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Sesaminol Glucosides Improve Cognitive Deficits and Oxidative Stress in SAMP8 Mice

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Abstract The objective of this study was to investigate the effects of sesaminol glucosides (SG) on age-related cognitive deficits in senescence-accelerated mice P8 (SAMP8). Male SAMP8 (9 month-old) were randomly divided into 3 groups and received diets containing 0, 0.25, or 0.5% SG for 12 weeks. Step-through latency of the SAMP8 control group was higher than that of the senescence-accelerated resistant mice (SAMR) group, whereas it was lowered in the SG-supplemented group on the passive-avoidance test. In the Morris water maze, the escape latency of the SAMP8 control group was increased and recovered in the 0.5% SG-supplemented group. The SG supplementation significantly decreased thiobarbituric acid reactive substance (TBARS) levels in brains of the SAMP8. On the other hand, catalase, superoxide dismutase, and glutathione peroxidase activities in brains of the SG supplemented group decreased compared with the SAMP8 control group. These results suggest that SG could attenuate cognitive deficits caused by aging through its antioxidant capacity.

Keywords: senescence-accelerated mice P8, aging, cognitive deficit, oxidative stress, sesaminol glucoside

Introduction

The senescence-accelerated mouse (SAM), a murine model of accelerated aging, was established by Takeda *et al.* (1). One of the many mouse sub-strains that are used to study Alzheimer's disease (AD) and aging is the SAMP8 (2). The SAMP8 shows age-related deterioration in memory and learning, expression of amyloid precursor protein (APP), AD energy metabolism, abnormal circadian rhythms, and emotional alterations (3-5). Also, several studies have reported that the increase of lipid peroxidation in SAMP8 (6,7). Thus, SAMP8 is a good rodent model for aging-related cognitive impairment and oxidative stress and is used as model for studying β-amyloid (Aβ) mediated effects in cognitive decline (8).

The oxidative stress theory of aging postulates that the oxidative effects of free radicals on biomolecules, such as lipids, proteins, and DNA, are responsible for the functional deterioration associated with aging (9,10). In fact, excessive production of free radicals and imbalances between free radicals and antioxidant defense systems are thought to be significantly related to the process of aging (11). However, human have several effective antioxidant defense systems against oxidative stress. Non-enzymatic (e.g., vitamin C, vitamin E, and glutathione) and enzymatic antioxidant defense mechanisms [e.g., catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx)] work through removing free radicals and/or suppressing chain reaction. Dietary antioxidants are considered beneficial because of their potential protective effects against the pathogenesis of multiple diseases associated with oxidative stress (12).

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Sesame seeds (*Sesamum indicum* Linn) are known as a beneficial food to health in Asian countries (13). Sesame seeds contain large quantities of lignan glucosides, such as sesaminol glucosides (SG), pinoresinol glucosides, and sesamolinol glucosides (14). SG, which is hydrophilic antioxidant, has been reported to decrease susceptibility to oxidative stress in hypercholesterolemic rabbits (15). Kang *et al.* (16) reported that the inhibitory effect of sesaminol on Cu^{2+}-induced low density lipoprotein (LDL)-oxidation. In addition, Lee *et al.* (17) reported that SG has protective effects against Aβ-induced apoptotic cell death in cultured PC12 cells through an antioxidant mechanism. Considering this facts, it is hypothesized that SG exerts a protective effect in aging-related cognitive deficits.

Therefore, this study using SAMP8 investigated focusing on whether SG supplementation could improve cognitive deficits and oxidative stress because aging is the major risk factor for AD and human brain changes through the overall life cycle.

Materials and Methods

Sample preparation Sesame seeds were purchased from Kyungdong Oriental market (Seoul, Korea). Sesaminol glucosides (SG) isolated from sesame seeds were prepared by the method described by Katsuzaki *et al.* (14). Defatted sesame flour was extracted with distilled water (1:10, w/v) for 1 hr at 95°C and filtered. The crude SG extract was loaded onto an open column (2.5×100 cm) packed with Diaion HP 20 (Mitsubishi Chemical, Tokyo, Japan). The column was eluted with 60% ethanol and concentrated under reduced pressure. The residues were freeze-dried and stored at −80°C until use.

The fraction was analyzed for SG content using an analytical high performance liquid chromatography (HPLC) fitted with a UV detector. The condition of HPLC was:

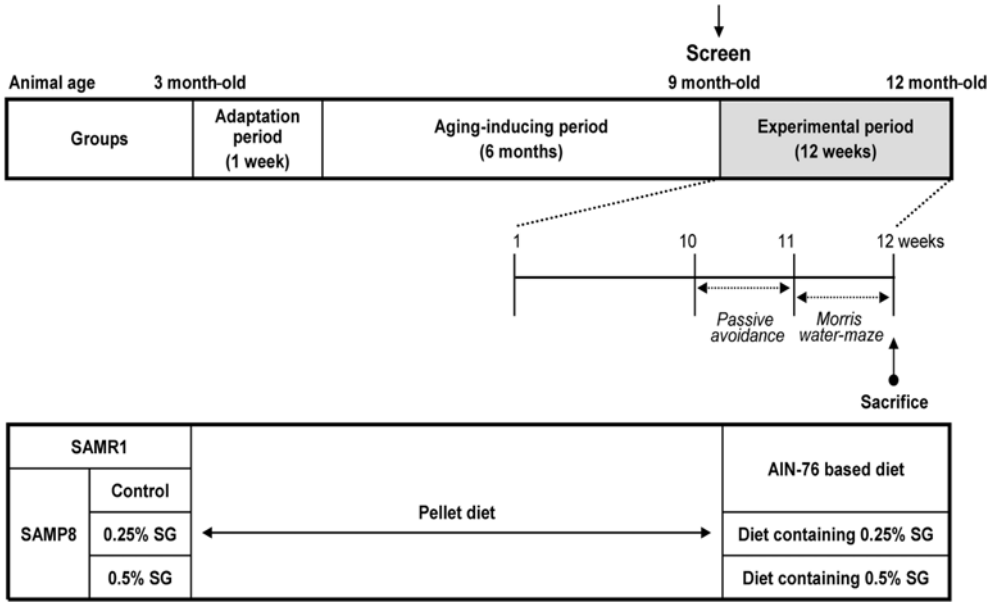

Fig. 1. Schematic of the experimental schedule.

column, Hypersil-100 C18 (4.6×250 mm, 5 μm particle size): mobile phase, methanol/water=10:90 and linear gradient to methanol/water=90:10 (v/v) in 65 min: flow rate, 0.8 mL/min: wavelength for detection (UV-2075; Jasco, Tokyo, Japan), 280 nm. Also, identification of SG was achieved by ^{1}H-nuclear magnetic resonance (NMR) and liquid chromatography-mass spectrometry (LC-MS). The contents of SG were 1,400 mg/100 g defatted sesame flour.

Animals and diets Male SAMP8 (3 month-old) and SAMR (age-matched control, 3 month-old) were obtained from the Korean Research Institute of Chemical Technology. All experiments were performed in compliance with the guide for the care and use of laboratory animals recommended by Korea Food Research Istitute. The schematic of the experimental schedule is shown in Fig. 1. Animals were housed for 6 months under standard conditions (temperature, 20-22°C; lighting cycle, 12/12-hr; humidity, 45%) and allowed free access to commercial diet and tap water. After all mice (9 month-old SAMP8) were screened in terms of cognitive function by passive avoidance, the mice that had step-through latency greater than 120 sec were considered to be mildly cognitive-impaired and were randomly divided into 3 groups (n=12/group). During the experimental period, mice were fed either normal or experimental diets containing either 0.25 or 0.5% SG based on AIN-76 diet for 12 weeks. At the end of the experimental period, the animals were deprived of food overnight and sacrificed; the brain tissue was then removed and frozen at −80°C for the analysis of lipid peroxide content and antioxidant enzyme activity.

Passive avoidance test Passive avoidance was tested at the end of the feeding experiment using a 2-compartment shuttle chamber (256000 Series; TSE Systems, Midland, Germany), 1 illuminated and 1 dark, equipped with a grid floor and shock generator. For the acquisition trials, each mouse was placed in the lighted compartment and the time prior to entering the dark compartment was recorded. When the mouse entered the dark compartment, the door was immediately closed and it received an inescapable shock (0.3 mA, 3 sec). During the next trial, the mouse was again placed in the lighted compartment and the time prior to entering the dark compartment was measured (with a maximum of 300 sec) as the step-through latency.

Morris water maze task The Morris water maze task was slightly modified from the Morris method (18). The water maze pool was a round-shaped tank, 100 cm in diameter and 35 cm in depth. The pool was filled with water to 15 cm deep, at 23°C and rendered opaque by the addition of powdered milk. A transparent platform was positioned inside the tank, and its top was 2 cm below the water surface in the target quadrant of the maze. Each mouse was given 2 trials/day for 4 consecutive days to find the hidden platform. On the 5th day, the animals tested for spatial memory retention in the water maze.

Measurement of thiobarbituric acid reactive substance (TBARS) levels The brain tissue was homogenized in 10 volumes of 0.1 M potassium phosphate (pH 7.2) buffer. After centrifugation for 10 min at 600×g, the TBARS levels in the supernatant were estimated according to the method of Ohkawa *et al.* (19).

Measurement of antioxidant enzymatic activity The brain tissue was homogenized in 10 volumes (w/v) of ice-cold 0.25 M sucrose-0.5 mM ethylenediamine tetraacetic acid (EDTA) buffer and centrifuged at 600×g for 10 min at 4°C. The supernatant was used for the assay of catalase activity. Subsequently, the supernatant was centrifuged at 10,000×g for 30 min at 4°C and used as the enzyme source of SOD and GPx. Catalase activity was measured using the method of Aebi (20). SOD activity was assayed according to Marklund and Marklund (21). GPx activity was measured according to Lawrence and Burk (22). Protein concentration

was determined by the method of Lowry *et al.* (23) with bovine serum albumin as a standard.

Statistics All results are expressed as the mean±standard error (SE). The data were analyzed by an one-way analysis of variance (ANOVA) using the SAS program 9.1.3 and the differences between experimental groups were evaluated using Duncan's multiple range test at the $p<0.05$ level.

Results and Discussion

SG improved cognitive deficits in SAMP8 The SAMP8 mouse strain has been widely accepted as an animal model of senile dementia. Learning and memory deficits begin 4 months after birth and worsen with aging (24). In the present study, the effect of SG on age-related learning and memory impairments were investigated in the passive avoidance and Morris water maze tasks in the SAMP8 animal model. Changes in the step-through latency in the passive avoidance test are shown in Fig. 2A and 2B. There was a significant difference between the SAMR1 group and the age-matched SAMP8 control group (257.6±22.4 vs. 59.5±12.3), respectively (Fig. 2A). Compared with the screening data, the step-through latency of the SAMP8 control mice decreased from 115.9 to 59.5 sec, while those of the SG-supplemented group increased to 125 and 142 sec in the 0.25 and 0.5% SG groups, respectively (Fig. 2B). Step-through latency of the SAMP8 mice fed the SG diet significantly increased in a dose-dependent manner (3.5 fold increase in the 0.5% SG group compared with the SAMP8 control group). Changes in the escape latency in the Morris water maze are shown in Fig. 2C. In the probe trials, the escape latencies of the group fed the diet containing 0.5% SG became shorter than that of the SAMP8 control group, similar to the latency level of the SAMR1 group. These results are in line with data from Corney *et al.* (25) in which chronic supplementation of a spin-trapping compound, *N*-*tert*-butyl-α-phenylnitrone, improved the loss of temporal and spatial memory in aged gerbils. In addition to this result, antioxidant carotenoids, capsanthin, and lycopen attenuated the age-related learning impairment in SAMP8 mice (26). SG has been reported to be potent antioxidants (27,28). In addition, SG has been shown to protect against the β-amyloid peptide (Aβ)-induced oxidative stress and apoptosis signals in neuronal cells (17). Moreover, Um *et al.* (29) previously found that defatted sesame flour containing SG also improves memory impairments associated with increases in lipid peroxide levels in SAMP8 mice. Thus, SG may contribute to attenuation of memory and learning impairment during the aging progress in SAMP8 mice.

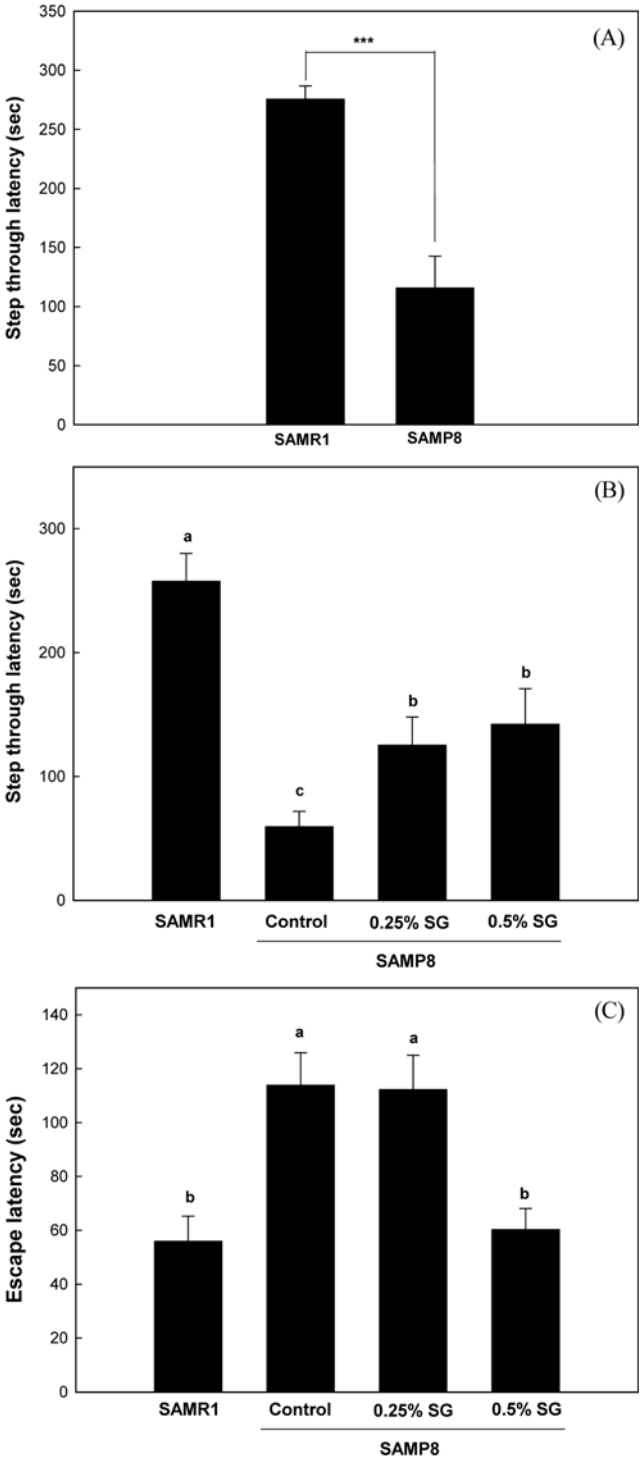

Fig. 2. Effects of sesaminol glucosides (SG) on aging-related cognitive deficits in SAMP8 animals. A, passive avoidance. In the screening step, step-through latencies of SAMR1 and SAMP8 groups were measured at 9 months of age. (***$p<0.001$ compared to age-matched SAMR1 group); B, passive avoidance. After mice were fed each experimental diet, step-through latencies were measured at 12 months of age; C, Morris water maze. Bars with different letters are significantly different at $p<0.05$ by Duncan's multiple range test.

Effect of SG on lipid peroxide levels and antioxidant enzymatic activity The brain is highly sensitive to oxidative damage because it is very rich in polyunsaturated fatty acids and has low antioxidants. Accumulated evidence indicates that free radicals play an important role in neurodegeneration-associated cognitive deficits in both aging and AD (30). However, oxidative stress can be blocked or delayed by various antioxidants, including antioxidant enzyme (11,31). In this study, the effects of SG on the lipid peroxidation and the activities of antioxidant enzymes in the brain are evaluated and showed in Fig. 3 and Table 1. As shown in Fig. 3, the TBARS levels in the

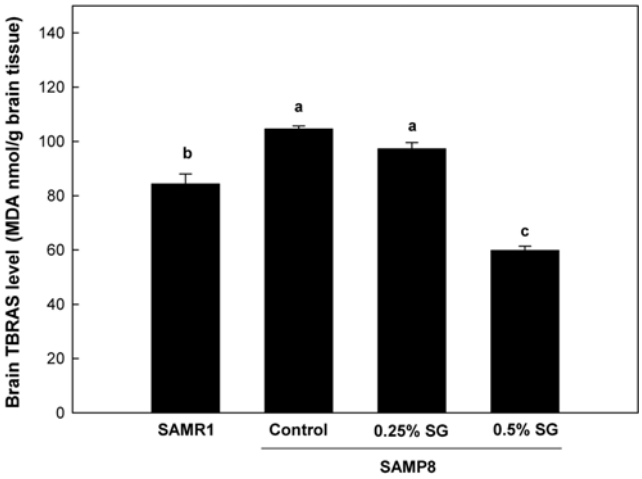

Fig. 3. Effects of sesaminol glucosides (SG) on lipid peroxide levels in brains of SAMP8 animals. Bars with different letters are significantly different at $p<0.05$ by Duncan's multiple range test.

brain tissue of the SAMP8 control group significantly increased compared with the SAMR1 group. The increase in the TBARS levels in brain was significantly attenuated by 0.5% SG supplement. The catalase activity of the SAMP8 control group was significantly higher than that of the SAMR1 group. However, this increase was significantly attenuated in brains of mice fed either the 0.25 or 0.5% SG diet. Similarly, SOD activity was also found to be significantly decreased in brains of the SG-supplemented group. GPx activity was significantly elevated in brain tissues of the SAMP8 control group, but reduced in the 0.5% SG group (Table 1).

These results are consistent with those of Hussain *et al.* (32) who showed that overall antioxidant enzyme activities tend to increase with aging. Siqueira *et al.* (33) also reported that increases in oxidative damage and higher SOD activities were observed in the cerebella of aged rats. Accordingly, the results showed significantly elevated SOD, catalase, and GPx activities in the brain, possibly reflecting compensatory rise in the antioxidant response to serve oxidative damage. Dietary SG supplementation also decreased lipid peroxide levels and antioxidant enzyme activities. According to Osawa *et al.* (39) study, SG is metabolized to sesaminol, an effective antioxidant, by intestinal microflora and then incorporated via lymphatic absorption into the cardiovascular system, transported to peripheral tissues. SG was also shown to have antioxidant activity in the *in vitro* systems (13). Kang *et al.* (15) reported that defatted sesame flour containing SG inhibits lipid peroxidation in hypercholesterolemic rabbits. It is possible that SG acts as an antioxidant through antioxidant enzyme independent mechanisms.

It appears that increases in lipid peroxides and imbalances among antioxidant enzymes accelerate with aging, resulting in exposure to additional oxidative stress. Additionally, previous studies support the notion that oxidative stress is a fundamental cause of cognitive impairment (40,41). It has been also reported that a reduction in spatial cognition during the water maze task correlates with an increased amount of malonaldehyde, an end product of free radical reactions, in the cerebral cortex of aged mice, and the administration of antioxidants improve memory impairment in older mice and gerbils (34-37). Yasui *et al.* (11) showed that increases in lipid peroxides and deficits in learning and memory seen in SAMP8 animals can be reduced by the administration of acetyl-L-carnitine, and Saganuma *et al.* (26) also reported same results. Another study has shown that aged rats given antioxidant-rich diet improved cerebellar physiology and learning ability (38). These findings suggest that the reduction of oxidative stress by SG supplementation may improve learning and memory impairment in SAMP8 animals.

In summary, chronic supplement of SG markedly improves age-related cognitive deficits and this effect is mediated by the antioxidant properties of SG. Future studies should determine the detailed molecular mechanism in SG responsible for protecting against cognitive impairment.

Acknowledgments

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Table 1. Activities of antioxidant enzymes in brains of SAMP8 animals

Group		Catalase (unit/min/mg protein)	SOD (unit/min/mg protein)	GPx (nmol/min/mg protein)
SAMR1		1.6±0.1[b1)]	1.8±0.2[b]	15.9±1.2[b]
SAMP8	Control	2.1±0.2[a]	3.0±0.2[a]	22.0±1.8[a]
	0.25% SG	1.6±0.1[b]	1.9±0.1[b]	15.1±0.5[b]
	0.5% SG	1.5±0.1[b]	1.1±0.2[c]	15.0±0.5[b]

[1)]All values are mean±SE (n=12); Different letters in a column are significantly different at $p<0.05$ by Duncan's multiple range test.

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