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Original article

Neuroprotective Effects of Saraswatharishtam Against Glutamate-Induced Oxidative Stress in SH-SY5Y Neuroblastoma Cells

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Abstract

This study investigates the neuroprotective effects of various concentrations of Saraswatharishtam (SAM) against glutamate-induced oxidative stress in SH-SY5Y neuroblastoma cells. By dividing cells into experimental groups, we aim to determine the efficacy of SAM in pre-treatment scenarios, with one group receiving glutamate exposure without SAM as a negative control. Key methodologies include MTT and LDH assays to assess cell viability, JC-1 assays to evaluate mitochondrial membrane potential, cholinesterase inhibition assays, and measurements of intracellular calcium levels. Furthermore, we analysed the expression of critical genes associated with neuroprotection, such as AKT, p38, ERK, and alpha-synuclein, using RT-PCR and Western blotting techniques. One-way ANOVA was employed to compare results across groups. This research seeks to establish the relationship for SAM in mitigating oxidative stress, potentially identifying optimal concentrations for therapeutic applications. The findings could elucidate the molecular mechanisms underlying SAM-mediated neuroprotection, contributing to the development of novel strategies for treating neurodegenerative disorders linked to oxidative damage. This study aligns with the Sustainable Development Goal 3 (SDG 3: Good Health and Well-Being) by exploring affordable, traditional neuroprotective strategies that may contribute to improved prevention and management of neurodegenerative disorders. Ultimately, this work aims to enhance our understanding of SAM's role in neuronal health and its potential as a protective agent against excitotoxicity.

Keywords: *Neurodegenerative disorders (ND), Saraswatharishtam (SAM), Oxidative stress*

Introduction:

Neurodegenerative disorders (ND) represent a diverse group of debilitating conditions characterized by the progressive loss of structure or function of neurons [1] These disorders, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS), impose a significant burden on affected individuals and healthcare systems worldwide [2] Central to the pathology of ND is oxidative stress (OS), a condition where cellular antioxidant defences are overwhelmed by reactive oxygen species (ROS), leading to neuronal damage and death [3] The accumulation of oxidative stress and excitotoxicity, primarily mediated by glutamate, an essential excitatory neurotransmitter in the central nervous system, plays a pivotal role in these disorders [4]. Excessive glutamate levels can induce neuronal injury and death through oxidative mechanisms that surpass the capacity of cellular antioxidant defenses, highlighting the importance of understanding these pathways for developing effective neuroprotective strategies.

Glutamate is crucial for normal brain function; however, under pathological conditions, it can contribute to neurodegeneration [4]. Elevated glutamate levels lead to excitotoxicity, calcium dysregulation, mitochondrial dysfunction, and ultimately cell death, mechanisms implicated in various neurodegenerative disorders [5]. Current therapeutic strategies often focus on symptomatic relief rather than addressing underlying disease

mechanisms. For instance, donepezil, a cholinesterase inhibitor, is used primarily in Alzheimer's disease to enhance cholinergic transmission [6]. However, concerns regarding its long-term efficacy and side effects underscore the need for alternative therapies, including herbal medicines. In this context, traditional Ayurvedic formulations have gained attention for their potential neuroprotective properties. One notable formulation, Saraswatharishtam (SAM), is a fermented herbal remedy recognized for its cognitive enhancement and restorative effects on the nervous system.

Saraswatharishtam (SAM) is a traditional Ayurvedic formulation comprising 18 medicinal plants and minerals, recognized for its neuroprotective properties in the management of neurological conditions. Previous studies have highlighted SAM's role in enhancing cognitive function, reducing oxidative stress, and modulating neurotransmitter levels [7,8] Despite its traditional use, scientific validation of SAM's neuroprotective effects, particularly against glutamate-induced oxidative stress, remains limited. This study aims to address this gap by evaluating the relationship of Saraswatharishtam in mitigating glutamate-induced oxidative stress using SH-SY5Y neuroblastoma cells. Through a comprehensive array of cellular and molecular assessments, including viability assays, cytotoxicity markers, mitochondrial function analysis, cholinesterase inhibition assays, intracellular calcium dynamics, and gene expression profiling, this research seeks to elucidate the potential neuroprotective mechanisms of SAM. By establishing optimal therapeutic concentrations and providing insights into underlying molecular pathways, this study aims to contribute valuable data towards SAM's potential as a therapeutic agent for neurodegenerative disorders. Finally, this work could pave the way for integrating traditional knowledge with modern therapeutic strategies, offering new hope in the fight against neurodegeneration. Neurodegenerative diseases represent a growing global public health challenge, particularly in ageing populations, with significant social and economic consequences. In this context, the present study contributes to the Sustainable Development Goal 3 (SDG 3: Good Health and Well-Being) by investigating neuroprotective strategies that may support brain health, reduce disease burden, and promote sustainable, accessible healthcare solutions through evidence-based traditional medicine.

Materials & Methods:

Study Design:

Cell Line and Culture Conditions

Human neuroblastoma SH-SY5Y cells were obtained from the National Centre for Cell Sciences (NCCS), Pune, India, were cultured in DMEM/F-12 medium supplemented with 10% fetal bovine serum (FBS) and 1% non-essential amino acids (NEAA). The cells were subcultured thrice and maintained at 37°C in a humidified atmosphere with 5% CO^2 [9].

Experimental Design:

SH-SY5Y neuroblastoma cells will bewere divided into six experimental groups:

- Group I (Negative control): Untreated SH-SY5Y cells.
- Group II (Glutamate-treated): Cells exposed to 1 mM glutamate to induce oxidative stress.
- Groups III (SAM-treated): Cells pre-treated with Saraswatharishtam (SAM) (50 µg/mL) for 4 hours followed by glutamate exposure.
- Groups IV (SAM-treated): Cells pre-treated with Saraswatharishtam (SAM) (100 µg/mL) for 4 hours followed by glutamate exposure.
- Group V (DONEPEZIL-treated) Cells treated with Donepezil (1.5mM)

Viability Assay - MTT Assay

Cell viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay as followed by previous study [10]. After treatments, MTT solution was added to cells and incubated for 4 hours. Formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. Viability was calculated as a percentage relative to untreated control cells.

Measurement of Reactive Oxygen Species (ROS)

Intracellular ROS levels were assessed using the fluorescent probe DCFH-DA (dichlorodihydrofluorescein diacetate). Changes in fluorescence intensity were monitored at excitation/emission wavelengths of 485 nm/535 nm using a fluorescence microscope [11].

Lactate Dehydrogenase (LDH) Release Assay

Cytotoxicity was evaluated by measuring LDH release from damaged cells using the Promega CytoTox 96 assay kit [12]. Absorbance was read at 490 nm, and LDH release was quantified as a percentage relative to total LDH content.

Mitochondrial Membrane Potential (MMP) Analysis

MMP was determined using the JC-1 assay. Cells were stained with JC-1 dye, and the ratio of red (aggregates)/green (monomers) fluorescence intensity was measured using a fluorescence microplate reader. Changes in MMP were expressed as a percentage of control [13].

Intracellular Calcium Ion Measurement

Intracellular calcium levels were measured using a confocal laser-scanning microscope after loading cells with a fluorescent calcium indicator. Changes in fluorescence intensity were quantified as F/F0 ratios, where F0 represents initial fluorescence intensity [14].

Cholinesterase Inhibition Assay

The inhibitory activity of SAM on acetylcholinesterase (AChE) was determined using Ellman's assay [15]. SAM at concentrations of 50 µg/mL and 100 µg/mL was incubated with AChE, and absorbance was measured at 405 nm using a UV-Visible spectrophotometer.

Gene Expression Analysis

Total RNA was extracted from treated and untreated SH-SY5Y cells using Bio Basic Inc. RNA was reverse transcribed to cDNA, which was then amplified by PCR using gene-specific primers. Gene expression levels of AKT, p38, ERK, and alpha-synuclein were analyzed by gel electrophoresis and quantified using densitometry [16].

Statistical Analysis

Data were analyzed using Sigma Plot 13 (Systat Software, USA). Mean values and standard errors were calculated for each parameter. Statistical significance between groups was determined by one-way analysis of variance (ANOVA), followed by post-hoc tests where appropriate. A p-value < 0.05 was considered statistically significant. All experiments were performed in triplicate. Data are expressed as Mean ± SEM (n = 3). *, **, *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively vs. glutamate-treated group; @@@ indicates $p < 0.001$ vs. negative control. ### indicates $p < 0.001$ vs. SAM treated group. Symbols (@, #) also represents a significant difference when compared with the glutamate-induced group and was used in certain graphs to avoid overcrowding or repetition of symbols.

Results:

Cell Viability Assay

This study aimed to evaluate the neuroprotective effects of Saraswatharishtam (SAM) against glutamate-induced oxidative stress in SH-SY5Y neuroblastoma cells, addressing specific objectives focused on understanding SAM's potential therapeutic mechanisms. SAM, a polyherbal formulation from Ayurveda, demonstrated significant neuroprotective properties as assessed by the MTT assay. Treatment with SAM markedly enhanced cell viability in SH-SY5Y cells exposed to glutamate-induced toxicity, with higher concentrations (100 µg/mL) showing superior protective effects compared to lower doses (50 µg/mL). This concentration-dependent response suggests that SAM's efficacy in mitigating neuronal damage correlates with its dosage, highlighting its potential as a neuroprotective agent.

Intracellular reactive oxygen species (ROS) levels, induced by glutamate exposure, were substantially reduced following SAM treatment, as illustrated in Figure 1 and graph 1. SAM effectively attenuated glutamate-induced ROS generation (similar to the standard drug Donepezil as shown in group V), indicating its potent antioxidant activity in neuronal cells under oxidative stress conditions.

Lactate Dehydrogenase (LDH) Assay

The LDH assay revealed a significant increase in LDH release in glutamate-treated cells compared to the control group ($p < 0.001$). In contrast, pre-treatment with SAM resulted in a marked decrease in LDH levels at both concentrations (50 µg/mL: $p < 0.01$; 100 µg/mL: $p < 0.001$), suggesting that SAM effectively preserved cell membrane integrity and reduced cytotoxicity. Furthermore, SAM pre-treatment significantly decreased lactate dehydrogenase (LDH) release, a marker of cytotoxicity, compared to glutamate-treated cells (Graph 2) alike the standard drug Donepezil which showed a significant reduction in the percentage of cytotoxicity.

Mitochondrial Membrane Potential (MMP)

Mitochondrial membrane potential was significantly reduced in the glutamate group, indicating mitochondrial dysfunction ($p < 0.001$). Pre-treatment with SAM notably restored MMP, with the 100 µg/mL concentration demonstrating the highest restoration compared to the glutamate-only group ($p < 0.001$), suggesting a protective effect on mitochondrial function. This protective effect was consistent with SAM's ability to preserve mitochondrial membrane potential (MMP) (Graph 3), crucial for cellular energy production and function. SAM maintained MMP even at higher concentrations, suggesting its role in mitigating mitochondrial dysfunction associated with oxidative stress.

Graph:1 Effect of SAM on glutamate induced free radical (ROS) generation in SH-SY5Y cells

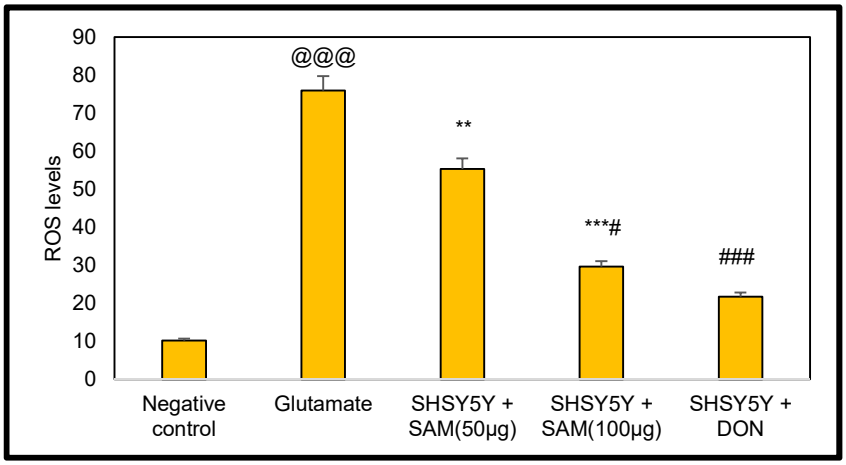
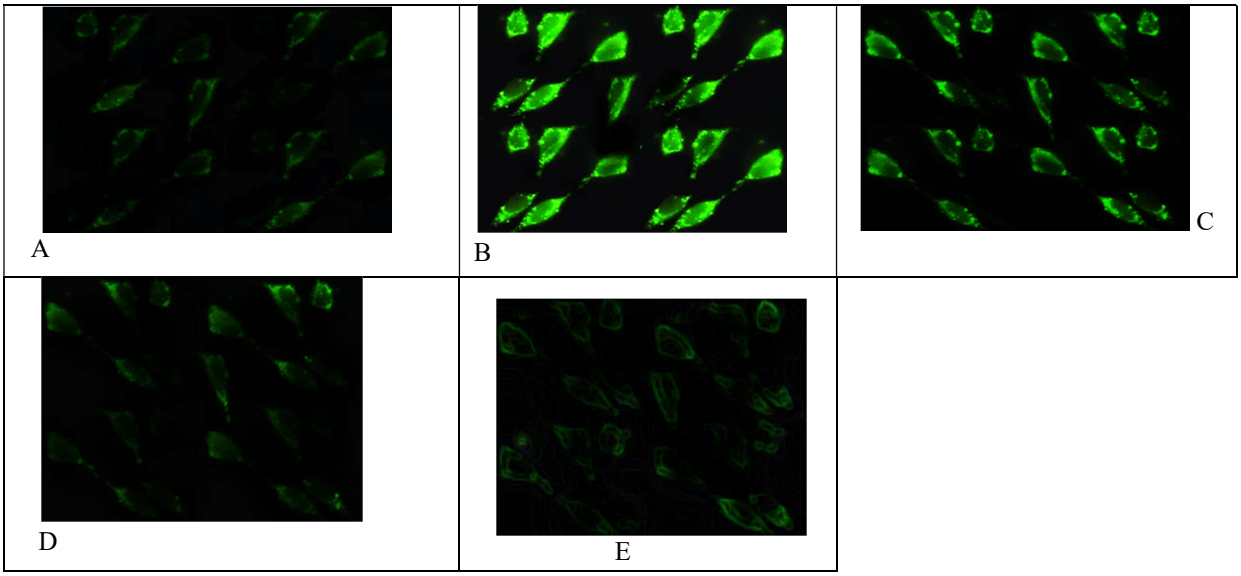
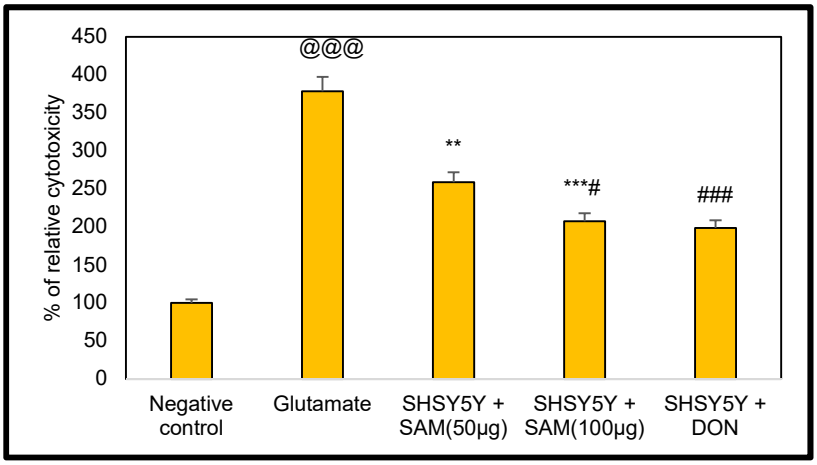


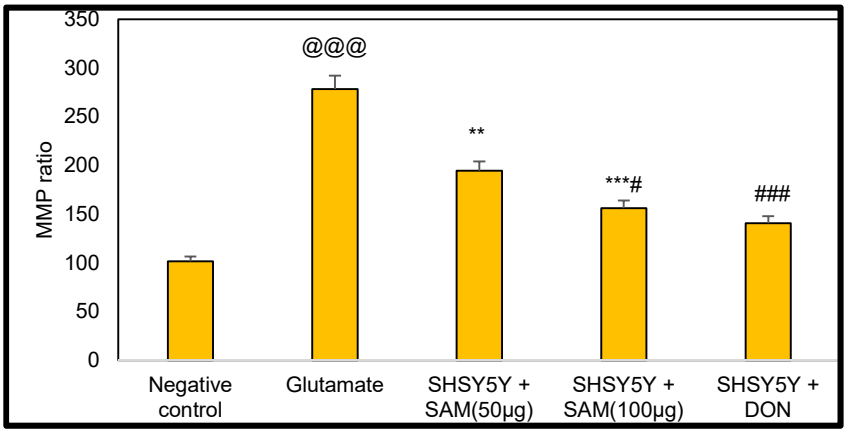
Figure:1 Image A- Negative Control; B- GLUTAMATE induced; C - SAM (50µg); D -SAM (100µg); E-DON treated



Graph 2: Cell membrane integrity (LDH release assay)



Graph 3: Mitochondrial membrane potential



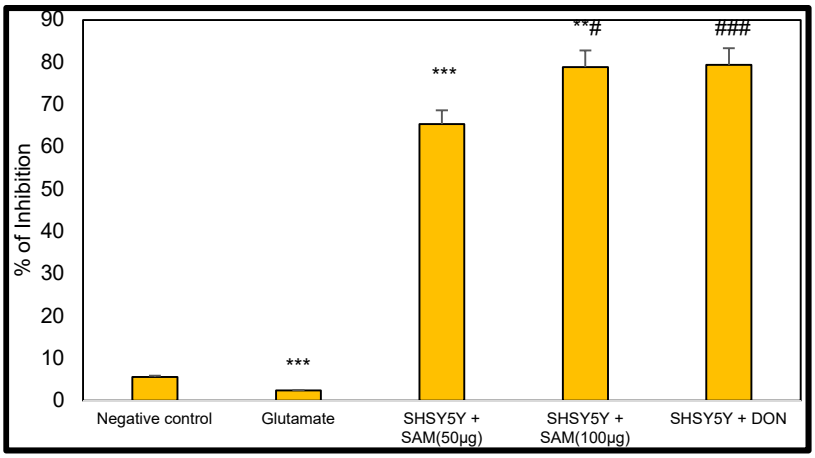
SAM also demonstrated inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes (Graph 4), pivotal in cholinergic neurotransmission and cognition. This finding supports SAM's potential in enhancing cholinergic function, which is often impaired in neurodegenerative diseases.

Intracellular Calcium Levels

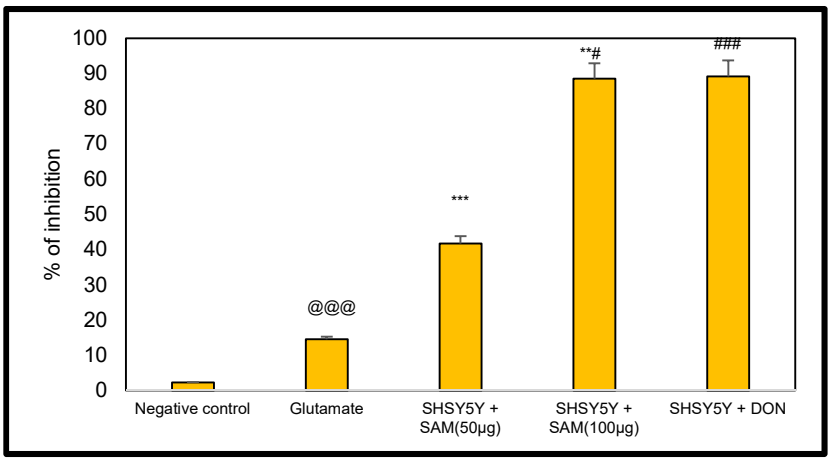
Flow cytometry analysis indicated a significant increase in intracellular calcium levels in the glutamate group compared to the control ($p < 0.001$). Pre-treatment with SAM effectively reduced calcium levels, with the 100 µg/mL group showing a decrease comparable to the control ($p < 0.001$) and the group treated Donepezil also showed a similar change.

Moreover, SAM treatment significantly reduced intracellular calcium levels elevated by glutamate exposure (Graph 5), indicating its role in regulating calcium homeostasis critical for neuronal survival and function.

Graph 4a: Assay of acetylcholinesterase inhibitory activity

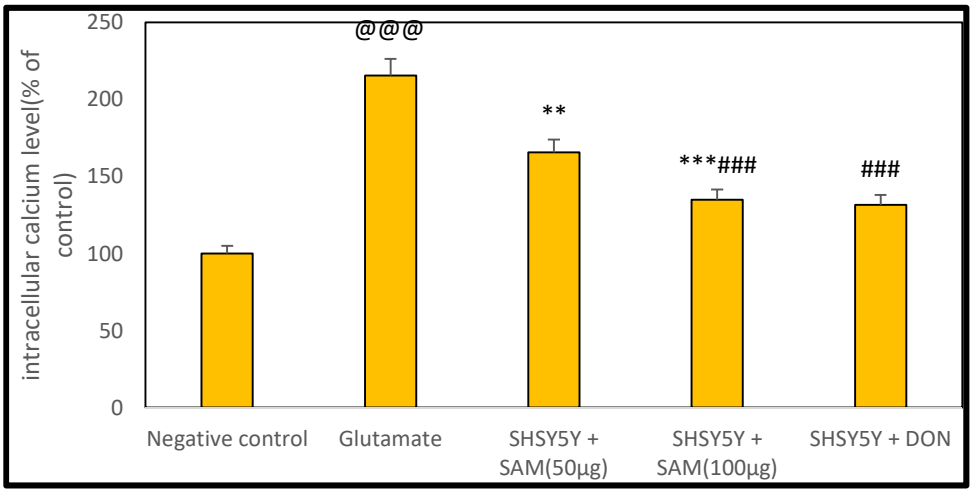


Graph 4b: Assay of butyrylcholinesterase inhibitory activity



The Graphs 4a & b showed the effect of SAM on AChE and BuChE inhibitory activity correspondingly. The cells treated with glutamate offers less inhibitory activity on acetylcholinesterase and butyrylcholinesterase. Whereas the inhibition was more predominant in the cells treated with different doses of SAM and the standard drug Donepezil.

Graph 5: Estimation of Intracellular Calcium Levels



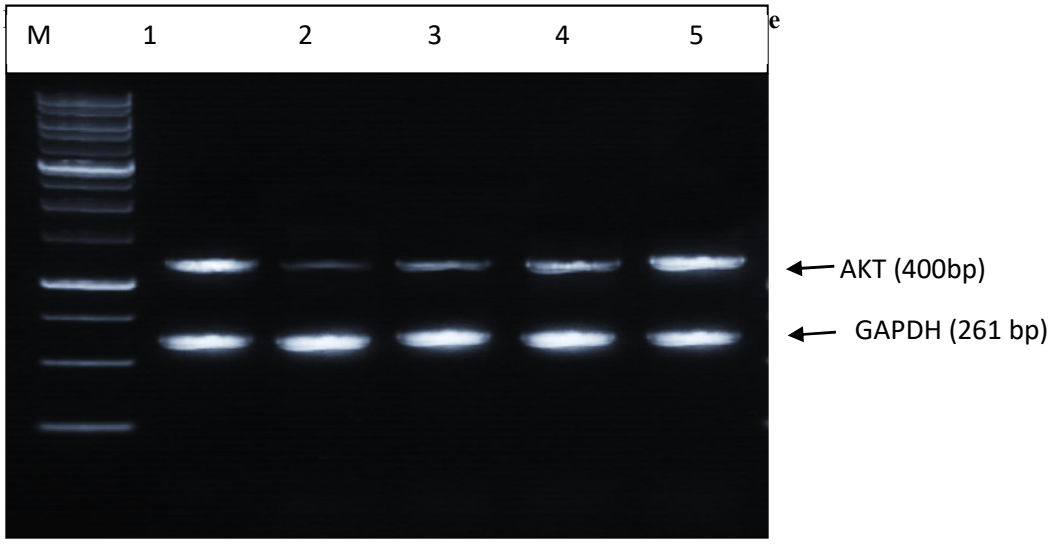
Cholinesterase Inhibition Assay

Cholinesterase activity was significantly higher in glutamate-treated cells compared to the negative control ($p < 0.001$). Pre-treatment with SAM at both concentrations significantly decreased cholinesterase activity, with the 100 µg/mL concentration resulting in a 40% reduction ($p < 0.001$). Graph 5 shows that the cells are treated with glutamate showed a marked increase in the intracellular calcium levels when compared to the negative control. The groups treated with SAM with two different doses showed a significant decrease in the intracellular calcium levels than the controls. Increase in the dose of SAM showed a concurrent decrease in the calcium.

Gene Expression Analysis

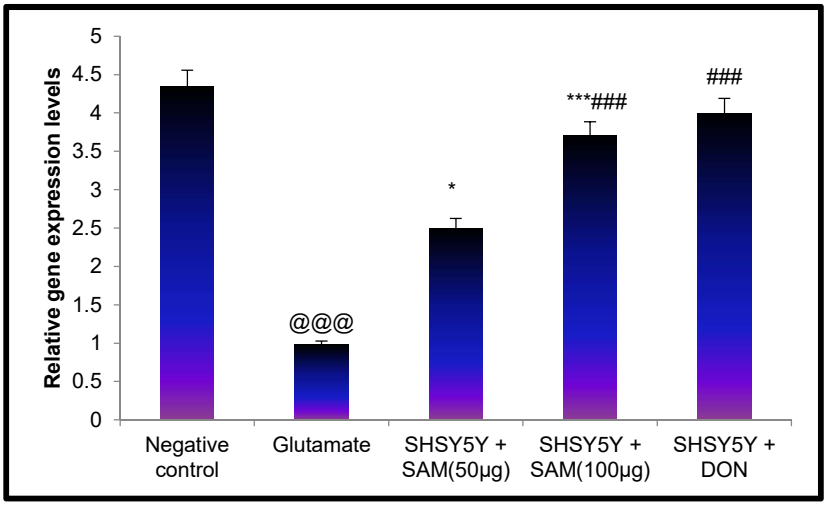
Quantitative PCR analysis revealed that glutamate treatment significantly upregulated the expression of pro-apoptotic genes (p38 and alpha-synuclein) and downregulated neuroprotective genes (AKT and ERK) compared to the control group ($p < 0.001$). In contrast, SAM pre-treatment restored the expression of AKT and ERK while reducing the expression of p38 and alpha-synuclein at both concentrations (50 µg/mL: $p < 0.05$; 100 µg/mL: $p < 0.001$).

Gene expression analysis revealed SAM's ability to modulate neuroprotective genes under oxidative stress conditions. SAM treatment upregulated the expression of AKT (figure 2 and Graph 6), p38 (figure 3 and Graph 7), ERK (Figure 4), and attenuated alpha-synuclein expression (Figure 5), which are involved in cell survival, antioxidant defense, and protein aggregation pathways. These results suggest that SAM exerts its neuroprotective effects through multiple mechanisms, including anti-oxidative, anti-apoptotic, and cholinergic pathway modulation.



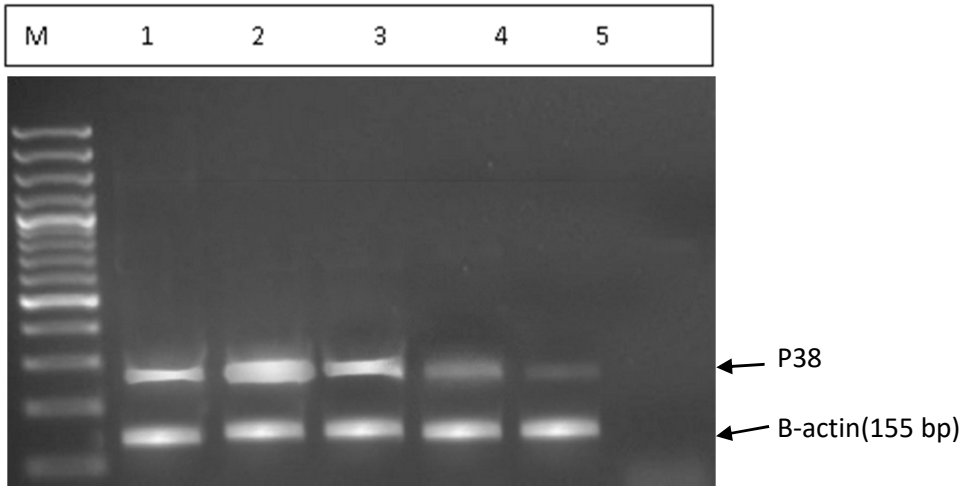
Lane 1 -Marker Lane; Lane 2- Negative control; Lane 3- Glutamate induced; Lane 4 –SHSY5Y+SAM(50µg); Lane 5 – SHSY5Y+SAM(100 µg)); Lane 6 - SHSY5Y + Donepezil. As indicated in Figure 2, the glutamate-incubated group had lower levels of AKT gene expression than the negative control group and there was a progressive increase in AKT gene expression in the SAM-treated group. When compared to the glutamate-incubated group, donepezil demonstrated an increase in the expression of AKT.

Graph 6:Effect of Saraswatarishtam on the expression of AKT gene



The expression of AKT gene was measured using GAPDH as an internal control. Each bar represents the Mean ± SEM of three independent experiments (Graph 6).

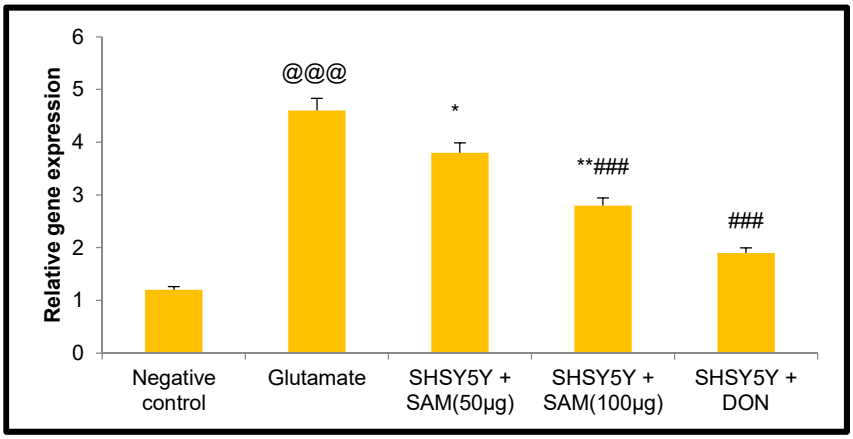
Figure 3: Effect of Saraswatarishtam on the expression of p38 gene



Lane 1 -Marker Lane; Lane 2- Negative control; Lane 3- Glutamate induced; Lane 4 –SHSY5Y+SAM(50µg); Lane 5 – SHSY5Y+SAM(100 µg) and Lane 6 - SHSY5Y + Donepezil.

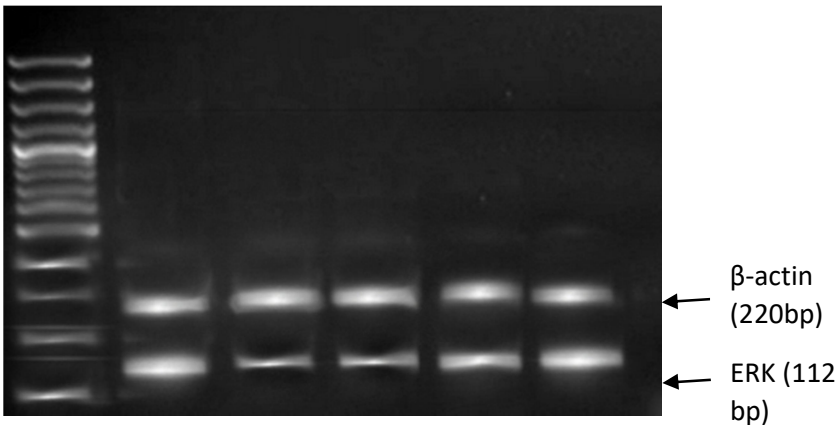
Increased p38 gene expression in the glutamate-incubated group as compared to the negative control group, as seen in Figure 3 When compared to the glutamate-incubated group, the expression of p38 in the SAM-treated group showed a progressive decrease with dose and standard Donepezil.

Graph 7: Effect of Saraswatarishtam on the expression of p38 gene



Graph:7 The expression of p38 gene was measured using β-actin as an internal control. Each bar represents the Mean ± SEM of three independent experiments. Due to its capacity to suppress the expression of antioxidant genes, p38 was significantly downregulated in our study by glutamate-induced oxidative stress, which resulted in a considerable buildup of ROS after exposure to low or moderate levels of H_2O_2.

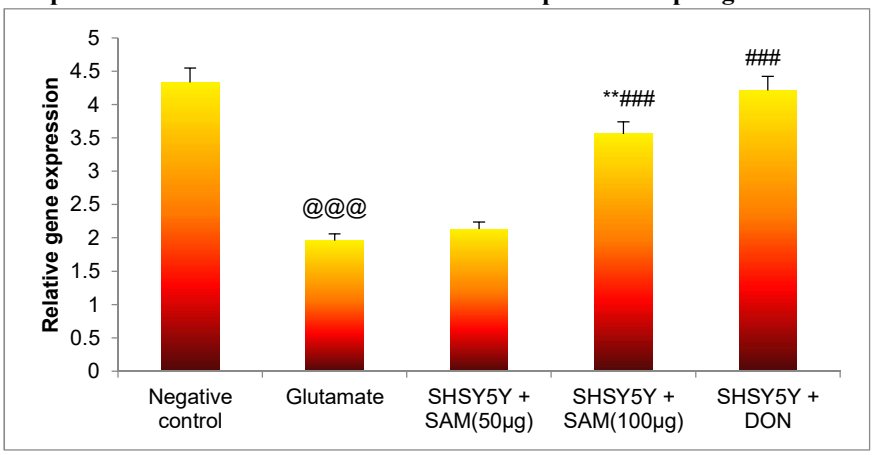
Figure 4: Effect of Saraswatarishtam on the expression of ERK gene



Lane 1 -Marker Lane; Lane 2- Negative control; ımate induced; Lane 4 – SHSY5Y+SAM(50µg); Lane 5 – SHSY5Y+SAM(100 µg) and Lane 6 - SHSY5Y + Donepezil.

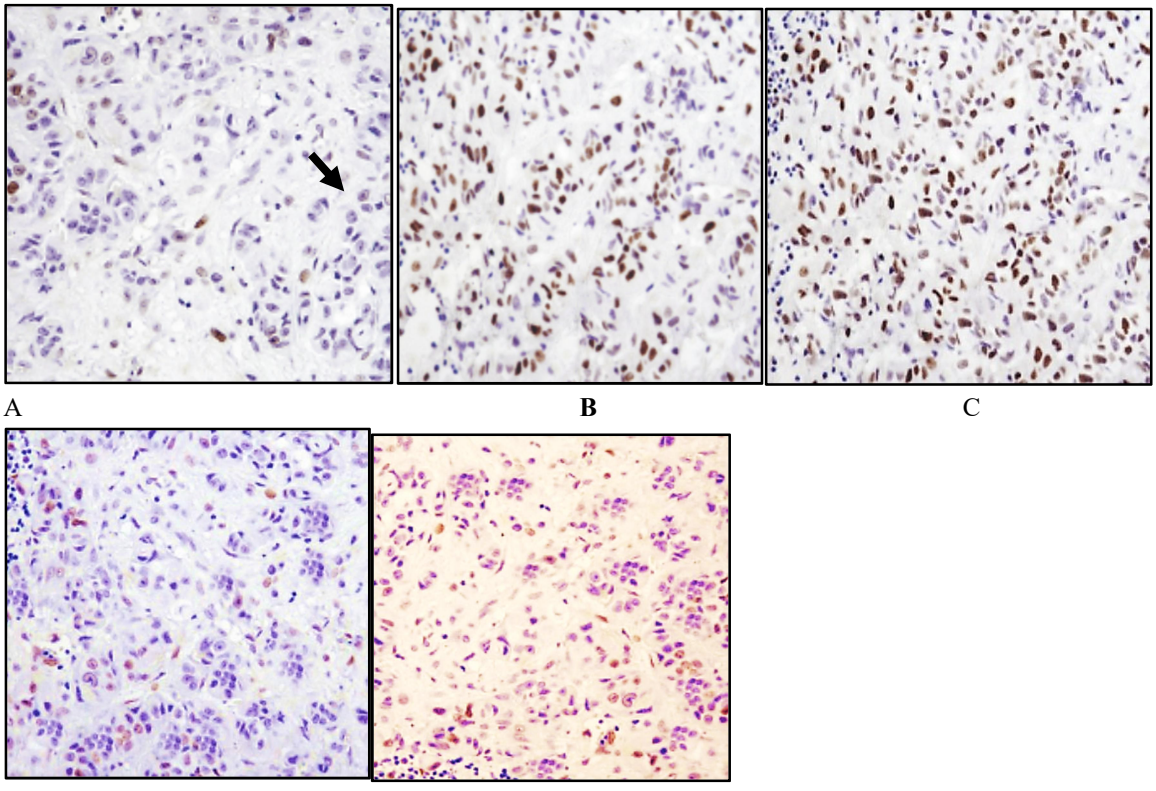
ERK gene expression was lower in the glutamate-incubated group compared to the negative control group, as shown in Figure 4. When compared to the glutamate-incubated group, the expression of ERK in the SAM-treated group increased gradually with increasing doses and standard Donepezil also increased this expression.

Graph 7: Effect of Saraswatarishtam on the expression of p38 gene



Graph:8 The expression of p38 gene was measured using β-actin as an internal control Each bar represents the Mean ± SEM of three independent experiments.

Figure 5: α-synuclein expression by immunohistochemistry

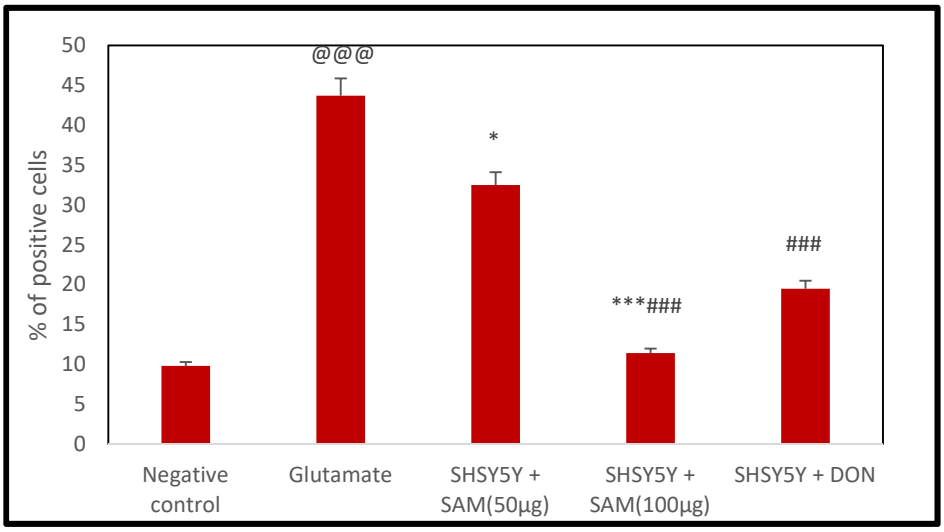


A B C

D E

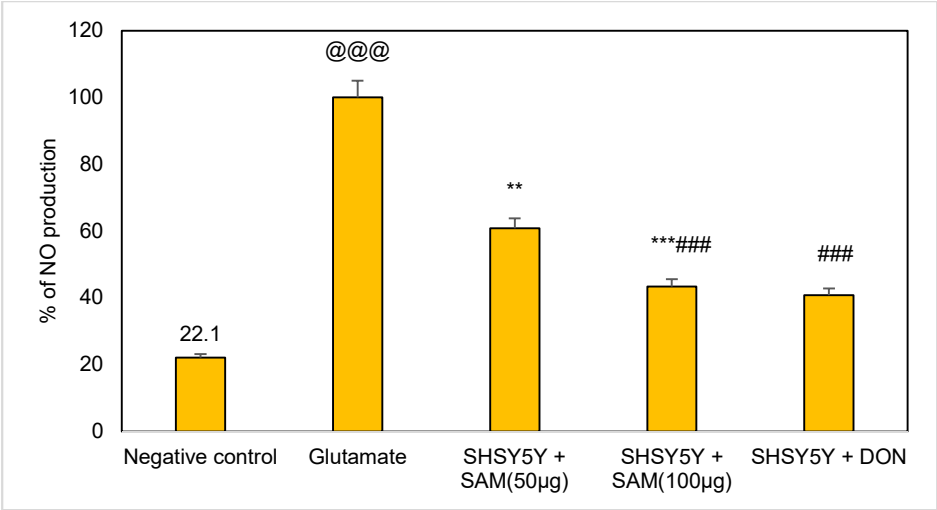
Image A- Negative Control; B- GLUTAMATE induced; C - SAM (50µg); D -SAM (100µg); E - Donepezil. magification-400x, scale bar-50µm. In SH5YHY cells that had been stimulated with glutamate, the positivity for -synuclein was predominately present. Immunostaining for -synuclein (arrow mark denotes dark brown color positive expression) was less intense in the cells of samples A, C and E. However, the sample D showed far less favourable expression.

Graph 9: Quantitative analysis of protein expression by Image analysis software.



The glutamate-induced oxidative stress group had considerably higher levels of -synuclein expression. The SAM-treated animals, however, displayed a noticeable down-regulation (Graph 9).

Graph 10: Percentage of NO production



The percentage of nitrous oxide production was studied among the experimental groups. When compared to glutamate incubated group the nitrous oxide production was significantly reduced among the cells treated with Saraswatharishtam.

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Figure 6: Tyrosine kinase expression by immunohistochemistry

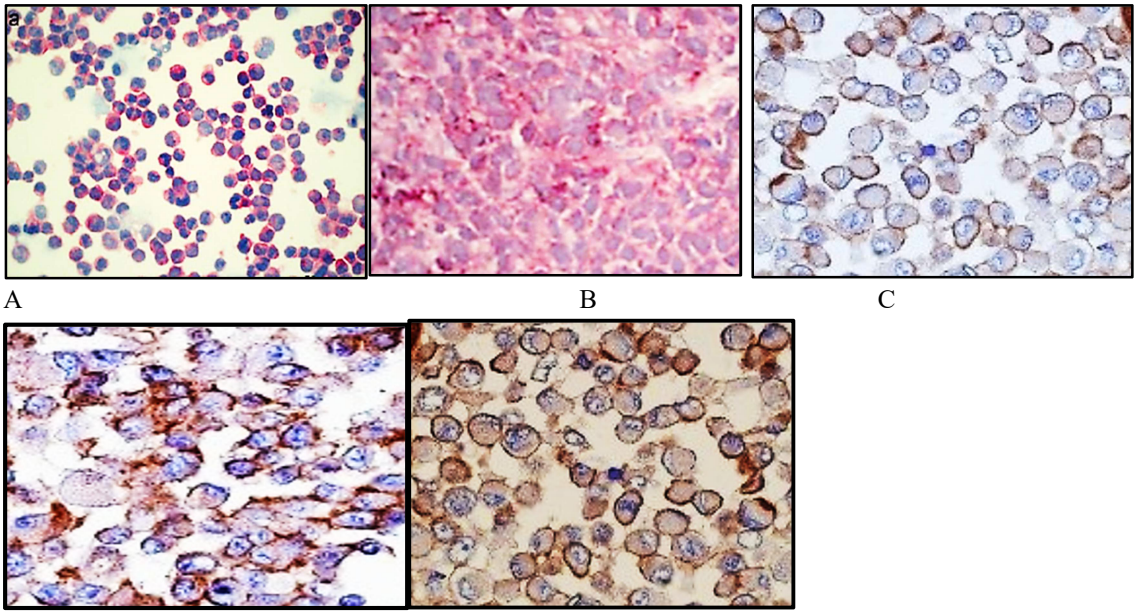
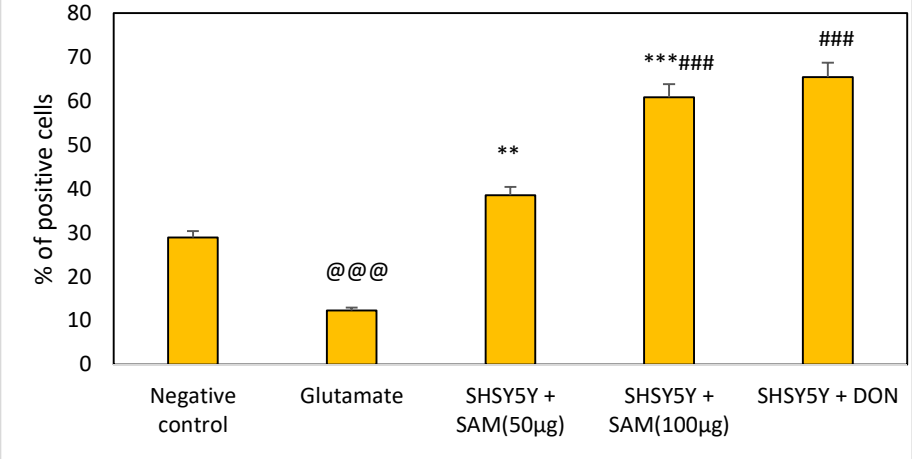


Image A- Negative Control; B- GLUTAMATE induced; C - SAM (50µg); D -SAM (100µg); E - Donepezil. magification-400x, scale bar-50µm. The majority of SH5YHY cells that showed positive immunoreactivity for tyrosine kinase were present. Tyrosine kinase immunostaining was more intense in the cells of samples C, D and E (arrow mark denotes dark brown color positive expression). However, sample B showed far less favorable expression (glutamate induce group).

Graph 11: Quantitative analysis of tyrosine kinase protein expression by Image analysis software.



Overall, these findings underscore Saraswatharishtam (SAM) as a promising neuroprotective agent against glutamate-induced oxidative stress in neuronal cells. The dose-dependent efficacy observed in enhancing cell viability, reducing cytotoxicity, preserving mitochondrial function, modulating cholinergic pathways, regulating calcium levels, and influencing neuroprotective gene expression supports further exploration of SAM's therapeutic potential in treating neurodegenerative disorders.

Discussion:

The present study demonstrates that Saraswatharishtam (SAM) exerts significant neuroprotective effects against glutamate-induced oxidative stress in SH-SY5Y neuroblastoma cells. The results indicate that SAM can mitigate cellular damage and improve neuronal viability in the face of excitotoxicity, which is a key feature of various neurodegenerative disorders.

Oxidative stress plays a pivotal role in the pathogenesis of neurodegenerative diseases, and glutamate is a major contributor to this process. Glutamate-induced oxidative stress is implicated in neurodegenerative diseases due to its role in disrupting cellular calcium homeostasis, promoting nitric oxide (NO) synthesis, increasing acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity, generating reactive oxygen species (ROS), and triggering apoptotic pathways [17]. Our findings that glutamate exposure drastically reduces cell viability and increases LDH release align with previous research highlighting the toxic effects of excessive glutamate. The protective effects observed with SAM pre-treatment suggest that the formulation may enhance the cellular antioxidant capacity, potentially through its bioactive constituents. The observed restoration of mitochondrial membrane potential further supports the hypothesis that SAM helps maintain mitochondrial integrity, which is critical for neuronal survival.

SAM demonstrated neuroprotective properties, as evidenced by its ability to enhance cell viability in SH-SY5Y cells exposed to glutamate-induced toxicity. Higher concentrations of SAM (100 µg/mL) exerted superior protective effects compared to lower doses (50 µg/mL), indicating a clear dose-response relationship in mitigating glutamate-induced neuronal damage. These findings are consistent with previous reports suggesting SAM's efficacy in combating oxidative stress and preserving neuronal function [18].

Glutamate toxicity is often associated with increased LDH release, a marker of cytotoxicity reflecting compromised cell membrane integrity [19] Our study revealed that SAM treatment significantly reduced LDH release in SH-SY5Y cells exposed to glutamate similar to the standard drug Donepezil, indicating its ability to maintain cellular membrane stability and prevent neuronal cell death. This protective mechanism may involve SAM's antioxidant properties and its capacity to mitigate mitochondrial dysfunction, critical factors in apoptotic pathways triggered by glutamate-induced oxidative stress [20,21] Furthermore, SAM exhibited potent inhibitory activity against AChE and BChE enzymes in vitro. This inhibition suggests SAM's potential in enhancing cholinergic neurotransmission, which is impaired in neurodegenerative disorders like Alzheimer's disease [22]. The presence of active compounds such as eugenol and serine in SAM likely contributes to its neuroprotective effects by modulating cholinergic pathways and promoting neuronal health [23].

The increase in intracellular calcium levels due to glutamate exposure is another significant finding, as dysregulated calcium homeostasis can lead to mitochondrial dysfunction and cell death. SAM's ability to lower intracellular calcium levels indicates a protective mechanism that may involve the modulation of calcium signalling pathways, reducing excitotoxicity and preserving neuronal function. Calcium dysregulation plays a pivotal role in glutamate-induced neurotoxicity, leading to mitochondrial dysfunction and apoptotic cell death [24]. In our study, SAM treatment effectively attenuated the rise in intracellular calcium levels induced by glutamate exposure, thereby preserving neuronal survival. This protective effect of SAM on calcium homeostasis underscores its potential as a therapeutic agent for neurodegenerative diseases characterized by calcium-mediated neuronal damage. Moreover, the cholinesterase inhibition results suggest that SAM may enhance cholinergic signaling, which is often compromised in neurodegenerative conditions like Alzheimer's disease. By reducing cholinesterase activity, SAM may help improve neurotransmitter availability, supporting cognitive function and overall neuronal health.

The gene expression analysis adds a critical dimension to our understanding of SAM's mechanisms. The upregulation of pro-apoptotic genes (p38 and alpha-synuclein) in response to glutamate, along with the downregulation of neuroprotective genes (AKT and ERK), underscores the cellular stress that occurs during excitotoxicity. SAM's ability to reverse these expression changes suggests that it may act on key signaling pathways involved in cell survival and apoptosis, particularly through the activation of the AKT and ERK pathways, which are known for their roles in promoting neuronal survival and reducing oxidative stress. Gene expression analysis revealed that SAM upregulated the expression of AKT, p38, and ERK genes while attenuating alpha-synuclein expression in SH-SY5Y cells under oxidative stress conditions. These genes are involved in cell survival, antioxidant defense, and protein aggregation pathways, suggesting that SAM exerts its neuroprotective effects through multifaceted molecular mechanisms [25].

These findings underscore the potential of SAM demonstrates promising neuroprotective properties against glutamate-induced oxidative stress in SH-SY5Y neuroblastoma cells. The observed efficacy in enhancing cell viability, reducing cytotoxicity, inhibiting cholinesterase activity, regulating calcium homeostasis, and modulating neuroprotective gene expression supports SAM's potential as a therapeutic intervention for neurodegenerative disorders. The effects of SAM were similar to the effects of the standard drug Donepezil and

in some vital parameters like alpha synuclein expression and intracellular calcium levels, the effects were better than the standard drug. Nevertheless further studies are warranted to elucidate the specific active compounds within SAM responsible for these effects and to explore its efficacy in in vivo models of neurodegeneration. Additionally, clinical investigations are needed to assess the safety and therapeutic potential of SAM in human subjects, paving the way for its integration into contemporary treatment paradigms. This research supports SDG 3: Good Health and Well-Being by providing experimental evidence for neuroprotective interventions that may contribute to improved management of neurodegenerative disorders and promotion of neurological health.

Conclusion:

This study demonstrates that Saraswatharishtam (SAM) exhibits significant neuroprotective effects against glutamate-induced oxidative stress in SH-SY5Y neuroblastoma cells. The findings indicate that SAM enhances cell viability, preserves mitochondrial function, and reduces cytotoxicity and dysregulated calcium levels associated with excitotoxicity. Furthermore, SAM modulates key signaling pathways by restoring the expression of neuroprotective genes while inhibiting pro-apoptotic factors. The results highlight that SAM effectively reduces cytotoxicity markers, such as LDH release, inhibits acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes, and regulates intracellular calcium levels. These findings suggest that SAM acts through multiple mechanisms to protect neuronal cells from oxidative damage. The observed response underscores SAM's potential as a therapeutic agent for neurodegenerative disorders, emphasizing the need for further investigation into its clinical applications and possible synergies with existing treatments. This research paves the way for integrating traditional Ayurvedic remedies into modern neuroprotective strategies, offering hope for improved management of neurodegenerative diseases.

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