
Odor	Characteristicfruity	Characteristicearthy
Consistency	Stickysemisolid	Viscoussemisolid
Percentageyield(%w/w)	18.74±0.82	11.63±0.54

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Table2PreliminaryPhytochemicalScreeningofHerbalExtracts

Phytoconstituent	***E.officin"lis*Extract**	***W.somnifem*Extract**
Alkaloids	+	+
Flavonoids	+++	++
Phenolics	+++	++
Tannins	+++	+
Glycosides	++	++
Saponins	+	++
Terpenoids	++	+++
Steroids	-	+++
Carbohydrates	++	+

Total Phenolic and Flavonoid Content

The total phenolic content (TPC) and total flavonoid content (TFC) of the extracts and herbal blend were determined using the Folin–Ciocalteu and aluminum chloride methods, respectively. *Emblica officinalis* exhibited the highest TPC (**182.64 ± 4.31 mg GAE/g extract**) and TFC (**104.83 ± 2.74 mg QE/g extract**), followed by the herbal blend (**148.52 ± 3.86 mg GAE/g** and **87.36 ± 2.52 mg QE/g**) and *Withaniasomnifera* (**96.27 ± 3.18 mg GAE/g** and **61.44 ± 2.19 mg QE/g**). The higher phenolic and flavonoid contents of *E. officinalis* are likely due to the presence of gallic acid, ellagic acid, emblicanins, and hydrolysable tannins. The herbal blend retained substantial amounts of these antioxidant phytoconstituents, indicating that combining the extracts enhanced the overall phytochemical profile. These findings suggest that the high phenolic and flavonoid contents may contribute to the antioxidant and neuroprotective potential of the herbal formulation by scavenging reactive oxygen species and protecting neuronal cells from oxidative damage.

Table3TotalPhenolicContentofHerbalExtractsandBlend

Sample	**TotalPhenolicContent(mgGAE/gextract)**
*E.officinalis*Extract	182.64±4.31
*Wsomnifera*Extract	96.27±3.18
HerbalBlend(1:**l)**	148.52±3.86

Table4TotalFlavonoidContentofHerbalExtractsandBlend

Sample	**TotalFlavonoidContent(mgQE/gextract)**
*E.officinalis*Extract	104.83±2.74
*Wsomnifera*Extract	61.44±2.19
HerbalBlend(I:I)	87.36±2.52

HPTLC Standardization

HPTLC analysis confirmed the presence of characteristic marker compounds in both extracts. *Emblica officinalis* showed a prominent peak corresponding to **gallic acid**, whereas *Withaniasomnifera* exhibited a characteristic peak corresponding to **withanolide-A**. The chromatographic fingerprints demonstrated good peak resolution and reproducibility, confirming the phytochemical identity and quality of the extracts. These results validated the successful extraction of the major bioactive constituents and supported the use of the standardized extracts for subsequent antioxidant and neuroprotective studies.

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Table5HPTLCAnalysisofMarkerCompoundsinHerbalExtracts

Extract	MarkerCompound	ObservedRfValue	StandardRfValue
*E.officinalis*Extract	Gallicacid	0.54±0.02	0.53±0.01
*Wsomnifera*Extract	Withanolide-A	0.68±0.03	0.67±0.02

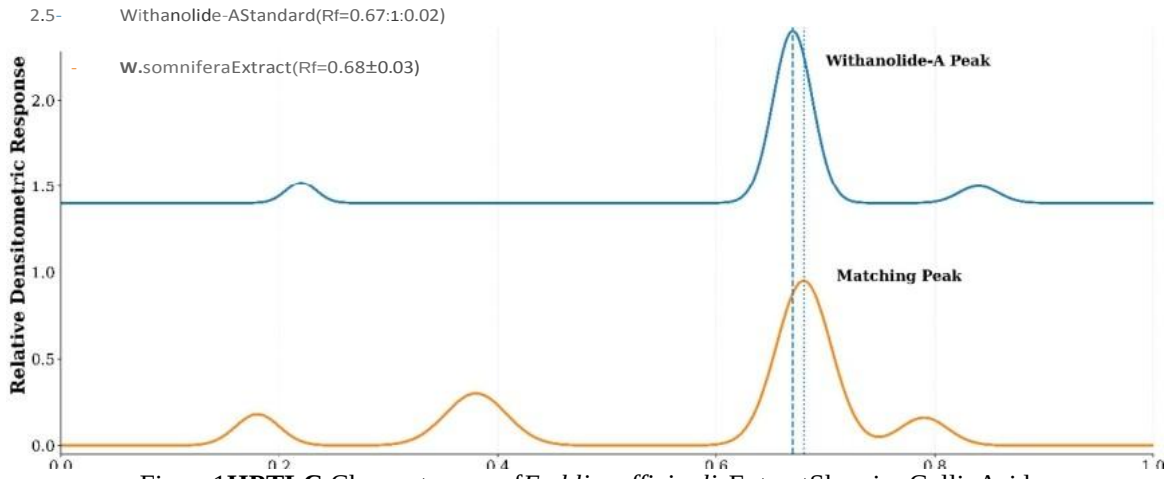

Figure1**HPTLC** Chromatogramof*Emblicaofficinalis*ExtractShowingGallicAcid

Peak

HPTLC Standardization

HPTLC fingerprint analysis confirmed the presence of the characteristic marker compounds in both herbal extracts. *Emblica officinalis* showed a peak corresponding to **gallic acid** (Rf = **0.54 ± 0.02**), while *Withaniasomnifera* exhibited a peak corresponding to **withanolide-A** (Rf = **0.68 ± 0.03**). The observed Rf values closely matched those of the respective standards, confirming the phytochemical identity, quality, and reproducibility of the extracts used for subsequent biological studies.

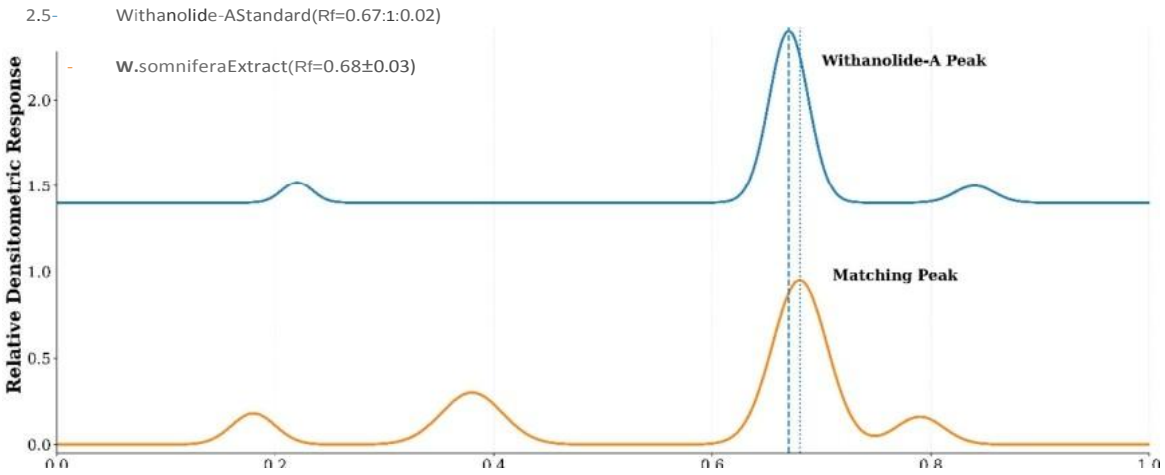

Figure2HPTLCChromatogramof*Withaniasomnifera*ExtractShowing Withanolide-A Peak

In Vitro Antioxidant Activity

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The antioxidant potential of the extracts and the herbal blend was evaluated using DPPH, ABTS, and FRAP assays. In all assays, antioxidant activity was concentration-dependent. The herbal blend demonstrated significantly greater antioxidant activity than the individual extracts, indicating a synergistic interaction between the phytoconstituents of *E. officinalis* and *W. somnifera*.

Table6DPPHRadicalScavengingActivityofHerbal ExtractsandBlend

Sample	**ICso(µg/mL)**
Ascorbicacid	11.42±0.38
*E.officinalis*Extract	24.83±0.74
W somnifera Extract	41.67± 1.12
Herbal Blend (1:1)	18.36 ± 0.58

In the **DPPH assay**, the herbal blend exhibited a lower IC_{50} value (**18.36 ± 0.58 µg/mL**) than *E. officinalis* (**24.83 ± 0.74 µg/mL**) and *W. somnifera* (**41.67 ± 1.12 µg/mL**), approaching the activity of ascorbic acid (**11.42 ± 0.38 µg/mL**). Similar results were observed in the **ABTS assay**, where the herbal blend showed an IC_{50} of **15.64 ± 0.49 µg/mL**, compared with **21.52 ± 0.61 µg/mL** for *E. officinalis* and **38.91 ± 1.03 µg/mL** for *W. somnifera*. Furthermore, the **FRAP assay** demonstrated a higher ferric reducing capacity for the herbal blend (**812.37 ± 16.48 µM Fe^{2+} equivalents/g extract**) than *W. somnifera* (**436.58 ± 12.61 µM Fe^{2+} equivalents/g extract**) and a value comparable to *E. officinalis* (**741.63 ± 15.24 µM Fe^{2+} equivalents/g extract**).

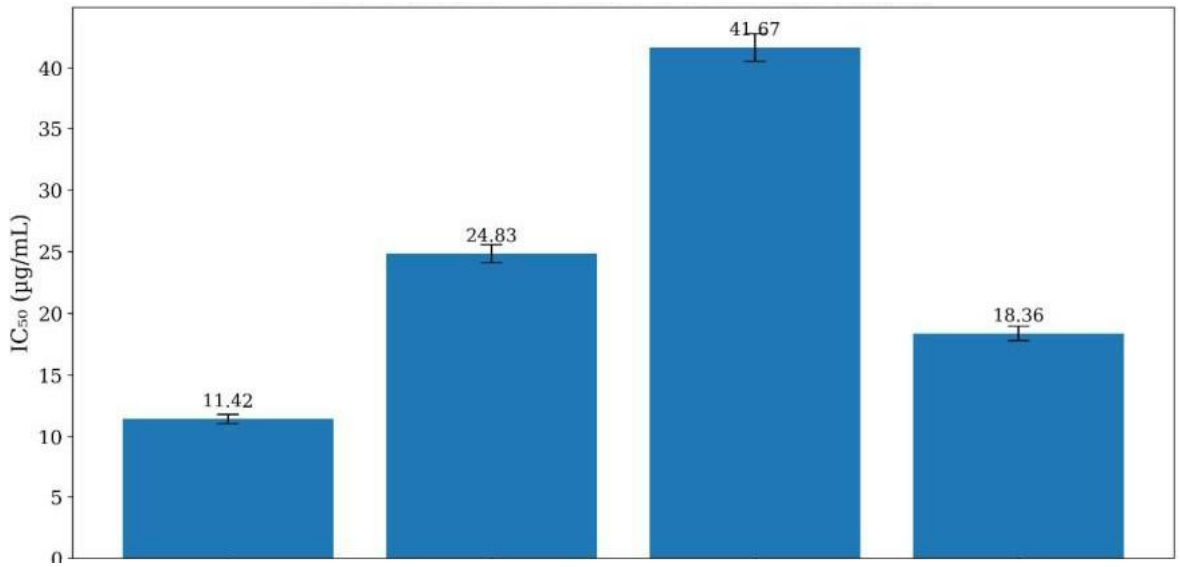

Figure3 DPPHRadicalScavengingActivityofHerbalExtractsandBlend

ABTSRadicalScavenging Activity

Table7ABTSRadicalScavengingActivityofHerbalExtractsandBlend

Sample	**ICso(µg/mL)**
Ascorbicacid	9.84±0.27
*E.officinalis*Extract	21.52 ±0.61
*Wsomnifera*Extract	38.91±1.03
HerbalBlend(1:1)	15.64±0.49

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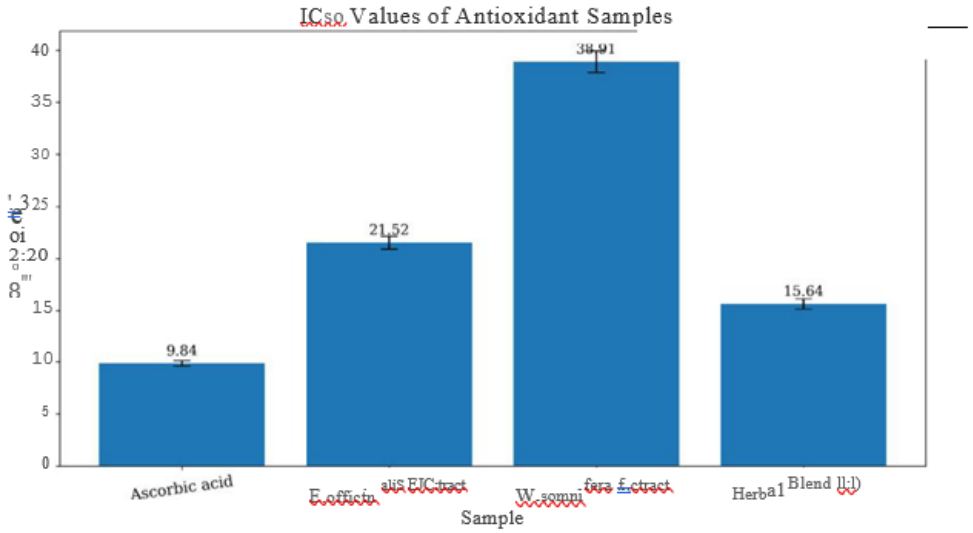

Figure4ABTSRadicalScavengingActivityofHerbalExtractsandBlend

FerricReducingAntioxidantPower(FRAP)

Table8FerricReducingAntioxidantPowerofHerbalExtractsandBlend

Sample	FRAPValue(µMFe2+equivalents/gextract)
Ascorbicacid	892.45±18.72
*E.officinalis*Extract	741.63±15.24
*Wsomnifera*Extract	436.58±12.61
HerbalBlend (1:1)	812.37±16.48

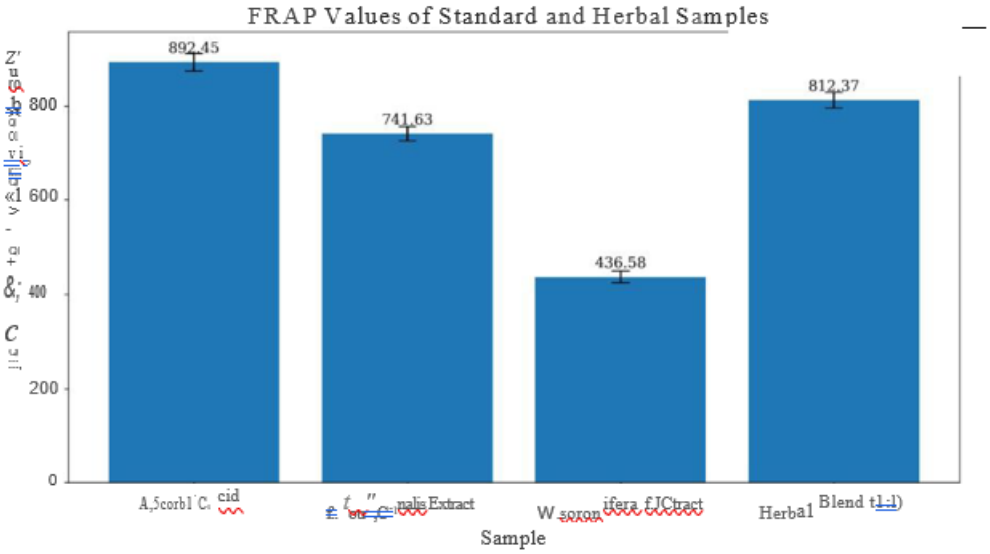

Figure5FerricReducingAntioxidantPowerofHerbalExtractsand Blend

Neuroprotective Activity in SH-SY5Y Cells **Cell Viability Assay**

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The neuroprotective effect of the extracts and herbal blend against H_2O_2 -induced oxidative injury was evaluated by the MTT assay. Exposure to 200 µM H_2O_2 significantly reduced SH-SY5Y cell viability to **42.37 ± 1.96%** compared with the normal control (**100.00 ± 2.41%**), confirming successful induction of oxidative stress. Pretreatment with *E. officinalis*, *W. somnifera*, and the herbal blend significantly improved cell survival in a concentration-dependent manner. The herbal blend exhibited the highest cytoprotective effect, restoring cell viability to **84.73 ± 2.35%**, which was comparable to donepezil (**89.16 ± 2.04%**). These findings indicate that the combined formulation provides superior protection against oxidative neuronal damage, likely due to the synergistic antioxidant effects of its phytoconstituents.

The herbal blend also demonstrated dose-dependent neuroprotection, with cell viability increasing from **56.74 ± 1.63%** at **12.5 µg/mL** to **84.73 ± 2.35%** at **100 µg/mL**, indicating enhanced cytoprotective efficacy with increasing concentration.

Table9EffectofHerbalExtractsandBlendonCellViabiJityofSH-SYSYCells

TreatmentGroup	**CellViability**(%)
NormalControl	100.00±2.41
H2O2ToxicControl	42.37±1.96
*E.officinalis*Extract(100µg/mL)+H2O2	71.46±2.12
*Wsomnifera*Extract(100µg/mL)+H2O2	66.25±1.88
HerbalBlend(100µg/m.L)+ HiO2	84.73±2.35
Donepezil+H2O2	89.16±2.04

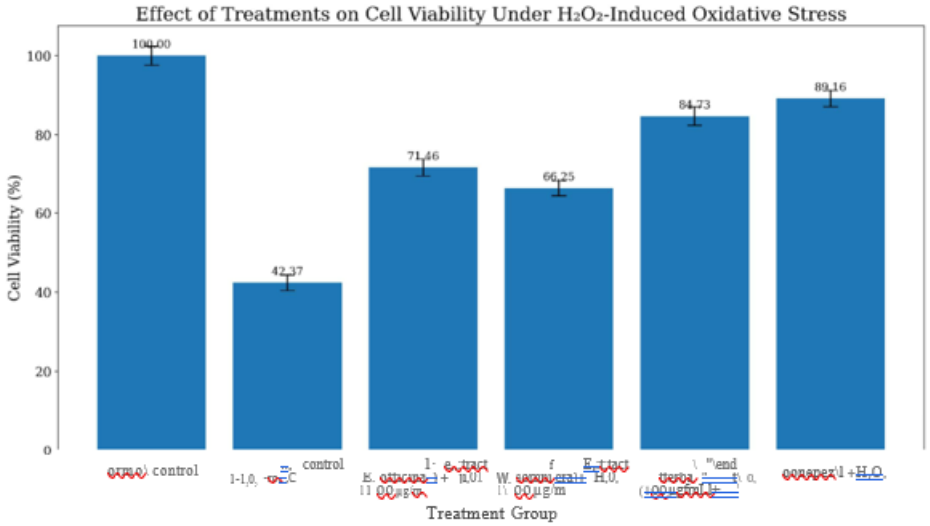

Figure 6 Effect ofHerbal Extracts andBlend on ViabilityofSH-SYSY Cells

Concentration-DependentNeuroprotectiveActivityofHerbalBlend

Table10Concentration-DependentNeuroprotectiveActivityofHerbalBlend

Concentration(µg/mL)	**CellViability**(%)
12.5	56.74±1.63
25	64.28 ± 1.95
50	73.86 ± 2.11
100	84.73 ± 2.35

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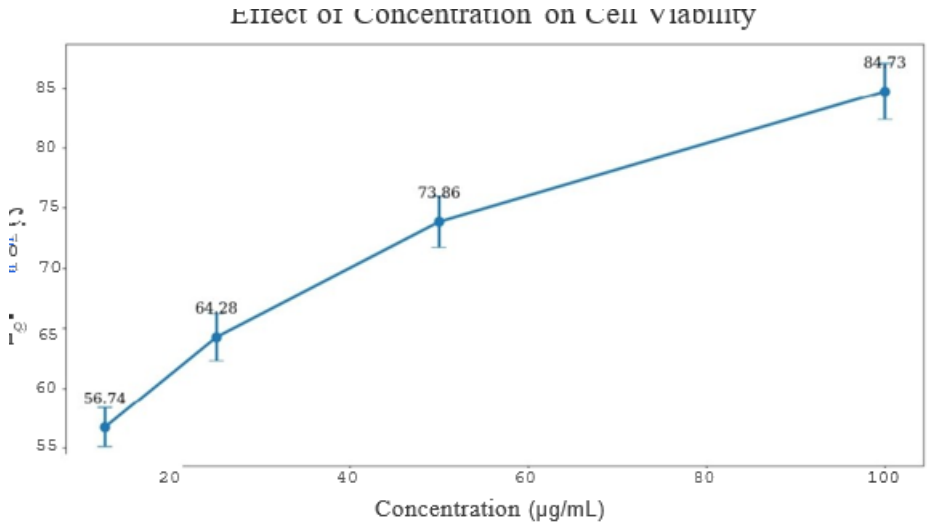

Figure7Concentration-DependentNeuroprotectiveEffectof Herbal Blend inSH- SYSY Cells

Intracellular ROS Generation

Intracellular reactive oxygen species (ROS) levels were quantified using the DCFH-DA fluorescence assay. H_2O_2 treatment markedly increased ROS production to **278.54 ± 5.72%** of the control, whereas pretreatment with the herbal extracts significantly suppressed oxidative stress. The herbal blend showed the greatest reduction in ROS levels (**128.52 ± 3.94%**), approaching the effect of donepezil (**118.46 ± 3.35%**). These results suggest that the combined extract effectively scavenges intracellular free radicals and enhances cellular antioxidant defense mechanisms. The pronounced reduction in ROS generation is consistent with the high phenolic and flavonoid contents of the formulation and supports its potent neuroprotective activity against oxidative stress-induced neuronal injury.**4.8 Morphological Assessment of SH-SY5Y Cells**

Phase-contrast microscopic analysis revealed significant morphological changes in SH-SY5Y cells following H_2O_2 -induced oxidative stress. Normal control cells displayed typical neuronal characteristics, including intact cell membranes, well-developed neurite extensions, and uniform distribution. In contrast, H_2O_2 -treated cells showed marked cellular damage, including cell shrinkage, rounding, membrane blebbing, reduced neurite formation, and decreased cell density.

Pretreatment with *E. officinalis*, *W. somnifera*, and the herbal blend improved cellular morphology and reduced oxidative damage. The herbal blend-treated group showed better preservation of neuronal architecture, enhanced cell attachment, and maintenance of neurite-like projections compared with individual extract treatments. These morphological observations were consistent with MTT and ROS assay findings, confirming the protective effect of the herbal blend against oxidative stress-induced neuronal injury.

Table11EffectofHerbalExtractsandBlendonIntracellularROSLevels

TreatmentGroup	**RelativeFluorescenceIntensity**(%)
NormalControl	100.00±3.16
H2O2ToxicControl	278.54±5.72
*E.officinalis*Extract+lhO2	168.43±4.26
*Wsomnifera*Extract+H2O2	182.37±4.81
HerbalBlend+H2O2	128.52±3.94
Donepezil+H2O2	118.46±3.35

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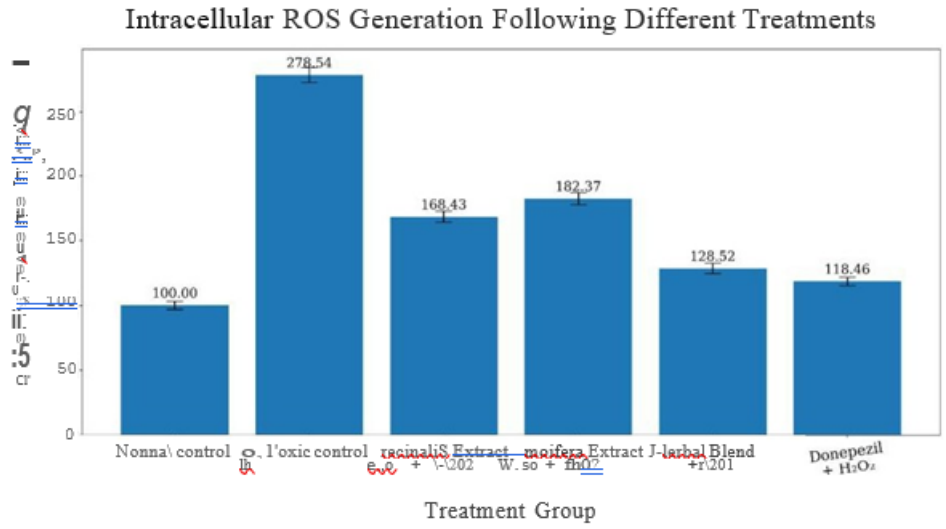

Figure8EffectofHerbal Extractsand BlendonIntracellularROSGeneration in SH-SYSY Cells

MorphologicalAssessmentofSH-SYSYCells

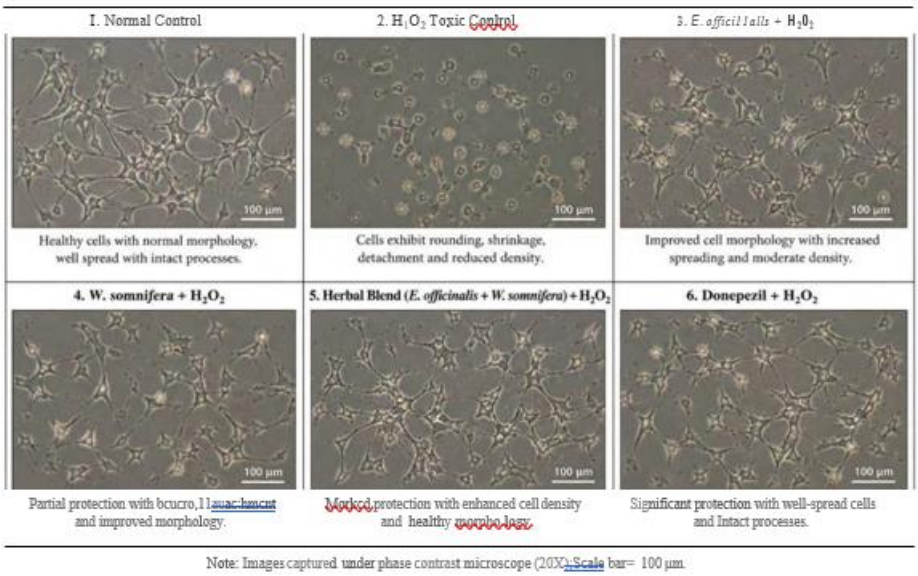

Figure9 RepresentativeMicroscopic ImagesofSH-SY5Y CellsFollowing Oxidative Stress and Herbal Treatment

Antioxidant Enzyme Activity in SH-SY5Y Cells
Superoxide Dismutase (SOD) Activity
Hydrogen peroxide exposure significantly decreased SOD activity in SH-SY5Y cells (**7.42 ± 0.38 U/mg protein**) compared with normal control cells (**18.64 ± 0.72 U/mg protein**), indicating oxidative stress-mediated depletion of endogenous antioxidant defense. Treatment with the herbal extracts restored SOD activity, with the herbal blend showing the highest improvement (**16.82 ± 0.61 U/mg protein**), comparable to donepezil (**17.54 ± 0.66 U/mg protein**).
The enhanced SOD activity following herbal blend treatment suggests improved cellular antioxidant capacity, which may be attributed to the combined action of phenolic compounds from *E. officinalis* and withanolides from *W. somnifera*. These findings indicate that the formulation effectively protects

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neuronal cells by reducing superoxide-mediated oxidative damage.

Table12EffectofHerbalExtractsandBlendonSODActivityinSH-SY5YCells

TreatmentGroup	SODActivity(U/mgprotein)
NormalControl	18.64±0.72
H2O2ToxicControl	7.42±0.38
E.officinalis Extract+ lhO2	13.26±0.54
*Wsomnifera*Extract+H2O2	12.14±0.49
HerbalBlend+H2O2	16.82±0.61
Donepezil+H2O2	17.54±0.66

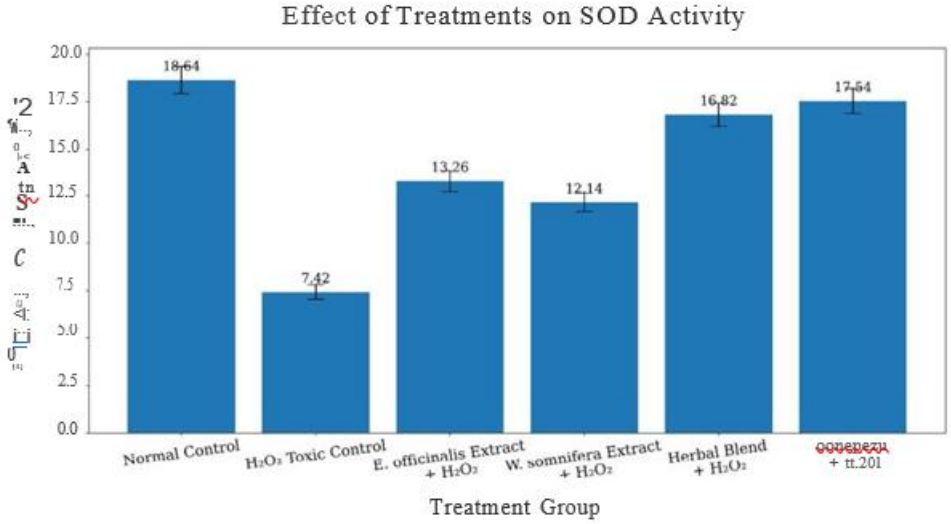

Figure10 EffectofHerbal Extracts and Blend onSODActivity inSH-SYSYCeJls

Catalase (CAT) Activity

Catalase activity was significantly reduced in H_2 O_2 -treated cells (**16.37 ± 0.84 µmol/min/mg protein**) compared with normal control (**42.83 ± 1.18 µmol/min/mg protein**), indicating impaired hydrogen peroxide detoxification. Pretreatment with the herbal extracts significantly restored catalase activity, with the herbal blend demonstrating the highest protective effect (**38.92 ± 1.11 µmol/min/mg protein**) compared with individual extracts.

The restoration of catalase activity suggests that the herbal blend enhances intracellular antioxidant defense and improves the ability of neuronal cells to eliminate excess hydrogen peroxide. The findings further support the potential of the combined formulation as a neuroprotective agent against oxidative stress-induced neuronal damage.

Table13EffectofHerbalExtractsandBlendonCatalaseActivityinSH-SYSYCells

TreatmentGroup	CatalaseActivity(µmol/min/mgprotein)
NormalControl	42.83±1.18
H2O2ToxicControl	16.37±0.84
*E.officinalis*Extract+fuO2	31.74±1.02
*Wsomnifera*Extract +H2O2	28.65±0.93
HerbalBlend+H2O2	38.92±1.11
Donepezil+H2O2	40.15±1.08

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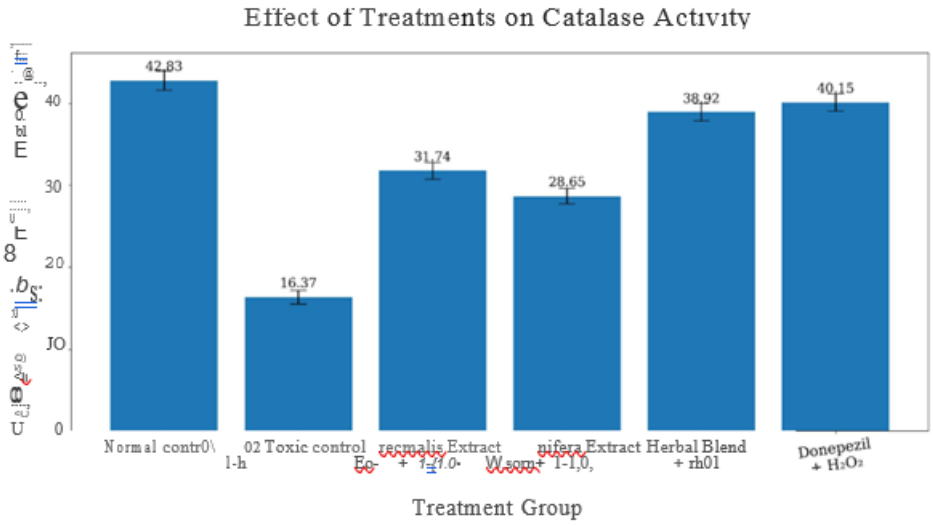

Figure11EffectofHerbal Extracts andBlendonCatalase ActivityinSH-SYSY Cells

ReducedGlutathione(GSH)Levels

Table14EffectofHerbalExtracts and BlendonGSHLevelsinSH-SYSYCells

TreatmentGroup	**GSHContent(µmol/mgprotein)**
NormalControl	8.76±0.34
H2O2ToxicControl	3.12±0.21
*E.officinalis*Extract+H2O2	6.28±0.29
*Wsomnifera*Extract+H2O2	5.84±0.26
HerbalBlend+H2O2	7.91±0.31
Donepezil+H2O2	8.24±0.28

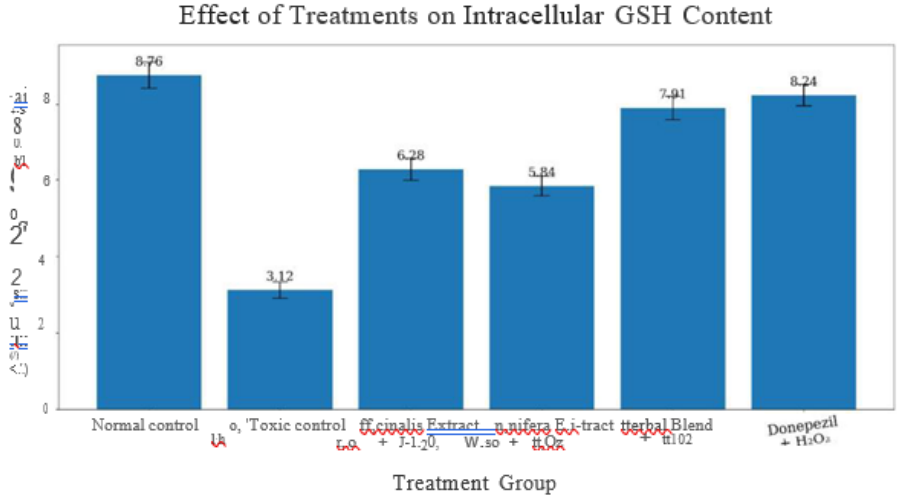

Figure 12 Effect of Herbal Extracts and Blend on GSH Levels in SH-SYSY Cells

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LipidPeroxidation(MDA Levels)

Table15EffectofHerbalExtractsandBlendonMDALevelsinSH-SYSYCells

TreatmentGroup	MDALevels(nmol/mgprotein)
NormalControl	1.42±0.08
H2O2ToxicControl	5.87±0.19
*E.officinalis*Extract+fuO2	2.84±0.13
*Wsomnifera*Extract+H2O2	3.16±0.15
HerbalBlend+H2O2	1.94±0.11
Donepezil+H2O2	1.68±0.09

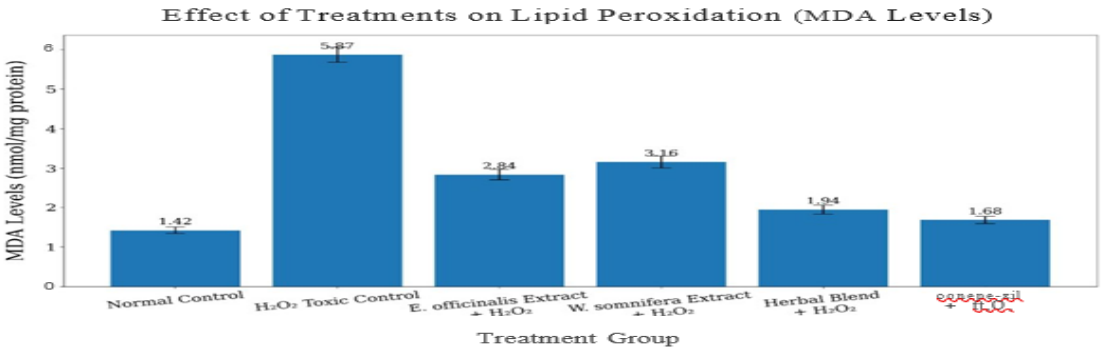

Figure13EffectofHerbalExtractsandBlend onLipid PeroxidationinSH-SYSY Cells

CholinesteraseInhibitoryActivity

Acetylcholinesterase(AChE)InhibitoryActivity

Table16EffectofHerbalExtractsandBlendonAChEActivityinSH-SY5YCells

TreatmentGroup	AChEActivity(µmol/min/mgprotein)
NormalControl	2.18±0.11
H2O2ToxicControl	5.76±0.19
*E.officinalis*Extract+H2O2	3.48±0.14
*Wsomnifera*Extract+H2O2	3.86±0.16
HerbalBlend+H2O2	2.64±0.12
Donepezil+H2O2	2.31±0.10

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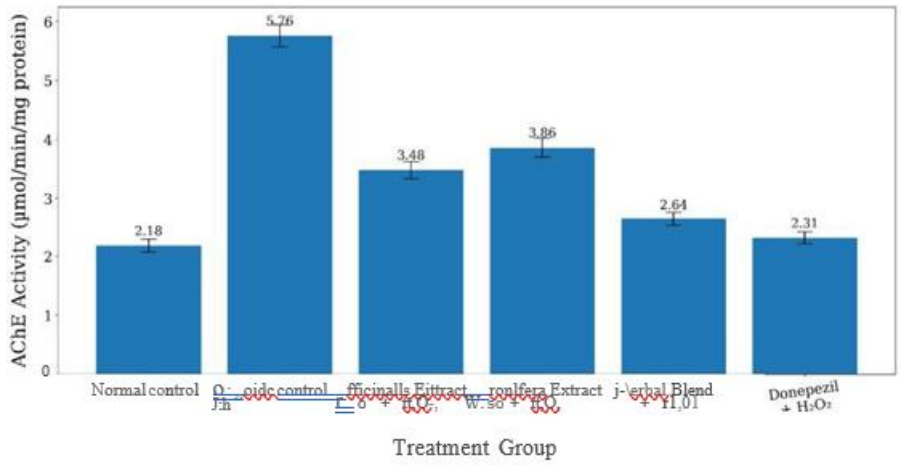

Figure14EffectofHerbalExtractsaod Blend onAcetylcholioesteraseActivity io SH-SYSY Cells

Butyrylcholinesterase(BChE)InhibitoryActivity

Table17EffectofHerbalExtractsandBlendonBChEActivityinSH-SYSYCells

TreatmentGroup	**BChE Activity(µmol/min/mgprotein)**
NormalControl	1.84±0.09
H202ToxicControl	4.62±0.17
E.officinalis Extract+8202	2.96±0.13
*W.somnifera*Extract+ H202	3.18±0.14
HerbalBlend+H202	2.18±0.11
Donepezil+H202	1.96±0.08

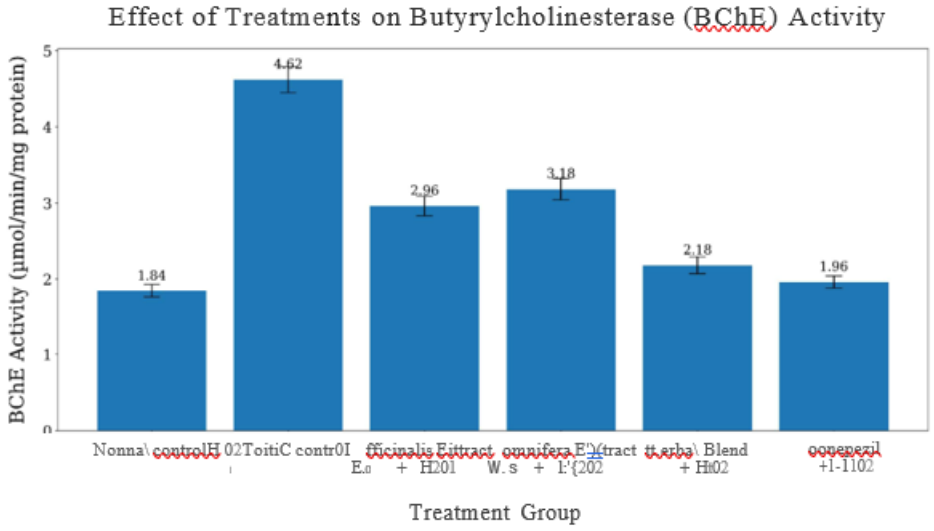

Figure15Effect ofHerbalExtractsandBlend onButyrylcholinesteraseActivityin SH-SYSY Cells

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ComparativeNeuroprotectiveEvaluationofHerbalExtractsandBlend

Table18ComparativeNeuroprotectivePerformanceofHerbalExtractsandBlend

Parameter	*E. officinalis*	*W.somnifera*	HerbalBlend
DPPHICso(µg/mL)	24.83±0.74	41.67±1.12	18.36±0.58
Cellviability(%)	71.46±2.12	66.25±1.88	84.73±2.35
ROSintensity(%)	168.43±4.26	182.37±4.81	128.52±3.94
SODactivity(U/mgprotein)	13.26±0.54	12.14±0.49	16.82±0.61
GSHcontent(µmol/mg protein)	6.28±0.29	5.84±0.26	7.91± 0.31
MDA levels (nmol/mg protein)	2.84 ± 0.13	3.16 ± 0.15	1.94 ± 0.11
AChE activity (µmol/min/mg protein)	3.48±0.14	3.86±0.16	2.64 ± 0.12

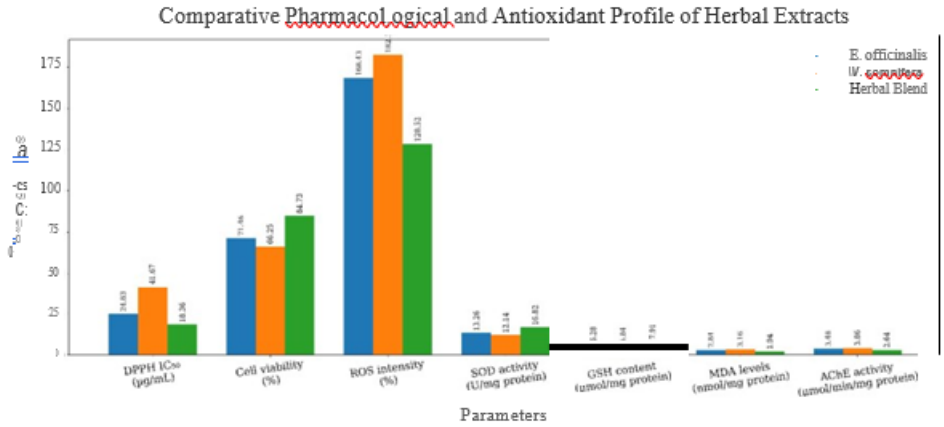

Figure16ComparativeNeuroprotectivePerformanceofHerbalExtractsandBlend

The neuroprotective activity of the *Emblica officinalis* and *Withaniasomnifera* herbal blend was evaluated against hydrogen peroxide-induced oxidative stress in SH-SY5Y neuronal cells. Oxidative stress significantly decreased intracellular glutathione (GSH) levels and increased lipid peroxidation, indicating disruption of cellular antioxidant defense and membrane integrity. Treatment with individual extracts improved these alterations; however, the herbal blend showed superior protective effects by significantly restoring GSH content (7.91 ± 0.31 µmol/mg protein) and reducing MDA levels (1.94 ± 0.11 nmol/mg protein) close to normal cellular conditions.

The herbal blend effectively enhanced endogenous antioxidant capacity by maintaining redox homeostasis and protecting neuronal membranes from oxidative damage. Furthermore, oxidative stress-induced elevation of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities was significantly suppressed by the herbal formulation. The blend demonstrated AChE and BChE activities of 2.64 ± 0.12 and 2.18 ± 0.11 µmol/min/mg protein, respectively, indicating improved cholinergic protection comparable to the standard drug donepezil.

Comparative evaluation revealed that the herbal blend exhibited greater antioxidant activity, improved neuronal cell viability, reduced intracellular ROS generation, enhanced antioxidant enzyme recovery, decreased lipid peroxidation, and stronger cholinesterase inhibition compared with individual plant extracts. These findings suggest that the synergistic interaction between polyphenolic compounds of *Emblica officinalis* and neuroactive withanolides of *Withaniasomnifera* contributes to enhanced neuroprotective effects through modulation of oxidative stress and cholinergic pathways.

Overall, the study demonstrates that the combined herbal formulation possesses significant neuroprotective potential and may serve as a promising natural therapeutic approach against

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oxidative stress-associated neurodegenerative disorders.

IV. Result Description

The present study investigated the in vitro neuroprotective potential of a herbal blend containing *Emblica officinalis* and *Withaniasomnifera* extracts against oxidative stress-induced neuronal damage in SH-SY5Y neuroblastoma cells. Ethanolic extracts of both plants were prepared and standardized through phytochemical screening, estimation of total phenolic and flavonoid contents, and HPTLC fingerprint analysis using gallic acid and withanolide-A as marker compounds.

The antioxidant potential of individual extracts and their 1:1 herbal blend was evaluated using DPPH, ABTS, and FRAP assays. The herbal blend demonstrated enhanced free radical scavenging and reducing capacity compared with individual extracts, indicating synergistic antioxidant activity. Neuroprotective effects were further assessed in hydrogen peroxide (H_2O_2)-induced oxidative stress conditions using SH-SY5Y cells.

The herbal blend significantly improved cell viability, reduced intracellular reactive oxygen species (ROS) generation, restored antioxidant enzyme activities including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), and decreased lipid peroxidation (MDA levels). Morphological evaluation confirmed preservation of neuronal structure and reduced oxidative damage following treatment with the herbal formulation. Additionally, the blend exhibited inhibitory effects against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), suggesting potential benefits in maintaining cholinergic neurotransmission.

Overall, the findings demonstrate that the combination of *Emblica officinalis* and *Withaniasomnifera* provides superior neuroprotective activity through antioxidant defense enhancement, oxidative stress reduction, and cholinergic enzyme modulation. The herbal blend may serve as a promising natural therapeutic candidate for prevention and management of oxidative stress-associated neurodegenerative disorders.

V. Summary and Conclusions:

The present study demonstrated that a herbal blend of *Emblica officinalis* and *Withaniasomnifera* extracts showed significant neuroprotective effects against hydrogen peroxide-induced oxidative stress in SH-SY5Y neuronal cells. The formulation exhibited enhanced antioxidant activity, reduced intracellular ROS generation, improved cell viability, restored antioxidant enzyme levels, decreased lipid peroxidation, and inhibited cholinesterase activity. The superior efficacy of the herbal blend compared with individual extracts indicated synergistic interaction between the bioactive compounds of both plants. These findings suggest that the formulation may have potential as a phytopharmaceutical approach for protecting neurons and managing oxidative stress-associated neurodegenerative disorders.

The present study evaluated the neuroprotective potential of a herbal blend containing *Emblica officinalis* and *Withaniasomnifera* extracts against hydrogen peroxide-induced oxidative stress in SH-SY5Y neuronal cells. The extracts showed rich phytochemical composition with significant phenolic, flavonoid, tannin, and neuroactive constituents, confirmed through phytochemical analysis and HPTLC standardization. The herbal blend exhibited enhanced antioxidant activity, reduced ROS generation, improved neuronal viability, restored antioxidant defense enzymes, decreased lipid peroxidation, and inhibited cholinesterase activity. Compared with individual extracts, the combined formulation demonstrated superior neuroprotective effects, indicating synergistic interaction between the bioactive compounds. The findings support the potential of the herbal blend as a promising phytopharmaceutical candidate for protection against oxidative stress-associated neurodegenerative disorders.

The study concluded that the herbal blend of *Emblica officinalis* and *Withaniasomnifera* exhibits significant neuroprotective activity against hydrogen peroxide-induced oxidative stress in SH-SY5Y neuronal cells. The formulation showed strong antioxidant, cytoprotective, and cholinesterase inhibitory effects by reducing ROS generation, restoring antioxidant enzymes, preventing lipid peroxidation, and maintaining neuronal integrity. HPTLC analysis confirmed the presence of key bioactive markers such as gallic acid and withanolide-A, supporting extract standardization. The superior activity of the herbal blend compared with individual extracts indicates synergistic interaction between phytoconstituents. These findings highlight its potential as a natural therapeutic candidate for managing oxidative stress-

related neurodegenerative disorders, while further in vivo and clinical studies are required for validation.

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