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Neuroprotective Activity of Bromelain in Alzheimer's Disease: an in Vitro Study on Neuroblastoma Cell Line

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ABSTRACT
Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, largely attributed to the accumulation of amyloid-beta (Aβ) plaques and tau tangles. Current pharmacological treatments primarily focus on AChE inhibitors, which, while beneficial, are limited by side effects and only provide symptomatic relief rather than halting the disease progression. This underscores the urgent need for alternative therapeutic strategies that are both effective and safe. Recent studies suggest that natural compounds may offer therapeutic potential against AD. This in vitro study investigates the anti-Alzheimer's activity of bromelain, a proteolytic enzyme derived from Ananas comosus (pineapple), in human neuroblastoma cell line (SH-SY5Y). We treated SH-SY5Y cells with varying concentrations of bromelain and assessed its effects on Aβ aggregation and cell viability using MTT assay. Our findings reveal that bromelain significantly reduces Aβ aggregation indicating its potential to mitigate key pathological feature of AD. Furthermore, bromelain treatment was associated with enhanced cell viability. The results suggest that bromelain exerts neuroprotective effects through its ability to modulate protein aggregation,positioning it as a promising candidate for further investigation in AD therapeutics. These findings underscore the need for additional studies to elucidate the mechanisms underlying bromelain's protective effects and its potential applications in clinical settings for AD management.

Keywords: Alzheimer's disease, bromelain, neuroblastoma, amyloid-beta,cell viability, in vitro, neuroprotection.

I. INTRODUCTION

Alzheimer's disease (AD) is the most common type of dementia and can be defined as a slowly progressive neurodegenerative disease characterized by neurotic plaques and neurofibrillary tangles as a result of amyloid-beta peptide's (Aβ) accumulation in the most affected area of the brain, the medial temporal lobe(The hippocampus and its adjacent para-hippocampal cortex, entorhinal cortex, and perirhinal cortex) and neocortical structures.[1]The pathophysiology of AD is associated with a variety of factors, including the extracellular deposition of β-amyloid (Aβ) plaques, accumulation of intracellular neurofibrillary tangles, oxidative neuronal damage, and inflammatory cascades. It is widely believed, however, that an increase in the production of the Ab peptide, the main component of amyloid plaques, is central to the pathogenesis of the disease.[2]

Alzheimer's disease (AD) is the most common type of dementia, affecting at least 27 million people and corresponding from 60 to 70% of all dementia's cases.[3]Currently, treatment for Alzheimer's disease does not impact the progression or underlying pathology of the disease. However, with medication and other therapies, steps are taken to increase the quality of life of those with AD. Current therapeutics are based off the Cholinergic Theory, which attributes a decrease in cholinergic neurotransmission to a decline in cognitive function.[4]Bromelain, a proteolytic enzyme extracted from pineapple, has demonstrated anti-inflammatory, antioxidant, and anti-aggregation properties.[5]This study aims to explore the neuroprotective effects of bromelain in an in vitro model of AD, focusing on its ability to reduce Aβ aggregation and enhance cell viability in SH-SY5Y cells.[1]

II. MATERIALS AND METHODS

1.SELECTION OF PLANT

Ananas comosus(pineapple) plant was selected for bromelain extraction as it havehigh concentration of proteolytic enzyme bromelain.

2. COLLECTION AND AUTHENTICATION

Ripe pineapple fruit was collected from a local market in Thodupuzha, Kerala, and authenticated by Dr. Saju Abraham, HOD,

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Newman College, Thodupuzha. The fruit was selected based on its freshness and maturity to ensure optimal bromelain content.

3. EXTRACTION OF CRUDE BROMELAIN

The pineapple fruit and peel were separated and cut into small pieces and was processed for the extraction of enzyme. Fruit pieces were weighed and grinded to extract the juice. After extracting the juice, crude enzyme from the fruit remaining was further extracted. The juice was filtered using whatman filter paper. The filtrate was centrifuged at 10,000 rpm for 10 minutes to remove insoluble materials. The supernatant was collected and stored at 4°C.It was used as crude extract of bromelain enzyme from pineapple fruit.[6]

4. PHYTOCHEMICAL SCREENING

The crude bromelain extract was subjected to protein-specific tests :

- Biuret Test
- Millon's Test
- Ninhydrin Test[7,8]

5.GELATIN DEGRADATION TEST

The proteolytic activity of bromelain was assessed using a gelatin degradation test.Gelatin powder was dissolved in distilled water and allowed to solidify in test tubes. The crude bromelain extract was added to the gelatin, and the degradation of gelatin was observed after 1-2 hours.[9]

6. IN VITRO STUDY

SH-SY5Y neuroblastoma cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum, L-glutamine, sodium bicarbonate, and antibiotics (penicillin, streptomycin, and amphotericin B). The cells were maintained at 37°C in a humidified 5% CO2 incubator. Two-day-old confluent monolayers of cells were trypsinized, and the cell suspension was seeded into 96-well plates at a density of 5x10^3 cells per well.

To induce toxicity, the cells were treated with 10 µM of amyloid-beta (Aβ) for one hour. Afterward, the cells were treated with varying concentrations of bromelain (1.5 µg/ml, 3.1 µg/ml, 6.25 µg/ml, 12.5 µg/ml, and 25 µg/ml) and incubated for 24 hours. Untreated control cells and Aβ-treated control cells were also maintained for comparison.

7. CYTOTOXICITY EVALUATION

Cell viability was assessed using the MTT assay. After 24 hours of treatment, the medium was removed, and 30 µL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well. The plates were incubated for 4 hours at 37°C, after which the formazan crystals formed were dissolved in dimethyl sulfoxide (DMSO). The absorbance was measured at 540 nm using a microplate reader. The percentage of cell viability was calculated using the formula:

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ Viability } = \frac{(\text{Mean OD Samples })}{(\text{Mean OD of Control group })} \text{ x } 100$$

Morphological changes in the cells were observed under a phase-contrast microscope to assess cytotoxicity. Changes such as cell rounding, shrinkage, granulation, and vacuolization were noted as indicators of cellular damage.[10,11]

III. RESULTS AND DISCUSSION

1.EXTRACTION OF CRUDE BROMELAIN

Fig.1 Sliced pineapple

Fig.2 Crude bromelain extract

2.PHYTOCHEMICAL SCREENING

EXPERIMENT	INFERENCE
1.Biuret test Add 1 mL of 5% sodium hydroxide and 2 drops of 1% copper sulphate solution to 1 mL of the sample.	Positive
2.Millon's Test Add Millon's reagent to the sample and boiling it for 30 seconds, followed by the addition of sodium nitrite	Positive
3.Ninhydrin Test Add Ninhydrin reagent to the sample and heating it in a water bath,	Positive

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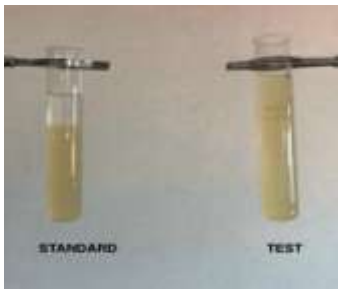
3.GELATIN DEGRADATION TEST

The gelatin solution has turned from semi-solid to liquid and has become more turbid, this indicates that the bromelain has degraded the gelatin protein showing its activity.



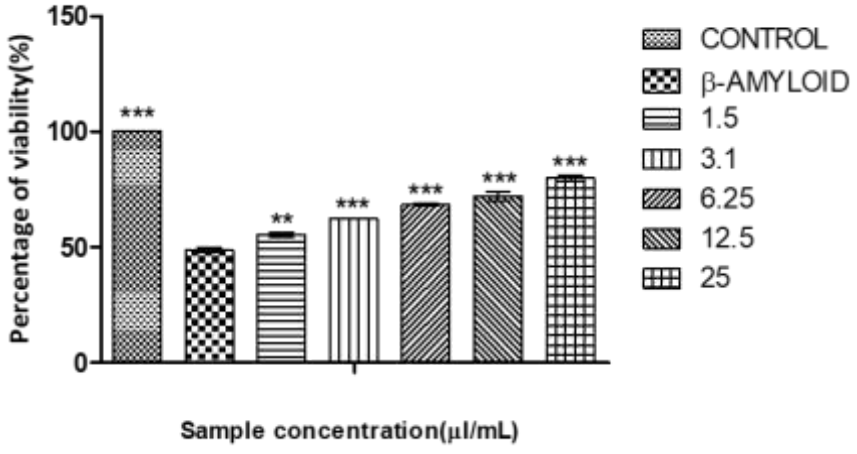
4.IN VITRO NEUROPROTECTIVE EFFECTS

The MTT assay revealed that bromelain significantly improved cell viability in SH-SY5Y cells exposed to Aβ-induced toxicity. At the highest concentration (25 µg/ml), bromelain increased cell viability to 79.90%, compared to 48.96% in the Aβ-treated control group. The study demonstrated a dose-dependent neuroprotective effect, with higher concentrations of bromelain providing greater protection against Aβ-induced toxicity.

Microscopic observations confirmed that bromelain-treated cells exhibited reduced morphological changes, such as cell rounding and vacuolization, compared to Aβ-treated controls. These findings suggest that bromelain mitigates Aβ-induced neuronal damage, potentially through its anti-aggregation and neuroprotective properties.

STATISTICAL ANALYSIS

Cell line - SHSY-5Y									
SAMPLE CODE-PFEB1									
	OD1	**OD2**	**OD3**	**Percentage viability 1**	**Percentage viability 2**	**Percentage viability 3**	**Average**	**Stdev**	**Std error**
Control	0.3774	0.3861	0.3865	100	100	100	100	0	0
Beta Amyloid	0.1928	0.1836	0.1866	51.0864	47.5524	48.2794	48.9728	1.8662	1.07745
1.5	0.2163	0.2054	0.2148	57.3132	53.1982	55.5757	55.3625	2.06554	1.19254
3.1	0.2342	0.2415	0.2406	62.0562	62.5486	62.251	62.2852	0.24798	0.14317
6.25	0.2561	0.2674	0.2639	67.859	69.2567	68.2794	68.465	0.71707	0.414
12.5	0.2854	0.2768	0.2655	75.6227	71.6913	68.6934	72.0025	3.4751	2.00635
25	0.3115	0.3026	0.3047	82.5384	78.3735	78.8357	79.9159	2.28293	1.31805



Statistical analysis using ANOVA and Dunnett's test confirmed the significant neuroprotective effects of bromelain. The results align with the hypothesis that natural compounds like bromelain could offer a safe and effective alternative to current pharmacological treatments for AD.

IV. CONCLUSION

This study demonstrates that bromelain exhibits significant neuroprotective effects in an in vitro model of Alzheimer's disease. By reducing Aβ aggregation and enhancing cell viability, bromelain shows promising activity as a therapeutic agent for Alzheimer's disease. However, further research is needed to validate these findings in animal models and clinical trials. Future studies should also explore the mechanisms underlying bromelain's neuroprotective effects and its potential use in combination therapies.

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