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Original Article

Neuroprotection and Oxidative Stress Reduction by *Bacillus coagulans*: An *in vitro* Comparison with Escitalopram

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ABSTRACT

Background: Neurodegenerative and psychiatric disorders are often linked to oxidative stress and mitochondrial dysfunction, necessitating novel therapeutic strategies. *Bacillus coagulans*, a probiotic known for its role in gut-brain axis modulation, has been investigated for its potential neuroprotective and antioxidant properties. Assessing its effects on neuronal cell viability and oxidative stress may provide valuable insights into its therapeutic potential. This study aims to evaluate the cytotoxicity and oxidative stress modulation of *Bacillus coagulans* in SH-SY5Y neuroblastoma cells. **Materials and Methods:** Escitalopram was used as a standard to compare its potential neuroprotective and antioxidant effects. Cytotoxicity studies on SH-SY5Y were assessed by MTT Assay at 5 different concentrations. Oxidative stress levels were analyzed using ROS (DCFDA) to evaluate antioxidant activity. Escitalopram was used as a reference standard for comparative analysis. **Results:** *Bacillus coagulans* exhibited minimal cytotoxicity at all tested concentrations, with SH-SY5Y cell viability remaining high and comparable to untreated controls. The MTT assay confirmed a concentration-dependent response, but no significant toxicity was observed at physiologically relevant doses. In ROS assays, *Bacillus coagulans* treatment led to moderate suppression of ROS generation in SH-SY5Y cells exposed to oxidative stress, with antioxidant effects closely matching those of escitalopram. Fluorescence imaging and quantification revealed that *Bacillus coagulans* effectively attenuated oxidative stress without compromising cell viability. **Conclusion:** The study provides insights into the effects of *Bacillus coagulans* on neuronal cell survival and oxidative stress modulation. Observed cytotoxicity and antioxidant activity suggest its potential neuroprotective role, supporting its relevance in neurological health. The findings highlight the neuroprotective and antioxidant potential of *Bacillus coagulans* in SH-SY5Y cells. Its ability to regulate oxidative stress may contribute to novel therapeutic approaches for neurodegenerative and psychiatric disorders, warranting further investigation.

Keywords: *Bacillus coagulans*, Oxidative stress, SH-SY5Y, Escitalopram.

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INTRODUCTION

Neuropsychiatric disorders are a wide range of conditions that target the brain and mind, affecting mood, cognition, behavior, and general mental health. Anxiety, depression, schizophrenia, bipolar disorder, and neurodegenerative diseases like Alzheimer's disease and Parkinson's disease are examples of neuropsychiatric disorders. Neuropsychiatric disorders have multifactorial and complex pathophysiology, in most cases including genetic, environmental, and neurobiological Elements. Historically, treatment for these disorders has centered on medication and psychotherapy. But recently, there has also been increasing interest in the contribution of the gut-brain axis, i.e., the modulation of neurological health and the causation of neuropsychiatric diseases by gut microbiota (Trushina and McMurray, 2007; Yadav *et al*., 2023).

Probiotics, defined as live microorganisms conferring health benefits to the host, have demonstrated potential in mitigating oxidative stress and modulating neuroinflammation. Specific strains exhibit inherent antioxidant properties, such as scavenging

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free radicals and enhancing host antioxidant enzyme activities, thereby contributing to neuronal protection. Additionally, probiotics can influence the gut-brain axis by producing neuroactive compounds and modulating systemic inflammation, suggesting their therapeutic potential in neurodegenerative conditions (Begum *et al*., 2022).

Bacillus coagulans, a spore-forming, lactic acid-producing bacterium, has been recognized for its gastrointestinal benefits, including alleviation of irritable bowel syndrome symptoms and enhancement of immune function. Emerging evidence suggests that *Bacillus coagulans* may exert neuroprotective effects through its antioxidant capabilities and modulation of the gut-brain axis. However, comprehensive studies evaluating its direct impact on neuronal cells and oxidative stress are limited (Savignac *et al*., 2015).

This study aims to investigate the neuroprotective and antioxidant potential of *Bacillus coagulans* in SH-SY5Y neuroblastoma cells, utilizing escitalopram as a standard for comparison. By assessing cytotoxicity and oxidative stress modulation, this research seeks to elucidate the therapeutic viability of *Bacillus coagulans* in neurodegenerative disease contexts.

MATERIALS AND METHODS

Assessment of Cytotoxicity of *Bacillus coagulans* using MTT Assay

Cell Line and Culture Conditions

Human neuroblastoma SH-SY5Y cell line was utilized for the cytotoxicity assessment. Cells were maintained in DMEM-F12 (Himedia, #AL127A) supplemented with 10% fetal bovine serum (Himedia, #RM10432) and 1% antibiotic-antimycotic solution containing penicillin and streptomycin (Himedia, #A001A). Cultures were incubated at 37°C in a humidified atmosphere with 5% CO_2 in a Memmert incubator (Germany).

Experimental Design

SH-SY5Y cells were seeded at a density of 1×10^4 cells per well in a 96-well plate (Corning, USA) and allowed to adhere overnight. *Bacillus coagulans* was prepared at concentrations of 6.25, 12.5, 25, 50, and 100 µM, and cells were exposed to these concentrations for 48 hr.

MTT Assay Protocol

Following the incubation period, the medium was aspirated, and 20 µL of MTT reagent (Himedia, #4060) was added to each well to achieve a final concentration of 0.5 mg/mL. Plates were wrapped with aluminum foil and incubated at 37°C for 3 hr. Post incubation, the formazan crystals were dissolved by adding 100 µL of DMSO (Sigma, #PHR1309), and the absorbance was recorded at 570 nm using an ELX-800 microplate reader (BioTek, USA). The IC_{50} value was calculated using a linear logarithmic equation: Y=Mln(x) + C, where Y was set at 50, and M and C values were obtained from the viability graph (American Type Culture Collection, 2010; Mosmann, 1983).

Evaluation of Oxidative Stress-Induced Reactive Oxygen Species (ROS) Generation

Induction of Oxidative Stress and Treatment

SH-SY5Y cells were seeded in a 96-well glass-bottom plate (Corning, USA) at 1×10^4 cells per well and incubated overnight for adherence. The cells were then treated with *Bacillus coagulans* (S1) at 50 µM for 48 hr. After pre-treatment, the medium was replaced with 200 µL of fresh medium containing 50 µM hydrogen peroxide (H_2O_2) as an oxidative stress inducer and incubated for 1 hr at 37°C.

ROS Detection Using H_2DCFDA Assay

After oxidative stress induction, the cells were washed twice with 1X Phosphate-Buffered Saline (PBS, Himedia, #TL1006) to remove residual compounds. The cells were then incubated with 100 µL of 10 µM H_2DCFDA dye (Invitrogen, #D399) in PBS at 37°C for 30 min in the dark. After incubation, the wells were gently washed twice with PBS to remove excess dye.

Hoechst Staining and Imaging

Following ROS staining, cells were incubated with 100 µL of 2 µg/mL Hoechst 33258 solutions (Sigma, #94403) in PBS for 15 min at 37°C in the dark. After incubation, excess stain was removed by washing the wells with PBS. The plate was then imaged using a Zeiss Axiovert 5 confocal microscope at 20X magnification to detect fluorescence intensity indicative of intracellular ROS levels.

Controls and Data Analysis

The experimental setup included the following controls:

- Negative control: SH-SY5Y cells cultured in medium without the test compound or stress inducer.
- Stress-induced control: SH-SY5Y cells treated with 50 µM H202 alone.
- Standard control: Cells treated with 20 µM escitalopram (Ma *et al*., 2017; Martinez *et al*., 2020).

Fluorescence intensity data for ROS levels were quantified and statistically analyzed using GraphPad Prism. All experiments were conducted in triplicate, and results were expressed as Mean±Standard Deviation. Statistical significance was determined using one-way ANOVA followed by Tukey's *post hoc* test (Martinez *et al*., 2020).

RESULTS

MTT Assay

Morphological analysis of SH-SY5Y cells following *Bacillus coagulans* exposure revealed a concentration-dependent cytotoxic effect. Cells treated at increasing concentrations showed subtle changes in morphology when compared with the untreated control, while escitalopram (standard) also demonstrated similar dose-dependent effects (Figure 1).

Quantitative viability assessment using the MTT assay confirmed that *Bacillus coagulans* exhibited minimal cytotoxicity across all tested concentrations. Cell viability remained consistently above 80% at physiologically relevant doses, with the highest viability observed at lower concentrations. Detailed numerical data from the assay are provided in Table 1.

A regression analysis of the dose-response curve demonstrated a strong logarithmic correlation (R^2=0.9909), further validating the concentration-dependent response of *Bacillus coagulans*

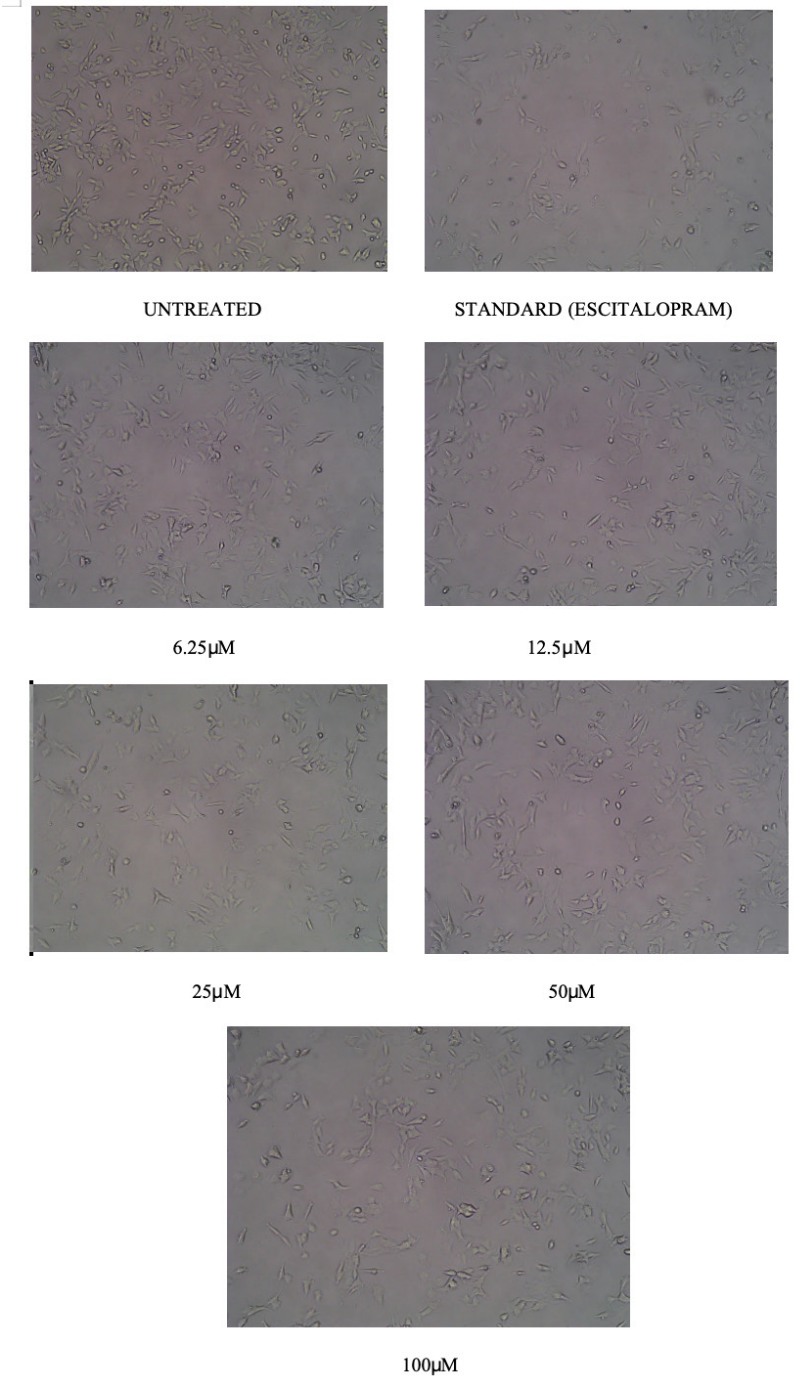

Figure 1: The morphological analysis indicated dose-dependent cytotoxicity in SH-SY5Y cells post-MTT assay (see Figure 1). Morphological analysis of cells post-MTT assay showing dose-dependent cytotoxicity of test compound compared to untreated control and escitalopram standard. MTT Assay Data Table after Incubation of 48 hr.

Table 1: Quantitative viability data from the MTT assay is presented in Table 1.

Concentration Unit: µM	Incubation: 48 hr							
Concentration	Blank	Untreated	Standard	6.25	12.5	25	50	100
Abs Reading 1	0.011	0.877	0.637	0.848	0.823	0.79	0.748	0.713
Abs Reading 2	0.011	0.876	0.639	0.847	0.828	0.79	0.749	0.717
Mean Abs	0.011	0.8765	0.638	0.8475	0.8255	0.79	0.7485	0.715
Mean Abs (Sample-Blank)	0	0.8655	0.627	0.8365	0.8145	0.779	0.7375	0.704
Standard Deviation	0.0055	0.000707107	0.001414214	0.000707107	0.0035355	0	0.000707	0.0028284
Standard Error	0.003889087	0.0005	0.001	0.0005	0.0025	0	0.0005	0.002
Cell Viability%		100	72.4367418	96.6493564	94.107452	90.005777	85.21086	81.340266

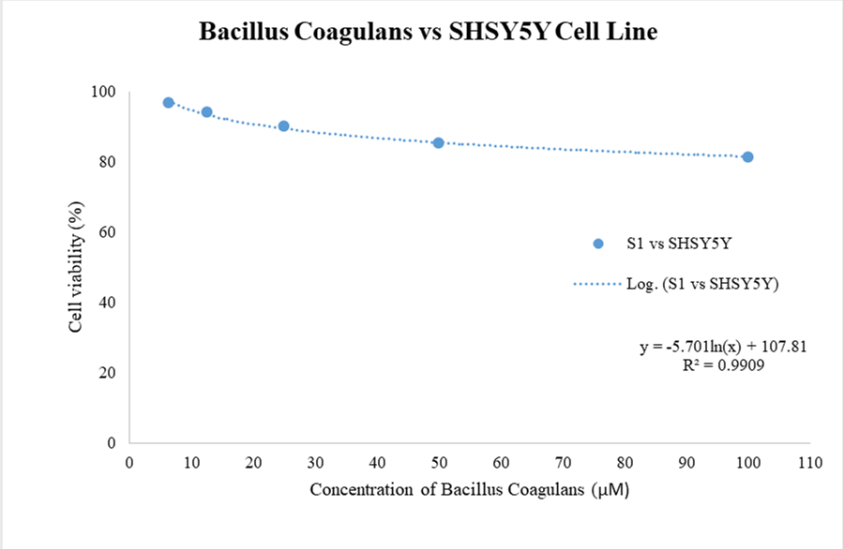

Figure 2: A logarithmic regression analysis of dose-response confirmed a strong correlation with an R^2 of 0.9909 (Figure 2). Dose-dependent cytotoxic effect of *Bacillus coagulans* on SHSY5Y cell line with logarithmic regression fit (R^2=0.9909) (S1 reffered to *Bacillus coagluans*).

in SH-SY5Y cells (Figure 2). Notably, after 48 hr of incubation, no significant toxicity was observed at the recommended concentrations, underscoring the favorable safety profile of *Bacillus coagulans* when compared to the untreated control and escitalopram (Figure 3).

ROS studies

Oxidative stress assessment using the H_2DCFDA assay revealed increased fluorescence in *Bacillus coagulans*-treated cells compared to untreated controls. This suggested the induction of moderate ROS levels, while escitalopram treatment demonstrated a slightly stronger suppression of ROS generation (Figure 4).

Quantitative analysis of fluorescence intensity supported these findings, showing that *Bacillus coagulans* significantly reduced oxidative stress in SH-SY5Y cells challenged with hydrogen peroxide. The antioxidant effect was closely comparable to that of escitalopram, highlighting its potential neuroprotective capacity (Figure 5).

DISCUSSION

The current study explored the neuroprotective and antioxidant effects of *Bacillus coagulans* in SH-SY5Y neuroblastoma cells, positioning it against escitalopram as a reference standard. The results contribute to a growing body of literature suggesting that probiotics, beyond their gastrointestinal benefits, may have significant roles in modulating neuronal health and protecting against oxidative stress-induced damage (Esposito *et al.*, 2000).

The MTT assay demonstrated that *Bacillus coagulans* exhibits minimal cytotoxicity at tested concentrations, indicating a favourable safety profile for neuronal applications. This aligns with prior studies that emphasize the biocompatibility of probiotic strains in cellular models when administered at physiological doses (Scudiero *et al.*, 1988).

The observed concentration-dependent viability of SH-SY5Y cells, without significant reduction at therapeutic levels, underscores the neuroprotective potential of *Bacillus coagulans*.

Oxidative stress is a well-established contributor to the pathogenesis of neurodegenerative and psychiatric disorders, where elevated ROS leads to neuronal dysfunction and apoptosis. In this context, the moderate ROS suppression observed following *Bacillus coagulans* treatment is particularly noteworthy. While escitalopram-a known neuroprotective antidepressant-served as a comparative standard, *Bacillus coagulans* demonstrated comparable reductions in ROS levels, suggesting its potential as an antioxidant agent capable of modulating oxidative pathways, possibly through mitochondrial regulation or up-regulation of antioxidant enzymes (Li *et al*., 2021).

Although *Bacillus coagulans* showed slightly less ROS suppression than escitalopram, it maintained a robust antioxidant profile. Considering its ability to influence the gut-brain axis-by producing neuroactive metabolites and modulating systemic inflammation-its benefits may extend beyond direct ROS scavenging (Yadav *et al*., 2023; Banerjee *et al*., 2021).

These systemic effects, including immune modulation and improved gut barrier function, are particularly relevant for neuropsychiatric applications.

Moreover, the *in vitro* results might only partially reflect the full scope of its therapeutic potential. *In vivo*, the gut-brain communication pathways involving immune, endocrine, and neuronal signaling can amplify *Bacillus coagulans*' effects, suggesting broader translational implications in neurodegenerative conditions (Begum *et al*., 2022; Kaur *et al*., 2021).

Nonetheless, this study is limited by its *in vitro* design. Further investigation into the molecular pathways, mitochondrial targets,

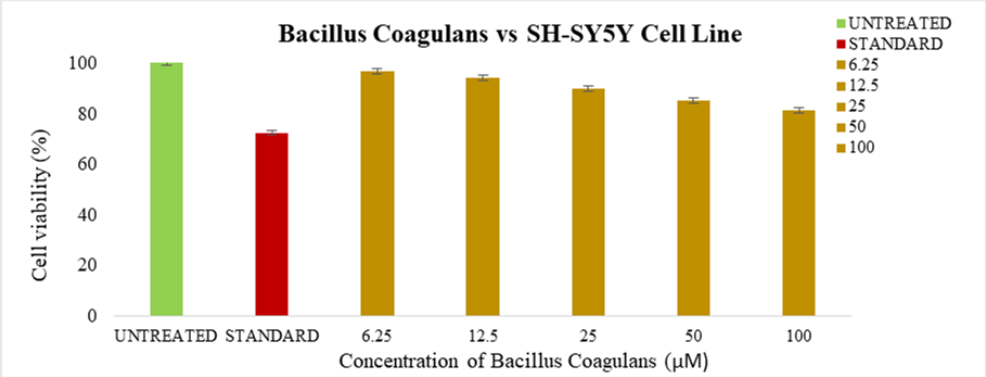

Figure 3: After 48 hr, Bacillus coagulans showed no significant toxicity at recommended concentrations (Figure 3). Mean % cell viability of SH-SY5Y cells after exposing to *Bacillus coagulans* for 48 hr. SH-SY5Y cells were treated with recommended concentrations of *Bacillus coagulans* along with negative and positive controls for an incubation of 48 hr. The experiment results showed that, after 48 hr of incubation, the compound S1 was non- toxic, against the SH-SY5Y cells, at the recommended concentrations.

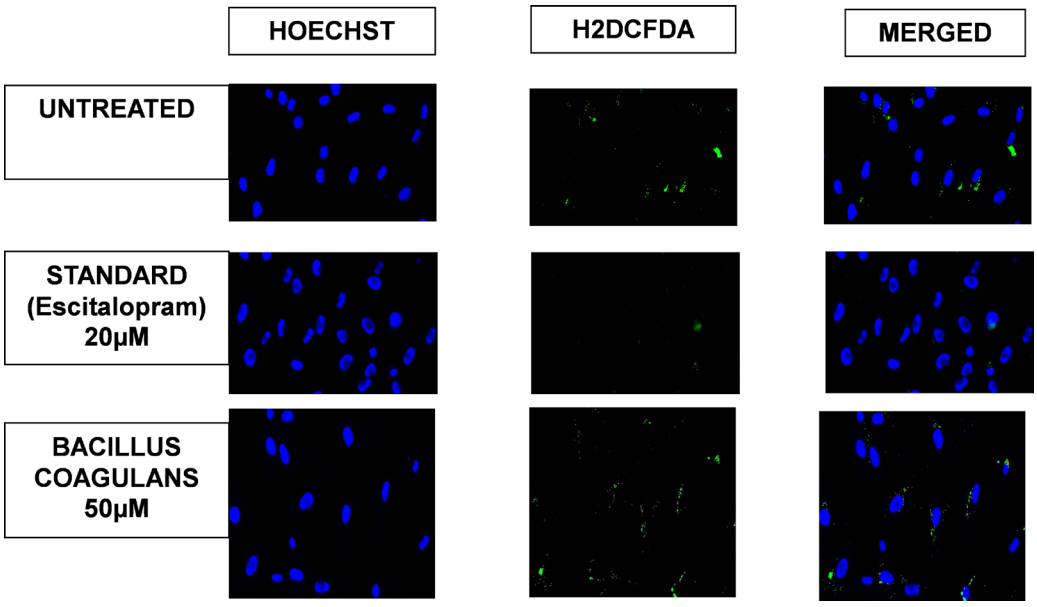

Figure 4: ROS detection using H_2DCFDA revealed higher fluorescence in *Bacillus coagulans*-treated cells, suggesting elevated ROS (Figure 4). ROS analysis using H2DCFDA staining shows increased fluorescence intensity in *Bacillus coagulans*-treated SHSY5Y cells compared to untreated and standard (escitalopram) groups, indicating elevated ROS generation.

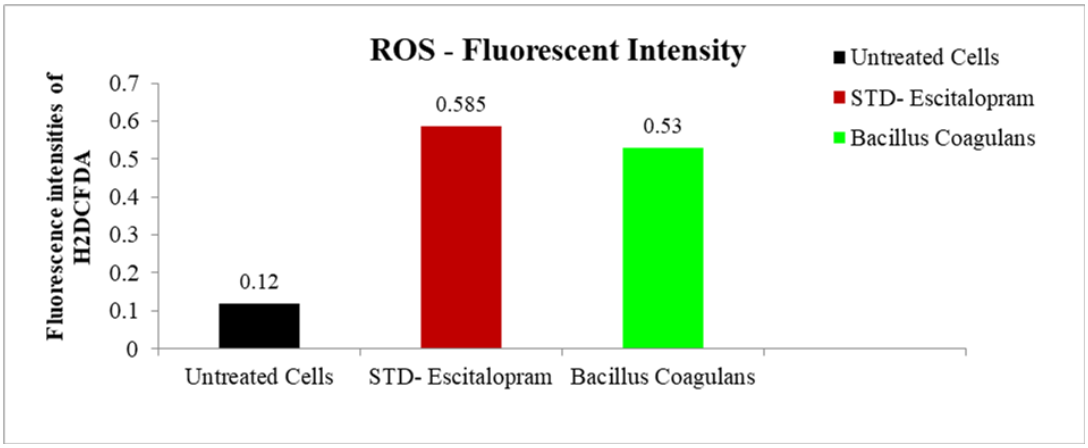

Figure 5: Quantitative fluorescence intensity analysis confirmed that *Bacillus coagulans* induced moderate ROS levels comparable to escitalopram (Figure 5). SHSY5Y cells were treated with recommended concentration of the test compounds (*Bacillus coagulans* and Standard (Escitalopram)) along with negative control for an incubation of 48 hr. The ROS assay showed that *Bacillus coagulans* showed moderate ROS generation, close to the Standard (Escitalopram).

and gene expression involved in *Bacillus coagulans*-mediated neuroprotection is warranted. Additionally, *in vivo* studies are essential to validate these findings and assess clinical relevance. Future directions may include combinational therapy approaches involving *Bacillus coagulans* and conventional neuro-therapeutics for synergistic outcomes (Martinez *et al*., 2019).

CONCLUSION

The present study investigated the neuroprotective and antioxidant potential of *Bacillus coagulans* using SH-SY5Y neuroblastoma cells as a model system, with escitalopram serving as the comparative standard. Through MTT assay, *Bacillus coagulans* demonstrated a concentration-dependent cytotoxicity profile, with no significant toxicity observed at therapeutic concentrations, suggesting its safety for neuronal application. Furthermore, the evaluation of intracellular oxidative stress via ROS (H_2DCFDA) assay revealed that *Bacillus coagulans* exhibited a moderate yet consistent ability to attenuate oxidative stress levels, comparable to that of escitalopram, a well-established antidepressant.

The findings from this *in vitro* study underscore the capacity of *Bacillus coagulans* to preserve neuronal cell viability and reduce reactive oxygen species under oxidative insult induced by hydrogen peroxide. This highlights its dual functionality as both a neuroprotective and anti-oxidative agent. Given its established role in modulating the gut-brain axis, these cellular effects may reflect a broader systemic potential for *Bacillus coagulans* in the management of neuropsychiatric and neurodegenerative disorders.

The implications of this research align with current trends toward exploring micro biota-based therapeutic strategies. Probiotics such as *Bacillus coagulans* may offer a novel, safe, and biologically active adjunct in the treatment of disorders associated with oxidative stress and neuronal damage, including depression, anxiety, and Parkinson's disease.

Nonetheless, while these preliminary findings are promising, further research is essential to delineate the exact molecular mechanisms involved, assess long-term effects, and validate efficacy through *in vivo* and clinical studies. Future exploration may also investigate combinational strategies integrating *Bacillus coagulans* with conventional therapeutics for synergistic neuroprotective outcomes.

In conclusion, *Bacillus coagulans* emerges as a promising candidate in the evolving field of neuro-probiotics, warranting deeper exploration for its potential translational application in neuro-therapeutics.

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ABRREVIATIONS

ANOVA: Analysis of Variance; **ATCC:** American Type Culture Collection; **DAPI:** 4′,6-diamidino-2-phenylindole (not explicitly mentioned but implied by Hoechst usage); **DMEM-F12:** Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12; **DMSO:** Dimethyl Sulfoxide; **ELISA:** Enzyme-Linked Immunosorbent Assay (referenced indirectly through assay usage, common in context); **H_2DCFDA:** 2′,7′-Dichlorodihydrofluorescein Diacetate; **H_2O_2:** Hydrogen Peroxide; **IC_{50}:** Half Maximal Inhibitory Concentration; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; **PBS:** Phosphate-Buffered Saline; **ROS:** Reactive Oxygen Species; **SD:** Standard Deviation; **SH-SY5Y:** Human Neuroblastoma Cell Line; **UV:** Ultraviolet (common in absorbance reading context, though not explicitly named).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study demonstrates that the probiotic *Bacillus coagulans* has significant neuroprotective and antioxidant effects in a laboratory model of oxidative stress. Using human neuronal cells, researchers found that *Bacillus coagulans* effectively reduced harmful Reactive Oxygen Species (ROS) and maintained high cell viability without toxicity. These results confirm its dual ability to mitigate oxidative damage and support neuronal survival. Given the established role of *Bacillus coagulans* in gut-brain axis communication, these direct cellular benefits suggest its potential as a therapeutic agent for neuropsychiatric conditions like depression. The study concludes by proposing future research into combination therapies, where *B. coagulans* could work alongside conventional neurological drugs to achieve synergistic benefits for brain health.

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