Prev. Nutr. Food Sci. 2018;23(3):196-205
https://doi.org/10.3746/pnf.2018.23.3.196
pISSN 2287-1098 · eISSN 2287-8602

Spatial Memory and Antioxidant Protective Effects of Raisin (Currant) in Aged Rats

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ABSTRACT: Diets rich in fruits and vegetables can prevent age-related diseases. This research was conducted to evaluate the effects of raisin consumption on the spatial memory and morphometric parameters of brain tissue in aging rats. Old rats (20 months of age) were divided into 2 groups: control and raisin, with 6 rats in each group. The raisin group received 6 g of raisins daily in addition to their food and water for 90 days. After treatments, all animals were evaluated by behavioral tests to assess spatial memory and learning alongside other tests including the ferric reducing antioxidant power (FRAP), malondialdehyde, and histological examinations. The results showed that there are significant differences in the Morris water task and passive avoidance learning of behavioral tests and biochemical tests (FRAP and thiobarbituric acid reactive substances) between the two groups. The histological study indicated that the cell count of the hippocampus, the diameter of the lateral ventricle, and area of the corpus callosum in the raisin group changed in comparison with the control group but they were not significant. The results demonstrated that raisins significantly raise antioxidant levels in blood and promotes cognitive and motor performance in aging rats.

Keywords: spatial memory, antioxidant, aging, raisin, currant

INTRODUCTION

The elderly population is increasing due to advancements in health and healthcare facilities (1). Aging decreases the brain's functioning capacity which could lead to dementia (2). A minimum of 4 areas of activities including memory, visual-spatial skills, personality, and cognition are affected by aging (3). Aging causes several pathological changes in the nervous system which include: degeneration of certain nerve cells, forming tangles of neurofibrillary, and decreasing nerve fibers (4,5). In addition, synaptic degradation and degeneration of cells in the hippocampus area are other histopathological changes induced by aging. It is evident that inflammation, genetic factors, and oxidative stress enhance the aging process. Antioxidant levels decrease with aging, unlike free radicals which increase. In fact, there is a direct relationship between free radical levels and aging (6,7). Moreover, there are behavioral and neuronal alterations in the normal aging process. Motor and cognitive changes occur in elderly people as a result of reduction in neuronal activity. Furthermore, memory deficits in cognitive tasks including decreased spatial learning, and deficits in motor functions such as loss of balance, muscle strength, and coordination have been reported in aged humans and animals (8). Fiber rich diets (fruits and vegetables) may prevent age-related diseases (9,10). Polyphenols in extracts of blueberry, strawberry, and black currant have antioxidant and anti-inflammatory properties that play a significant role in reducing age-related motor and cognitive disorders (11). Flavonoids also play a similar role and are found in plants which prevent learning and memory deficits during aging (12). These compounds are also found in fruits such as pomegranates, blueberries, apples, grapes, and strawberries (13). The *Vitis vinifera* family, such as raisins and black grapes, are native in many regions of Iran containing the richest phenolic compounds (14). Raisins have been examined in several studies due to their rich phenolic levels (15). Most phenols in grapes are concentrated in their seeds. Black grapes have a high

Received 12 April 2018; Accepted 4 July 2018; Published online 30 September 2018
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levels of flavonoids and polyphenols, which have antioxidant properties (16). It has been shown that antioxidant and phenolic contents of grapes and dried grapes (raisins) have enhanced potential in human health (17). Among the dried fruit products, raisins have the highest total phenolic concentrations and antioxidant activity (18). The amount of antioxidants including polyphenols, phenolic acids, and tannins is higher in raisins compared to their juice due to the amount of sugar and beneficial minerals such as potassium, calcium, and iron (19). High performance liquid chromatography analysis showed that 4 varieties of raisins have significant levels of a variety of phenolic contents and antioxidant activity (20). Although, several studies have been conducted on the effects of wine and grape juice on the aging process, the health effects of raisins or dried grapes are still not fully understood. Recently, we showed the protective effect of raisins against spatial memory impairment and oxidative stress in an Alzheimer's disease rat model (21). The present study was conducted to evaluate the effects of raisin consumption on the spatial memory and morphometric parameters of the brain in aging rats.

MATERIALS AND METHODS

Animals and study design

Twelve male Wistar rats (20 months of age), raised at the local vivarium (animal house of Babol University of Medical Sciences, Babol, Islamic Republic of Iran), were randomly chosen and used in this study. All animals were maintained on a 12-h light/dark cycle (lights on at 7:30 am), temperature (23°C), and humidity (60%) with *ad libitum* access to food and water. The rats were also assigned to 2 groups, and each group consisted of 6 rats: group I (control) and group II (raisins recipient). The raisin group received 6 g raisin (Maviz) mixed with daily normal chow for 90 days. Black raisins were obtained from the Takestan vinery in Islamic Republic of Iran. Procedures in this study were performed in accordance with the National Institute of Health (NIH) Guide to the Care and Use of Laboratory Animals and were approved by the Babol University of Medical Sciences Animal Care Committee (Ethics committee number: 2809).

Body weight

Body weight of all animals was monitored at 2 time points, before and after the experiment.

Biochemical assessment

Blood samples were collected from all animals via retro-orbital venous sinus in heparinized disposable syringes for biochemical investigation including the ferric reducing antioxidant power (FRAP) and malondialdehyde (MDA) assays upon completion of the treatment. Blood samples were collected from rats at a specified day and time (after 8 h of fasting) and were centrifuged at 3,000 rpm for 10 min for serum collection. The blood serum was then kept in the biochemistry laboratory freezer at −20°C.

Total antioxidant capacity

The FRAP aasay is one of the simplest procedures to evaluate the antioxidant status. This test was performed using a spectrophotometric method (22).

MDA concentration

A well-known method used to evaluate the status of the antioxidant is the thiobarbituric acid reactive substances (TBARS) test using spectrophotometry. The test is based on the measurement of the levels of MDA. MDA is one of the auxiliary compounds of oxidation of fatty acids such as linoleic acid with at least three double bonds. Employing this procedure, a MDA molecule reacts with two molecules of thiobarbituric acid. The reaction results in the removal of two water molecules and formation of a pink color complex with maximum absorption at 532 nm (23).

Behavioral assessment

After 3 months, when the rats were 23 months of age, all rats were tested for spatial performance and passive avoidance learning (PAL).

Morris water task (MWT)

In order to evaluate spatial performance of the rats before and after receiving raisins, the rats were tested in 8 trials per day for 9 consecutive days (8 days for working memory and one day for reference memory or probe trial) within the moving hidden platform version of the MWT. The training and testing procedures were previously published in detail (24). Briefly, the MWT comprised a black pool (150 cm diameter) filled with water (23±1°C) to about 25 cm to the top of the wall. The pool was situated in a room rich with distal cues, which remained unobstructed throughout the duration of the experiment. During all hidden platform trials, a platform was submerged 1∼1.5 cm under the water surface. Each trial commenced with the rat being placed in the pool at one of the four cardinal compass positions around the perimeter of the pool according to a pseudo-random sequence. The maximum duration of each swim trial was 60 s. In the moving hidden platform version of MWT, the platform is moved to a new location every odd day. In other words, the platform remains in the same position for 2 consecutive days. Since the location of the hidden platform was different every 2 days, all odd days were called learning days, and even days were called Memory days. On the ninth day, a probe test was performed in order to determine the extent to which the rats had learned the location of the platform. The platform was removed from the pool, and the rats were allowed to swim freely for 1 min. The percentage of time that the animals spent in each quadrant of the task was recorded (25).

A video tracking system (HVS Image 2020, HVS Image Ltd., Thornborough, Buckinghamshire, UK) was used to record and analyze the movements of the animals including latency and path length and speed within MWT.

PAL

PAL is a classic fear-motivated test used to assess learning and memory in small laboratory animals such as mice and rats. PAL requires the animal to learn how to escape a situation in which an aversive stimulus (such as a foot-shock) was delivered. In the current experiment, the classic fear-motivated passive avoidance test was carried out using a shuttle box that comprises 2 compartments: dark and light chambers, which are separated by a guillotine door. The testing for PAL follows 3 stages.

Compatibility stage: On the first day of training, each rat was acquainted with the device for 5 min, the window was open between the 2 boxes, and animal moved freely inside the shuttle box.

Acquisition stage: After 24 h of the compatibility stage, the rats were placed in a light chamber for 2 min while the other chamber was kept dark. In this stage, both the light and dark chambers were connected by a closed guillotine door. At the end of the period, the chamber was lit and the guillotine door was opened. The time elapsed by the rats to move from the light chamber to the dark chamber called the initial latency (IL) was calculated. When animals moved into the dark box, an electrical foot shock was delivered via the floor grid in the dark compartment.

Memory stage: This stage was performed 24 h after the second stage, and it is similar to the previous step with the exception that when animals moved into the dark compartment, they did not receive any shock. The time spent for moving into the dark chamber called step-through latency (STL) was measured. If the animal did not enter the dark room within 300 s, the trial was terminated (26).

Histologic evaluation

Following all behavioral assessments, the animals were sacrificed using a mixture of ketamine (Rotexmedica GmbH, Trittau, Germany) and xylazine (Sanofi, Paris, France) under the hood. Perfusion was carried out via the left cardiac ventricle employing saline (NaCl 0.9%) and 10% formalin fixative solution (Merck KGaA, Darmstadt, Germany). Brains were immediately removed and immersed in 10% formalin solution for 72 h before sectioning. When dehydration was manually completed and paraffin templates were made from the brains, sagittal and coronal sectioning was performed using the rotary microtome (Leitz 1512 Leitz, Wetzlar, Germany) at a thickness of 5 microns.

Morphometric measurements

The cerebellum was separated from the rest of the brain with a scalpel. The brain was divided into 2 equal hemispheres with a midsagittal cut from the corpus callosum. The corpus callosum and lateral ventricle were photographed using a stereotaxic microscope to measure the area of the corpus callosum and diameter of the lateral ventricle by Motic Image Plus ver. 2.0 (Motic, Xiamen, China).

Staining

The sagittal and coronal brain sections (5 microns in thickness) were produced in serial form by a microtome and stained with hematoxylin and eosin and Cresyl violet acetate.

Hippocampus cell count

The number of neurons (Motic Image Plus ver. 2.0, Motic) and histological architecture in the dentate gyrus and regions of the hippocampus (CA1 and CA3) were evaluated.

Statistical analysis

Data analysis was performed using SPSS ver. 23.0 (SPSS Inc., Chicago, IL, USA). The data analysis for the first 8 days of the MWT was conducted using repeated measures analysis of variance (ANOVA). Analysis of probe trial function (percentage of time spent in target quadrant) and other measures were carried out using independent sample t-tests with $P<0.05$ set as the significance level.

RESULTS

MWT

The results of all behavioral measures before and after receiving raisins including latency to locate the hidden platform, swimming path length, probe trial performance for both groups along with the difference between learning, and memory days in both groups are shown in Fig. 1.

Latency: Fig. 1A illustrates that latency to locate the hidden platform in MWT declined from day 1 to day 8 signifying that all rats spent the same time to find the hidden platform during different days. Results also indicated a better spatial function on the memory day compared to learning days. Repeated measures ANOVA revealed a significant effect of day [$F(7, 4)=28.38$, $P<0.05$], platform location [$F(1, 10)=377.04$, $P<0.05$], trial [$F(7, 4)=14.62$, $P<0.05$] but not group [$F(1, 10)=0.004$, $P>0.05$].

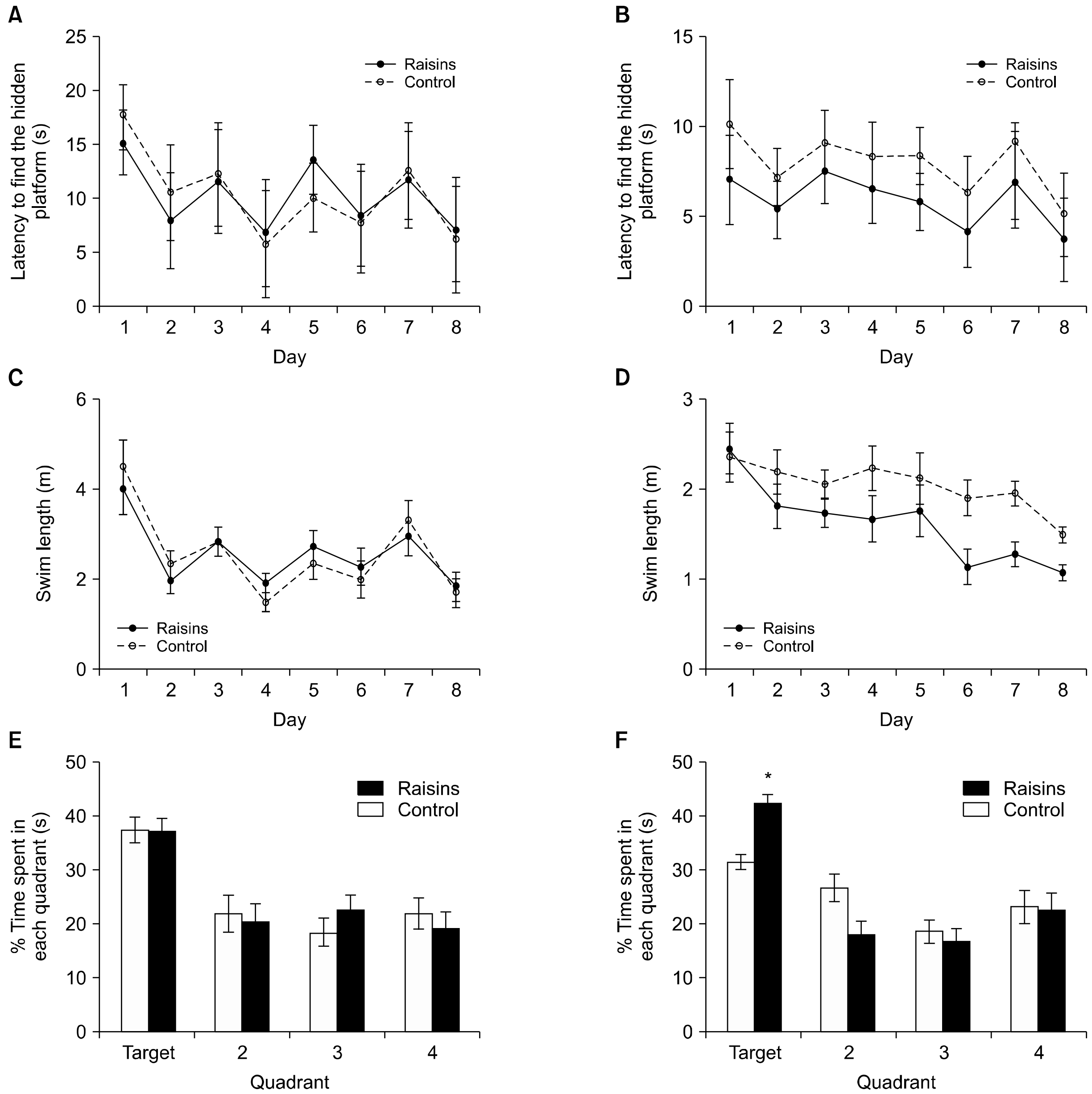

Fig. 1. Morris water task (MWT) of latency (A) before and (B) after receiving raisins, swim length (C) before and (D) after receiving raisins, and probe trial (E) before and (F) after receiving raisins. Error bars signify mean±standard error of the mean for each group. *$P<0.05$.

The results revealed that all animals were able to acquire and retrieve the spatial information in a similar manner before receiving raisins.

Swim length: Results of the mean distance traveled by rats in both groups to find the hidden platform revealed that all rats had progressively spent a shorter distance from the first to the last day. There was no significant difference ($P>0.99$; Fig. 1C) between groups in terms of swim length.

Probe trial: Results indicated that the percentage of time spent in each quadrant during the probe trial (the first 30 s) were similar in both groups as shown in Fig. 1E. Hence, no significant difference was observed between the 2 groups ($P=0.95$).

In summary, pre-test examination of the latency, swim length, and probe function during spatial navigation showed no differences between the raisin and control groups.

After receiving raisins

All animals in both groups were tested within MWT after receiving raisins.

Latency: Fig. 1B illustrates that the raisin group, spent less time finding the hidden platform in MWT compared to the control group. Repeated measures ANOVA indicated a significant effect of group [$F(1, 10)=29.87$, $P<0.05$], platform location [$F(1, 10)=1,319.66$, $P<0.05$] and trial [$F(7, 4)=8.153$, $P<0.05$] while insignificant of day [$F(7, 4)=5.41$, $P=0.061$]. These results showed that raisins could enhance spatial performance in terms of the time

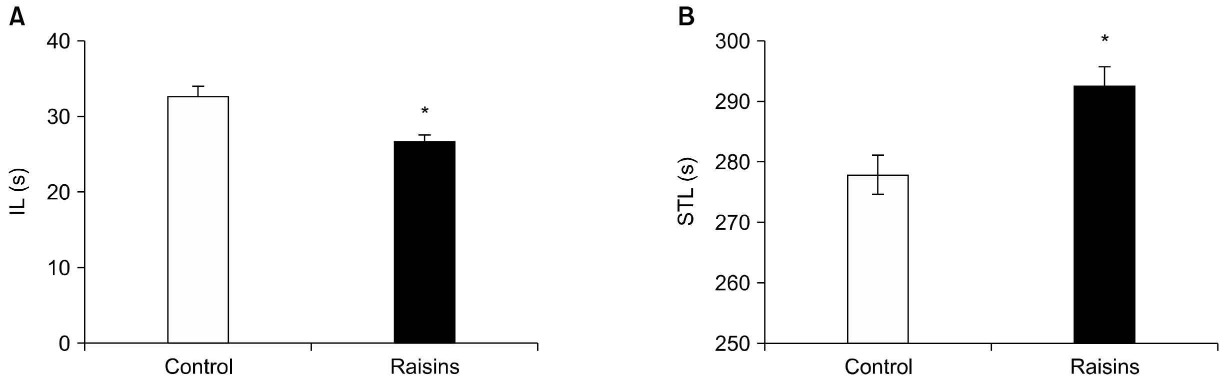

Fig. 2. Testing in passive avoidance learning test to assess short-term or long-term memory. (A) initial latency (IL) and (B) step-through latency (STL). Error bars signify mean±standard error of the mean for each group. *$P<0.05$; independent sample t-test.

spent finding the hidden platform in MWT.

***Swim length*:** Also, the rats that received raisins during the past 90 days had shorter swim length compared to the controls. ANOVA revealed significant differences between the 2 groups [$F(1, 10)=15.77$, $P<0.05$; Fig. 1D].

***Probe trial*:** Results showing the percentage of time spent in each quadrant during the first half of the probe trial (30 s duration) are depicted in Fig. 1F. ANOVA revealed that rats in the raisin group spent a considerable proportion of their time searching in the target quadrant of the MWT in which the platform had previously been presented [$F(1, 11)=50.48$, $P<0.05$].

Passive avoidance test

Fig. 2A and 2B show a summary of IL and STL within the PAL test for both groups. Results revealed that raisin rats had significantly better performance in terms of the IL and STL when compared with control rats (both $P<0.05$; independent sample t-test).

FRAP

Investigation of the blood plasma of all animals to assess FRAP indicated that daily consumption of raisins significantly increased the plasma antioxidant power ($P<0.05$; Fig. 3A).

MDA

Plasma biochemical assessment using TBARS revealed that raisins significantly reduced the amount of MDA and decreased the levels of lipid peroxidase (LPO) in the raisin group compared to the control group ($P<0.05$; Fig. 3B).

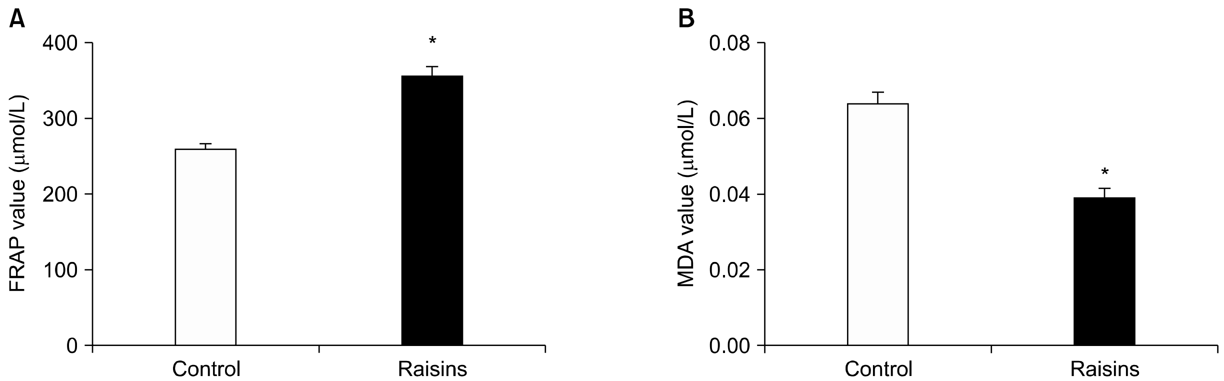

Fig. 3. Evaluation of the effect of raisin on the blood antioxidant status (A) ferric reducing antioxidant power (FRAP) and (B) malondialdehyde (MDA). Error bars signify mean±standard error of the mean for each group. *$P<0.05$.

Table 1. Mean body weight of rats in the control and raisin groups

Groups	Mean±SE		Mean difference	P-value
	Control	Raisins		
Primary body weight	279.16±4.31	277.16±3.04	2	0.398
Termination body weight	276.16±3.04	298.66±2.9*	22	0.05

SE, standard error.

Body weight

There were no differences in body weight in the pre-test session. However, changes in body weight after daily consumption of raisins indicated a significant difference between the groups ($P<0.05$) as rats who received raisins across 90 days gained weight (Table 1).

Hippocampal morphology and cell number

Typically, changes in the shape of neurons that occur in the hippocampus along with irregularity in the neuronal form and a reduction in the number of the pyramidal and granular neurons are signs of normal aging brain, and could be observed in the brains of the control rats (Table 2). Conversely, neurons in the hippocampus of raisin rats were more regular and more rounded in shape with higher numbers than the control rats (Fig. 4, 5, and 6). However, no significant difference was observed between the groups ($P>0.05$).

Anatomical measures

The lateral ventricle diameter in the old rats that consumed raisins daily was less affected by the normal aging process and were more compact compared to the control rats. Furthermore, the area of the corpus callosum in the raisin group was greater compared to the control group. However, no significant differences were found between the groups ($P>0.05$; Table 3).

Table 2. Effect of raisins on the number of neurons in different regions of the hippocampus

Groups	Mean±SE		Mean difference	*P*-value
	Control	Raisins		
DG	107.5±5.14	122±7.62	14.5	0.485
CA1	244.5±20.23	281.3±8.72	12.3	0.062
CA3	83.5±5.23	95.83±3.85	36.8	0.283

SE, standard error.
DG, dentate gyrus; CA1 and CA3, regions of the hippocampus.

DISCUSSION

The present experiment showed that daily consumption of raisins for 90 days enhances spatial learning and memory in aged rats. Also, morphometric alterations occurred in the corpus callosum and hippocampus in old rats who received raisins daily which provides further evidence for the neuroprotective effects of *Vitis vinifera*, specifically in elderly vulnerable populations.

Normal aging is usually associated with impairment in cognitive and motor behaviors (27). This result is possibly caused by increased oxidative stress, which results in motor deficits during aging (28,29). The progression of abnormal function and programmed cell death (apoptosis) in certain areas of the central nervous system such as the hippocampus occurs faster than in other brain regions (30).

Nutrition and healthy diets, especially foods containing antioxidants, are critical factors to prevent motor disorders and age-related memory decline in the elderly (31). Therefore, the foods with antioxidant properties appear to primarily improve cognitive performances in older adults. Grape and its dried variations such as raisins possess phenolic antioxidants beneficial for health. It has been suggested that oxidative stress associated with destructive oxidation reactions can be prevented by dietary

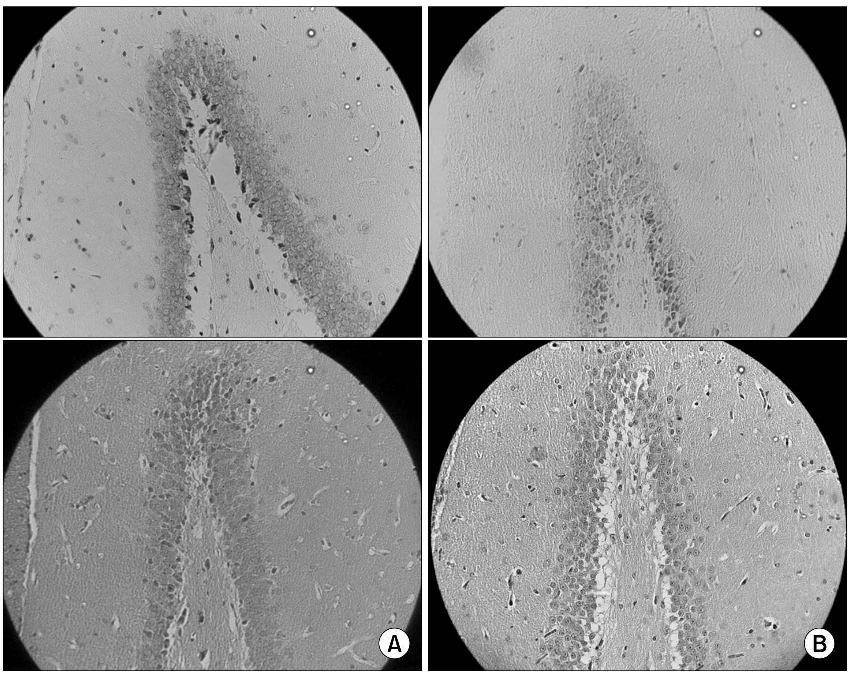

Fig. 4. Dentate gyrus of (A) raisins group and (B) control group. Neurodegeneration, morphological changes of neurons and neuronal loss occurred more in the control group. Nissle and hematoxylin and eosin stainings (×20).

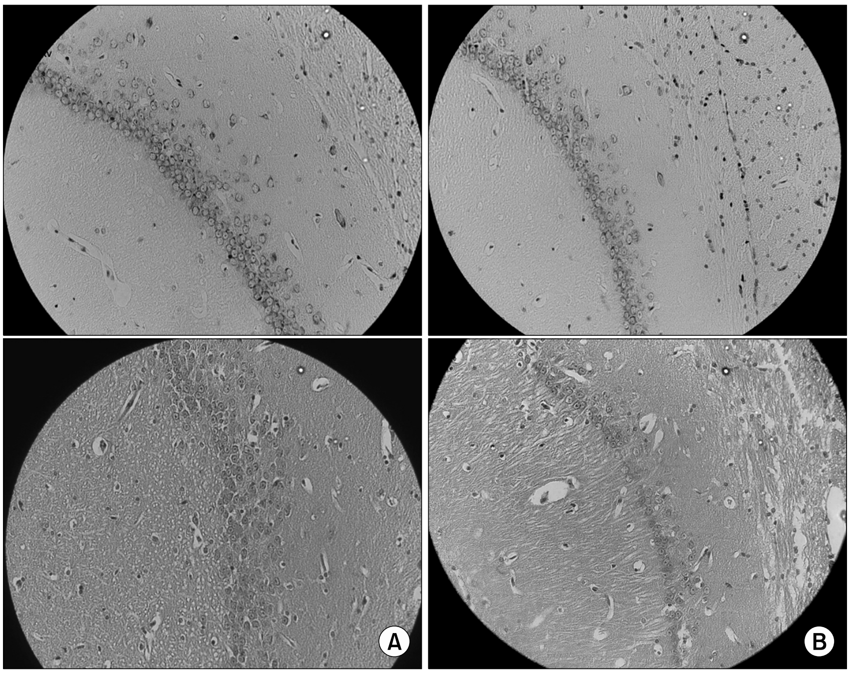

Fig. 5. CA1 area in (A) raisins group and (B) control group. The control group displayed more neuronal loss, neurodegeneration, and morphological changes. Nissle and hematoxylin and eosin stainings (×20).

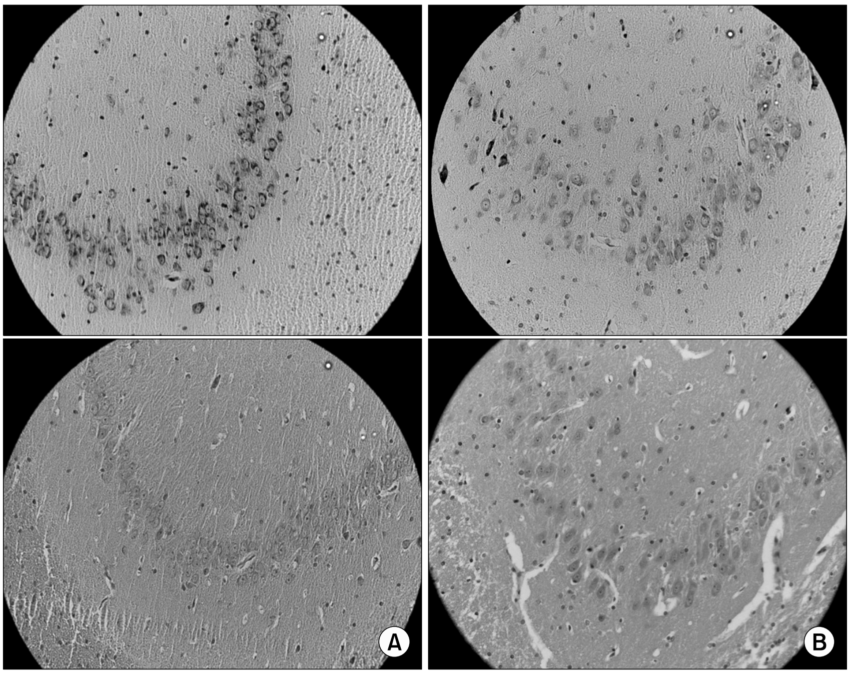

Fig. 6. CA3 area in (A) raisins group and (B) control group. Neuronal changes such as neuronal loss, neurodegeneration, and morphological changes have been observed to occur in the control group more than the raisin group. Nissle and hematoxylin and eosin stainings (×20).

supplements rich in antioxidants such as dried black grape (32). Antioxidants seem to play a vital role in preventing structural damages when free radicals created by reactive oxygen species begin to damage cells via their chain reactions. It has been proposed that consumption of diets rich in antioxidants such as strawberries, walnuts, and black currant juice can lower age-related cognitive declines and the risk of developing neurodegenerative diseases (33,34). In the present study, the antioxidant levels of blood serum from the raisin group that received raisins for 90 days had significantly increased compared to the control rats. In addition, the average concentration of LPO or MDA was significantly reduced in the raisin group compared with the control group. These results are in agreement with the objectives of the present study and validate that oral administration of raisins increased antioxidant capacities of blood serum in raisin-recipient rats and reduced oxidative stress.

Raisins contain protein, carbohydrates, fats (lipids), fiber, sugar, calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, and vitamins such as vitamin K, E, B6, niacin, and riboflavin. The present study proposed that daily consumption of raisins can be associated with increase in body weight and it could be due to increase in the level of food consumption (35).

Also, it has been previously shown that grape juice en-

Table 3. Effect of raisins on corpus callosum and diameter of lateral ventricle in the brain of rats

Groups	Mean±SE		Mean difference	*P*-value
	Control	Raisins		
Area of the corpus callosum	70.51±2.17	72.68±1.12	2.17	0.066
Diameter of the lateral ventricle	2.35±0.26	1.83±0.17	0.52	0.51

SE, standard error.

hances memory function in rats within the passive avoidance learning test. This notion was supported by the increased latency in the first trial of PAL moving into the dark room, total time spent in bright room, and reduction in the total time spent in the dark room (36). The antioxidant compounds such as polyphenols, flavonoids, and resveratrol found in grape juice were suggested to be the major factors responsible for improving memory function (37). These results are consistent with studies that have shown that a long-term use of antioxidants will reduce cognitive impairments caused by normal aging in animals (38). In this study, the rats that were treated by oral administration raisins (raisin group) displayed better performance in the PAL test during the initial delay time and cross delay time compared with the control group. Furthermore, a study on the effect of grape seed extract on spatial memory in old male rats by MVT indicated that the test rats treated with grape seed extract had spent significantly less time finding the hidden platform compared to the control rats. Probe function, which mainly reflects reference memory, was also improved by the consumption of the grape extract (39). These results suggest that consumption of grape seed extract as a dietary supplement increases the spatial memory performance in rats and also prevents memory decline caused by normal aging by protecting the central nervous system against damaging oxidizing agents and free radicals (39). Therefore, the results of the present experiment provide further evidence for previous findings that showed that consumption of antioxidant-rich foods prevent structural degeneration and cognitive deficits in old animals. Precisely, the daily consumption of raisins improves both working and reference memory functions in the present study. Since normal aging is associated with increased free radicals and reactive oxygen species, they could cause necrosis, cell damage, and programmed cell death (apoptosis) in the elderly (40). Pyramidal cells mostly found in the cerebral cortex, the hippocampus, and the amygdale have been shown to be influenced by oxidative stress (41). The use of antioxidants can therefore reduce cell death by reducing cellular damage. Antioxidants are substances that eliminate free radicals and compounds which cause cell damage by disrupting the responsible mechanisms (40). Decreasing changes in the shape of neurons in the hippocampus as well as the decline of abnormal variations and irregularities in the arrangement of the neurons paired with the increased number of pyramidal and granular neurons in raisin rats emphasize the neuroprotective role of high rich foods and antioxidants in the brain structure and function compared to the control group. Moreover, our results suggest that antioxidants found in *Vitis vinifera* may prevent cells from physical destruction, apoptosis, and degeneration that normally occur as a result of aging which lead to memory impairment.

Many lesion studies conducted on the effect of polyphenols on brain neurogenesis have also linked the post-lesion recovery to neuroprotective properties of antioxidants after brain injury. For instance, male rats exposed to a diet rich in antioxidant polyphenol compounds for 40 days showed a significant increase in the number of new cells in the subgranular region and subventricular zone (SVZ) (42). In addition, a study on a mouse model of Alzheimer's disease showed that behavioral improvement in old mice was associated with a 70% increase in cellular proliferation in the SVZ of the brain (43). In the present study, the lateral ventricle diameter of rats in the raisin group was found to be smaller with less death rate and degeneration compared to control rats. Moreover, raisins are a good source of boron, a trace element necessary for brain function, such as hand-eye coordination, memory, and attention. It is suggested that boron plays a role in some normal mechanisms in the body, such as enzyme reaction, cell membrane function, and hormone metabolism (44). It has been reported that low boron intake influences the behavioral functions of the body and has a poor performance in psychomotor skills, perception, cognitive functions, attention, and memory (45).

Also, the corpus callosum plays a vital role in the integrity of the cerebral hemispheres and hemispheric functions (46). Furthermore, several studies have indicated that corpus callosum is one of the brain areas that more susceptible to the age-related atrophy (47). In this study, raisin-recipient rats had larger corpus callosums compared with control rats, which suggest that antioxidant-rich foods provide anti-aging characteristics and to some extent protect brain neurons against degeneration that normally occurs due to aging. In the present study, histological changes were observed but were not significant, whereas behavioral and biochemical changes were significant. Therefore, it can be concluded that biochemical and behavioral changes occur earlier than histological changes in the brain tissue.

In conclusion, the results of the present study indicated that daily consumption of black dried grape as a food supplement increases the concentration of antioxidants in the blood and reduces lipid peroxidation in old rats. Apparently, spatial performance and passive avoidance learning were improved by the diet rich in antioxidants found in *Vitis vinifera*. Our findings here reemphasize the fact that essential nutrients (e.g. antioxidants) may have great potential in delaying the onset of age-related normal alterations and provide further evidence to establish a comprehensive approach to geriatric health and rehabilitation.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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