

Neuroprotective Activity of Ethanolic Extract of Red Ginger Containing 6-Shogaol on Scopolamine-Induced Memory Impairment in Alzheimer's Mice

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Red ginger contains 6-shogaol, which has a neuroprotective effect and is crucial in several brain diseases, including Alzheimer's. This study investigated the neuroprotective effect of red ginger extract (RGE) containing 6-shogaol on scopolamine-induced memory loss in mice. Male Balb/C mice (n = 30; 6–7 weeks old) were divided into six groups: normal, Alzheimer, drug control (donepezil), and RGE (100, 200, and 400 mg/kg BW). In the acute experiment, the mice were treated 60 minutes before the test, followed by 1 mg/kg of scopolamine 30 minutes later. Thirty minutes later, the mice were placed individually in a Y-maze to observe spontaneous alteration activity (SAA). In the chronic experiment, the mice were treated once daily for seven days. On days 8–14, the mice were administered scopolamine and treatment. SAA was observed every 3–4 days. On day 15, malondialdehyde (MDA) and acetylcholinesterase (AChE) levels in the serum and brain were determined. RGE treatment 400 mg/kg BW, containing 1.664 µg of 6-shogaol per 100 mg of ethanol RGE, reduced memory loss better than the other two doses. RGE successfully decreased MDA and increased AChE in the serum and brain. RGE also showed effectively to improve memory in Alzheimer's disease.

Keywords: Alzheimer; memory loss; neuroprotective; red ginger extract; 6-shogaol.

Alzheimer's disease, one of the neurodegenerative diseases, is a general manifestation of dementia.¹ Usually, Alzheimer's disease, which affects almost 40 billion people worldwide, is characterized by cognitive dysfunction and memory loss in the elderly.² Disease progression is related to damage to the structure and function of the hippocampus and neocortex, which are responsible for cognitive and memory action.³

Several pathways contribute to the pathophysiology of Alzheimer's disease, such as loss of synapses, deposition of β -amyloid plaque, neuron fibrillation changes, and hyperphosphorylation of tau protein.³ All changes in Alzheimer's disease activate the N-methyl-D-aspartate receptor (NMDAR) and oxidative stress accumulation. However, β -amyloid induces exocytosis of NMDAR due to an increase in calcium influx and oxidative stress.⁴ NMDAR

hyperactivity, intracellular calcium accumulation, and oxidative stress also contribute to tau protein dysfunction.⁵

The high concentration of acetylcholine found in synapses indicates the cholinergic system's increased function in Alzheimer's disease. Some drugs that block acetylcholinesterase (AChE) have the potential to reduce the loss of memory in Alzheimer's disease. AChE inhibitors can enhance neural function by increasing acetylcholine (ACh) levels.⁶ Some of the drugs that work as acetylcholinesterase inhibitors, such as donepezil, rivastigmine, and tacrin, are used minimally due to their side effects and hepatotoxicity.^{7,8} Even though preclinical studies have shown positive outcomes, anti-Alzheimer drugs' activity in clinical practice is still debated, and finding other substances with new targets is essential.⁹

Ginger has anti-inflammation, antioxidant, and anticancer effects, and 6-shogaol is one of the vital compounds present in ginger responsible for these effects.^{10,11} The amount of 6-shogaol in red ginger is higher than in other variants of ginger. Previous studies have shown that 6-shogaol has the potential as a neuroprotector due to its antioxidant and anti-inflammatory properties.¹²⁻¹⁴ In addition, 6-shogaol can minimize NMDAR expression and restore sciatic nerve damage in diabetic mice with neuropathy.^{15,16}

This study tried to find the effect of red ginger extract (RGE) containing 6-shogaol as a neuroprotector on scopolamine-induced memory loss in mice. A previous study showed that scopolamine was a muscarinic acetylcholine receptor antagonist and widely used in Alzheimer's models. We evaluated the effect of different RGE doses to potentially prevent and treat learning and memory deficiency in a mouse model of Alzheimer's disease. We also examined whether RGE treatment improves scopolamine-induced memory impairment by conducting Y-maze tests in short- and long-term induction.

MATERIALS AND METHODS

This study was conducted in the Faculty of Pharmacy, University of Jember Indonesia. All experiment was completed after 12 months (2020 – middle 2022).

Extraction

The rhizomes of *Zingiber officinale* var *Rubrum* (red ginger) were harvested by the U.P.T. Laboratorium Herbal Medica Batu Malang, East Java, Indonesia (074/226A/102.7/2020) with aged 6–8 months. Plants were dried at room temperature. The rhizomes were ground, and the dried powder (0.5 kg) was macerated thrice for 24 h in 96% ethanol (1:5). The residue was evaporated using a rotary evaporator to obtain a thick extract; the yield was 20.134%.

Determination of 6-Shogaol in RGE using high-performance liquid chromatography

Analysis was performed using high-performance liquid chromatography (HPLC) using Cecil Adept CE4300 equipped with an autoinjector sampler programmed at 10 iL capacity per injection. Chromatographic separations were performed on an RPC-18 column (150 mm × 4.6 mm). The mobile phase (combination of acetonitrile and water) was seen in Table 1. The chromatographic run time was 400 min at 25°C, with a 1 mL/min flow rate. All chromatograms were monitored at 254 nm. The amount of 6-shogaol was counted using a calibration curve. This method was followed previously study by Jazokaite *et al.*¹⁷ with some modifications.

Determination of acetylcholinesterase (AChE) inhibitory activity

The AChE inhibitory activity of the extract was determined using Ellman's method¹⁸ with some modifications. Briefly, AChE (type VI-S lyophilized powder, 518 U/mg solid, 844 U/mg protein) was diluted using 50 mM of Tris-HCl buffer (pH 8.0). The solution was mixed between 100 iL of 3 mM 5,5-Dithio-bis-(2-nitrobenzoic acid) (DTNB) in Tris-HCl buffer, 20 iL of 0.26 U/mL of AChE, and 40 iL of buffer (50 mM Tris, pH 8.0), 20 iL of samples (RGE in various concentrations (0–1000 ig/mL); 6-shogaol (0–500 ig/mL); and donepezil as a control (0–250 ig/mL) in buffer). Samples were incubated at 25°C for 15 min and the absorbances were determined at 412 nm using a microplate reader (ELx800; Dialab GmbH; Austria). The absorbance was measured every 5 min for 20 min to monitor the ACh hydrolysis. All reactions were performed in triplicate. The percentage of inhibition was calculated using the following formula:¹⁹

Inhibition (%) = $[(E - S)/E] \times 100$, where E was the activity of the enzyme without the test compound and S was the activity of the enzyme with the test compound.

Animals and ethics statements

Thirty male Balb/C mice (20–30 g, 6–7 weeks old) were provided and certified by the Faculty of Pharmacy, University of Jember, Jember, Indonesia. All mice were maintained in standard-clean 30 × 20 cm² cages with free access to pellet and water ad libitum. They lived at a temperature of 25°C ± 1°C in a 12 h/12 h light/dark cycle.

Before using animals, all tests involving animals were previously approved by the Ethical Committee of Medical Research, University of Jember (997/UN25.8/KEPK/DL/2020).

Scopolamine-induced acute memory loss

The 30 mice were randomly divided into six groups: normal control, Alzheimer's (no treatment), drug control (donepezil), RGE 100 mg/kg body weight (BW), RGE 200 mg/kg BW, and RGE 400 mg/kg BW. After overnight fasting, each group was administered treatment as follows: normal group (1% sodium carboxymethyl cellulose), Alzheimer group (1% sodium carboxymethyl cellulose), drug control group (1 mg/kg BW of donepezil), RGE100 group (100 mg/kg BW of RGE), RGE200 group (200 mg/kg BW of RGE), and RGE400 group (400 mg/kg BW of RGE). After 1 h, 25 mice were intraperitoneally injected with 1 mg/kg BW of scopolamine in sterile normal saline; the normal control group received a normal saline injection. Finally, 30 min later, all mice were placed individually in a Y-maze to observe spontaneous alteration activity.

Scopolamine-induced long-term memory loss in Alzheimer's disease

We randomly divided 30 mice into six groups, as described in Section 2.6. On days 8–14, 25 mice were intraperitoneally injected with 1 mg/kg BW of scopolamine in sterile normal saline 1 h after treatment; the normal control group received a normal saline injection. Finally, 30 min later, all mice were placed individually in a Y-maze to observe spontaneous alteration activity on days 0, 3, 7, 10, and 14.

Determination of spontaneous alteration activity

Spontaneous alternation activity was assessed using a single, dark acrylic Y-maze with each arm measuring 35 cm (length) × 6 cm

(width) × 18 cm (height). Each mouse was placed individually at the end of one arm and allowed to move freely for 8 min. The behavior of each mouse was recorded using a webcam (Jete W7, Indonesia). The arms of the maze were cleaned between sessions using 70% ethanol. An entry was considered complete when a mouse's entire body, including the tail, was entirely within the arm. The number of entries per arm was calculated during 8 min. The number of alterations was defined as the ability of a mouse to enter the three arms consecutively, for example, A-B-C, B-C-A, or C-A-B. The percentage of alterations of each mouse was calculated as (total number of arms entered " 2) × 100.²⁰

Preparation of serum and brain homogenates

On day 15, all mice were euthanized. Their brain and blood were obtained for further tests. The blood was centrifuged at 4000 rpm for 15 min, and the serum was stored at –80°C for additional tests. The whole brain was quickly removed and cleaned using chilled normal saline on ice. The brain was weighed to determine its relative weight before further examination. A 200 mg homogenate of brain samples was homogenized using a micro pestle (Axygen; cat. #PES-15-B-SI; USA) on ice, and the homogenized tissue was centrifuged at 4000 rpm for 15 min at 4°C. Finally, the homogenate was placed at –80°C to measure various biochemical parameters.²¹

MDA assay in the serum and brain

Malondialdehyde (MDA) levels in the serum and brain were measured by the method of Ohakawa *et al.*²² The principle of this method was MDA reacted with thiobarbituric acid (TBA) to produce a colored complex, which was measured using a spectrophotometer at 532 nm (UVD-2950; Labomed, Inc., Los Angeles, CA, USA). The result was expressed in μM.

Protein in the serum and brain

Total protein in the serum and brain were calculated using protein kits (Fluitest® TP; Germany) according to the manufacturer's instructions. The total protein concentration was analyzed at 546 nm using a spectrophotometer (UVD-2950; Labomed, Inc.). The result was expressed in g/L.

AChE in the serum and brain

According to the manufacturer's instructions, the AChE concentrations in the serum

and brain were assessed using ELISA kits (Bioassay Technology Laboratory, cat. #E1347Mo, China). AChE activity was measured by the method of den Blaauwen *et al.*²³ using a colorimetric reagent kit (Bioassay Technology Laboratory, cat. #E1347Mo, China) according to the kit's instructions. Each assay was performed in duplicate, and the results were expressed as ng/mL.

Histopathology of the hippocampus

The histopathologic assessment was performed on the brains of 2–3 mice randomly selected from each group. The brains were immediately fixed in 10% phosphate-buffered formaldehyde, embedded in paraffin using a tissue processor (Tissue-Tek VIP 5 Jr; TEC 5 EM J-2 5233; TEC 5 CM J-2 5234; Sakura Finetek Japan), and 5 μ m longitudinal sections were cut. The sections were stained with hematoxylin and eosin (H&E) and examined using a microscope (Olympus CX33; Olympus, Japan).

Data analysis

All data were reported as the mean \pm standard error of the mean (SEM). Statistically significant differences between groups were assessed using SPSS Programme version 20. Data were analyzed with one-way analysis of variance (ANOVA), followed by least significant difference (LSD) post hoc tests. A significant difference between groups was considered when $p < 0.05$.

RESULTS AND DISCUSSION

6-shogaol concentration in RGE

The calibration curve was plotted from 6-shogaol in the range of 5-500 ppm and resulted in a 0.999 correlation coefficient which means linear. The linear regression was $y = 6.2572x - 1.5962$, where y is the response as the peak area and x is the concentration in ppm (Fig. 1C). From this equation, the 6-shogaol content was $4.1235\% \pm 0.1920\%$ w/w mg in 100 mg of ethanol RGE. Figs 1A and 1B showed consecutively the chromatograms of 6-shogaol and the ethanol RGE.

Determination of acetylcholinesterase inhibitory activity

AChE is an enzyme responsible for ACh metabolism. The AChE inhibitory activity of RGE and 6-shogaol was tested using donepezil as a drug control. Both RGE and 6-shogaol showed lower AChE inhibitory activity than donepezil, while

6-shogaol showed better AChE inhibitory activity than RGE. The median inhibitory concentration (IC_{50}) indicated the potency of AChE inhibition (Table 3). A low IC_{50} indicated better AChE inhibitory activity.

Data are expressed as the mean \pm SEM. Statistical analysis used one-way ANOVA followed by the LSD test. Different subscript letters are considered significantly different between groups using a 95% confidence interval. AChE, acetylcholinesterase; IC_{50} , median inhibitory concentration; RGE, red ginger extract; SEM, standard error of the mean; ANOVA, analysis of variance; LSD, least significant difference.

Spontaneous alteration activity after scopolamine-induced acute memory loss using a Y-maze

In the Y-maze test, the Alzheimer group showed a significantly decreased total number of entries per arm (16.0 ± 4.45), the total number of alterations (12.25 ± 4.55), and percentage alteration ($38.49\% \pm 13.40\%$) compared to the normal control group (Table 4; $p < 0.05$). RGE treatment changed the behavior of mice after a single dose of scopolamine. The RGE400 group showed the maximum total number of entries per arm, total number of alterations, and percentage of alteration. These values were the same for the drug control group.

Data are expressed as the mean \pm SEM of 4–5 mice. Statistical analysis used one-way ANOVA followed by the LSD test. Different subscript letters are considered significantly different between groups using a 95% confidence interval. CMC-Na, sodium carboxymethyl cellulose; RGE, red ginger extract; BW, body weight; SEM, standard error of the mean; ANOVA, analysis of variance; LSD, least significant difference.

Spontaneous alteration activity after scopolamine-induced Alzheimer's disease using a Y-maze

After 7 days of scopolamine administration, the memory behavior of mice changed. The number of entries per arm, the total number of alterations, and the percentage of alteration significantly decreased in the Alzheimer group day by day compared with the normal control group (Figure 2A–C and Table 5; $p < 0.05$). RGE administration (7 days before and 7 days during scopolamine administration) successfully reversed

the alteration percentage as an effect of memory loss due to scopolamine (Figure 2C). On day 14, the RGE400 group showed the best improvement in scopolamine-induced memory loss by an increase in the total number of entries per arm (928.75 ± 4.66), the total number of alterations (20.75 ± 3.14), and percentage alteration ($73.79\% \pm 9.06\%$) compared to the Alzheimer group ($p < 0.05$). This improvement was the same in the drug control group ($p > 0.05$), except for the total number of alterations, indicating that 400 mg/kg BW of RGE treatment leads to better activity than donepezil.

Data are expressed as the mean \pm SEM of 4–5 mice. Statistical analysis used one-way ANOVA followed by the LSD test. Different subscript letters considered the significant difference between groups using a 95% confidence interval. CMC-Na, sodium carboxymethyl cellulose; RGE, red ginger extract; BW, body weight; SEM, standard error of the mean; ANOVA, analysis of variance; LSD, least significant difference.

Determination of relative brain weight and protein concentration

Scopolamine induced a significant increase in protein levels in both serum and brain. Table 6 shows the effect of different doses of RGE on serum and brain protein levels. RGE in different doses and donepezil both resulted in a significant increase ($p < 0.05$) in serum and brain protein levels compared to the Alzheimer group.

Data are expressed as the mean \pm SEM of 4–5 mice. Statistical analysis used one-way ANOVA followed by the LSD test. Different subscript letters were considered significantly different between groups using a 95% confidence interval. CMC-Na, sodium carboxymethyl cellulose; RGE, red ginger extract; BW, body weight; SEM, standard error of the mean; ANOVA, analysis of variance; LSD, least significant difference.

Determination of MDA levels in the serum and brain

As seen in Table 7, the MDA concentration in the brain was seen lower than in serum.

Table 1. Mobile-phase-exchange stage until 40 minutes.

Time (min)	0	5	25	28	30	35	37	40
Water (%)	100	90	50	0	0	30	100	100
Acetonitrile (%)	0	10	50	100	100	70	0	0

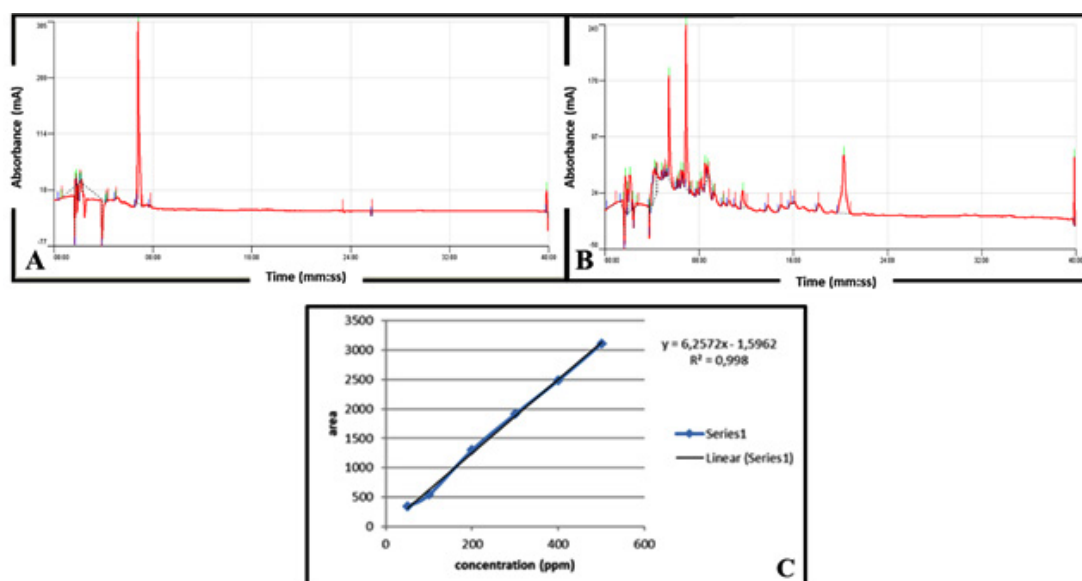


Fig. 1. Chromatogram of 6-shogaol (A) and RGE (B) using HPLC in 254 nm. Calibration curve of 6-shogaol (C) was determined by the equation $y = 6.2572x - 1.5962$. RGE, red ginger extract; HPLC, high-performance liquid chromatography

Scopolamine administration for 7 days increased MDA levels in both serum ($29.99 \pm 2.12 \mu\text{M}$) and brain ($19.03 \pm 1.04 \mu\text{M}$) in Alzheimer's group compared to the normal control group ($p < 0.05$). It explained that scopolamine caused the accumulation of ROS and brought a higher level of MDA. Administration of RGE was successful to reduce MDA concentration in both the brain and serum. The optimum dose of RGE which successfully reduced the MDA levels in the serum and brain was 400 mg/kg BW. This reduction was the same in the drug control group, donepezil ($p > 0.05$).

Data are expressed as the mean \pm SEM of 4-5 mice. Statistical analysis used one-way ANOVA followed by the LSD test. Different subscript letters were considered significantly different between groups using a 95% confidence interval. CMC-Na, sodium carboxymethyl cellulose; RGE, red ginger extract; MDA, malondialdehyde; BW, body weight; SEM, standard error of the mean; ANOVA, analysis of variance; LSD, least significant difference.

Determination of AChE levels in the serum and brain

Scopolamine injection significantly decreased AChE concentration in the brain ($12.90 \pm 0.78 \text{ ng/mL}$) and serum ($12.80 \pm 1.19 \text{ ng/mL}$) in Alzheimer's group compared to the normal group

($p < 0.05$). The activity of RGE in decreasing AChE concentration depended on the dose. The RGE dose 200 mg/kg BW and 400 mg/kg BW groups showed the optimum dose to reduce AChE levels in the serum and brain compared to RGE dose 100 mg/kg BW. The potency of RGE to minimize AChE levels was the same as the drug control group after scopolamine administration ($p < 0.05$), as seen in Table 8.

Data are expressed as the mean \pm SEM of 4-5 mice. Statistical analysis used one-way ANOVA followed by the LSD test. Different subscript letters considered the significant difference between groups using a 95% confidence interval. CMC-Na, sodium carboxymethyl cellulose; RGE, red ginger extract; AChE, acetylcholinesterase; BW, body weight; SEM, standard error of the mean; ANOVA, analysis of variance; LSD, least significant difference.

Histology of the hippocampus of mice with Alzheimer's disease posttreatment

Repeated scopolamine administration resulted in disorganization, a decrease in thickness, as well as the density of pyramidal cells in cornu ammonis (CA)1 and CA3 subregions and granular cells of the dentate gyrus (DG) hippocampal subregions (Fig. 3B). RGE administration resolved the morphology of the hippocampus (Figs 3D-F;

Table 2. AChE inhibitory activity (IC_{50}) of RGE and 6-shogaol

No.	Compound	<i>n</i>	$\text{IC}_{50} \pm \text{SEM}$
1	Red ginger extract	4	2938.55 ± 307.60^a
2	6-shogaol	3	361.18 ± 5.91^b
3	Donepezil	4	5.38 ± 0.87^c

Table 3. Spontaneous alteration activity of each group after scopolamine-induced acute memory loss

Group	Treatment	Total number of entries per arm (\pm SEM)	Total number of alterations (\pm SEM)	Percentage alteration (\pm SEM)
Normal	CMC-Na 1%	46.5 ± 6.41^a	32.25 ± 4.55^a	61.02 ± 2.92^a
Alzheimer	CMC-Na 1%	16.0 ± 4.45^b	7.25 ± 3.64^b	38.49 ± 13.40^b
Donepezil	1 mg/kg BW	41.5 ± 11.26^a	20.75 ± 4.97^a	64.22 ± 8.46^a
RGE	100 mg/kg BW	23.5 ± 2.36^b	13.75 ± 3.20^b	61.80 ± 7.04^a
RGE	200 mg/kg BW	32.75 ± 3.79^a	24.75 ± 3.75^a	80.02 ± 4.45^a
RGE	400 mg/kg BW	25.0 ± 5.64^b	18.25 ± 4.13^b	66.81 ± 3.15^a

3J–L). The neurons showed a uniform size and were well arranged. Each neuron also performed a rounded central vesicular nucleus with a prominent nucleolus in the RGE400 group (Fig. 3L). These

effects were better than those in RGE100 and RGE200 groups (Figs. 3J–K). This morphology was also seen in the normal control (Fig. 3G) and drug control group (Fig. 3I).

Table 4. Spontaneous alteration activity of each group after scopolamine-induced Alzheimer's disease on day 14.

Group	Treatment	Total number of entries per arm on day 14 (\pm SEM)	Total number of alterations on day 14 (\pm SEM)	Percentage alteration on day 14 ($\%$ \pm SEM)
Normal	CMC-Na 1%	21.75 \pm 2.10 ^a	12.50 \pm 1.19 ^a	71.04 \pm 11.78 ^a
Alzheimer	CMC-Na 1%	5.25 \pm 1.65 ^b	4.25 \pm 1.93 ^b	26.78 \pm 15.53 ^b
Donepezil	1 mg/kg BW	13.75 \pm 1.31 ^c	11.75 \pm 2.32 ^a	81.25 \pm 11.97 ^a
RGE	100 mg/kg BW	25.25 \pm 4.75 ^a	16.25 \pm 2.69 ^a	65.12 \pm 12.01 ^a
RGE	200 mg/kg BW	28.75 \pm 2.84 ^a	16.00 \pm 1.41 ^a	64.22 \pm 4.31 ^a
RGE	400 mg/kg BW	28.75 \pm 4.66 ^a	20.75 \pm 3.14 ^c	73.79 \pm 9.06 ^a

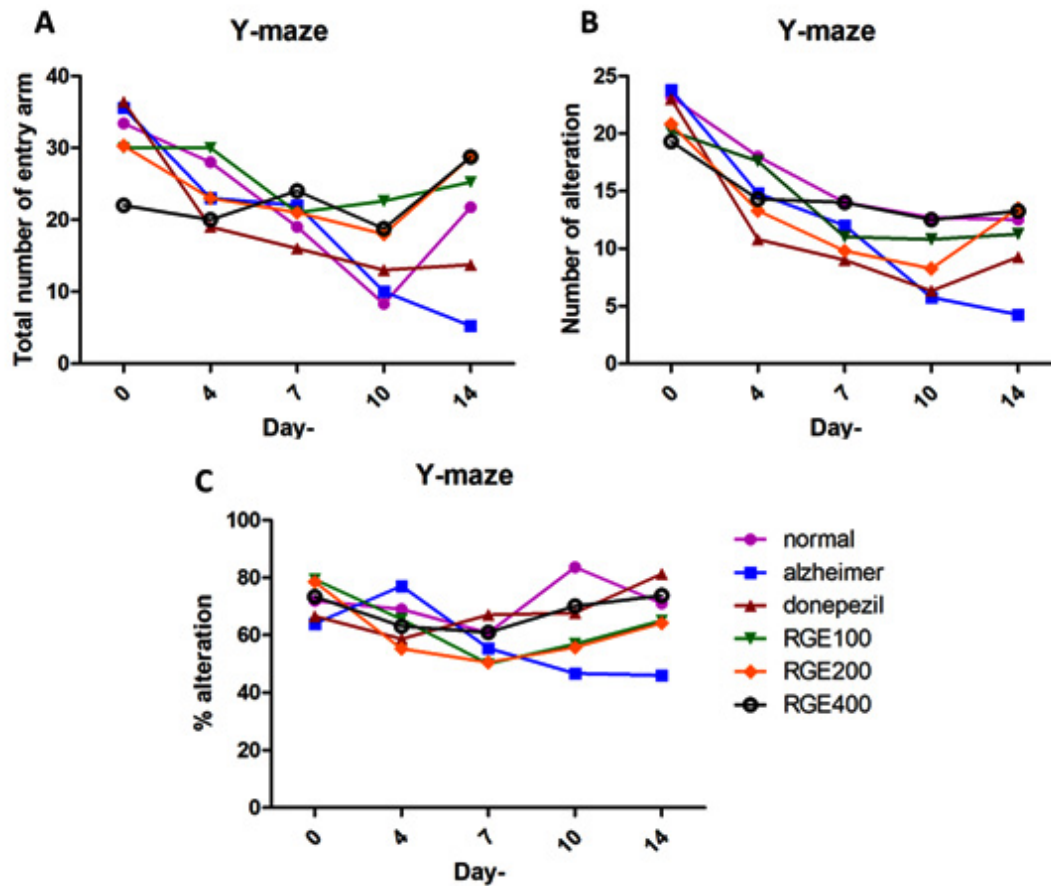


Fig. 2. Spontaneous alteration activity of each group after scopolamine-induced Alzheimer's disease on days 0, 4, 7, 10, and 14. RGE, red ginger extract.

DISCUSSION

Scopolamine disturbs learning and memory functions and further leads to impairing the learning acquisition in both short- and long-term memories.²⁴ Scopolamine also causes brain connectivity alterations, similar to those reported in Alzheimer's disease.²⁵ Furthermore, cholinergic neurons in the central nervous system were known to involve in mediating memories of both humans and animals in the short- and long-term.^{6,8} Many previous studies have found a relationship between the cholinergic system hypothesis and the function of learning, memory, and cognition.

The disturbance of the cholinergic system causes a critical role in the early stage of Alzheimer's disease.²⁶⁻²⁸

The spontaneous alternation activity score in the Y-maze test, that we used in our study, indicates the condition of working (short-term) memory.²⁹ Scopolamine-induced reduction in the spontaneous alternation score increases after RGE treatment. RGE treatment improves the memory of mice with Alzheimer's disease, as shown by the total number of entries per arm, the total number of alterations, and the percentage of alteration in this study.

Table 5. Effects of RGE administration on brain weight and protein levels during scopolamine-induced memory loss in Alzheimer's disease

Group	Treatment	Brain weight (g ± SEM)	Relative brain weight (% ± SEM)	Brain protein (g/dL ± SEM)	Serum protein (g/dL ± SEM)
Normal	CMC-Na 1%	0.36 ± 0.01	1.90 ± 0.13	1.43 ± 0.32 ^a	7.17 ± 0.18 ^a
Alzheimer	CMC-Na 1%	0.41 ± 0.02	1.76 ± 0.07	0.64 ± 0.04 ^b	5.39 ± 0.29 ^b
Donepezil	1 mg/kg BW	0.34 ± 0.11	1.72 ± 0.06	1.22 ± 0.22 ^a	6.94 ± 0.65 ^a
RGE	100 mg/kg BW	0.40 ± 0.02	1.46 ± 0.20	1.09 ± 0.26 ^a	6.16 ± 0.49 ^a
RGE	200 mg/kg BW	0.43 ± 0.01	1.38 ± 0.05	1.60 ± 0.33 ^a	6.31 ± 0.29 ^a
RGE	400 mg/kg BW	0.42 ± 0.01	1.52 ± 0.16	1.85 ± 0.23 ^c	7.22 ± 0.28 ^a

Table 6. Effects of RGE administration on MDA and protein levels during scopolamine-induced memory loss in Alzheimer's disease

Group	Treatment	Serum MDA(μM ± SEM)	Brain MDA(μM ± SEM)
Normal	CMC-Na 1%	17.37 ± 0.59 ^a	11.68 ± 1.58 ^a
Alzheimer	CMC-Na 1%	29.99 ± 2.12 ^b	19.03 ± 1.04 ^b
Donepezil	1 mg/kg BW	6.57 ± 2.04 ^c	4.16 ± 0.86 ^c
RGE	100 mg/kg BW	20.13 ± 1.70 ^a	17.05 ± 1.66 ^b
RGE	200 mg/kg BW	17.37 ± 1.36 ^a	15.37 ± 1.57 ^b
RGE	400 mg/kg BW	9.32 ± 2.36 ^c	4.57 ± 2.40 ^c

Table 7. Effects of RGE administration on AChE levels during scopolamine-induced memory loss in Alzheimer's disease.

Group	Treatment	Serum AChE(ng/mL ± SEM)	Brain AChE(ng/mL ± SEM)
Normal	CMC-Na 1%	21.35 ± 2.08 ^a	17.15 ± 0.57 ^a
Scopolamine	CMC-Na 1%	12.80 ± 1.19 ^b	12.90 ± 0.78 ^b
Donepezil	1 mg/kg BW	19.95 ± 2.65 ^a	18.10 ± 1.69 ^a
RGE	100 mg/kg BW	18.95 ± 1.08 ^a	14.95 ± 1.03 ^c
RGE	200 mg/kg BW	18.00 ± 1.41 ^a	18.35 ± 0.84 ^a
RGE	400 mg/kg BW	19.55 ± 2.63 ^a	19.70 ± 1.63 ^a

We found that RGE treatment inhibits AChE from metabolizing ACh. An increase in ACh would restore the neurotransmitter homeostasis in the brain and improve learning and memory

functions.^{27,30} Our result indicated a relationship between AChE levels in the serum and brain. This is interesting because our *in vitro* results implied that the activity of red ginger oil in inhibiting AChE

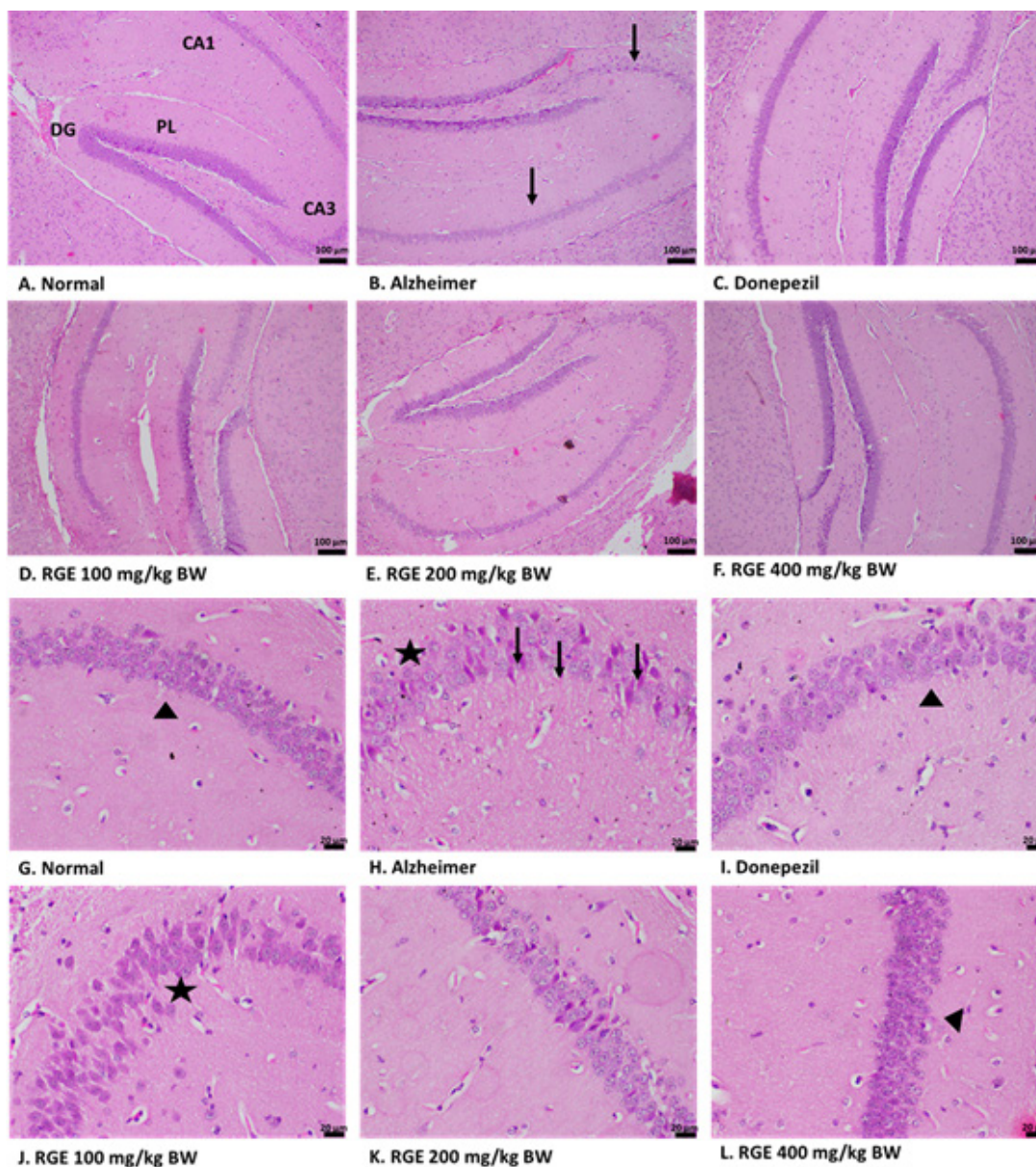


Fig. 3. Histology of the hippocampus of mice with Alzheimer's disease after different treatments: (A,G) normal control, (B,H) Alzheimer, (C,I) drug control (donepezil), (D,J) RGE100, (E,K) RGE200, and (F,L) RGE400 groups. H&E staining, 100 \times magnification (A–F) and 400 \times magnification (G–L). Alzheimer's disease caused neuronal degeneration in the hippocampus (arrow, B). Normal control, drug control, and RGE400 groups showed uniform, regularly arranged neurons (triangle), which was different from the Alzheimer and RGE100 groups (star). In the Alzheimer group (B), almost all neurons were shrunken, with hyperchromatic nuclei (arrow). RGE, red ginger extract; PL, pyramidal layer; DG, dentate gyrus; PL, pyramidal layer; CA, cornu ammonis; RGE, red ginger extract; H&E, hematoxylin, and eosin.

is relatively weak compared to donepezil, while our *in vivo* results showed that the activity of RGE to block AChE is the same as that of donepezil. We think further studies are required because *in vitro* and *in vivo* tests use different approaches with different results.

In addition to increasing the function of the cholinergic system, oxidative stress also plays a major role in neurodegenerative disorders such as Alzheimer's disease.³¹ Oxidative stress, or damage such as protein oxidation, lipid oxidation, DNA oxidation, and glycolysis, actively induces changes in learning and memory in Alzheimer's disease.³² There are two possible conceptual approaches to the treatment of Alzheimer's disease. The first is to prevent the onset of the disease, reduce the secondary pathologies or slow the progression of the disease, leading to the arrest or even repair of neuronal damage after the onset of the disease. The second is to provide symptomatic treatment to treat tertiary cognitive symptoms of the disease and to protect patients from further cognitive decline.³³

Learning, memory, and cognition dysfunction induced by scopolamine administration in animal models is associated with changes in reactive oxygen species production and antioxidant enzyme expression.^{24,34,35} We found increased MDA levels in both the serum and brain of mice with Alzheimer's disease. RGE treatment reduced these MDA levels and increased memory function, which was tested using the Y-maze. Elevated MDA levels have been found in the brain, cerebrospinal fluid, blood, and urine of Alzheimer's disease patients.³⁶ One limitation of this study was that we did not check any other antioxidant enzyme since this is the first report of RGE activity in Alzheimer's disease.

RGE treatment at doses of 400 mg/kg BW had the best effect on memory function improvement through the AChE and MDA pathway. This dose of the ethanolic extract was equal to 16.49–24.74 mg of 6-shogaol. Another neuroprotector study on 6-shogaol also determined a similar result: 15–20 mg/kg BW of 6-shogaol could improve nerve function in mice.^{12,15,37}

CONCLUSION

RGE treatment at a dose of 400 mg/kg BW is effective in reducing memory loss in Alzheimer's disease by increasing the total number of entries

per arm, the total number of alterations, and the percentage of alteration. RGE, which contains 6-shogaol inhibits AChE activity, increases protein levels, and reduces MDA levels in both serum and brain. RGE also improves the morphology of the hippocampus of Alzheimer's mice.

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Conflict of Interest

The authors declare no conflict of interest.

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