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Research Article

DEVELOPMENT OF ALTERNATIVE NANOFOOD MATERIALS AS NUTRIENT DELIVERY SYSTEM (NDS) FOR IMPROVING COGNITIVE IMPAIRMENT (AND) USING AFFINITY BEAD TECHNOLOGY (ABT)

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ABSTRACT

Aims: Cognitive function impairment, which increased with the aging population, underscored the need for enhanced acetylcholine synthesis and synaptic function in the hippocampus for effective therapeutic strategies. In this study, we explored the development of alternative nanofood materials and their therapeutic potential as Nutrient Delivery System (NDS) for improving cognitive impairment using edible bird's nest (EBN)-derived sialic acid, KJM-SA1, using Affinity Beads Technology (ABT). **Study design:** Commercialization of brain function-improving health functional food from sialic acid based on affinity bead technology (Stepping Stone Startup Project) From May 2021 to August 31, 2024, the Ministry of SMEs and Startups of the Republic of South Korea supported the design and research of the experiment (Commercialization of brain function-improving health functional food from sialic acid based on affinity bead technology, Stepping Stone Startup Project). **Place and Duration of Study:** Samples were provided by KJMBio Co., Ltd. and KJMAloe Co., Ltd. from May 2021 to August 31, 2024, and basic and animal experiments were conducted at the Department of Food and Nutrition, Nanofood Research Institute, Seoul National University, Department of Chemical & Biomolecular Engineering, KAIST, and Department of Biomedical Engineering, UNIST. **Methodology:** We investigated the effects of KJM-SA1 on cell viability in SH-SY5Y cells, its antioxidant activity, and its impact on cognitive functions in scopolamine-induced mice through Morris Water Maze and Passive Avoidance tests. **Results:** Our results demonstrated that KJM-SA1 not only enhanced SH-SY5Y cell viability and exhibited significant antioxidant properties but also improved cognitive functions in scopolamine-induced mice. This improvement was associated with changes in the expression of genes crucial for ace-tylcholine production and synaptic operation in the hippocampus, including acetylcholinesterase (AChE), choline acetyltransferase (ChAT), and brain-derived neurotrophic factor (BDNF). **Conclusion:** These results suggest that KJM-SA1 may be a promising NDS for cognitive impairment and an alternative food and therapeutic agent for the development of nanofood materials, which requires further studies.

Keywords: cognitive impairment, sialic acid, edible bird's nest, affinity bead technology (ABT), Nutrient Delivery System (NDS), alternative nanofoods.

INTRODUCTION

Cognitive function impairment, predominantly observed in the elderly, is a significant concern due to its potential to lead to severe depression and social isolation [1]. Acetylcholine (ACh) plays a crucial role in mediating cognitive functions as a major neuro-transmitter in the nervous system [2]. Acetylcholinesterase (AChE) degrades ACh into acetic acid and choline, reducing its availability [3]. While acetylcholinesterase inhibitors (AChEIs) are employed to counteract this process and enhance cognitive function, their use is marred by side effects such as diarrhea, vomiting, and hepatotoxicity [4]. This necessitates the exploration of safer therapeutic alternatives. Edible Bird's Nest (EBN), traditionally valued in Chinese cuisine, is crafted mainly from the saliva of swiftlets. The health-promoting potential of EBN, attributed to its rich composition of glycoprotein, calcium, carbohydrates, and other nutrients, varies based on factors like geographical origin and swiftlet species [5]. Sialic acid, a nine-carbon sugar, constitutes the primary carbohydrate component, representing 9%, in the composition of edible bird nests [6]. In the comprehensive review by Varki, A. [7], sialic acids are high-lighted as pivotal molecules involved in numerous physiological and pathological processes, emphasizing their integral role in both health and disease states. This underscores the versatile and significant impact of sialic acids across various biological systems, mediating critical functions from cell signaling to immune response modulation. Further-more, several studies have reported improvements in cognitive functions following the supplementation of sialic acid [8-10]. Affinity Beads Technology (ABT), an eco-friendly method using non-polar polymer beads, has been developed for the specific separation and purification of target components like sialic acid. KJM-278A-28, a polymer bead, has demonstrated efficacy in selectively adsorbing sialic acid with high purity and yield [11]. Utilizing this technology, KJM-SA1, a sialic acid isolate from EBN, has been produced by KJM Bio Ltd. KJM-SA1 is a lot number (Lot. No. KJM-SA1) that separates and purifies sialic acid by adsorption to KJM-278A-28 beads, and in a previous study, it was developed as an alternative nanofood material as a Nutrient Delivery System (NDS) for improving cognitive impairment [11-17]. This study aims to assess the potential cognitive function improvement effects of KJM-SA1.

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MATERIALS AND METHODS

SH-SY5Y cells:

SH-SY5Y human neuroblastoma cells were cultured in high-glucose Dulbecco's Modified Eagle's Medium (DMEM) supplied by Sigma-Aldrich Co., St. Louis, USA. The medium was enriched with 10% fetal bovine serum (FBS) provided by WELGENE Inc., Gyeongsangbuk-do, Korea, and 1% Penicillin-Streptomycin (P/S) solution from Gibco™, Paisley, Scotland, to inhibit bacterial growth. Cultures were maintained at a steady 37°C in a humidified incubator with 5% CO_2. Media were refreshed bi-daily to sustain optimal conditions, while cell confluency was closely monitored to prevent over-cultivation.

MTT assay:

Overnight, SH-SY5Y cells were seeded at a density of 1 × 10^5 cells/well in a 96-well plate. Subsequently, the cells were treated with varying concentrations of donepezil and KJM-SA1, in addition to 5 mM scopolamine, at 37°C for 24 hours. The concentration of 5 mM scopolamine was determined based on the results of preliminary tests. KJM-SA1 was obtained from KJM Bio Ltd., while scopolamine and donepezil were sourced from Sig-ma-Aldrich Co., St. Louis, USA. Donepezil was used as the positive control to counteract scopolamine-induced cellular damage [18]. Following this, the cells were exposed to a 3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) solution (DuchefaBiochemie, Amsterdam, Netherlands) for 4 hours. After removing the MTT solution, 100 µL of dimethyl sulfoxide (DMSO) supplied by Sigma-Aldrich Co., St. Louis, USA, was added to each well to dissolve the formazan crystals. The absorbance was measured at 570 nm and 630 nm using a Synergy HT microplate reader (BioTek Instruments, Winooski, VT, USA). The mean optical density (OD) of the control cells was considered as representing 100% viability.

Antioxidant activity:

The antioxidant capacity was assessed via the DPPH radical scavenging method, based on Blois' method [19]. KJM-SA1 was solubilized in 95% ethanol at various concentrations and incubated with 0.1 mM DPPH solution in 95% ethanol for 30 minutes in a dark environment. Ascorbic acid, dissolved in 95% ethanol, acted as the standard control. Absorbance readings were taken at 530 nm using a Synergy HT microplate reader (BioTek Instruments, Winooski, VT, USA). The DPPH radical scavenging activity was calculated with the formula: ((control absorbance - sample absorbance) / control absorbance) × 100. The IC50 value, indicative of the concentration needed to neutralize 50% of DPPH radicals, was determined from these measurements.

Mouse:

Male Institute of Cancer Research (ICR) mice, aged 6 weeks, were obtained from Orient Bio, Inc. (Seongnam, Korea) and acclimatized to laboratory conditions for 1 week be-fore experimentation. They were housed in a temperature (23.0 ± 2.0°C) and humidity (50.0 ± 5.0%) controlled environment with a 12-hour light/dark cycle. Mice had free access to food and water throughout the study. All procedures were approved by the Animal Care and Use Committee of Kyung Hee University [KHUASP-21-405], ensuring ethical treatment and care of the animals.

Morris Water Maze (MWM)

The MWM test was conducted to assess spatial learning and memory in scopolamine-induced mice, based on the method described by Vorhees *et al.,* [20]. The test in-volved a circular pool filled with opaque water containing milk, where a hidden platform was placed just below the surface. Mice were trained over 5 days to find the platform. On the day of testing, mice received an intraperitoneal injection of scopolamine (1 mg/kg) to induce cognitive impairment, followed by oral administration of KJM-SA1 (300 mg/kg) 30 minutes before the MWM test. The latency to find the hidden platform was recorded, providing a measure of the mice's spatial memory, and learning capability.

Passive avoidance (PA)

The PA test, conducted in a chamber (50 × 15 × 40 cm) divided into illuminated and dark sections, was used to evaluate the mice's learning and memory based on their natural aversion to brightly lit areas. During training, mice were conditioned to associate the dark compartment with an aversive stimulus—a 0.2 mA electric shock delivered through the grid floor for 2 seconds. Response latency, the time taken by the mouse to enter the dark compartment from the light compartment, was measured. The mice were intraperitoneally administered with 1 mg/kg of scopolamine 1 h before the test, and orally administered with 300 mg/kg of KJM-SA1 30 min before PA test.

Quantitative real-time polymerase chain response (RT-qPCR)

RT-qPCR was performed to analyze gene expression changes. The total RNA in hippocampus was extracted using Trizol reagent (Invitrogen, Carlsbad, CA), and quantitatively evaluated with NanoDrop 2000 (Thermo Fisher Scientific). Complementary DNA (cDNA) was synthesized using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Korea Ltd., Seoul, Korea) according to the manufacturer's protocol. RT-qPCR was performed using SYBR Green Master mix (Applied Biosystems, Thermo Fisher Scientific Korea Ltd., Seoul, Korea). The primer sequences for PCR are shown in Table 1. Conditions for PCR consisted of one cycle at 95°C for 10 min; followed by 45 cycles at 95°C for 10 seconds and at 60°C for 10 seconds, and at 72°C for 20 seconds, and, lastly, one cycle at 72°C for 10 min. Gene expression levels were calculated using the -2ΔΔCt method [21] with normalization by the expression change of β-Actin.

Table 1. Primer sequences for RT-qPCR.

Gene	Forward primer	Reverse primer
AChE	5'-CATCTTTGAACACCGTGC-3'	5'-GTCGTATTATATCCCAGCCC-3'
ChAT	5'-CCGGTTTATTCTCTCCACC-3'	5'-TCTAAAGACCTCAGAGGGAG-3'
BDNF	5'-CTAAGGTCTAGGATGGAGGT-3'	5'-GTAAAGTACAGACGGGACTC-3'
β-Actin	5'-GAAGAGCTATGAGCTGCCTGA-3'	5'-TGATCCACATCTGCTGGAAGG-3'

Histological analysis

Histological changes were confirmed by evaluating the distribution of neurons by Nissl staining. The dissected hippocampus of mouse was fixed with 4% paraformaldehyde in PBS for 24h, and then embedded in paraffin blocks. Tissue sections were cut to a thickness of 4 µm and mounted on glass slides. After drying for 30 min, deparaffinization was performed using Xylene and descending grades of absolute alcohol. The sections were stained using cresyl violet solution, and dehydration was performed using ascending grades of absolute alcohol. Before covering the sections with coverslips, the sections were cleared in xylene. Histological images were observed

using a fluorescence micro-scope (Olympus 1 x 70; Olympus Co., Tokyo, Japan).

Statistical analysis

Data were processed and analyzed using SigmaPlot software (Systat Software Inc., USA), ensuring rigorous statistical examination. Results were presented as the mean ± standard error of the mean (SEM). The significance of experimental outcomes was deter-mined through one-way Analysis of Variance (ANOVA). To further investigate significant findings, post-hoc analysis was performed using Tukey's Honestly Significant Difference (HSD) test.

RESULTS

Effect of KJM-SA1 on SH-SY5Y cell viability:

Cell viability within SH-SY5Y human neuroblastoma cells was assessed using the MTT assay, as depicted in Figure 1. Preliminary tests revealed that the cell viability significantly decreased as the concentration of scopolamine (SCO) increased, with 5 mM scopolamine showing the most substantial reduction in cell survival rates. Consequently, 5 mM of scopolamine was chosen for use in this experiment. Exposure to 5 mM scopolamine at 37°C for 24 hours led to a notable decline in cell viability (###p < 0.001). In contrast, treatment with donepezil (DPZ) exhibited a neuroprotective effect, countering the scopolamine-induced damage in SH-SY5Y cells at concentration of 1 µM (*p < 0.05) and 10 µM (**p < 0.01). Additionally, treatment with KJM-SA1 resulted in a marked increase in cell viability at concentrations of 10 µg/mL (*p < 0.05) and 100 µg/mL (***p < 0.001), indicating a dose-dependent protective effect.

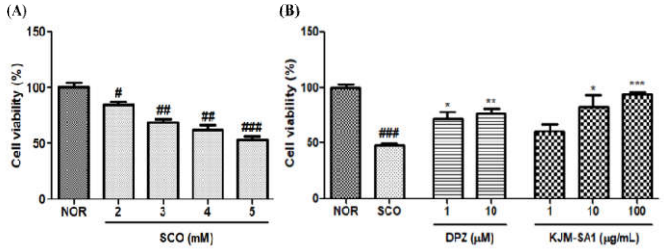

Figure 1. Cell viability in MTT assay. (A) SCO-induced SH-SY5Y cell damages. (B) Recovery of SCO-induced SH-SY5Y cell damages by DPZ or KJM-SA1. 'NOR' represented the normal group, 'SCO' referred to the control group treated with scopolamine, and 'DPZ' denoted the positive control group treated with donepezil with 5 mM SCO. 'KJM-SA1' represented the KJM-SA1 treated group with 5 mM SCO. Data are presented as mean ± standard error of the mean (SEM). #p < 0.05, ##p < 0.01 and ###p < 0.001 (NOR vs. SCO). *p < 0.05, **p < 0.01 and ***p < 0.001 (SCO vs. DPZ or SCO vs. KJM-SA1).

Effect of KJM-SA1 on antioxidant activity:

The antioxidant activity of KJM-SA1 was quantified using the DPPH radical scavenging assay across a range of concentrations, extending up to 1,000 µg/mL, as depicted in Figure 2. Ascorbic acid, employed as the standard reference due to its well-documented antioxidant properties, exhibited IC50 values of 9.074 µg/mL, serving as a benchmark for comparison. In this assay, KJM-SA1 demonstrated notable antioxidant activity, with an IC50 value of 75.14 µg/mL. This indicates that KJM-SA1, while less potent than ascorbic acid, still possesses significant free radical scavenging capabilities. Further result revealed that the antioxidant efficacy of KJM-SA1 increased in a dose-dependent manner, suggesting that its radical scavenging potential is concentration-dependent.

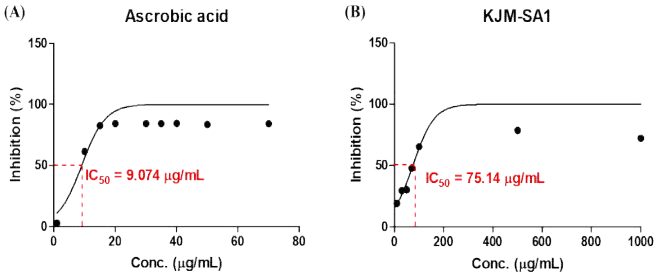

Figure 2. Scavenging activity of DPPH radical by KJM-SA1. (A) Inhibition of ascorbic acid on DPPH radical. (B) Inhibition of KJM-SA1 on DPPH radical. Values are the average of triplicate experiments and data are presented as mean ± standard error of the mean (SEM).

Effect of KJM-SA1 on Morris Water Maze (MWM) test:

In the MWM test (Figure 3A), mice (n=10 per group) were allowed to swim for up to 120 seconds to locate a hidden platform submerged just below the water surface. The es-cape latency, the time required for a mouse to find the platform, was recorded. If a mouse failed to locate the platform within the 120-second timeframe, the escape latency was noted as 120 seconds. Over the course of the trials, a decrease in the time taken to find the platform was observed, indicating learning and memory capabilities. The mice treated with scopolamine (SCO) showed a significant increase in escape latency compared to the normal control (NOR), with ##p < 0.01 or ###p < 0.001, highlighting a deterioration in cognitive function. Conversely, the group treated with KJM-SA1 demonstrated a notable reduction in escape latency by days 3-5, bringing it close to the levels observed in the NOR group (**p < 0.01).

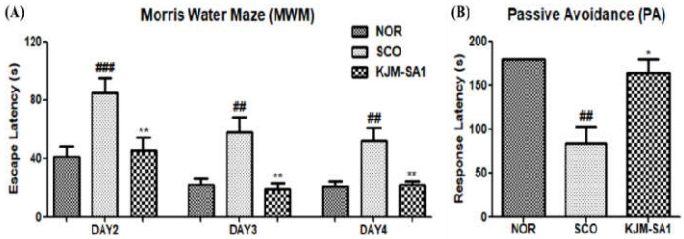

Figure 3. Behavior test for assessment of learning and memory. (A) Escape latency of MWM test. (B) Response latency of PA test. 'NOR' represented the normal group, 'SCO' referred to the group intraperitoneally treated with scopolamine, and 'KJM-SA1' denoted the group intraperitoneally treated with scopolamine and orally treated with KJM-SA1. Data are presented as mean ± standard error of the mean (SEM). ##p < 0.01 and ###p < 0.001 (NOR vs. SCO). *p < 0.05 and **p < 0.01 (SCO vs. KJM-SA1).

Effect of KJM-SA1 on Passive Avoidance (PA) test:

In the PA test (Figure 3B), mice (n=10 per group) were trained to associate entering the dark compartment with receiving a 0.2 mA electric shock for 2 seconds. The response latency, the time from the start of the trial to the entry into the dark compartment, was measured. If a mouse did not enter the dark compartment within 180 seconds, the latency time was set at 180 seconds as the maximum threshold. The scopolamine-treated group (SCO) displayed a significant reduction in response latency compared to the normal control group (NOR), with ##p < 0.01, indicating impaired learning and memory. In contrast, treatment with KJM-SA1 significantly increased the response latency (*p < 0.05), suggesting an

improvement in memory retention and a reversal of the negative effects induced by scopolamine.

Effect of KJM-SA1 on gene expression in the hippocampus:

The impact of scopolamine (SCO) on hippocampal function and the potential neuro-protective effects of KJM-SA1 was investigated (Figure 4). SCO treatment led to an upregulation of acetylcholinesterase (AChE) gene expression (##p < 0.01) and a downregulation of choline acetyltransferase (ChAT) (#p < 0.05) and brain-derived neurotrophic factor (BDNF) (##p < 0.01) expressions in the hippocampus of mice, indicating impaired cholinergic neurotransmission and potential cognitive decline. In contrast, administration of KJM-SA1 significantly attenuated the SCO-induced increase in AChE expression (***p < 0.001) and elevated BDNF expression (*p < 0.05), suggesting a restoration of synaptic function and neuronal survival. Although there was an observed increase in ChAT expression following KJM-SA1 treatment, the change did not reach statistical significance.

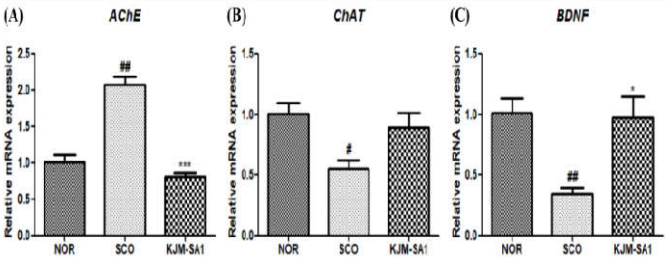

Figure 4. Effects of scopolamine and KJM-SA1 on gene expressions. (A) acetylcholinesterase (AChE), (B) choline acetyltransferase (ChAT), and (C) brain-derived neurotrophic factor (BDNF) in the Hippocampus. 'NOR' represented the normal group, 'SCO' referred to the control group treated with scopolamine, and 'KJM-SA1' denoted the group administrated KJM-SA1 with SCO. Data are presented as mean ± standard error of the mean (SEM). #p < 0.05 and ##p < 0.01 (NOR vs. SCO). *p < 0.05 and ***p < 0.001 (SCO vs. KJM-SA1).

Effect of KJM-SA1 on Histological distribution of neurons:

The restoration capability of neuronal density by KJM-SA1 was assessed through histological examination using Nissl staining within the hippocampal region, as depicted in Figure 5. The analysis revealed a pronounced reduction in neuronal density in the hippocampus of mice subjected to scopolamine (SCO) exposure, underscoring the neuro-degenerative effect of SCO. In stark contrast, treatment with KJM-SA1 markedly improved neuronal distribution within the hippocampus, indicative of its potential to counteract SCO-induced neuronal damage.

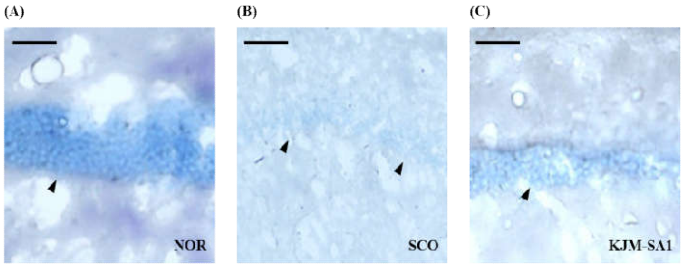

Figure 5. Histological images using Nissl staining within the hippocampal region. (A-C) Neuronal density in the hippocampus of the normal (NOR), scopolamine (SCO), KJM-SA1 treated group, respectively. The arrows indicated the hippocampus stained with cresyl violet and the scale bar represented 100 µm.

DISCUSSION

Human neuroblastoma SH-SY5Y cells were extensively utilized as an in vitro model for neurological research, owing to their relevance to human neuronal properties [22]. Scopolamine (SCO) disrupted cholinergic neurotransmission by elevating acetylcholinesterase (AChE) activity, an enzyme that degrades acetylcholine (ACh) into acetic acid and choline [23]. In this study, the impact of KJM-SA1 on cell viability damaged by SCO expo-sure was assessed using the MTT assay in SH-SY5Y cells. The results indicated that SCO exposure significantly diminished cell viability, underscoring its neurotoxic potential. Conversely, donepezil, an FDA-approved reversible inhibitor of AChE for the treatment of mild to moderate Alzheimer's disease, markedly ameliorated the SCO-induced reduction in cell viability, highlighting its neuroprotective capabilities. Furthermore, KJM-SA1 demonstrated a significant protective effect against SCO-induced neurotoxicity, as evidenced by the dose-dependent increase in cell viability at concentrations of 10 µg/mL (*p < 0.05) and 100 µg/mL (***p < 0.001). This outcome implied that KJM-SA1 might have had neuroprotective attributes capable of mitigating the adverse impacts of scopolamine on neuronal cells.

Antioxidant activity plays a pivotal role in combating neurological disorders in the brain, as increased levels of free radicals are centrally involved in the pathogenesis of these conditions [24]. The hippocampus is highly susceptible to oxidative stress, making the study of antioxidant interventions crucial for protecting this brain region [25]. The antioxidant capacity of KJM-SA1 was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. This assay measured the ability of antioxidants to donate a hydrogen atom to the nitrogen radical of DPPH, neutralizing its free radical activity [26]. In this experiments, ascorbic acid, known for its potent free radical neutralizing effects, served as the standard reference [27] with an IC50 value of 9.074 µg/mL. KJM-SA1 exhibited a dose-dependent inhibition of DPPH radicals, achieving an IC50 value of 75.14 µg/mL. This indicated that while KJM-SA1 had a notable antioxidant activity, it was less potent than ascorbic acid in this specific assay. Nonetheless, the demonstrated capability of KJM-SA1 to scavenge DPPH radicals underscored its potential as an antioxidant agent, which could be particularly beneficial in mitigating oxidative stress-related damage in the hippocampus.

The Morris Water Maze (MWM) test served as a benchmark for assessing the hippo-campus's role in spatial memory and learning by recording the time required for mice to locate a hidden platform within a specific pool [28]. Complementarily, the passive avoidance (PA) test gauged the impact of compromised hippocampal function by observing the mice's reaction to aversive stimuli, such as electric shocks. Notably, mice with hippocampal lesions exhibited a diminished capacity to avoid areas associated with negative experiences, reflecting a breakdown in passive avoidance learning [29]. A critical aspect of both experimental setups was the pre-training phase, which acclimatized the mice to the task, ensuring they persisted in their search for the platform [30] and exhibited a preference for safe zones over shock-associated areas. In this study, the SCO-treated group demonstrated a significant increase in escape latency in the MWM test (##p < 0.01) and a reduction in response latency in the PA test (##p < 0.01), indicating a decline in spatial learning and memory retention compared to the normal group. Conversely, mice treated with KJM-SA1 exhibited markedly improved performance, with reduced escape latency (**p < 0.01) and extended response latency (*p < 0.05) relative to the SCO-treated group. These outcomes underscored the efficacy of KJM-SA1 in mitigating the cognitive deficits induced by SCO, as evidenced by its positive

influence on both spatial learning in the MWM test and memory retention in the passive avoidance task.

The hippocampus, situated beneath the cerebral cortex within the temporal lobe, plays a pivotal role in the limbic system's learning and memory processes [31]. In this study, the effects of KJM-SA1 on the gene expression levels of acetylcholinesterase (AChE), choline acetyltransferase (ChAT), and brain-derived neurotrophic factor (BDNF) in the hippocampus of SCO-challenged mice were assessed using RT-qPCR. AChE, crucial for ACh hydrolysis, when overexpressed, particularly in the cortex and hippocampus, could lead to synaptic loss and consequent cognitive decline [32]. ChAT, responsible for ACh synthesis, had its reduced activity linked to cholinergic system malfunctions characteristic of Alzheimer's disease [33]. BDNF, a key neurotrophin, supported neuronal survival and synaptic formation, crucial for learning and memory adaptability [34]. RT-qPCR re-sults revealed a significant increase in AChE gene expression (##$p < 0.01$) and notable de-creases in ChAT (#$p < 0.05$) and BDNF (##$p < 0.01$) expressions in SCO-treated mice relative to normal group. Remarkably, KJM-SA1 administration led to a significant reduction in AChE expression (***$p < 0.001$) and an increase in BDNF expression (*$p < 0.05$) when com-pared to SCO-challenged mice. Although KJM-SA1 also enhanced ChAT expression in SCO-treated mice, this change was not statistically significant. Hence, KJM-SA1 demonstrated a significant modulatory effect on genes related to acetylcholine synthesis and synaptic function in the hippocampus, suggesting its potential therapeutic value in ad-dressing cognitive dysfunctions associated with cholinergic deficits.

The loss of neurons had been established to correlate with cognitive function impairment, as highlighted by Spalding *et al.,* [35]. Nissl staining, which selectively stained the soma of neurons and glial cells with cresyl violet, serves as a method to ascertain the neuronal distribution within brain structures. In this study, this technique revealed a marked reduction in neuronal distribution within the hippocampus of mice subjected to SCO-induced neurodegeneration, in comparison to normal group. Intriguingly, treatment with KJM-SA1 appeared to mitigate this effect, as evidenced by a notable restoration in the distribution of neurons that were previously compromised by SCO exposure. This finding underscored the potential of KJM-SA1 in counteracting SCO-induced neuronal loss in the hippocampus, thereby implicating its therapeutic efficacy in preserving cognitive functions compromised by neuronal degeneration.

CONCLUSIONS

This study provided compelling evidence that KJM-SA1, a sialic acid extracted from Edible Bird's Nest (EBN) using Affinity Beads Technology (ABT), has the potential to be developed as alternavtve nanofood material as a Nutrient Delivery System (NDS) for improving cognitive impairment in previous studies, and has significant potential as a neuroprotective agent for cognitive dysfunction. KJM-SA1 demonstrated notable efficacy in enhancing cell viability, exhibiting antioxidant properties, and improving cognitive functions in a scopolamine-induced model of neurodegeneration. Importantly, KJM-SA1's capacity to modulate key gene expressions, such as acetylcholinesterase (AChE), choline acetyltransferase (ChAT), and brain-derived neurotrophic factor (BDNF), which were integral to neuronal health and synaptic function, underscored its therapeutic potential. These findings suggested that KJM-SA1 could serve as a promising, viable, and safe alternative to conventional AChE inhibitors, potentially offering a solution to mitigate the adverse effects commonly associated with existing treatments for cognitive disorders. The observed neuroprotective effects of KJM-SA1, coupled with its ability to reverse cognitive deficits in a neurodegeneration model, warrant further investigation into its mechanisms of action and potential clinical applications in treating cognitive impairments.

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