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The Effect of an 8-Week Germinated Black Rice Extract Consumption on Cognitive Functions in Healthy Middle-Aged and Elderly Volunteers: A Randomized Controlled Trial

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Abstract

The population of older people has been increasing rapidly worldwide. It was found that the neurons in the brains of the elderly were deteriorating, which has resulted in decreased learning and memory. The prevention of these conditions has therefore received more attention. The objective of this study was to evaluate the effects of germinated black rice extract (GBR) on learning and memory in middle-aged and elderly subjects. Twenty-four subjects aged 45 to 70 were randomized into 2 groups, a control and an experimental group who received placebo versus GBR (1,000 mg/day) for eight weeks. A computerized battery test was performed at weeks 4 and 8. One of the neurotransmitter biomarkers for learning and memory assessment is the inhibition of acetylcholinesterase (AChE) and monoamine oxidase (MAO) in plasma. The results showed that 4 weeks after GBR consumption, the working memory was improved by decreasing the response time, attentional control, and memory speed. Moreover, at week 8, there was a significant improvement in response time (speed of memory) and quality of memory compared to the placebo group. At the end of the study period, the findings revealed that AChE and MAO levels were statistically significantly lower in the intervention group than in the control group. Moreover, no alteration in biochemical parameters of the liver and renal function was observed, which could confirm the safety of this formula. Therefore, GBR could promote learning and memory. The possible mechanism might have occurred primarily via the suppression of AChE and MAO levels.

Keywords: Black rice, *Oryza sativa* L., Acetylcholinesterase, Monoamine oxidase, Working memory

Introduction

Recently, the population of older people has been increasing rapidly worldwide. The number of elderly persons in Thailand has been growing rapidly and consistently. According to reports, 16.73 % of Thailand's population comprises senior citizens. Moreover, the percentage of senior citizens in the Northeast is 16.04, which is higher than the national average [1]. The correlation between this aging trend and the prevalence of memory impairment has been demonstrated [2]. Cognitive capability declines with increasing age. Since cognitive decline is costly in financial, personal, and societal terms, it is recognized as a significant health and social problem. The prevention of these ailments has received significantly more emphasis. The accumulation of oxidative damage to lipids, proteins, and nucleic acids has been linked to cognitive impairment in older adults, according to recent research [3,4], in addition to the susceptibility of various neurotransmitters to oxidative damage [5].

Furthermore, it has been linked to the hypofunction of cholinergic and monoaminergic systems, as evidenced by the lowered levels of acetylcholinesterase (AChE) and monoamine (MAO), the critical markers of cholinergic and monoaminergic functioning in various brain regions [6]. Since effective symptomatic therapies or preventive measures to complement the current approach have not yet been developed, creating new strategies and alternative treatment to prevent cognitive deterioration with age is still necessary. The previously mentioned function of the cholinergic system has provided the rationale for preventing and treating age-related cognitive decline using substances that enhance cholinergic function and promote working memory.

Traditional medical practices have used numerous plants to improve cognitive function in healthy individuals and those with disorders such as mild cognitive impairment (MCI) and Alzheimer's disease

(AD). *Oryza sativa*, L. indica or black rice, has been widely consumed in regions of Asia, including Thailand, since antiquity. The black rice is particularly processed according to the production process of the wisdom of Northeastern Thai people called Khao Hang (germinated rice from black rice). The paddy of black rice is soaked in water to enhance rice germination. This method enriches rice with nutrients, vitamins, minerals, and fiber and enhances the memory-enhancing GABA concentration [7,8]. Previous studies have shown its antioxidant, anti-inflammatory, neuroprotective, and memory-enhancing properties [9-12]. Based on this information, we hypothesized that an 8-week consumption of germinated black rice extract products would increase working memory and decrease acetylcholinesterase (AChE) and monoamine oxidase (MAO) in middle-aged and elderly healthy volunteers. Due to the lack of data on this topic, this study is designed to investigate the hypothesis.

Materials and methods

Plant material preparation and extraction

Oryza sativa, L. indica (black rice) was obtained from Amphoe Waritchaphum, Sakon Nakhon, Thailand, harvested in September-October. For the preparation, germinated rice from black rice was soaked in water at 27 ± 5 °C, then incubated for 48 h at room temperature (RT). Then, the water extract of germinated black rice was prepared using maceration technique for 24 h at room temperature. After being collected, the extracts were centrifuged at 3000 rpm for 10 min and filtered using Whatman no. 1 filter paper. The filtrates were dried in the oven (Memmert GmbH, USA) at 60 °C for 72 h and stored at −20 °C until use. Germinated black rice extract (GBR) containing 500 mg per capsule using a semi-automatic capsule filling machine (DTJ-CA, BB tech & Service LTD., Part.) with capsules of size 0. The quality of the GBR extract capsule was controlled by weight variation according to USP criteria [13].

Study design and population

This study was designed as a randomized, double-blind, placebo-controlled examination in order to collect data on the potential effects of consuming a product containing germinated black rice extract for eight weeks on working memory in 24 healthy middle-aged and elderly Thai participants. In addition, the possible underlying mechanisms were examined. The study was performed according to the Declaration of Helsinki (ethical principles for research involving human subjects), and all procedures were approved by the Human Research Ethics Committee, Rajamangala University of Technology Isan, under HEC-04-65-006. Moreover, this project was submitted to the Thai Clinical Trial Registry online (TCTR20230206003).

Sample size

The sample size calculation was performed using 80 % power and 95 % confidence level. This calculation was based on a similar study carried out on healthy volunteers [14]. The value of N was 10 per group. Additionally, we approximated the number of withdrawal cases to be around 20 %, resulting in 12 instances in each group.

Participants and interventions

A total of 24 healthy subjects aged between 45 and 70 years were recruited and screened for eligibility. A physician also screened participants for physical health to verify that they were in good health. Individuals receiving any medications or herbs that could have affected brain function, as well as individuals who had a prior diagnosis or history of stroke, heart disease, diabetes, gastrointestinal disease, cancer, central nervous system or psychiatric disorders, or traumatic brain injury, were excluded from the study. In addition, heavy smokers and alcoholics were eliminated from the study. Before participating in the trial, each subject was provided a written informed consent. Subjects were randomly divided into 2 groups: Placebo and intervention group, which received 500 mg 2 capsules daily of germinated black rice extract for 8 weeks.

Primary and secondary outcomes were assessed at the start of the intervention and eight weeks later. The primary outcome of this study was working memory, as measured by a computerized battery test consisting of word presentation, word recognition, picture presentation, picture recognition, simple reaction time, digit vigilance, choice reaction time, and spatial working memory. Acetylcholinesterase (AChE) and monoamine oxidase (MAO) were secondary outcomes. Clinical safety was evaluated from the screening phase until the end of the trial. **Figure 1** presents a schematic illustration of the subject intervention procedure.

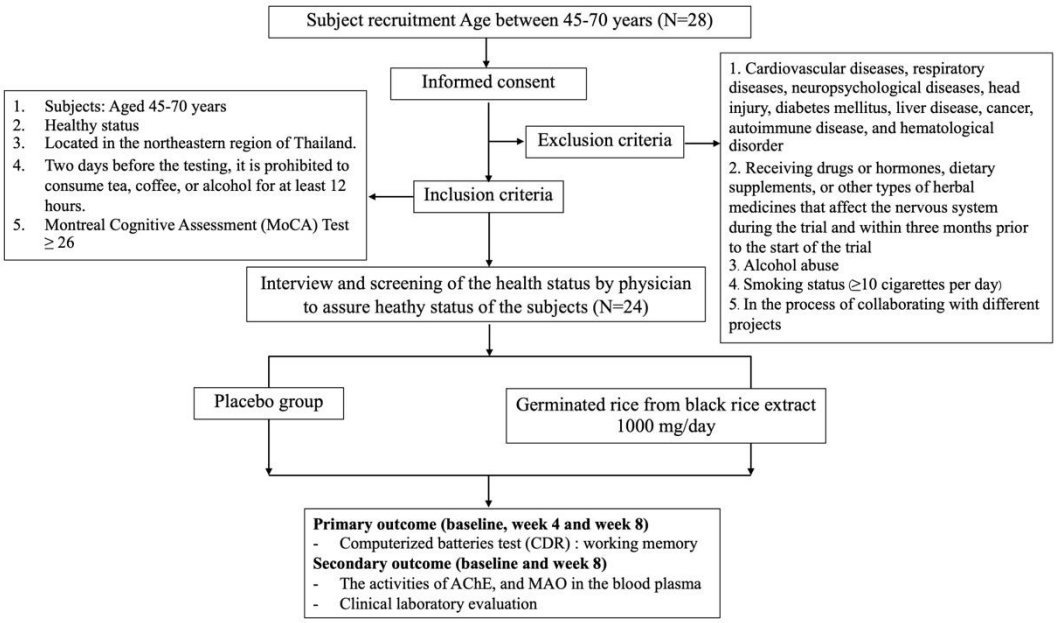

Figure 1 Schematic illustration of the subject intervention procedure.

Computerized battery test

This study's computerized assessment battery test was modified from the CDR computerized battery assessment performed in hundreds of European and North American preclinical studies. The test is sensitive to acute cognitive impairments as well as impairments affected by a wide range of substances. This study assessed 4 domains of working memory, including attention, continuity of attention, quality of memory, and speed of memory [15]. The responses to each task were recorded using a button with the words "yes" and "no" on a notebook computer with a high-resolution VGA color monitor. The selection procedure took around 30 min. The tests were administered in the following order:

Word presentation

The monitor sequentially displayed 15 words that were matched for frequency and definiteness for the subject to remember. Both the stimulus duration and inter-stimulus interval were 1 s.

Picture presentation

Twenty photographic images were displayed on the monitor at a speed of 1 every 3 s, with a stimulus duration of 1 s, for the subject to memorize.

Simple reaction time

The participants were required to rapidly press the 'yes' response button whenever the word 'yes' was displayed on the monitor. The inter-stimulus interval between 50 stimuli varied randomly between 1 and 3.5 s. Times of reaction were measured in milliseconds.

Digit vigilance task

A target digit was selected at random and displayed continuously to the right of the monitor screen. The participant was instructed to press the 'yes' button as rapidly as possible even when a digit in the series matched the target digit. The activity lasted 1 min and consisted of fifteen stimulus-target matching. The accuracy (percent), reaction time (milliseconds), and number of false alarms were the task measurements.

Choice reaction time

On the monitor, either the word 'no' or 'yes' was displayed, and the participant was instructed to press the corresponding button as rapidly as possible. There were 50 trials in which the stimulus word was selected randomly with equal probability using an inter-stimulus interval that varied randomly between 1 and 3.5 s. Reaction times (milliseconds) and accuracy (percent) were recorded.

Spatial working memory

Four of the nine windows of a visual representation of a house displayed on the screen were illuminated. Participants were instructed to memorize the locations of the lit windows. In each of the 36 consecutive presentations of the house, one of the windows was illuminated, and participants determined whether it matched one of the illuminated windows in the initial presentation. The participants responded as rapidly as possible by pressing the 'yes' or 'no' response button. Mean reaction times were assessed in milliseconds, and the accuracy of responses to original and novel (distracter) stimuli was recorded as percentages and used to calculate a percentage over chance performance score.

Numeric working memory

The individual was required to memorize 5 digits presented in serial order. The participant then responded as rapidly as possible to a series of 30 probe digits by pressing the 'yes' or 'no' button, depending on whether or not the digit appeared in the original sequence. This was conducted twice more with different stimuli and probe digits. Mean reaction times were measured in milliseconds, and the accuracy of responses to both original and novel (distracter) stimuli were recorded as percentages and used to calculate a percentage over chance performance score.

Clinical safety assessment

From the screening phase to the end of the trial, the clinical safety was evaluated via clinical examination, assessment of vital signs, body temperature, clinical laboratory parameters including biochemistry and hematology, subjective symptomatology, and observation of adverse events.

Biochemical assays

Assessment of acetylcholinesterase (AChE) activity

Acetylcholinesterase (AChE) has been recognized as a marker of cholinergic activity in the central nervous system [16]. The AChE activity in plasma was measured using a commercially available kit (Acetylcholinesterase Assay Kit, ab138873, Abcam) according to the manufacturer's instructions. Venous blood was drawn from each subject and centrifuged at 1300 g for 20 min at room temperature. In a 96-well plate, 50 µL of sample was mixed with 50 µL of assay buffer and incubated for 20 min at room temperature. The reaction was stopped with stop buffer. Following this step, an absorbance at 575 nm was recorded with a microplate reader (Infinite® 200 pro, Tecan, Männedorf, Switzerland). All data values were expressed as mU/mL.

Assessment of monoamine oxidase activity

Monoamine oxidase enzyme catalyze the oxidation of monoamines. There are 2 forms of MAO in humans: MAO-A and MAO-B and both are present in neurons and astrocytes. Due to the crucial role that MAOs play in the deactivation of neurotransmitters, MAO dysfunction (excess or deficient MAO activity) is believed to be the cause of a number of neurological diseases [17]. Therefore, in this study total monoamine oxidase activity in plasma was measured using a commercially available kit (Monoamine Oxidase Assay Kit, ab241031, Abcam) according to the instructions provided by the manufacturer. Based on the fluorometric detection of H_2O_2, which is one of the effects of the oxidation of the MAO substrate (Tyramine), the absorbance fluorescence was measured at wavelength (Ex/Em = 535/587 nm) (The SpectraMax® L Luminescence Microplate Reader, USA). All data values also express as µU/mg of protein.

Statistical analysis

The data were analyzed as mean ± standard deviation (SD), and an independent t-test was used to compare the differences between the primary and secondary outcomes in the experimental group and the control group. Statistical significance was regarded at p-value < 0.05.

Results and discussion

Participant characteristics

Twenty-four healthy participants were randomly assigned to either the placebo or experimental (received 1000 mg/day germinated black rice extract (GBR)) groups. As demonstrated in **Figure 1**, no one dropped out before the study completion. The results of the 8-week evaluation of vital signs in the GBR 1000 mg/day and placebo groups revealed no statistically significant difference between the 2 groups

(*p*-value > 0.05) as presented in **Table 1**. Moreover, there were no statistically significant differences between the GBR and placebo groups in terms of body weight and body mass index (*p*-value > 0.05).

Table 1 The characterization of subjects. Data were compared at baseline, 4-week, and 8-week study periods.

Cognitive domains	Test items		Placebo	GBR[1] 1,000 mg/day	*p*-value
Power of attention	Simple reaction (msec)	Baseline	906.63 ± 13.62	905.73 ± 18.66	0.47
		4-week	2065.05 ± 241.34	2025.16 ± 227.37	0.96
		8-week	827.59 ± 44.67	824.90 ± 40.21	0.97
	Digit vigilance (msec)	Baseline	696.09 ± 6.72	680.32 ± 14.56	0.58
		4-week	696.8 ± 21.80	609.06 ± 35.07*	0.04
		8-week	674.05 ± 17.20	653.46 ± 12.07	0.35
	Choice reaction (msec)	Baseline	1004.45 ± 7.60	1093.37 ± 11.91	0.79
		4-week	1049.20 ± 52.18	1060.43 ± 41.28	0.87
		8-week	1017.75 ± 43.51	1026.07 ± 25.11	0.88
Continuity of attention	Accuracy of Digit vigilance (%Accuracy)	Baseline	90.34 ± 0.39	81.765 ± 1.54	0.48
		4-week	65.78 ± 10.35	75.00 ± 12.74	0.58
		8-week	63.91 ± 5.31	84.47 ± 9.87	0.06
	Accuracy of Choice reaction (%Accuracy)	Baseline	98.15 ± 0.17	99.08 ± 0.09	0.66
		4-week	98.00 ± 0.76	99.33 ± 0.38	0.16
		8-week	97.73 ± 0.83	99.17 ± 0.39	0.13
Speed of memory	Word recognition (msec)	Baseline	2798.37 ± 139.57	2800.18 ± 129.12	0.48
		4-week	2065.05 ± 241.34	2025.16 ± 227.37	0.91
		8-week	1730.80 ± 106.58	1704.90 ± 112.94	0.87
	Picture recognition (msec)	Baseline	1896.37 ± 56.77	1889.86 ± 47.82	0.66
		4-week	1500.6 ± 124.62	1581.43 ± 239.57	0.75
		8-week	1630.23 ± 109.66	1383.50 ± 81.03	0.13
	Spatial memory (msec)	Baseline	2086.41 ± 42.05	2140.01 ± 61.05	0.81
		4-week	2044.89 ± 246.11	1385.75 ± 90.53*	0.03
		8-week	1953.24 ± 146.88	1484.09 ± 136.32*	0.03
	Numeric working memory (msec)	Baseline	1498.00 ± 81.24	1459.75 ± 95.72	0.81
		4-week	1328.76 ± 71.83	1393.79 ± 115.75	0.62
		8-week	1314.83 ± 72.09	1303.38 ± 38.39	0.89
Quality of memory	Accuracy of word recognition (%Accuracy)	Baseline	68.95 ± 0.74	62.05 ± 1.11	0.92
		4-week	77.76 ± 2.96	75.26 ± 5.12	0.66
		8-week	82.33 ± 2.57	89.72 ± 2.11*	0.04
	Accuracy of picture recognition (%Accuracy)	Baseline	77.74 ± 0.78	76.25 ± 0.67	0.74
		4-week	79.67 ± 4.10	75.83 ± 4.68	0.54
		8-week	77.33 ± 4.97	90.00 ± 2.38*	0.04
	Accuracy of spatial memory (%Accuracy)	Baseline	78.84 ± 0.82	76.95 ± 1.32	0.63
		4-week	72.44 ± 4.89	84.72 ± 1.75*	0.03
		8-week	81.21 ± 3.06	90.93 ± 3.15*	0.04
	Numeric working memory (%Accuracy)	Baseline	85.13 ± 1.27	82.31 ± 1.53	0.89
		4-week	84.44 ± 3.80	85.00 ± 4.83	0.93
		8-week	87.51 ± 2.96	87.50 ± 4.61	1.00

Note: Values are presented as mean ± SEM (n = 12/group). [1]Capsule containing of germinated black rice extract; [2]Blood pressure.

Comparison of intervention and placebo groups during the 8-week trial effect on working memory

In this study, the researchers measured working memory using the CDR battery test, a computer-aided measurement of working memory. These include word recognition, picture recognition, spatial working memory, numeric working memory, and digit vigilance. The findings of the eight-week variables for the GBR and placebo groups are presented in **Table 2**. The accuracy (%Accuracy) and response time (msec) from the CDR battery test at baseline did not significantly change statistically between the placebo and GBR groups. After a 4-week consumption period of GBR at a dose of 1000 mg daily, a significant reduction of response time in digit vigilance and spatial memory (*p*-value < 0.05 and *p*-value < 0.05,

respectively) was observed. A significant increase in %Accuracy in spatial memory (p-value < 0.05) was demonstrated. At the end of the study period (8 weeks), the group receiving capsules containing germinated black rice extract (GBR) demonstrated a significant reduction in the response time (msec) of the spatial memory test (p-value 0.05; compared to the placebo group). There was a significant increase in the %Accuracy in word recognition, picture recognition and spatial memory test compared with the placebo group (p-value < 0.05). Other variables were not significantly different between the 2 groups. (p-value > 0.05).

Table 2 Effect of germinated black rice extract at dose of 1,000 mg/day on response time and percent of accuracy response of each cognitive assessment test.

Characteristics	Baseline		*p*-value	4-week		*p*-value	8-week		*p*-value
	Placebo	GBR[1] 1,000 mg/day		Placebo	GBR[1] 1,000 mg/day		Placebo	GBR[1] 1,000 mg/day	
Age (year)	60.00 ± 0.37	61.40 ± 0.25	0.42	60.00 ± 0.37	61.40 ± 0.25	0.42	60.00 ± 0.37	61.40 ± 0.25	0.42
Heart rate (beats/min)	72.50 ± 0.45	70.29 ± 0.49	0.28	72.31 ± 0.60	75.54 ± 0.86	0.42	75.77 ± 0.86	76.08 ± 0.96	0.49
Respiratory rate (breaths/min)	19.85 ± 0.15	20.00 ± 0.00	0.43	20.00 ± 0.00	19.80 ± 0.20	0.51	20.00 ± 0.00	20.00 ± 0.00	0.25
Systolic BP (mmHg)	125.60 ± 0.83	128.08 ± 0.94	0.59	121.54 ± 0.87	129.38 ± 1.43	0.21	122.53 ± 0.86	123.92 ± 1.03	0.78
Diastolic BP[2] (mmHg)	75.87 ± 0.71	78.00 ± 0.65	0.59	78.23 ± 0.90	82.77 ± 0.86	0.32	74.31 ± 0.75	72.15 ± 0.54	0.52
Body weight (kg)	63.92 ± 1.27	65.16 ± 1.60	0.71	65.17 ± 0.71	65.76 ± 0.76	0.93	64.02 ± 0.74	64.45 ± 0.68	0.82
Body height (cm)	160.83 ± 1.12	159.00 ± 0.79	0.57	160.83 ± 1.12	159.00 ± 0.79	0.57	160.83 ± 1.12	159.00 ± 0.79	0.57
Body mass index (BMI)	24.64 ± 0.18	24.93 ± 0.14	0.51	24.61 ± 0.13	24.51 ± 0.09	0.75	24.94 ± 0.37	25.26 ± 0.20	0.83

Note: Values are presented as mean ± SEM (n = 12/group). Comparison of data was performed between at baseline 4-week, and 8-week study periods. [1]Capsule containing of germinated black rice extract. $^{*}p$-value < 0.05, $^{**}p$-value < 0.01, $^{***}p$-value < 0.001 respectively compared to the placebo group.

Effect of product containing germinated black rice extract (GBR) on biochemical parameters

Figures 2 and **3** illustrate the effect of GBR on AChE and MAO activities. In this study, the effect of GBR on AChE and MAO were assessed to indirectly indicate the effect of the available acetylcholine (ACh) and monoamine neurotransmitters on cholinergic and monoaminergic function, respectively. Compared to the placebo group, GBR treatment at a dose of 1000 mg per day for 8 weeks significantly decreased AChE and MAO in the blood plasma (p-value < 0.001).

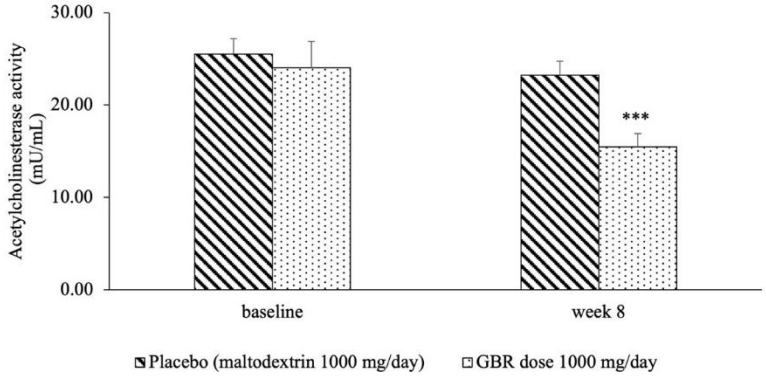

Figure 2 The effect of germinated black rice extract at dose of 1,000 mg/day on acetylcholinesterase activity in the blood plasma. Data are presented as mean ± SEM (n = 12/group). $^{***}p$-value < 0.001; compared to placebo group. GBR, Capsule containing of germinated black rice extract at dose of 1,000 mg/day.

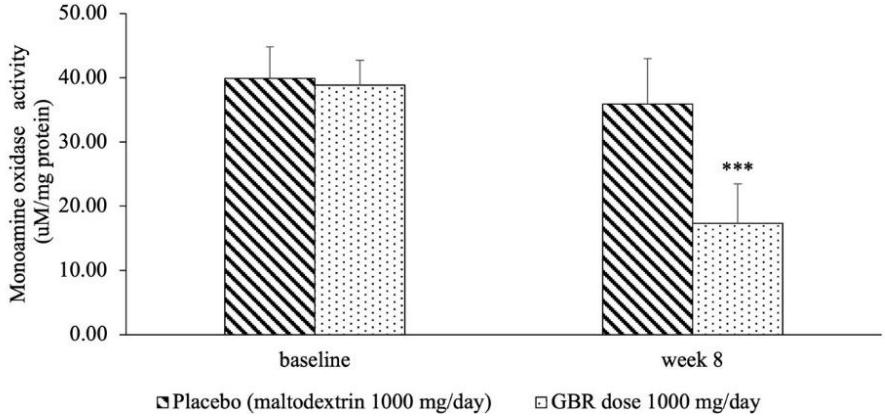

Figure 3 The effect of germinated black rice extract at dose of 1,000 mg/day on monoamine oxidase activity in the blood plasma. Data are presented as mean ± SEM (n = 12/group). $^{***}p$-value < 0.001; compared to placebo group. GBR, Capsule containing of germinated black rice extract at dose of 1,000 mg/day.

Clinical laboratory evaluation

All of the tests, including a complete blood count (CBC) test; kidney function tests consisting of the blood urea nitrogen (BUN) test and the creatinine (Cr) test; and liver function tests consisting of the Alanine aminotransferase (ALT) test and the Aspartate aminotransferase (AST) test, were performed at baseline, and week 8 of the treatment in order to determine if the administered treatments caused side effects. All patients in both groups exhibited no clinically significant abnormalities or alterations, as shown in **Tables 3 - 5**, and there were no statistically significant differences between the 2 groups (p-value > 0.05).

Table 3 Changes in the complete blood count (CBC) test of germinated black rice extract at dose of 1,000 mg/day.

Parameter	References	Baseline		*p*-value	8-week		*p*-value
		Placebo	GBR[1] 1,000 mg/day		Placebo	GBR[1] 1,000 mg/day	
Hb[2]	13.0 - 16.7 g/dL	13.18 ± 0.15	12.97 ± 0.25	0.39	12.59 ± 0.09	12.37 ± 0.13	0.67
HCT[3]	40.5 - 50.8 %	41.00 ± 0.16	40.84 ± 0.22	0.65	40.32 ± 0.23	39.69 ± 0.34	0.60
WBC[4]	4.6 - 10.6 $10^3/\mu L$	6.87 ± 1.29	7.63 ± 1.22	0.23	6.62 ± 0.21	6.62 ± 1.67	0.93
Platelets	173 - 383 $10^3/\mu L$	260.73 ± 3.22	230.07 ± 12.46	0.12	268.07 ± 13.96	236.62 ± 3.60	0.21
RBC[5]	4.7 - 6.2 $10^6/\mu L$	5.88 ± 0.05	5.71 ± 0.06	0.54	5.06 ± 0.05	4.86 ± 0.03	0.39
MCV[6]	80.0 - 97.0 fL	80.92 ± 0.62	80.27 ± 0.47	0.83	80.64 ± 0.69	81.58 ± 0.41	0.73
MCH[7]	25.2 - 32.0 pg	25.75 ± 0.21	25.47 ± 0.19	0.81	25.16 ± 0.23	25.40 ± 0.17	0.77
MCHC[8]	31.0 - 36.0 g/dL	31.86 ± 0.08	31.66 ± 0.09	0.66	31.16 ± 0.06	31.09 ± 0.09	0.96
RDW[9]	11.5 - 14.5 %	12.29 ± 0.08	12.25 ± 0.09	0.92	12.29 ± 0.09	12.02 ± 0.07	0.39
Neutrophils	40.0 - 75.0 %	56.00 ± 0.38	55.33 ± 0.31	0.73	52.29 ± 0.55	45.62 ± 1.04	0.16
Lymphocytes	20.1 - 44.5 %	35.40 ± 0.27	36.40 ± 0.27	0.50	37.79 ± 0.51	40.69 ± 0.50	0.35
Monocytes	2.0 - 10.0 %	5.60 ± 0.11	5.20 ± 0.06	0.42	6.21 ± 0.12	5.00 ± 0.09	0.07
Eosinophils	1.0 - 6.0 %	3.29 ± 0.20	3.20 ± 0.19	0.94	3.57 ± 0.19	2.85 ± 0.12	0.29

Note: Values are presented as mean ± SEM (n = 12/group). [1]Capsule containing of germinated black Hang rice extract, [2]Hemoglobin, [3]Hematocrit, [4]White blood count, [5]Red blood count, [6]Mean corpuscular volume, [7]Mean corpuscular hemoglobin, [8]Mean corpuscular hemoglobin concentration, [9]Red blood cell distribution width.

Table 4 Liver function of the volunteers receiving germinated black rice extract at dose of 1,000 mg/day. Values are presented as mean ± SEM.

Parameter	References	Baseline		*p*-value	8-week		*p*-value
		Placebo	GBR[1] 1,000 mg/day		Placebo	GBR[1] 1,000 mg/day	
ALT[2]	0-40 U/L	31.93 ± 3.78	23.93 ± 2.05	0.07	27.07 ± 3.83	23.46 ± 2.64	0.45
AST[3]	0-40 U/L	30.67 ± 3.40	27.53 ± 2.67	0.48	27.21 ± 2.67	26.31 ± 1.39	0.77

Note: Values are presented as mean ± SEM (n = 12/group). [1]Capsule containing of germinated black rice extract, [2]Alanine aminotransferase, [3]Aspartate aminotransferase.

Table 5 Renal Function of the volunteers receiving germinated black rice extract at dose of 1,000 mg/day.

Parameter	References	Baseline		*p*-value	8-week		*p*-value
		Placebo	GBR[1] 1,000 mg/day		Placebo	GBR[1] 1,000 mg/day	
BUN	8 - 24 mg/dL	11.51 ± 0.72	10.82 ± 0.41	0.62	12.85 ± 0.94	11.77 ± 1.05	0.45
CREATININE	0.5 - 1.2 mEq/L	0.72 ± 0.03	0.74 ± 0.03	0.52	0.68 ± 0.27	0.70 ± 0.31	0.56

Note: Values are presented as mean ± SEM (n = 12/group). [1]Capsule containing of germinated black rice extract, [2]Blood urea nitrogen.

Discussion

Previous studies have indicated that consuming herbal medicine and functional foods for 6 - 8 weeks can improve working memory and attention in healthy volunteers [15,18]. Therefore, we designed this study to collect data on the potential effect of germinated black rice extract (GBR) 8 weeks of consumption on working memory in middle-aged and elderly healthy volunteers. The evaluation of the power of attention, which includes the ability to pay attention and comprehend information, has gained much importance [19]. Utilized in simple reaction time, choice reaction time, and digit vigilance, the rate at which the subjects performed the test indicated how attentive they were to the stimulus. A faster reaction time suggests greater awareness of the stimulus. Therefore, the results of this study indicate that a daily dose of 1,000 mg of germinated black rice extract (GBR) enhances the effectiveness of attention. It was statistically significant instead of the placebo group for 4 consecutive weeks. The continuity of attention did not change during the simple reaction time, choice reaction time, or digit vigilance assessments.

A computerized battery test (CDR test) can also examine memory in addition to attention. At weeks 4 and 8, participants who received 1,000 mg of GBR daily shown statistically significant improvements in memorizing speed and spatial working memory accuracy. These changes suggest an improvement in memory quality and speed compared to the placebo group. According to the studies mentioned, consuming GBR can improve working memory functions, including attention, memory quality, and memory speed.

According to research published by Thukham-Mee *et al.* [6], working memory has a negative correlation with acetylcholinesterase (AChE) and monoamine oxidase (MAO) enzymes. In addition, previous studies have found that MAO and MAO-A are associated with working memory [20,21]. MAO inhibition has been reported to prevent memory impairment [22]. Therefore, the researcher evaluated the effect of consuming 1,000 mg of GBR per day on these parameters after 8 weeks of consumption. The results revealed that the plasma levels of acetylcholinesterase (AChE) and monoamine oxidase (MAO) were significantly decreased. Modulating neurotransmitter levels can profoundly influence brain function and behavior. Acetylcholine and monoamines, pivotal in learning and memory processes, offer promising avenues for enhancing memory performance. Recent research suggests that inhibiting AChE and MAO could potentially enhance memory by increasing the availability of these neurotransmitters. The recent findings support the outcomes of a computerized battery assessment, revealing that the GBR extract

can improve working memory capacities. However, we still need to further study the exact mechanism of action in GBR that enhances learning and memory.

Regarding the safety of consuming black germinated rice extract products, the baseline clinical chemistry values were renal function tests (blood urea nitrogen and creatinine), liver function tests (ALT and AST), and hematological data. There were no abnormalities or statistically significant differences between the placebo group and the black germinated rice extract group. Earlier evidence indicates that germinated black rice extract extracts are safe and non-toxic to volunteers.

Conclusions

This study demonstrates that the administration of germinated black rice extract at a daily dosage of 1,000 mg appears safe throughout the 8 weeks of the study. Furthermore, it shows promise for enhancing cognitive functions in middle-aged and older people. Our recent research suggests that consuming germinated black rice extract may further improve attention and memory, thereby leading to a better quality of life. The possible underlying mechanisms can also exert a positive modulation effect via the suppression effect on acetylcholinesterase (AChE) and monoamine oxidase (MAO). The current data show that consumption of germinated black rice extract has the potential to benefit healthy adults, suggesting a promising avenue for mitigating age-related cognitive decline. However, additional research is still required. Further exploration will investigate the effectiveness of GBR extract as a therapy and preventative approach for various diseases.

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