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마가목 수피 추출물의 콜린성 및 BDNF/CREB 신호 조절을 통한 인지 기능 개선

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Cognition-Enhancing Effects of *Sorbus commixta* Hedl. Bark Extract via Cholinergic and BDNF/CREB Modulation

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ABSTRACT

Background: This study aimed to investigate the memory-enhancing effects of *Sorbus commixta* Hedl. bark ethanol extract (SCBE) and its underlying mechanisms.
Methods and Results: Acetylcholinesterase (AChE) inhibitory activity was evaluated *in vitro* at concentrations ranging from 20 to 2000 μg/mL, and cognitive function was assessed using a scopolamine-induced memory impairment model in mice. SCBE significantly improved spontaneous alternation behavior from 42.20% in the scopolamine-treated group to 68.96% in the 500 mg/kg group, indicating a marked recovery of spatial working memory. SCBE inhibited AChE activity in a concentration-dependent manner. In addition, SCBE restored cholinergic function by increasing hippocampal acetylcholine levels from 0.82 to 1.31 nmol/mg protein and decreasing brain AChE activity from 35.69 to 23.64 mU/mg protein. Furthermore, SCBE enhanced brain-derived neurotrophic factor (BDNF) expression and cAMP response element-binding protein (CREB) phosphorylation, indicating the activation of neuroplastic signaling pathways associated with synaptic plasticity and memory formation. In addition, histological analysis revealed that SCBE improved neuronal staining intensity and structural organization in the hippocampus, thus supporting its neuroprotective effects and preservation of neuronal integrity.
Conclusions: SCBE improves cognitive function by modulating cholinergic transmission and the BDNF/CREB signaling pathway, revealing its potential as a functional medicinal crop resource for cognitive health.

Key Words **:** *Sorbus commixta*, Acetylcholine, Acetylcholinesterase, BDNF, Cognitive Impairment, CREB, Hippocampus, Neuroprotection, Scopolamine, Y-Maze

INTRODUCTION

The global prevalence of chronic neurodegenerative disorders has increased markedly, imposing a substantial public health burden (Liu *et al*., 2015; Baker and Petersen, 2018; Ma *et al*., 2019). Among them, Alzheimer's disease (AD) is characterized by progressive cognitive decline, particularly impairments in learning and memory, accompanied by neuronal loss, abnormal protein aggregation, and synaptic dysfunction.

Disruption of cholinergic neurotransmission is a key mechanism underlying memory impairment in AD and forms the basis of the cholinergic hypothesis (Bartus *et al*., 1982). Reduced acetylcholine (ACh) levels and elevated acetylcholinesterase (AChE) activity contribute directly to cognitive dysfunction, while oxidative stress and neuroinflammation further contribute to neuronal dysfunction (Islam, 2017). Although cholinesterase inhibitors such as donepezil and rivastigmine are used clinically, their limited efficacy and adverse effects highlight the need for safer, multi-target therapeutic strategies (Liu *et al*., 2015; Ma *et al*., 2019).

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In addition to cholinergic dysfunction, impairment of neurotrophic signaling is increasingly recognized as a critical factor in cognitive decline. Brain-derived neurotrophic factor (BDNF) and its downstream transcription factor CREB regulate synaptic plasticity and memory consolidation (Finkbeiner, 2000; Minichiello, 2009; Lu *et al*., 2013). Therefore, agents capable of modulating both cholinergic transmission and BDNF/CREB signaling may provide enhanced cognitive benefits.

Scopolamine is known to induce memory impairment by triggering cholinergic dysfunction, Aβ accumulation, and oxidative stress in the brain. Consequently, the scopolamine-induced animal model serves as a standard model for evaluating the memory-enhancing efficacy and underlying mechanisms of potential agents for the prevention and treatment of AD (Kim *et al*., 2020; Choi *et al*., 2021).

Sorbus commixta Hedl. (Rosaceae) is a deciduous broad-leaved small tree reaching heights of approximately 6–8 m (Chung *et al*., 2003; Lee *et al*., 2003). In Korea, it is primarily distributed across Gangwon State, Jeollanam-do, and Ulleung Island. It also grows naturally in mountainous regions at elevations of 500–1,200 m in Japan and parts of China, including the northeastern and northern provinces and Gansu (Song *et al*., 2007).

This species has a long history in East Asian medicine for treating inflammatory and respiratory disorders (Sohn *et al*., 2005; Kim *et al*., 2016). Phytochemical investigations have identified various bioactive compounds, such as flavonoids and triterpenoids, which exhibit potent antioxidant and anti-inflammatory properties (Agnieszka *et al*., 2020; Jin *et al*., 2020). Moreover, *S. commixta* extracts have been shown to suppress inflammatory mediators linked to neuronal injury (Yu *et al*., 2011; Ng *et al*., 2018), and its bark extract has demonstrated inhibitory effects on the proliferation of A549 lung cancer and HepG2 liver cancer cells (Lee *et al*., 2002).

Although recent studies reported the neuroprotective effects of *S. commixta* bark against Aβ-induced toxicity (Kim *et al*., 2022), the underlying mechanisms for its cognitive-enhancing effects remain largely elusive. Specifically, its role in modulating cholinergic neurotransmission and the BDNF/CREB signaling pathway has yet to be fully elucidated.

Therefore, the present study investigated the cognition-enhancing and neuroprotective effects of *S. commixta* bark ethanol extract (SCBE) using a scopolamine-induced memory impairment model in mice. We evaluated the AChE inhibitory activity of SCBE *in vitro* and examined its effects on cholinergic function, BDNF/CREB signaling, and hippocampal neuronal integrity *in vivo* to clarify the underlying mechanisms responsible for its cognitive benefits.

MATERIALS AND METHODS

1. Preparation of SCBE

S. commixta bark was purchased from a local herbal market (Baekje Herb Market, Daejeon, Korea). The bark was powdered and extracted three times with 70% (v/v) ethanol under mild shaking (JSSI-200C; JSR, Daejeon, Korea) at 25 ± 1℃ for 8 h. To obtain SCBE, the extracts were passed through Whatman No. 2 filter paper (8 μm; GE Healthcare, Chicago, IL, USA), followed by vacuum concentration at 60℃ and subsequent lyophilization. The extraction yield was 23.4% (w/w). The dried extract was stored at 4 ± 1℃ until use.

2. Acetylcholinesterase (AChE) inhibition assay

AChE inhibitory activity was determined using a colorimetric assay based on Ellman *et al*. (1961). Whole brains from mice not subjected to behavioral testing were homogenized using a tissue homogenizer (Ultra-Turrax T25; IKA, Staufen, Germany) in cold 100 mM sodium phosphate buffer (pH 8.0) and centrifuged at 14,000 × g for 25 min at 4℃. The supernatants were used as the enzyme source. The assay was performed in the reaction mixture composed of 100 mM sodium phosphate buffer (pH 8.0, 120 μL), 0.71 mM acetylthiocholine iodide (10 μL), 0.5 mM 5,5'-dithiobis(2-nitrobenzoic acid) (10 μL), and either the sample extract or donepezil (30 μL). Following a 30 min pre-incubation at 25℃, 30 μL of the enzyme source was added to initiate the reaction, and the optical density was monitored at 412 nm with a microplate reader (SpectraMax M2; Molecular Devices, San Jose, CA, USA).

3. Animals and experimental design

Male Institute of Cancer Research (ICR) mice were utilized for behavioral experiments following a 7-day acclimation period in a facility maintained at a constant temperature (23 ± 1℃) and 55 ± 5% relative humidity under a regulated 12-h light/dark cycle. Food and water were supplied ad libitum during the experimental period. Five-week-old mice were randomly assigned to six groups (n = 5 per group): normal control, scopolamine-treated, donepezil (5 mg/kg) + scopolamine, and SCBE (50, 100, or 500 mg/kg) + scopolamine. SCBE or donepezil was orally administered once daily for 2 weeks, and

scopolamine (2 mg/kg, i.p.) dissolved in saline was injected 30 min before behavioral testing to induce acute memory impairment. All groups except the normal control received scopolamine. Behavioral assessments were performed within 30 min after injection, and mice were euthanized by decapitation. All experimental protocols involving animals received official approval from the Institutional Animal Care and Use Committee (IACUC) at Kyung Hee University (Accession No. KHUASP (SE)-17-126-1). Furthermore, all procedures involving animal management and testing were conducted following the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (Publication No. 80-23, revised 1996).

4. Y-maze test

The Y-maze test was conducted to assess spatial working memory. The apparatus consisted of three black plastic arms ($40 \times 3 \times 12$ cm^3). Mice were individually introduced into one arm of the Y-maze and permitted to explore the apparatus freely for 8 min. A spontaneous alternation was recorded when the animal made successive entries into all three distinct arms (for instance, ABC, CAB, or BCA). The spontaneous alternation rate was determined as the ratio of alternation events to the total arm entries minus two and expressed as a percentage. Scopolamine (2 mg/kg, i.p.), dissolved in 0.9% saline, was administered 30 min before testing to induce memory impairment and was injected once daily for 14 consecutive days. The normal control group was administered an equal volume of 0.9% saline.

5. Measurement of ACh and AChE

ACh levels were measured in hippocampal homogenates using a commercial colorimetric assay kit (ab65345; Abcam, Cambridge, UK). The hippocampus from one hemisphere was homogenized on ice in assay buffer, and the optical density was measured at 570 nm using a microplate reader (SpectraMax M2; Molecular Devices, San Jose, CA, USA). ACh concentrations were quantified using standard curves and normalized to total protein (nmol/mg protein).

ACh levels were measured in the hippocampus, whereas AChE activity was assessed in the striatum, as these regions are known to reflect neurotransmitter levels and enzymatic activity of the cholinergic system, respectively.

AChE activity was determined in striatal homogenates using a colorimetric assay kit (ab138871; Abcam, Cambridge, UK), according to the manufacturer's instructions. Briefly, the striatum was homogenized on ice in lysis buffer. Absorbance was measured at 410 nm using a microplate reader (SpectraMax M2; Molecular Devices, San Jose, CA, USA). Enzyme activity was calculated using standard curves, normalized to total protein, and expressed as mU/mg protein.

6. Western blot analysis

Hippocampal tissues were homogenized in ice-cold RIPA lysis buffer supplemented with protease and phosphatase inhibitor cocktails (Sigma-Aldrich, St. Louis, MO, USA). The homogenates were centrifuged at 12,000 × g for 15 min at 4℃ to remove insoluble debris, and the supernatants were collected for further analysis. Protein concentrations were quantified using a Bradford protein assay with a microplate reader (SpectraMax M2; Molecular Devices, San Jose, CA, USA). Equal amounts of protein (30 μg per sample) were resolved on 10–12% SDS-polyacrylamide gels and subsequently transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Primary antibodies against BDNF (ab108319) and pro-BDNF (ab72440) were obtained from Abcam (Cambridge, UK), while antibodies against CREB (9198), phospho-CREB (p-CREB; 9197), and β-actin (3700) were obtained from Cell Signaling Technology (Danvers, MA, USA). After protein transfer, PVDF membranes were immersed in a blocking solution consisting of 5% skim milk prepared in TBS-T (Tris-buffered saline containing 0.05% Tween-20; Sigma-Aldrich, St. Louis, MO, USA) for 1 h at room temperature. The membranes were subsequently incubated with primary antibodies (1:1000 dilution; Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight. After extensive washing with TBS-T (Sigma-Aldrich, St. Louis, MO, USA), the membranes were treated with HRP-conjugated secondary antibodies (anti-rabbit or anti-mouse IgG; Cell Signaling Technology, Danvers, MA, USA) for 1 h at room temperature. Protein signals were detected using an enhanced chemiluminescence reagent (ECL; Thermo Fisher Scientific, Waltham, MA, USA) and captured with a chemiluminescence imaging system (Fusion Solo S; Vilber, Collégien, France). Densitometric analysis was performed using image analysis software, and band intensities were quantified using the predominant band corresponding to the expected molecular weight (~43 kDa for CREB and p-CREB; ~32 kDa for pro-BDNF; ~14 kDa for mature BDNF). Representative bands at the expected molecular weight were selected for analysis. All signals were normalized to β-actin.

7. Cresyl violet staining

Free-floating sections were incubated for 5 min in 1% cresyl violet acetate dissolved in tertiary distilled water, followed by sequential washing with 70–100% ethanol for 30 sec each and rinsing with tap water. The sections were then incubated for 3 min in xylene and mounted using DPX mounting medium. Microscopic images of the tissue sections were captured using a laser-scanning confocal microscope (K1-Fluo; Nanoscope Systems, Daejeon, Korea) in bright-field mode to obtain high-resolution images under conditions comparable to conventional light microscopy. Quantitative analysis was performed by measuring the optical density of cresyl violet staining in each region of interest [CA1, CA3, and dentate gyrus (DG)] using image analysis software. Regions of interest were defined based on anatomical landmarks, and staining intensity values were normalized to the normal group (set as 100%).

8. Statistical analysis

All experimental results were analyzed using one-way analysis of variance (ANOVA) with IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). When significant differences were detected, Tukey's multiple comparison test was applied for post hoc analysis. Groups sharing the same letter are not significantly different, whereas groups with different letters indicate significant differences at $p < 0.05$.

RESULTS

1. Inhibitory effects of SCBE on AChE activity (*in vitro*)

SCBE exhibited a concentration-dependent inhibitory effect on acetylcholinesterase (AChE) activity (Fig. 1). At 20, 100, 250, 500, 1000, and 2000 μg/mL, the inhibition rates were 8.12 ± 1.00%, 14.37 ± 2.50%, 28.50 ± 1.50%, 42.36 ± 1.83%, 73.20 ± 2.86%, and 73.55 ± 0.66%. Donepezil showed consistently potent inhibition across all tested concentrations (78.93 ± 0.73% to 87.40 ± 0.97%). A plateau in SCBE-mediated inhibition was observed at 1000–2000 μg/mL, although the maximal inhibition remained lower than that of donepezil (Fig. 1).

2. Effects of SCBE on scopolamine-induced cognitive impairment

Scopolamine administration significantly reduced spontaneous alternation in the Y-maze test (42.20 ± 1.31%) compared with the normal control group (69.10 ± 1.64%). The donepezil-treated group showed significantly increased alternation behavior (65.09 ± 3.52%) relative to the scopolamine group. SCBE treatment significantly attenuated scopolamine-induced deficits in a dose-dependent manner, with spontaneous alternation values of 59.75 ± 5.86%, 63.61 ± 4.66%, and 68.96 ± 1.51% at 50, 100, and 500 mg/kg, respectively. The 500 mg/kg SCBE group showed no significant difference compared with the normal control group (Fig. 2).

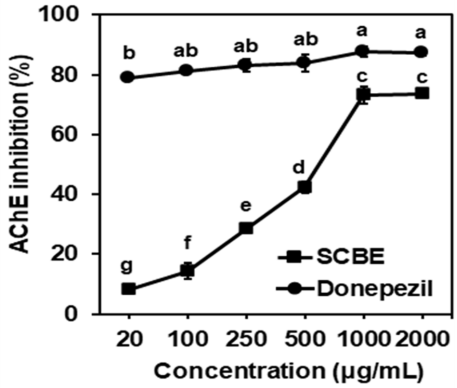

Fig. 1. Inhibitory effects of SCBE on AChE activity *in vitro*. SCBE and donepezil (20–2000 μg/mL). AChE inhibitory activity was measured using a colorimetric assay. Values are mean ± SD (n = 3). Statistical differences among groups were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. Different letters (a-g) indicate significant differences among groups at $p < 0.05$, whereas groups sharing the same letter are not significantly different.

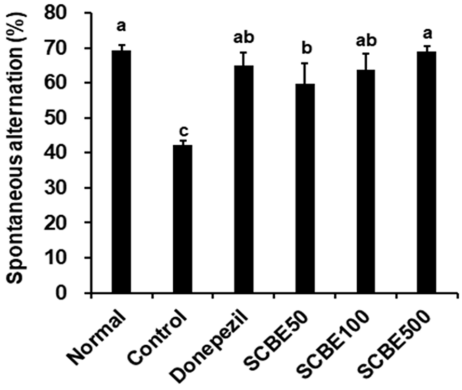

Fig. 2. Effect of SCBE on spontaneous alternation behavior in the Y-maze test in scopolamine-treated mice. Groups: saline (Normal); scopolamine (2 mg/kg, i.p.; Control); donepezil (5 mg/kg, p.o.) + scopolamine; and SCBE (50, 100, or 500 mg/kg, p.o.) + scopolamine. Values are mean ± SD (n = 3). Statistical differences among groups were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. Different letters (a-c) indicate significant differences among groups at $p < 0.05$, whereas groups sharing the same letter are not significantly different.

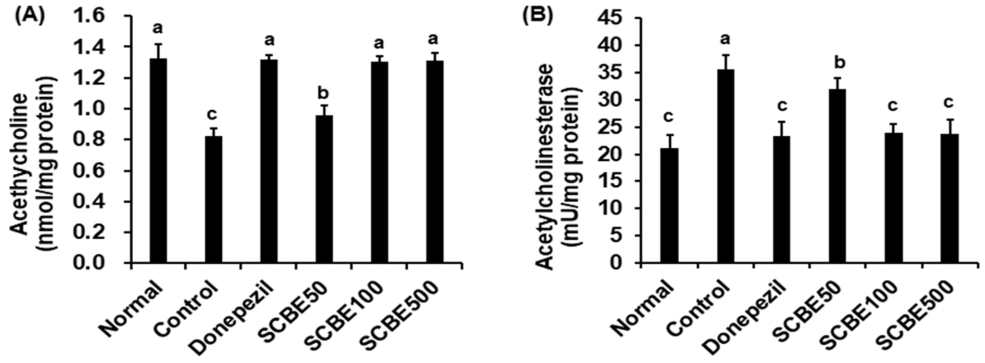

Fig. 3. Effect of SCBE on cholinergic markers in scopolamine-treated mice. Groups: saline (Normal); scopolamine (2 mg/kg, i.p.; Control); donepezil (5 mg/kg, p.o.) + scopolamine; and SCBE (50, 100, or 500 mg/kg, p.o.) + scopolamine. (A) Hippocampal ACh levels and (B) brain AChE activity. ACh levels are expressed as nmol/mg protein and AChE activity as mU/mg protein. Values are mean ± SD (n = 5). Statistical differences among groups were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. Different letters (a-c) indicate significant differences among groups at $p < 0.05$, whereas groups sharing the same letter are not significantly different.

3. Effects of SCBE on the cholinergic system in scopolamine-treated mice

Scopolamine administration significantly decreased hippocampal acetylcholine (ACh) levels (0.82 ± 0.05 nmol/mg protein) compared with the normal control group (1.33 ± 0.09 nmol/mg protein). Donepezil treatment increased ACh levels to 1.32 ± 0.03 nmol/mg protein. In the SCBE-treated groups, hippocampal ACh levels were 0.96 ± 0.06, 1.30 ± 0.04, and 1.31 ± 0.05 nmol/mg protein at doses of 50, 100, and 500 mg/kg, respectively. The 100 and 500 mg/kg SCBE groups showed no

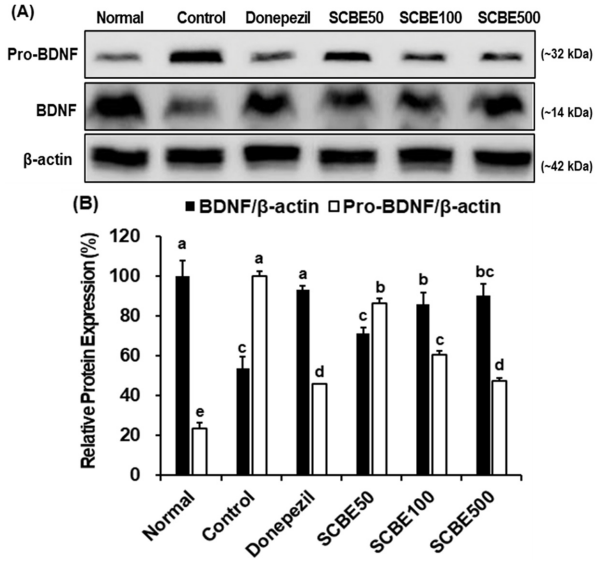

Fig. 4. Effect of SCBE on hippocampal BDNF expression in scopolamine-treated mice. Groups: saline (Normal); scopolamine (2 mg/kg, i.p.; Control); donepezil (5 mg/kg, p.o.) + scopolamine; and SCBE (50, 100, or 500 mg/kg, p.o.) + scopolamine. (A) Representative Western blots of pro-BDNF (~ 32 kDa), BDNF (~ 14 kDa), and β-actin. (B) Quantification normalized to β-actin. Values are mean ± SD (n = 3). Statistical differences among groups were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. Different letters (a-e) indicate significant differences among groups at $p < 0.05$, whereas groups sharing the same letter are not significantly different.

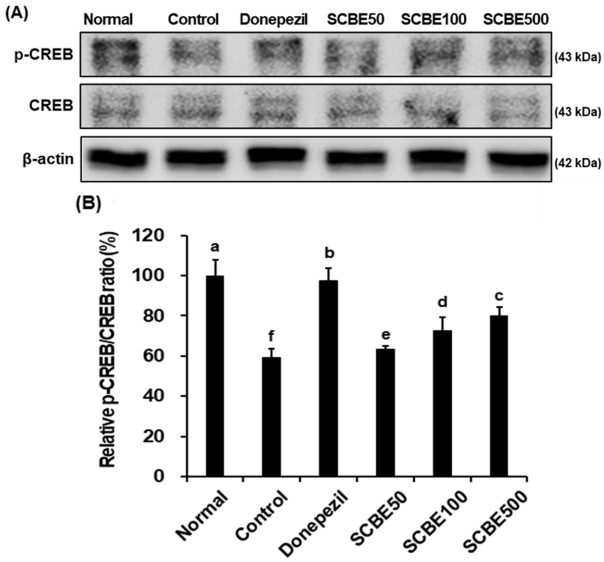

Fig. 5. Effect of SCBE on hippocampal CREB phosphorylation in scopolamine-treated mice. Groups: saline (Normal); scopolamine (2 mg/kg, i.p.; Control); donepezil (5 mg/kg, p.o.) + scopolamine; and SCBE (50, 100, or 500 mg/kg, p.o.) + scopolamine. (A) Representative Western blots of p-CREB (~ 43 kDa), CREB (~ 43 kDa), and β-actin. (B) p-CREB/CREB ratio. Values are mean ± SD (n = 3). Statistical differences among groups were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. Different letters (a-f) indicate significant differences among groups at $p < 0.05$, whereas groups sharing the same letter are not significantly different.

significant differences compared with the normal or donepezil-treated groups (Fig. 3A).

Conversely, scopolamine treatment significantly increased brain acetylcholinesterase (AChE) activity (35.69 ± 2.59 mU/mg protein) compared with the normal control group (21.14 ± 2.31 mU/mg protein). Donepezil treatment decreased AChE activity to 23.31 ± 2.68 mU/mg protein. SCBE administration reduced AChE activity in a dose-dependent manner to 31.95 ± 2.14, 24.01 ± 1.55, and 23.64 ± 2.61 mU/mg protein at 50, 100, and 500 mg/kg, respectively. The 100 and 500 mg/kg SCBE groups showed significantly lower AChE activity compared with the scopolamine-treated group (Fig. 3B).

4. Effects of SCBE on hippocampal BDNF expression

Scopolamine significantly decreased hippocampal BDNF protein expression to 53.78 ± 5.85% of the normal control level. SCBE increased BDNF expression in a dose-dependent manner to 64.67 ± 2.61%, 86.01 ± 6.01%, and 90.53 ± 5.63% at 50, 100, and 500 mg/kg, respectively. Pro-BDNF expression was also reduced by scopolamine (46.83 ± 4.37%). SCBE administration increased pro-BDNF levels to 46.95 ± 1.55%, 76.01 ± 2.63%, and 76.45 ± 2.45% at 50, 100, and 500 mg/kg, respectively. Pro-BDNF levels in the donepezil group were higher than those in the SCBE-treated groups, although all SCBE groups showed significant increases compared with the scopolamine-treated group (Fig. 4).

5. Effects of SCBE on hippocampal CREB activation

CREB phosphorylation, expressed as the p-CREB/CREB ratio, was significantly reduced by scopolamine (59.08 ± 4.41% of the normal control). SCBE treatment increased this ratio in a dose-dependent manner to 63.40 ± 1.76%, 72.77 ± 6.64%, and 79.87 ± 4.65% at 50, 100, and 500 mg/kg, respectively. Although the p-CREB/CREB ratio in the SCBE groups remained lower than that in the donepezil group (97.29 ± 6.47%), all SCBE-treated groups showed significantly higher values compared with the scopolamine-treated group (Fig. 5).

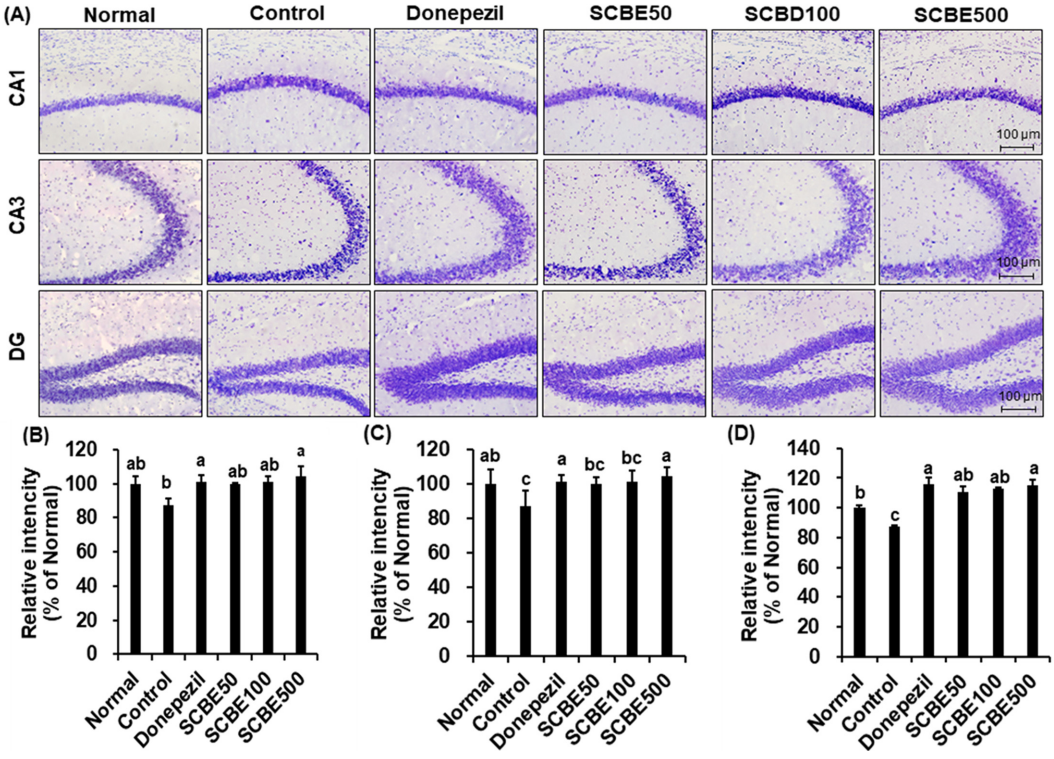

Fig. 6. Effects of SCBE on hippocampal neuronal morphology and staining intensity in scopolamine-treated mice. Groups: saline (Normal); scopolamine (2 mg/kg, i.p.; Control); donepezil (5 mg/kg, p.o.) + scopolamine; and SCBE (50, 100, or 500 mg/kg, p.o.) + scopolamine. (A) Representative cresyl violet-stained coronal hippocampal sections (-1.70 to -2.30 mm from bregma) showing CA1, CA3, and DG. Scale bar = 100 μm. (B-D) Staining intensity in CA1 (B), CA3 (C), and DG (D), expressed as % of the normal group (mean ± SD, n = 3). Statistical differences among groups were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. Different letters (a-c) indicate significant differences among groups at $p < 0.05$, whereas groups sharing the same letter are not significantly different.

6. Effects of SCBE on hippocampal neuronal morphology and staining intensity

Cresyl violet staining was performed to evaluate neuronal morphology and staining intensity in the CA1, CA3, and DG regions of the hippocampus. Compared with the normal group, the scopolamine-treated group showed reduced staining intensity and partial disruption of neuronal layer organization, without clear evidence of overt neuronal loss or degeneration under the present experimental conditions (Fig. 6).

Quantitative analysis showed that scopolamine significantly decreased staining intensity to 87.26 ± 3.73% in CA1, 81.60 ± 8.75% in CA3, and 87.31 ± 0.81% in DG, relative to the normal group (set at 100%). SCBE treatment modulated these histological alterations in a region-dependent manner. In CA1, staining intensity remained comparable to that of the normal group (SCBE50: 99.63 ± 0.71%; SCBE100: 101.23 ± 3.25%; SCBE500: 104.22 ± 5.82%) (Fig. 6A).

In CA3, SCBE partially restored staining intensity (SCBE50: 90.24 ± 4.11%; SCBE100: 91.84 ± 6.09%; SCBE500: 105.74 ± 5.38%), although the lower doses remained below or near the normal level (Fig. 6B).

In DG, SCBE increased staining intensity above the scopolamine-treated control level (SCBE50: 110.30 ± 4.30%; SCBE100: 112.86 ± 6.09%; SCBE500: 115.32 ± 3.72%), showing a pattern similar to that of the donepezil-treated group (116.12 ± 4.19%) (Fig. 6C).

At the highest dose (500 mg/kg), SCBE exhibited staining patterns and neuronal layer organization comparable to those of the donepezil-treated group across all hippocampal regions.

DISCUSSION

sThe present study suggests that SCBE ameliorated scopolamine-induced cognitive impairment through coordinated regulation of cholinergic neurotransmission and neuroplastic signaling pathways. SCBE exerts multiple mechanisms, including AChE inhibition and BDNF/CREB activation effects rather than targeting a single pathway, contributing to memory improvement.

Scopolamine markedly reduced spontaneous alternation behavior in the Y-maze (Fig. 2), reflecting impaired spatial working memory. This model induces transient cognitive deficits by disrupting central cholinergic signaling (Klinkenberg and Blokland, 2010). SCBE improved behavioral performance in a dose-dependent manner with the highest dose restoring alternation rates to near-normal levels (Fig. 2).

Restoration of cholinergic homeostasis represents a central mechanism underlying these effects. Elevated acetylcholinesterase (AChE) activity reduces synaptic acetylcholine (ACh) availability and contributes to cognitive decline (Bartus *et al*., 1982). SCBE inhibited AChE activity *in vitro* (Fig. 1) and reduced brain AChE activity *in vivo* (Fig. 3B), accompanied by recovery of hippocampal ACh levels (Fig. 3A). Together, these findings indicate that SCBE improves cholinergic neurotransmission by inhibiting AChE activity and increasing hippocampal ACh levels in scopolamine-treated mice. Although less potent than donepezil, SCBE exhibited biologically meaningful cholinesterase inhibition. Polyphenolic compounds are known to exhibit AChE inhibitory activity (Rice-Evans *et al*., 1997); however, the chemical composition of SCBE was not characterized in the present study. Therefore, the contribution of specific constituents remains to be elucidated.

SCBE also influenced neurotrophic signaling associated with synaptic plasticity. The BDNF/CREB axis regulates neuronal survival and memory consolidation (Finkbeiner, 2000; Minichiello, 2009; Lu *et al*., 2013). Scopolamine suppressed hippocampal BDNF expression (Fig. 4) and CREB phosphorylation (Fig. 5), whereas SCBE attenuated these reductions, partially restoring BDNF levels and the p-CREB/CREB ratio. SCBE appears to exert multi-target effects by modulating both cholinergic neurotransmission and BDNF/CREB-mediated neuroplastic signaling.

Histological findings further supported these results, demonstrating that SCBE dose-dependently ameliorated scopolamine-induced alterations in neuronal staining intensity and layer organization in the CA1, CA3, and DG regions (Fig. 6). These changes are consistent with improved neuronal integrity in the hippocampus. However, it should be noted that cresyl violet staining primarily reflects neuronal morphology and Nissl substance rather than direct evidence of cell death. Therefore, the observed changes are more appropriately interpreted as alterations in neuronal integrity and structural organization, rather than definitive evidence of neuronal loss. Further studies using apoptosis-related markers, such as TUNEL assay or cleaved caspase-3, are required to determine whether SCBE directly affects neuronal survival.

Several limitations should be noted. The scopolamine model reflects acute and reversible cholinergic dysfunction and does not fully mimic progressive Alzheimer's pathology (Klinkenberg and Blokland, 2010). In addition, ACh levels and AChE

activity were assessed in different brain regions (hippocampus and striatum, respectively), which may limit direct comparison of these parameters. Future studies should evaluate both markers within the same brain region to allow more direct interpretation. Moreover, the specific phytochemical constituents responsible for these effects remain unidentified. Further studies using disease-relevant models and phytochemical characterization are required.

In conclusion, SCBE may improve cognitive function by restoring cholinergic balance and partially reactivating BDNF/CREB-mediated neuroplastic signaling, accompanied by improved neuronal integrity. These findings support the potential of SCBE as a functional medicinal crop resource for cognitive health management.

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