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Original Article

Evaluating the Neuroprotective and Antioxidant Effects of Orgaheal™ Ashwagandha with Valerian Root caplet in Oxidative Stress Models: *In vitro* study

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ABSTRACT

Background: Ashwagandha (*Withania somnifera*), traditionally valued for enhancing stress resilience, is known for its neuroprotective and antioxidant effects. This study explores the Orgaheal™ Ashwagandha with Valerian Root, which includes Ashwagandha Root Powder, Ashwagandha Root Extract, and Organic Valerian Extract, for potential use in stress management and sleep enhancement. **Materials and Methods:** This study investigated the antioxidant, neuroprotective, and adaptogenic effects in aqueous extract of Ashwagandha Formula (AAE) using *in vitro* assays. Antioxidant activity was assessed via DPPH, FRAP, and other radical scavenging assays. Neuroprotective effects were evaluated in SH-SY5Y neuroblastoma cells exposed to Dex-induced oxidative stress, measuring cell viability, ROS, apoptosis, and protein expressions (BDNF, pTrkB, CREB). **Results and Discussion:** AAE demonstrated significant antioxidant activity, BChE inhibition (IC_{50}: 4.8 mg/mL), and neuroprotective effects, likely due to withanolides and valerenic acid. These compounds reduced ROS, apoptosis, and restored antioxidant levels. In SH-SY5Y cells under Dex-induced oxidative stress, AAE improved cell viability and morphology, reversing Dex-induced suppression of BDNF, pTrkB, and CREB (all $p<0.001$), thereby enhancing cellular resilience. **Conclusion:** Orgaheal™ Ashwagandha with Valerian Root, rich in withanolides and valerenic acid, offers potent antioxidant and neuroprotective effects, helping mitigate stress and enhance mental resilience.

Keywords: Antioxidant activity, Neuroprotection, Orgaheal™ Ashwagandha with Valerian Root, Oxidative stress, Valerenic Acid, Withanolides.

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INTRODUCTION

Ashwagandha (*Withania somnifera*), known as Indian ginseng, has been used in Ayurveda for over 3,000 years, revered for its ability to enhance vitality, reduce stress and support overall well-being (Patibandla *et al*., 2024). The name "ashwagandha" translates to "smell of the horse", which is reflective of the traditional belief that it imparts the vigor and strength of a horse. Originating from the Indian subcontinent, ashwagandha has been used to treat various conditions, including chronic fatigue, inflammation, and neurological disorders (Mishra *et al*., 2000). Historically, ashwagandha has been used extensively in Ayurveda practices as a rasayana, a rejuvenating herb that enhances longevity and vitality (Singh *et al*., 2011).

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In recent years, scientific interest has intensified around ashwagandha due to its reported antioxidant, neuroprotective and mood-modulating effects. One of the primary reasons for ashwagandha extensive use in traditional medicine is its adaptogenic properties (Mikulska *et al*., 2023). Adaptogen are natural compounds that assist the body in adapting to stress, balancing physiological functions and maintaining homeostasis. Ashwagandha adaptogenic effects are largely attributed to its rich phytochemical composition, which includes steroidal lactones (withanolides), alkaloids, saponins and flavonoids. These compounds work synergistically to modulate the body's stress response and improve mental and physical resilience (Salve *et al*., 2019).

Modern research into Orgaheal™ Ashwagandha with Valerian Root, including Ashwagandha Root Powder, Ashwagandha Root Extract and Organic Valerian Extract, emphasizes their adaptogenic properties and benefits for stress relief, mood support and sleep enhancement. The combination of these ingredients leverages both traditional and scientific knowledge to enhance therapeutic efficacy. The root powder offers a natural source

of ashwagandha active compounds, particularly withanolides, while the root extract provides a more concentrated form of these bioactive withanolides for enhanced therapeutic efficacy (Mikulska *et al*., 2023). Organic Valerian Extract (*Valeriana officinalis*), containing bioactive allergenic acid complements these effects by promoting relaxation and improving sleep quality (Bent *et al*., 2006). Together, these formulations aim to address contemporary issues of stress, mood instability and sleep disturbances through a blend of historical wisdom and modern science.

Stress is a major factor that contributes to a wide range of health issues, including anxiety, depression and cognitive impairment (Cohen *et al*., 2007). The HPA axis is essential in managing the body's stress response. When activated by stress, the HPA axis triggers the release of cortisol, the primary stress hormone (Herman and Cullinan, 1997). Chronic activation of this axis and prolonged exposure to elevated cortisol levels can lead to detrimental effects on the body includes, impairing cognitive function, weakening the immune system, and increasing the risk of chronic diseases (Mcewen, 2004). Ashwagandha has been shown to modulate the HPA axis, thereby reducing cortisol levels and alleviating stress. In addition to stress relief, ashwagandha is known for its mood-enhancing properties. Mental health disorders such as depression and anxiety are often linked to chronic stress and dysregulation of neurotransmitters like serotonin, dopamine and GABA. Ashwagandha bioactive compounds can influence these neurotransmitter systems, promoting a balanced mood and reducing symptoms of depression and anxiety (Pingali *et al*., 2013).

In vitro studies with SH-SY5Y human neuroblastoma cells show that ashwagandha mitigates the toxic effects of dexamethasone, a synthetic glucocorticoid that causes oxidative stress and apoptosis and also it improves cell viability and reduces apoptosis through its antioxidant properties and stress-response modulation. Assays such as the MTT assay and fluorescence microscopy with H2DCFDA staining are used to assess cell viability and Reactive Oxygen Species (ROS) levels. Flow cytometry further elucidates the biochemical pathways affected by ashwagandha, highlighting its adaptogenic and neuroprotective mechanisms.

In conclusion, ashwagandha adaptogenic properties, stress-relieving capabilities, mood-supporting and sleep enhancement benefits make it a valuable addition to modern therapeutic practices. Its ability to modulate the HPA axis, enhance neurotransmitter balance, and protecting against oxidative stress underscores its potential as a natural remedy for managing stress-related disorders and promoting mental well-being.

MATERIALS AND METHODS

Extraction

About 10 g of Orgaheal™ ashwagandha Valerian Root caplet is extracted with 100 mL of distilled water. The solution was heated for 1 hr, then cooled and centrifuged to obtain a clear supernatant. The supernatant was allowed to dry at room temperature to obtain the aqueous extract of Orgaheal™ ashwagandha Valerian Root caplet. The dried extract was then stored at 4ºC for future use.

In vitro antioxidant assay

Antioxidants are compounds that neutralize free radicals in the body, helping to prevent oxidative stress and cellular damage, which are linked to various chronic diseases and aging. The antioxidant capacity of Ashwagandha Aaqueous Extract (AAE) was evaluated through several assays, the DPPH radical scavenging assay, which measured absorbance at 517 nm to determine scavenging activity based on the reduction of purple color; the FRAP assay, assessing ferric ion reduction to ferrous ions with a maximum absorption at 593 nm; the nitric oxide scavenging assay, (Boora *et al*., 2014) which involved Griess reagent and measured absorbance at 546 nm; the superoxide radical scavenging assay, based on formazan formation and read at 560 nm; lipid peroxidation inhibition, involving TBA and absorbance measurement at 532 nm; the hydrogen peroxide scavenging assay, with absorbance at 510 nm; and the hydroxyl radical scavenging assay, which measured yellow color intensity at 412 nm (Mukhopadhyay *et al*., 2016). Scavenging activity for all assays was calculated using the formula,

$$\%\,Scavenging\ activity = \left[\frac{absorbance(control) - absorbance\ (sample)}{absorbance\ (control)}\right] * 100$$

In vitro cholinterase inhibition and kinetics

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) are key enzymes responsible for the breakdown of acetylcholine and butyrylcholine, which are crucial for maintaining neurotransmitter balance. The activity and inhibition of AChE and BChE enzymes were analyzed using similar protocols. Both assays involved incubating enzyme mixtures with specific buffers, substrates, and DTNB, followed by measuring the resulting yellow colour at 412 nm (Ellman *et al*., 1961). For inhibition studies, enzymes were incubated with different concentrations of AAE, and their activity was measured post-incubation. Kinetic analysis for both enzymes utilized Lineweaver-Burk plots to determine the inhibition type and Dixon plots to calculate the inhibitory constant (Ki).

MTT assay: Protection against dexamethasone induced toxicity in neuronal cell

The MTT assay assessed AAE's protection against dexamethasone toxicity in SH-SY5Y cells. The cells were first cultured and then exposed to various concentrations of the extract and

dexamethasone for a day. MTT reagent was added post-treatment and incubated for 3 hr. This reagent forms formazan crystals in viable cells. The crystals were subsequently dissolved in DMSO, and the absorbance was measured at 570 nm (Alley *et al.*, 1988).

$$\% \, Cell \ viability = \left[\frac{mean \ abs \ of \ treated \ cells}{mean \ abs \ of \ untreated \ cells}\right] \times 100$$

ROS generation: Fluorescence microscopy

Reactive Oxygen Species (ROS), which include hydroxyl radicals and peroxides, are naturally produced in cells but can increase under stress, causing oxidative damage and cell death. Using H2DCFDA, ROS levels were measured in SH-SY5Y cells treated for 1 day with control, Dex (1000 µM), or Dex-AAE (100 µg/mL) in high-glucose DMEM. Post-treatment, cells were stained with H2DCFDA and Hoechst 33342, then observed using a ZEISS LSM 880 fluorescence microscope (Kim and Xue, 2020). The fluorescent signal from DCF, indicating ROS presence, was analysed with ZEN Blue and Image J software.

Apoptosis: Fluorescence microscopy

The apoptosis assay for SH-SY5Y human neuroblastoma cells involves culturing the cells in 6-well plates and treating them with control, dexamethasone (1000 µM) and AAE (100 µg/mL) combined with dexamethasone for a day. Treated cells were harvested, ethanol-fixed, and stained with acridine orange and ethidium bromide to differentiate between viable and apoptotic cells (Oancea *et al.*, 2006). The stained cells are observed under a fluorescence microscope, using specific excitation and emission wavelengths for analysis. ImageJ software analyzes apoptosis, assessing AAE's protective effects.

Endogenous antioxidant level (Calorimetric assay)

The endogenous antioxidants assay for SH-SY5Y human neuroblastoma cells involves culturing the cells in high-glucose DMEM with serum and antibiotics, followed by treatment with control, dexamethasone (1000 µM) and AAE (100 µg/mL) for 1 day. After washing and lysing the cells with a lysis buffer, the lysate is centrifuged to obtain the supernatant, which is then analysed to measure specific endogenous antioxidants. Absorbance is measured using a spectrophotometer, and antioxidant levels are calculated based on standard curves (Weydert and Cullen, 2009).

Protein expression (Flow cytometry)

Protein expression, (Gray, 1997) which is the synthesis of proteins crucial for cellular function and stress response, was assessed to evaluate the effects of Dex and AAE on cellular pathways related to apoptosis and stress, thereby highlighting AAE's potential to protect neuronal health and support mood stability.SH-SY5Y cells maintained in high-glucose and seeded in 6-well plates, overnight incubated, and divided into three treatment groups: control, Dex (1000 µM) and AAE (100 µg/mL)+Dex. After 24 hr, cells were trypsinized, harvested, and centrifuged. They were then fixed with cold 70% ethanol, washed with PBS, and stained with the appropriate antibody. Following 30-min incubation in the dark, cells were analyzed using flow cytometry, adjusting for specific fluorophores based on their excitation and emission settings.

Statistical analysis

Statistical differences are expressed as mean±SD. One-way ANOVA was utilized to evaluate group differences, with Tukey's HSD test for *post-hoc* analysis. Statistical significance was set at a *p*-value less than 0.05. Data were analyzed using IBM SPSS Statistics (Version 29.0.2.0, 2023).

RESULTS

In vitro antioxidant assay

The AAE, rich in bioactive compounds such as with anolides from Ashwagandha and valerenic acid from Valerian, exhibited scavenging activity against DPPH, ABTS, superoxide, lipid peroxide and hydrogen peroxide radicals, as shown in Figure 1. The IC_{50} values for these activities are 663.29 µg/mL, 1106.25 µg/mL, 1059.16 µg/mL, 114.14 µg/mL and 494.23 µg/mL, are given in Table 1. Additionally, the extract demonstrated Ferric Reducing Antioxidant Powder (FRAP), with optical density increasing from 0.21 to 0.88 at 593 nm as the concentration of the extract increased, indicating potent antioxidant activity attributed to these bioactive compounds.

In vitro cholinterase inhibition and kinetics

The *in vitro* cholinesterase inhibition study using AAE containing withanolides and valerenic acid did not show inhibition against AChE, however inhibited BChE activity. At 5 mg/mL, AAE inhibited BChE activity by approximately 56%. The IC_{50} value for BChE inhibition is detailed in Table 2 and in Figure 2. Lineweaver-Burk (LB) plot indicated that AAE acts as a non-competitive inhibitor of BChE, reducing enzyme activity without changing substrate affinity, as shown in Figure 3. Dixon plot determined the inhibition constant (Ki) for AAE against BChE to be 1.67 mg/mL, also listed in Table 2.

MTT assay Protection against dexamethasone induced toxicity in neuronal cell

Treatment of neuronal cells (SH-SY5Y) with AAE did not affect cell viability up to 100 µg/mL, and cell morphology remained unchanged shown in Figure 4. In contrast, treatment with Dexamethasone (Dex) reduced cell viability and altered cell morphology, with 1000 µM Dex lowering viability to 20%. At this concentration, AAE provided protection against Dex-induced stress, increasing cell viability and maintaining normal cell morphology shown in Figure 4. These results suggest that AAE can protect neuronal cells from Dex-induced damage and relieve stress, supporting its use as a natural stress-relief agent. Statistical

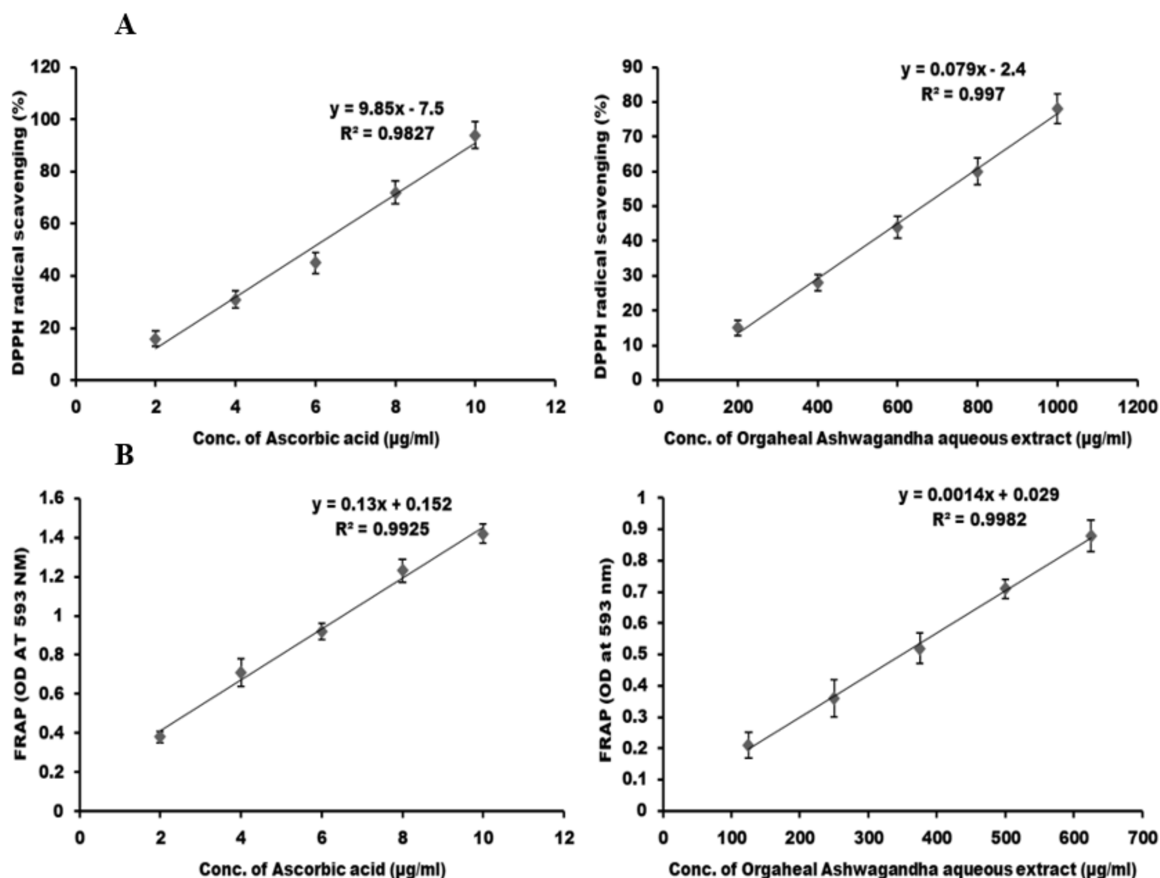
analysis using one-way ANOVA and Turkey's HSD test confirmed the significance of the findings [F (6,28)=15327.003, $p<0.001$].

ROS generation: Fluorescence microscopy

The study evaluated the impact of AAE on Reactive Oxygen Species (ROS) generation using fluorescence microscopy. The results revealed low ROS levels in Control, increased levels in the Dex, and a significant reduction in the Dex + AAE group Figure 5. Fluorescence microscopy images confirmed these findings, with minimal green fluorescence in Control group, high fluorescence in Dex group and reduced fluorescence in the Dex + AAE group shown in Figure 6. Statistical analysis (one-way ANOVA and Turkey's HSD test) supported the significance of these observations [F (2,6)=47.864, $p<0.001$], indicating that the

Table 1: *In vitro* antioxidant activity capacity of Orgaheal™ ashwagandha aqueous extract and standard drug.

Assay	Activity present/ absent	IC_{50} value	
		Orgaheal™ Ashwagandha Aqueous Extract	Standard
DPPH scavenging	Present	663.29 µg/mL	5.83 µg/mL (Ascorbic acid).
Nitric oxide scavenging	Present	1106.25 µg/mL	26.82 µg/mL (curcumin).
Superoxide radical scavenging	Present	1059.16 µg/mL	2.84 µg/mL (Ascorbic acid).
Lipid peroxidation inhibition	Present	114.14 µg/mL	106.19 µg/mL (Lipoic acid).
Hydrogen peroxide's radical scavenging	Present	494.23 µg/mL	49.33 µg/mL (Gallic acid).
Hydroxyl radical scavenging	Absent	-	-
FRAP	Present	OD increased from 0.21 to 0.88 at 593 nm.	OD increased from 0.38 to 1.42 at 593 nm (Ascorbic acid).



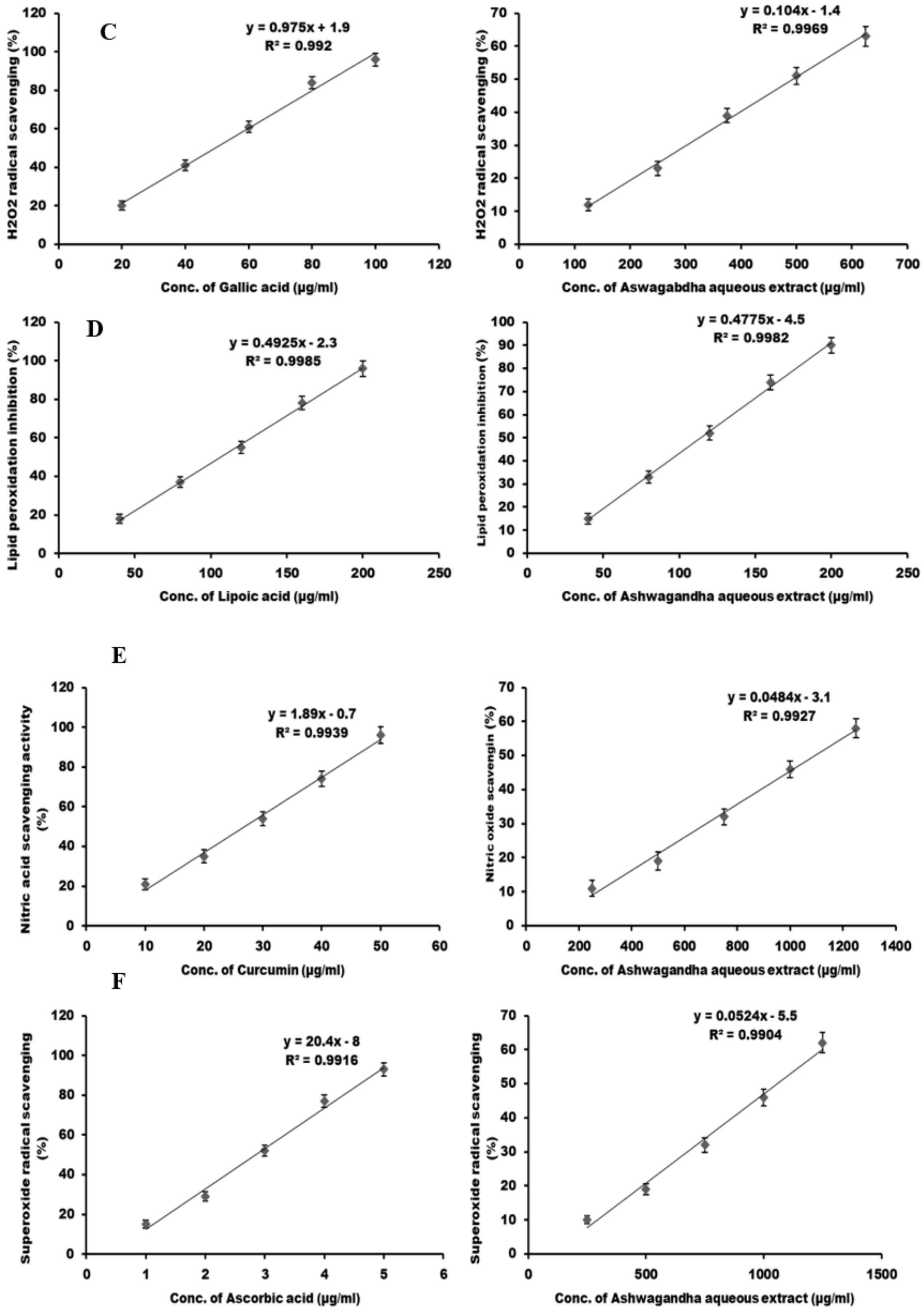


Figure 1: *In vitro* anti-oxidant assay of (A) DPPH scavenging assay, (B) FRAP, (C) Superoxide scavenging assay, (D) Lipid peroxidation inhibition, (E) Nitric oxide scavenging and (F) Superoxide radical scavenging.

bioactive compound in AAE effectively mitigates ROS generation and has potential antioxidant properties.

Apoptosis: Fluorescence microscopy

The fluorescence microscopy image in Figure 7 shows that AAE reduces apoptosis in neuronal cells exposed to dexamethasone. In Control, cells appear healthy with minimal apoptosis. The Dex Exposed group shows significant apoptosis with increased red fluorescence. In contrast, the AAE Treated + Dex Exposed group displays improved cell viability and reduced apoptosis, indicated elevated green fluorescence and reduced red fluorescence. These results suggest that AAE has potential stress-relief properties, protecting cells from dexamethasone-induced stress and implying benefits for managing stress-related conditions by maintaining cell health and reducing inflammation.

Endogenous antioxidant level (Calorimetric assay)

The effects of AAE on endogenous antioxidant levels in neuronal cells are shown in Figure 8. In the Control group, high levels of CAT, GSH, GPX and SOD indicate robust antioxidant capacity shown in Table 3. Dexamethasone exposure significantly reduces these levels, reflecting severe oxidative stress. However, due to the presence of withanolides and valerenic acid in AAE, the treatment restores antioxidant levels to near-normal values, mitigating dexamethasone-induced oxidative stress. Statistical analysis confirms the significance of these findings for CAT [F (2,6)=3760.767, $p<0.001$], GPX [F (2,6)=1822.832, $p<0.001$], GSH [F (2,6)=1631.540, $p<0.001$], and SOD [F (2,6)=3829.565, $p<0.001$]. These results highlight AAE's potent antioxidant properties and its potential as a natural therapeutic agent for combating oxidative stress-related conditions.

Protein expression (Flow cytometry)

The flow cytometry analysis reveals the impact of Ashwagandha Extract (AAE) on the expression levels of BDNF, pTrkB and CREB in SH-SY5Y cells. Histograms in Figure 9 show that untreated cells exhibit baseline levels of these proteins. However, treatment with Dexamethasone (Dex) significantly reduces the expression of BDNF, pTrkB, and CREB, indicating the inhibitory effect of dexamethasone. When cells are co-treated with AAE, the expression levels of these proteins are restored to near-control levels, suggesting that AAE mitigates the suppressive effects of dexamethasone. Figure 10 further supports these findings, showing a significant reduction in the percentage of cells expressing BDNF, pTrkB, and CREB with dexamethasone

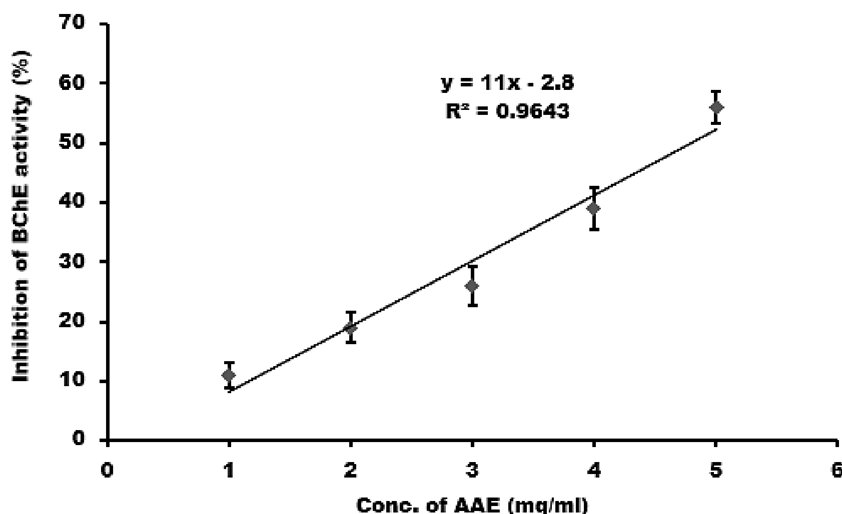


Figure 2: Inhibition of BChE by AAE.

Table 2: ***In vitro*** **AChE and BChE inhibition using AAE.**

Enzyme activity	IC_{50} (mg/mL)	Type of inhibition	Ki (mg/mL)
AChE	-	-	-
BChE	4.8	Non-competitive	1.67

Table 3: **Endogenous Antioxidant Levels in Neuronal Cells Treated with Dexamethasone and AAE.**

Culture condition	CAT (ng/mL)	GPx (pg/mL)	GSH (ng/mL)	SOD1 (pg/mL)
Control	11.16±0.35	1079.53±12.96	28.17±0.49	2699.99±151
Dex-1000 μM	1.66±0.1	113.73±6.75	3.11±0.28	174.88±5.33
Dex + AAE-100 μg/mL	8.83±0.08	593.14±30.62	20.92±0.77	1797.6±121.47

treatment. However, co-treatment with AAE significantly increases the percentage of cells expressing these proteins compared to dexamethasone alone. These results indicate that AAE has a protective effect against dexamethasone-induced suppression of BDNF, pTrkB and CREB, highlighting its potential therapeutic benefits in counteracting the negative impacts of dexamethasone on neuronal cells. For statistical analysis, BDNF [F (2,6)=8354.993, $p<0.001$], pTrkB [F (2,6)=3686.868, $p<0.001$] and CREB [F (2,6)=42564.992, $p<0.001$.

DISCUSSION

The comprehensive evaluation of Orgaheal™ Ashwagandha with Valerian Root and its effects on neuronal cells exposed to Dexamethasone (Dex) highlights its significant therapeutic potential, particularly in stress management and mood support. The *in vitro* antioxidant assays demonstrated that AAE has strong antioxidant properties. Similar to previous findings with Methanolic extracts, (Pal *et al.*, 2011) this aqueous extract showed significant scavenging activity against various radicals, including DPPH, ABTS, superoxide, lipid peroxide, and hydrogen peroxide. The IC_{50} values for these radicals indicated effective neutralization

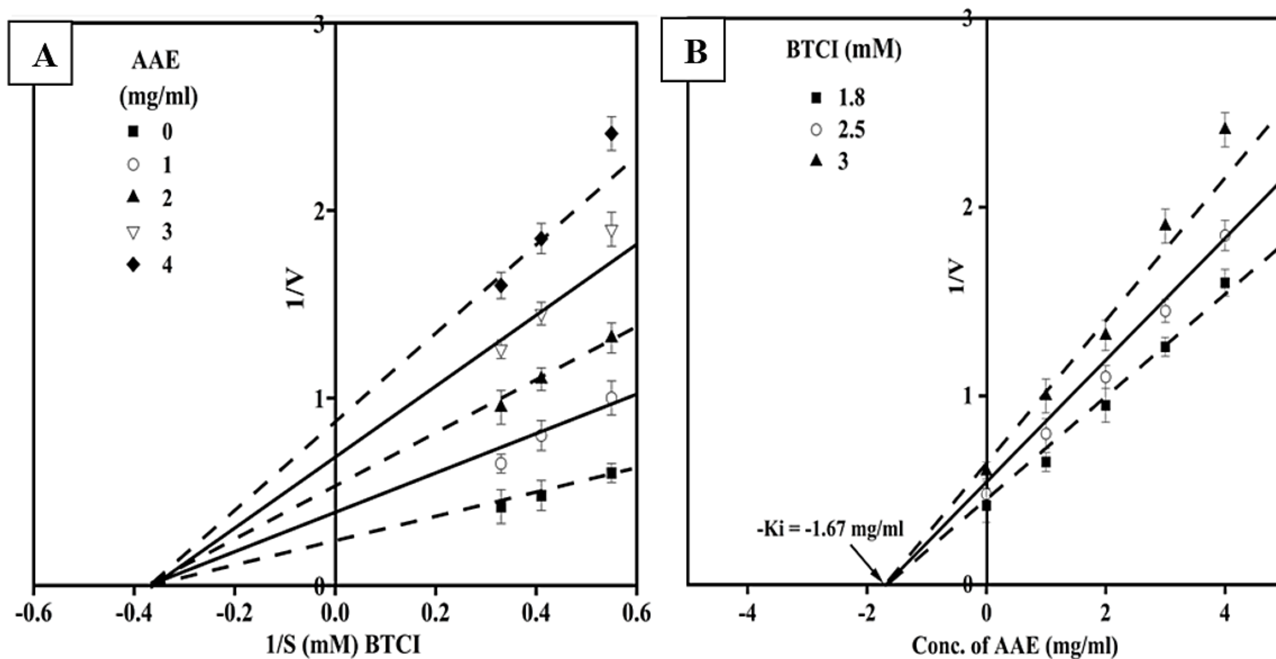
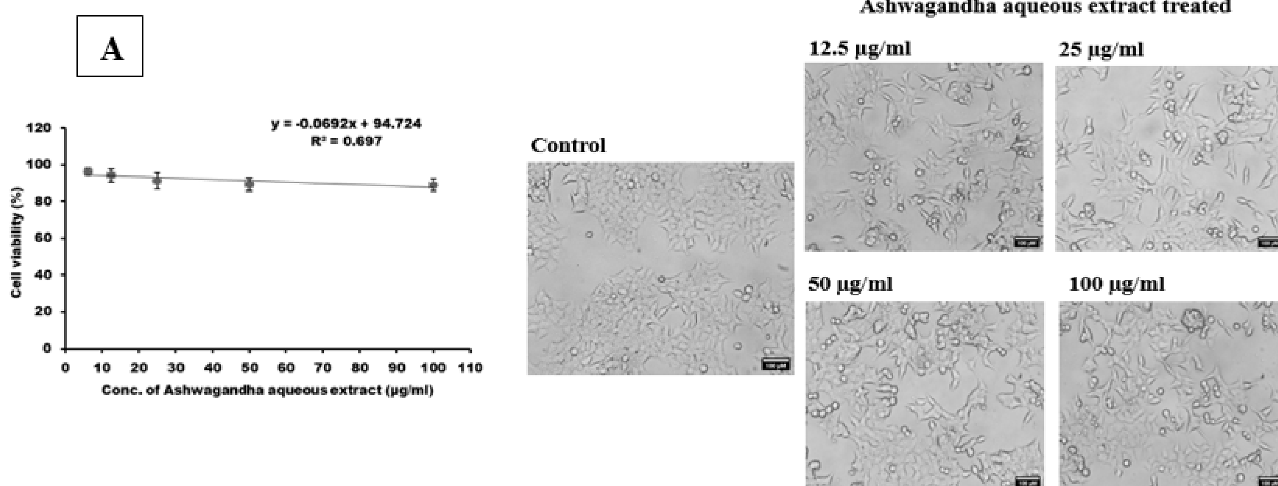


Figure 3: Inhibition Kinetics of Cholinesterase assay using AAE by Lineweaver Burk (LB) and Dixon plot includes: (A) LB plot of BChE inhibition, (B) Dixon plot of AChE inhibition.



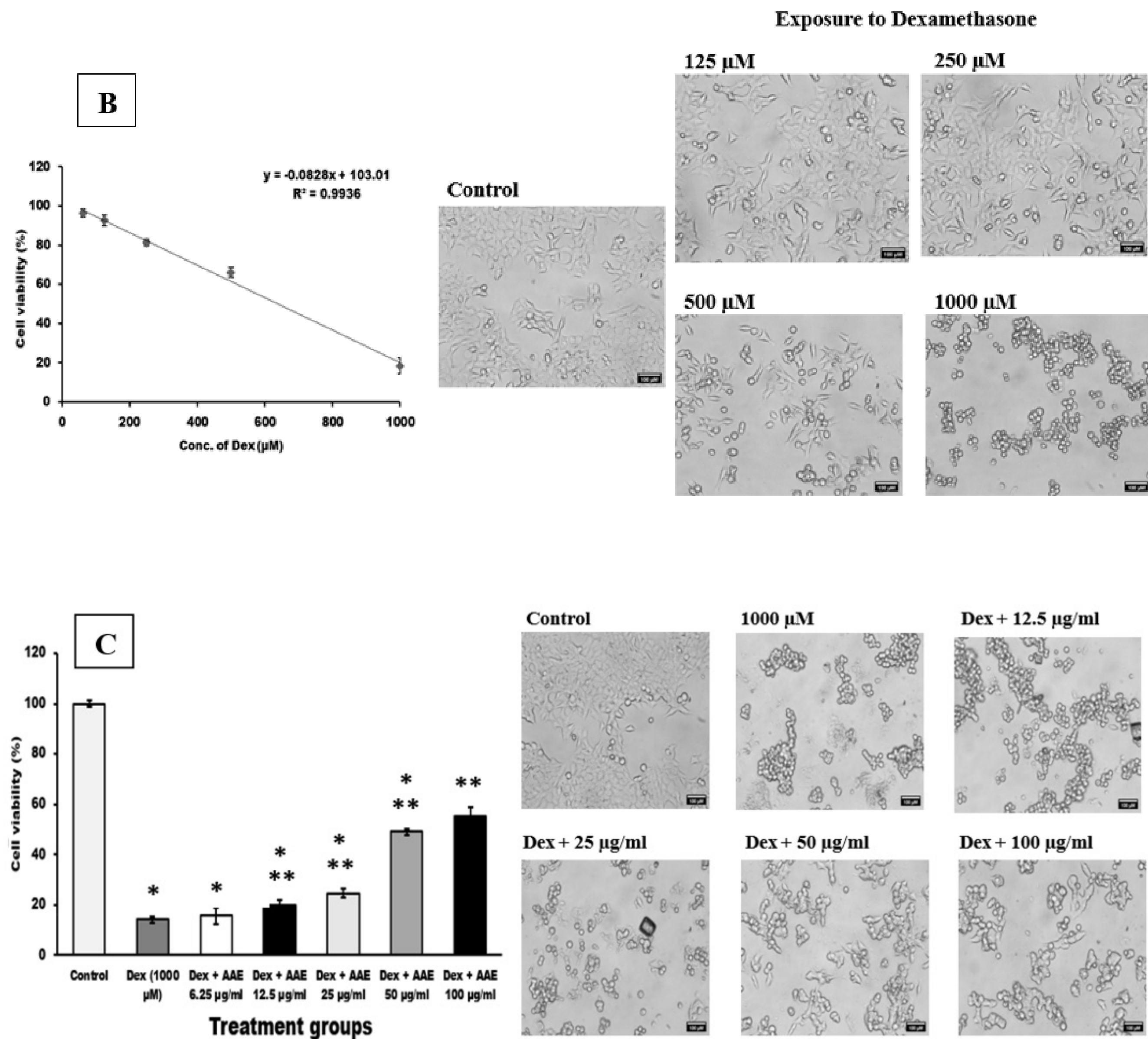


Figure 4: Effect of AAE and Dexamethasone on Cell Viability and Morphology (A) SH-SY5Y cells treated with ashwagandha aqueous extract (B) the SH-SY5Y cells treated with Dex (C) SH-SY5Y cells from various groups. The values are presented as mean±SD. * $p<0.001$ compared to the control group; ** $p<0.001$ between group.

of oxidative stressors. The Ferric Reducing Antioxidant Power (FRAP) assay further highlighted its electron-donating capacity, showing a significant increase in optical density with higher extract concentrations, underscoring its potential for protecting cells from oxidative damage. In contrast to previous studies showing significant AChE inhibition by ashwagandha root extracts (Pal *et al*., 2017; V. K. Singh *et al*., 2022), our study found that AAE selectively inhibits BChE with an IC_{50} of 4.8 mg/mL, without affecting AChE. This non-competitive inhibition, where the extract binds to BChE at a site other than the active site, suggests potential cognitive and neuroprotective benefits by modulating cholinergic pathways crucial for neurotransmission and cognitive functions. Previous research identified that the hydro alcoholic extract of *Withania somnifera* leaves exhibited significant cytotoxicity activity against MCF-7 cancer cells (Nema *et al*., 2013). In contrast, our study demonstrated that AAE offers neuroprotective effects by maintaining the viability and normal morphology of neuronal SH-SY5Y cells exposed to dexamethasone-induced toxicity. While this study highlighted the anticancer potential of ashwagandha, our research underscores its protective role against glucocorticoid-induced neuronal damage, suggesting its potential application in managing chronic stress and neurodegenerative conditions (Nema *et al*., 2013).

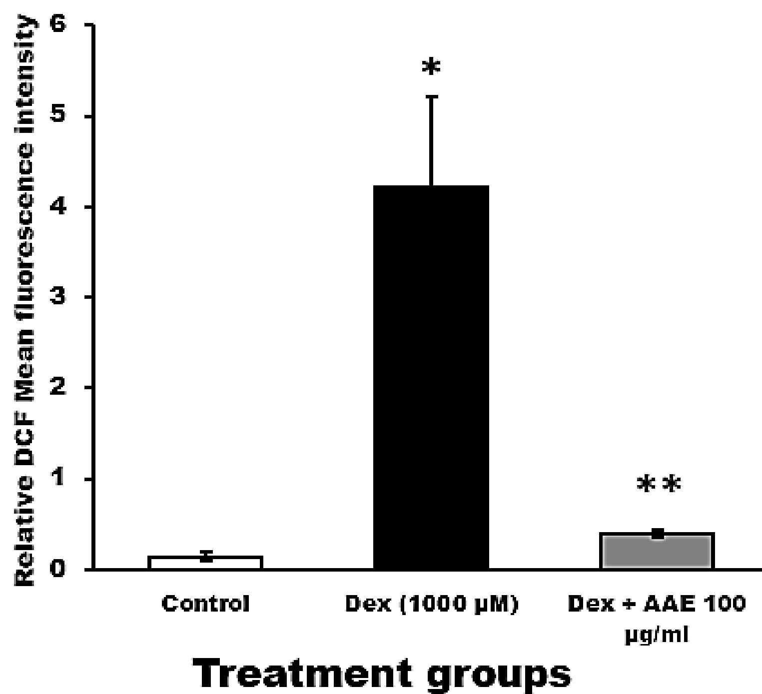


Figure 5: Effect of Dexamethasone and AAE on Reactive Oxygen Species (ROS) Generation. The values are presented as mean±SD. * $p<0.001$ compared to the control group; ** $p<0.001$ between groups.

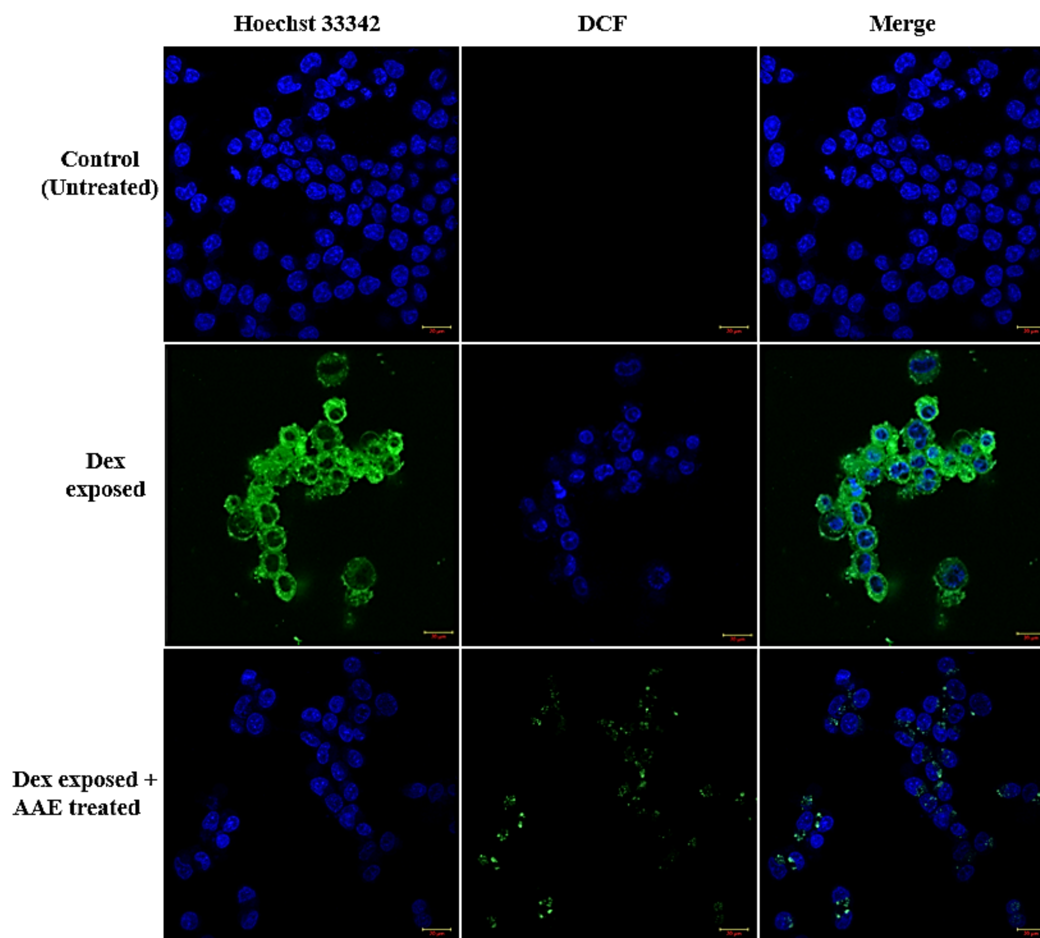


Figure 6: AAE's Protective Role Against Dexamethasone-Induced Stress in SH-SY5Y Cells.

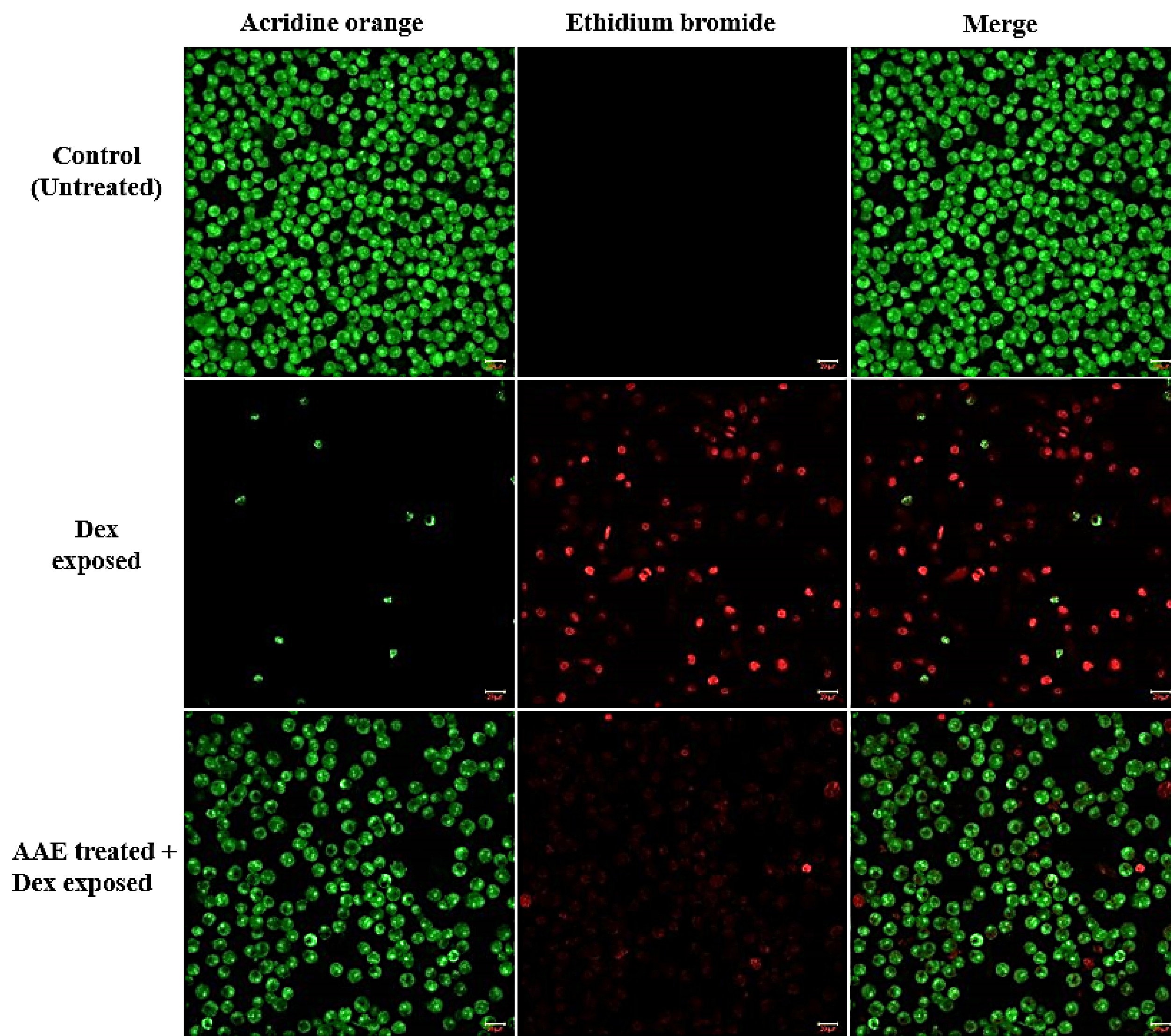


Figure 7: Effects of AAE on Dexamethasone (Dex)-induced Apoptosis in SH-SY5Y Cells.

A study on rats exposed to γ-radiation showed that Ashwagandha improved redox status by reducing ROS levels and oxidative stress in spleen and liver tissues, thereby protecting the antioxidant system from radiation-induced damage (Azab *et al.*, 2022). Similarly, our study using fluorescence microscopy found that AAE significantly reduced ROS levels and oxidative stress in neuronal SH-SY5Y cells exposed to dexamethasone. This demonstrates the extract's antioxidant effectiveness. For apoptosis reported that Withaferin A, an ashwagandha component, induces apoptosis useful in cancer research (Lee *et al.*, 2015). In contrast, our study on AAE showed it reduces apoptosis in neuronal cells exposed to dexamethasone, as indicated by decreased red fluorescence and increased green fluorescence. These findings suggest AAE has neuroprotective properties and can manage stress-induced oxidative damage.

Endogenous antioxidant has been demonstrated in previous study that the pre-treatment with Ashwagandha Root Extract (ARE) enhanced the activities of key antioxidant enzymes like SOD, GPx and CAT in hydrogen peroxide-challenged lymphocyte (Pal *et al.*, 2017). In contrast, our study found that AAE significantly restored levels of key antioxidants (CAT, GPX, GSH, and SOD) in dexamethasone-treated cells, highlighting its effectiveness in mitigating oxidative stress and enhancing cellular antioxidant defences. Protein expression showed that ashwagandha and withanolide A activate the BDNF receptor TrkB in hippocampal neurons, promoting neuronal survival (Chen and Russo-Neustadt, 2023). Our study further found that dexamethasone reduces the expression of crucial neuronal proteins (BDNF, pTrkB and CREB), but co-treatment with AAE restores these levels. This suggests that AAE supports neuronal health and counters dexamethasone-induced suppression, maintaining proteins essential for neuroprotection and cognitive function.

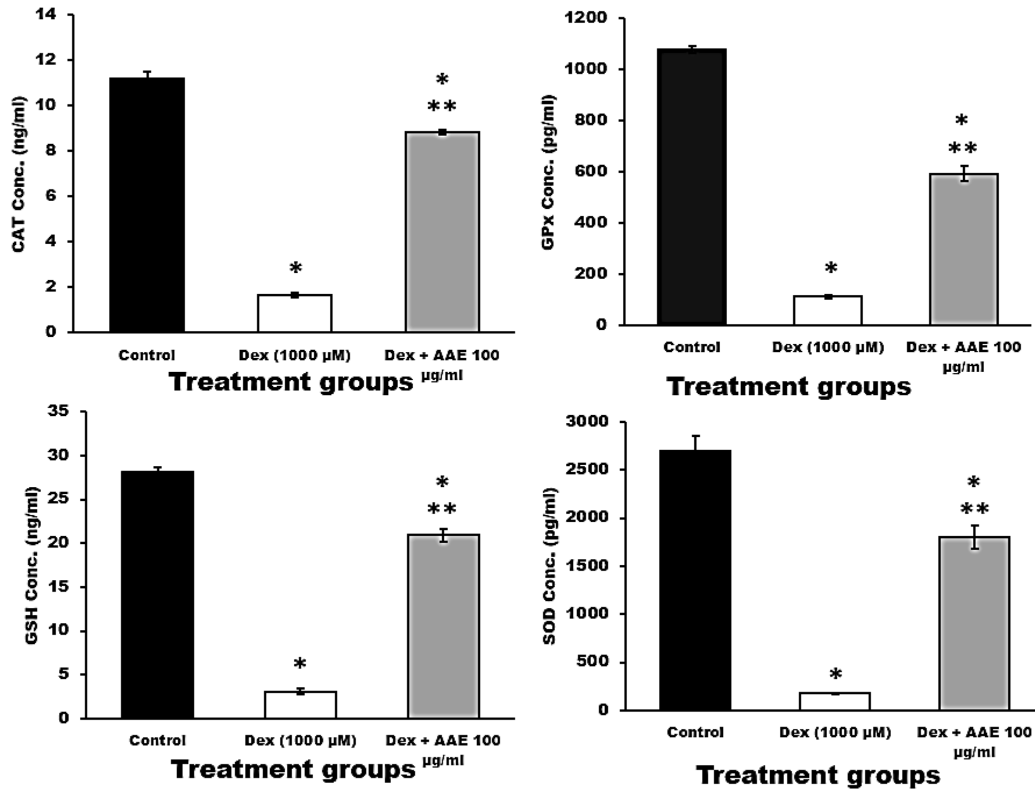


Figure 8: Endogenous Antioxidant Enzyme Levels in Neuronal Cells SH-SY5Y(A) Catalase, (B) Glutathione peroxidase, (C) Glutathione and (D) Superoxide dismutase in SH-SY5Y Cell line. The values are presented as mean±SD. * $p<0.001$ compared to the control group; ** $p<0.001$ between groups.

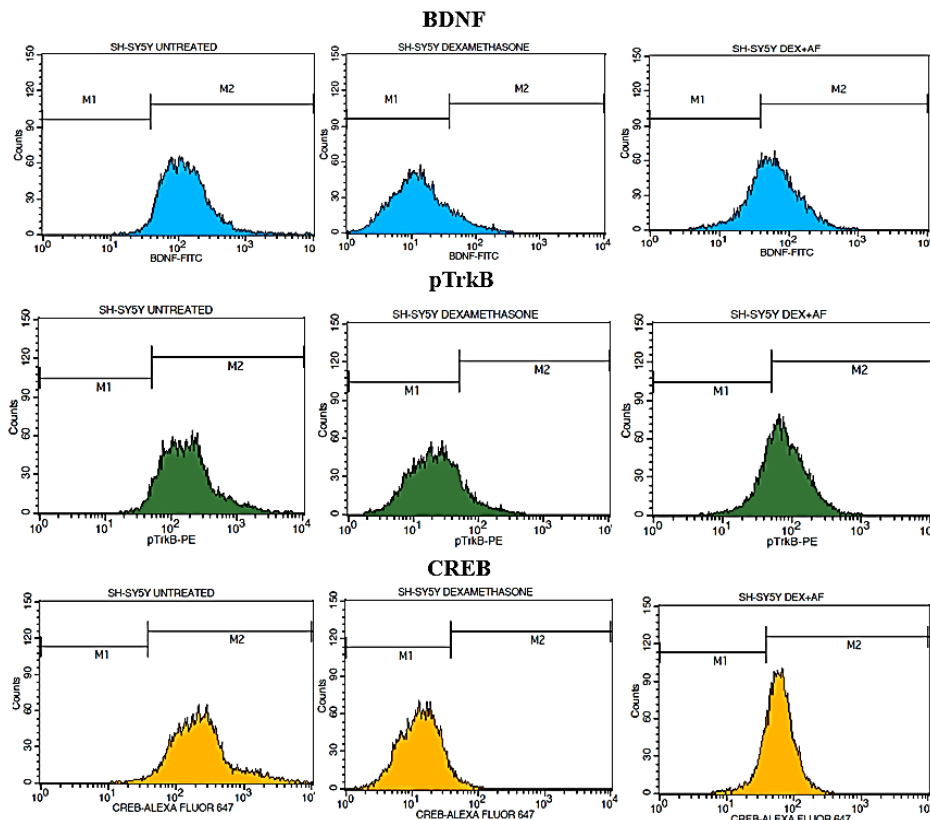


Figure 9: Flow Cytometry analysis of BDNF, pTrkB and CREB Protein Expression in SH-SY5Y Cells under different treatment conditions.

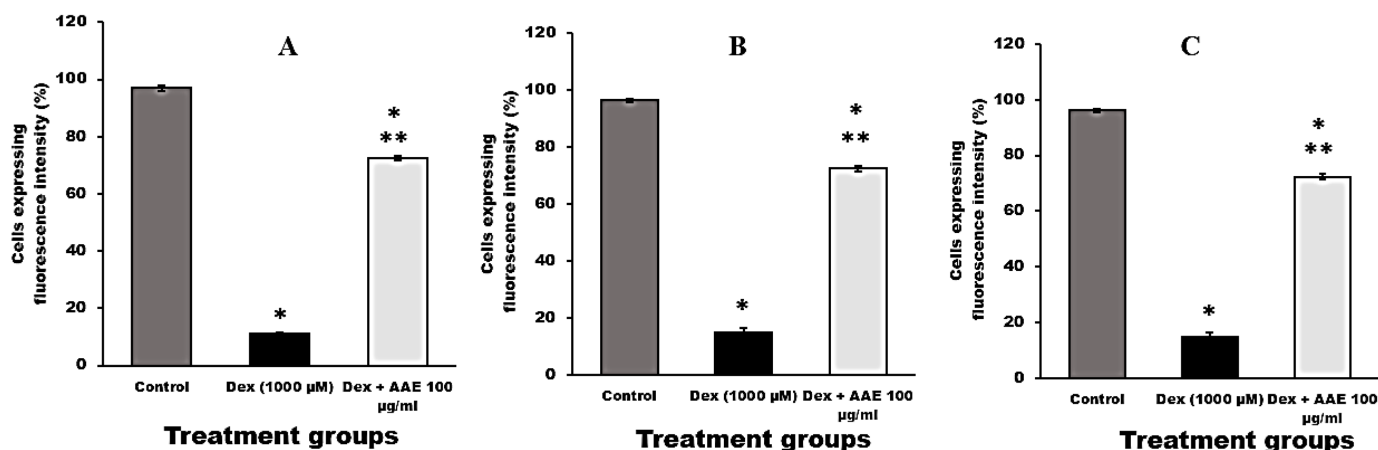


Figure 10: Effect of Dexamethasone and AAE on the Expression of (A) BDNF, (B) pTrkB and (C) CREB in SH-SY5Y Cells. The values are presented as mean±SD. * $p<0.001$ compared to the control group; ** $p<0.001$ between groups.

CONCLUSION

In conclusion, the Orgaheal™ Ashwagandha with Valerian Root, rich in bioactive compounds such as withanolides and valerenic acid exhibits potent antioxidant, neuroprotective and anti-stress properties in various *in vitro* assays. The presence of these bioactive compounds is responsible for AAE's selective inhibition of BChE, restoration of key antioxidants and support of essential neuronal proteins like BDNF highlight its potential in combating oxidative stress and neurodegenerative conditions. Orgaheal™ Ashwagandha with Valerian Root shows protective effects against apoptosis further underscore its promise as a natural remedy for enhancing cognitive function and managing stress-related damage. These findings suggest that Orgaheal™ Ashwagandha with Valerian Root could be a valuable addition to strategies aimed at promoting neurological health and well-being.

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FUNDING

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AAE: Ashwagandha Aqueous Extract; **ROS:** Reactive Oxygen Species; **PBS:** Phosphate buffer saline; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **FRAP:** Ferric Reducing Antioxidant Power; **GPx:** Glutathione Peroxidase; **GSH:** Glutathione; **CAT:** Catalase; **SOD:** Superoxide Dismutase; **HPA:** Hypothalamus-Pituitary-Adrenal; **IC_{50}:** Half Maximal Inhibitory Concentration; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **BDNF:** Brain-Derived Neurotrophic Factor; **pTrkB:** Phosphorylated Tropomyosin receptor kinase B and **CREB:** CAMP response element-binding protein; **H2DCFDA:** 2',7'-dichlorodihydrofluorescein diacetate.

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