Protective effects of blueberry drink on cognitive impairment induced by chronic mild stress in adult rats

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BACKGROUND/OBJECTIVES: Stress-induced cognitive impairment is related to the suppression of hippocampal neurogenesis that results from an increase of oxidative stress. Therefore, the aim of this study was to investigate the effects of administration of a blueberry drink, having a high antioxidant power, on the cognitive performance of adult rats exposed to chronic mild stress.

MATERIALS/METHODS: Twelve-week-old male Sprague-Dawley rats (n = 48) were randomly divided into four groups: control (CO), stress (ST), control + 5% blueberry drink (CO + B), and stress + 5% blueberry drink (ST + B). After eight weeks, the cognitive performance was assessed using a multiple T-maze water test. Levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and ascorbic acid were measured in the brain, and catecholamine concentrations were measured in plasma.

RESULTS: The brain weights of the rats from the ST and ST + B groups were significantly lower than those of the rats from the CO and CO + B groups. The cognitive performance of the ST group was impaired when compared to that of the CO group. This impairment was significantly improved by the blueberry drink supplementation (P < 0.05). The brain SOD and CAT concentrations were not influenced by the stress or by the blueberry drink. However, the brain levels of GPx and ascorbic acid were significantly lower in the ST group than those in the CO group and were increased by the blueberry drink supplementation. The plasma catecholamine concentrations were affected by chronic mild stress and by the blueberry drink. The plasma norepinephrine and dopamine concentrations were decreased by the chronic stress and improved by the blueberry drink supplementation. The plasma epinephrine level was only influenced by the stress.

CONCLUSION: These findings suggest that the blueberry drink may protect against the cognitive impairment induced by chronic mild stress.

Keywords: Blueberry, cognitive impairment, chronic stress, antioxidant, catecholamine

INTRODUCTION

Stress is defined as any situation capable of perturbing physiological or psychological homeostasis [1]. The response to stress involves the activation of the hypothalamic-pituitary-adrenocortical (HPA) axis, and psychological stress appears to be a more potent activator of the HPA axis. The HPA system, which releases glucocorticoids, and the sympathetic adrenergic system, which releases catecholamines, act as integrated units controlling stress responses [2]. It has been shown that psychological stress can induce dysfunctions in the nervous system, including cognitive impairment, anxiety, and insomnia [3]. Recent studies have shown that both intense acute and chronic stress can have detrimental neurocognitive effects [4,5], and repeated stress induces neurochemical and neuroanatomical changes in the brain, mainly in the HPA axis [6]. Memory and learning performances were also observed to decrease under chronic stress [7].

Oxidative stress, reflecting the accumulation of oxygen-containing free radicals, increases with aging and may play a critical role in age-related functional deficits of the brain [8,9]. The brain is particularly prone to oxidative damage owing to its high rate of oxygen consumption [10]. Therefore, it has been proposed that strategies improving the antioxidant defenses may prevent some of the effects of aging [11]. Research suggests that polyphenolic compounds contained in colorful fruits and vegetables exhibit potent antioxidant activity that reduces the age-related sensitivity to oxidative stress [12] and may be involved in protecting cognitive functions [13]. A large number of dietary supplements using flavonoid-rich foods have demonstrated beneficial effects on memory and learning in both animals and humans [14-16]. In particular, blueberries (fruits of Vaccinium uliginosum L and various other Vaccinium species) are one of several fruits with high levels of polyphenolic flavonoids and anthocyanins, which have a high antioxidant power [17]. Blueberry supplementation was effective in...
reversing cognitive declines in aged rats [18]. In aged rats fed blueberries for eight weeks, unmetabolized forms of anthocyanins were found in the cerebellum, hippocampus, cortex, and striatum [19]. Twelve-week blueberry supplementation in aged rats improved age-related deficits in cognitive and motor functioning [20].

Recently, the majority of studies have focused on the cognitive effects of flavonoid-rich foods in aged animals [13,21] and transgenic mouse models of Alzheimer's disease [20]. Only a few studies have investigated cognitive functions in young/adult rodents, and recent data suggest that flavonoids are capable of inducing cognitive improvements in young and healthy animals [22,23]. Dietary blueberry supplementation prevented deficits in the learning performance in adult rats and protected against neuronal loss induced by injections of kainic acid to the hippocampus [24] or by 56Fe particle irradiation [25]. It also improved the performance in memory tasks with protective effect on DNA in the hippocampus and cerebral cortex [26]. However, the effect of blueberries on the cognitive dysfunction induced by chronic mild stress has not been studied yet.

The aim of this study was to investigate the effects of eight-week administration of a blueberry drink on the cognitive performance in 12-week-old rats exposed to chronic mild stress. Loss of cognitive abilities during aging is a complex process that starts to become evident during middle age in rats (12-24-month-old), even in the absence of a specific neurodegenerative disease [27]. In the present study, a chronic mild stress model [28,29] was used to provide a realistic simulation of the stresses of daily life. After the rearing stage, the cognitive function of rats was assessed using a multiple T-maze water test. The levels of antioxidants (superoxide dismutase, catalase, glutathione peroxidase, and ascorbic acid) were measured in the brain, and catecholamine levels were measured in plasma.

MATERIALS AND METHODS

Materials

A freeze-dried 100% wild blueberry powder was purchased from Bactolac Pharmaceutical, Inc. (Hauppauge, NY, USA). A blueberry drink was prepared by adding the blueberry powder to fresh tap water (PurePlus, Inc., Incheon, Korea). Assay kits for catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). The standard of ascorbic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA), and all reagents used in ascorbic acid analysis were high-performance liquid chromatography (HPLC) grade. Catecholamines were measured using the 3-CAT plasma enzyme-linked immunosorbent assay (ELISA) kit from Labor Diagnostika (Nord, Nordhorn, Germany).

Animals and experimental design

Twelve-week-old male Sprague-Dawley rats (Hanlim Experimental Animal Laboratory, Seoul, Korea) weighing 470 ± 10 g were fed a laboratory diet. The animals were kept individually and housed in a temperature-controlled room (24 ± 1°C) with a relative humidity of 50-60% and a 12/12 h light/dark cycle (lights on at 6:00 a.m.). The rats had ad libitum access to food in the form of dry pellets (Purina, Nestle Purina PetCare Korea, Ltd., Seoul, Korea) and fresh tap water. The study protocol was approved by the Committee on Animal Welfare Regulations of Chung-Ang University (IRB 14-70).

After acclimation to laboratory conditions for one week, the rats were randomly divided into four groups (n = 12 per group): a control group (CO, no chronic mild stress/tap water), a blueberry drink only group (CO + B, no chronic mild stress/5% blueberry drink), a stress only group (ST, chronic mild stress/tap water), and a stress + blueberry drink group (ST + B, chronic mild stress/5% blueberry drink). To prepare a 5% blueberry drink, 5 g of the blueberry powder was mixed daily with 100 mL of tap water and then stirred with a magnetic stir bar under protection from light until completely dissolved. The blueberry drink was provided to the CO + B and ST + B groups instead of the regular drinking water, and the water bottles for the groups were wrapped in aluminum foil to protect from light in order to prevent the destruction of bioactive compounds. Feed and water consumption was recorded daily, and the rats were weighed weekly. A feed efficiency ratio (FER) was also calculated. All animals were maintained under the appropriate experimental conditions for eight weeks before performing the multiple T-maze water test.

Chronic mild stress model

The chronic mild stress model was slightly modified from those described previously for rats by Willner et al. [28] and for mice by Monleon et al. [29]. Briefly, the rats were subjected to the following stressors (one or two in any 24-h period): one period of food deprivation (8 h), one period of water deprivation (16 h), two periods of overnight illumination (12 h), one period of cage exchange (17 h), two periods of a 45° cage tilt (7 h/17 h), one period of soiled bedding (200 mL of water per cage; 17 h), or one period of no stress (24 h). Table 1 shows the timing and length of all the stressors used in the chronic mild stress protocol. All the individual stressors used have been classified as "mild" according to the Animals (Scientific Procedures) Act of 1986 (UK legislation) [28]. The stressors were scheduled throughout the eight weeks before the water maze test.

| Activity                  | Monday       | Tuesday    | Wednesday  | Thursday   | Friday     | Saturday   | Sunday     |
|---------------------------|--------------|------------|------------|------------|------------|------------|------------|
| Food deprivation          | 22:00 → 06:00|            |            |            |            |            |            |
| Water deprivation         |              |            |            | 17:00 → 09:00|            |            |            |
| Continuous light           |              |            |            |            | 18:00 → 06:00|            |            |
| Tilted cage               | 10:00 → 17:00|            |            |            |            | 20:00 → 13:00|            |
| Soiled cage               |              |            |            |            |            |            | 16:00 → 09:00|
| Cage exchange             |              |            |            |            |            |            | 18:00 → 11:00|
Table 2. Effects of the blueberry drink on body weight, brain weight, and water and food intake in adult rats under chronic mild stress conditions

| Group                     | Control (CO) | Stress (ST) | ANOVA(1) |
|---------------------------|--------------|-------------|----------|
| No blueberry              |              |             |          |
| Initial body weight (g)   | 471.09 ± 4.83| 472.97 ± 4.83| 0.8868   |
| Final body weight (g)     | 553.37 ± 7.96a| 553.69 ± 7.96a| 0.0176   |
| Brain weight (g)          | 2.18 ± 0.03a| 2.12 ± 0.03b| 0.0028   |
| Feed intake (g/day)       | 29.56 ± 0.39a| 28.30 ± 0.39c| 0.0134   |
| Feed efficiency ratio     | 0.09 ± 0.01b| 0.08 ± 0.01c| 0.0025   |
| Water intake (mL/day)     | 55.28 ± 1.35b| 57.69 ± 1.35c| 0.0254   |

Data are expressed as the mean ± SE of 12 animals per group.  
1) Significant differences between the chronic mild stress (ST) and blueberry drink (B) or interaction between these factors (ST × B) were tested by two-way ANOVA and expressed as P-values, followed by Fisher’s LSD test.  
2) Mean values with different superscript letters are significantly different (P < 0.05).
Fig. 1. Effects of the blueberry drink on the mean time to reach the goal in the multiple T-maze water test on two days in adult rats under chronic mild stress conditions. (A) mean time to reach the goal on the first day of the multiple T-maze water test and (B) mean time to reach the goal on the second day of the multiple T-maze water test. The data are expressed as the mean ± SE of 12 animals per group. Significant differences between the stress (ST) and blueberry drink (B) or interaction between these factors (ST × B) were tested by two-way ANOVA and expressed as P-values, followed by Fisher’s LSD test. Different letters represent statistical differences between the means (P < 0.05).

Fig. 2. Effects of the blueberry drink on the total number of errors in the multiple T-maze water test on two days in adult rats under chronic mild stress conditions. (A) Total number of errors on the first day of the multiple T-maze water test and (B) total number of errors on the second day of the multiple T-maze water test. The data are expressed as the mean ± SE of 12 animals per group. Significant differences between the stress (ST) and blueberry drink (B) or interaction between these factors (ST × B) were tested by two-way ANOVA and expressed as P-values, followed by Fisher’s LSD test. Different letters represent statistical differences between the means (P < 0.05).

The brain SOD, CAT, GPx, and ascorbic acid concentrations

The effects of the chronic mild stress and blueberry drink on the brain SOD, CAT, GPx, and ascorbic acid levels are shown in Table 3. The brain SOD and CAT concentrations were not affected by the stress or by the blueberry drink. The GPx and ascorbic acid concentrations were significantly increased by the blueberry drink supplementation. The chronic mild stress only affected the brain ascorbic acid level. The concentrations of GPx and ascorbic acid in the ST group were significantly lower than those in the ST + B group (P < 0.05).

Table 3. Effects of the blueberry drink on brain superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and ascorbic acid levels in adult rats under chronic mild stress conditions

| Group | Control (CO) | Stress (ST) | ANOVA 1) |
|-------|--------------|-------------|----------|
|       | No blueberry (CO) | Blueberry (CO + B) | No blueberry (ST) | Blueberry (ST + B) | ST | B | ST × B |
| SOD (U/g) | 20.55 ± 6.85 | 20.68 ± 6.53 | 21.27 ± 7.22 | 20.63 ± 6.85 | 0.4421 | 0.4493 | 0.4377 |
| CAT (mmol/min/g) | 405.04 ± 32.59 | 415.67 ± 31.07 | 361.94 ± 34.35 | 412.65 ± 32.59 | 0.4848 | 0.3542 | 0.5434 |
| GPx (nmol/min/mL) | 153.43 ± 7.01abc | 165.45 ± 7.36a | 135.53 ± 7.36b | 170.35 ± 6.71a | 0.3665 | 0.0021 | 0.1171 |
| Ascorbic acid (μg/g) | 97.53 ± 4.45abc | 117.42 ± 4.45a | 84.24 ± 5.46b | 106.31 ± 4.45ab | 0.0136 | < 0.0001 | 0.8191 |

Data are expressed as the mean ± SE of 12 animals per group.
1) Significant differences between the stress (ST) and blueberry drink (B) or interaction between these factors (ST × B) were tested by two-way ANOVA and expressed as P-values, followed by Fisher’s LSD test.
2) Mean values with different superscript letters are significantly different (P < 0.05).
of body weight) were consumed by the CO + B and ST + B groups; therefore, the blueberry drink in the current study improved liquid drinking by the stressed animals. Thus, the water intake in the ST + B group did not differ from that in the CO + B group. The plasma dopamine concentration was increased by the blueberry drink supplementation from 364.57 pg/mL in the ST group to 765.80 pg/mL in the ST + B group. No significant interactions between the chronic mild stress and blueberry drink were observed with regard to the plasma catecholamine concentrations.

**DISCUSSION**

Recently, there has been an increase in diseases related to psychological stress, and thus, more attention has been given to the prevention of stress-induced injuries. Stress is associated with the activation of the HPA axis. The hippocampus is critical for the learning, consolidation, and retrieval of declarative memories. It is also important for the formation of spatial memory [33]. Thus, the stress-induced impairment of learning and memory is related to the suppression of hippocampal neurogenesis, and one of the reasons why chronic stress suppresses hippocampal neurogenesis is that it increases the oxidative stress [34]. Research has focused on whether ingestion of antioxidants leads to the reduction of oxidative stress and to the improvement in impaired cognitive function. Therefore, this study investigated the antioxidant activities and neuroprotective effects of a blueberry drink on the cognitive impairment induced by chronic mild stress in Sprague-Dawley adult rats.

Exposure to stress situations can influence the feeding behavior, which results in changes of body weight of rats [35]. In this study, the ST and ST + B groups showed a reduced feed intake and a decreased body weight. The blueberry drink did not restore the feed intake, nor did it ameliorate the body weight loss caused by the chronic stress. The water intake in the ST group was significantly lower than that in the other groups, which might have resulted from water deprivation (16 h/week) under the mild stress conditions. However, the liquid intake in the ST + B group did not differ from that in the CO and CO + B groups; therefore, the blueberry drink in the current study improved liquid drinking by the stressed animals. Thus, similar amounts of the blueberry powder (5.76 and 5.55 g/kg of body weight) were consumed by the CO + B and ST + B groups, respectively, in this study, although the ST + B group had a period of liquid deprivation during mild stress.

Various studies have used the multiple T-maze test for analyzing spatial memory in mice and rats [36,37]. The advantage of the multiple T-maze test is that it uses a more complex choice system, with multiple choice points, and thus, can be used to test both reference and working memories [38]. Elizalde et al. [39] showed that the mice exposed to chronic mild stress showed long-lasting behavioral effects and recognition memory deficits as demonstrated by a significant increase in the swimming latency time compared to that in controls. Chen et al. [1] showed that the total time and number of mistakes in stressed groups in a water maze test increased in comparison to those in a control group. In this study, the adult rats from the ST group showed a significant increase in the mean time needed to reach the goal and in the number of errors, which indicates that the chronic mild stress used in this study induced cognitive impairment.

Studies have suggested that polyphenolic compounds may be involved in protecting cognitive functioning through their antioxidant activities [13]. Positive effects of polyphenolic consumption include direct effects on signaling, shown to enhance neuronal communication, the ability to buffer against excess calcium, the enhancement of neuroprotective adaptations, and the reduction of stress signals [40]. The major classes of berry phenolic compounds include flavonoids such as flavonols, flavanols, and anthocyanins, condensed tannins, hydrolysable tannins, stilbenoids, and phenolic acids [41]. It appears that berry fruits mediate signaling pathways involved in inflammation and cell survival, in addition to enhancing neuroplasticity, neurotransmission, and calcium buffering, all of which lead to attenuation of age- and pathologic-related deficits in behavior [10]. In this study, the cognitive impairment induced in adult rats by the chronic mild stress was improved by the supplementation of the blueberry drink. The mean time to reach the goal and the number of errors in the multiple T-maze test were significantly lower in the ST + B group than those in the ST group and similar to the results shown by the CO group. Thus, our findings indicate that dietary supplementation with a blueberry drink may protect and increase the spatial working memory and learning ability of adult rats exposed to chronic psychological stress. This is similar to the results of other studies that reported protective effects of blueberries on cognitive impairment induced by injections of kainic acid to the hippocampus [24] and by 56Fe particle irradiation [25].

The metalloproteins SOD, CAT, and GPx provide the first line of antioxidant defense against reactive oxygen species through
enzyme-catalyzed dismutation of O$_2$ to H$_2$O$_2$, which is further reduced to oxygen and water [42]. Chronic stress has been shown to increase the vulnerability of the brain, particularly the hippocampus, by altering the antioxidant capacity through the mediation of glucocorticoids. Papandreou et al. [43] have shown that after short-term supplementation with blueberry powder, a significant antioxidant enhancement was observed in adult mouse brains. In the current study, although no effects were observed on SOD and CAT, the brain GPx level was significantly lowered by the chronic mild stress and restored by the supplementation of the blueberry drink. Ascorbic acid can act as an antioxidant and oxygen radical scavenger, and has been detected in high amounts in brain tissue. Moreover, it has been shown that ascorbic acid acts as a neuromodulator of both glutamate- and dopamine-mediated neurotransmission and is an essential co-factor in the synthesis of norepinephrine and many neuropeptides [44]. It has been shown that short- and long-term supplementation with ascorbic acid results in positive effects on memory and passive avoidance learning in rats [45]. In our study, the concentration of ascorbic acid in the brains of the rats from the ST group was significantly lower than in those from the other groups; however, dietary supplementation with the blueberry drink significantly improved the ascorbic acid content in the brain.

It is well known that the sympathetic nervous system is closely involved in the regulation of the stress response. Psychological stress activates the sympathetic nervous system, and, in turn, catecholamines are released from the sympathetic nerve terminal and the adrenal medulla. Catecholamines include norepinephrine, epinephrine, and dopamine, which are involved in the modulation of the body's cognitive and emotional state and other psychoactivities. Norepinephrine and dopamine levels decrease upon experiencing psychological stress [1]. In the current study, the levels of norepinephrine and dopamine in the plasma of the ST group were lower than those in the plasma of the CO group, and these deficits were significantly ameliorated by the blueberry supplementation. Furthermore, the catecholamine levels in the CO + B group were slightly higher than those in the CO group. A significant decrease in the GPx level was detected in the ST group in this study; therefore, chronic mild stress may produce oxidants [35]. Increases in oxidative stress and hydrogen peroxide attenuated the dopamine release in the dorsal striatum and decreased the basal dopamine levels [46]. Thus, the supplementation of blueberry, having a high antioxidant power, possibly ameliorated the oxidative damage induced by the stress, which resulted in a significant improvement of the dopamine level in the ST + B group in this study.

Based on the compositional analysis, 1 g of the blueberry powder in this study contained 19.96 mg of total polyphenols in gallic acid equivalents (data not shown). The approximately 5.5 g/kg of body weight of the blueberry powder consumed daily by the rats equated to the amount of polyphenols in 53 g of fresh whole blueberries, based on the polyphenol content [47]. The amount of raw blueberries given to the animals (53 g) would correspond to approximately 515 g of raw blueberries for a human adult weighing 60 kg, using the formula for a human equivalent dose based on the body surface area [48]. Further, the amount of the blueberry powder, 5.5 g/kg of body weight of the rats, would correspond to 0.88 g/kg of body weight of human adults according to the equation [48]. Future studies should investigate different concentrations of blueberry drinks to maximize the protective effects of blueberry on the cognitive impairment caused by chronic mild stress.

In conclusion, our findings suggest that blueberry supplementation may protect against the cognitive impairment induced by chronic psychological stress. These results may be due to the antioxidant and neuroprotective effects of a blueberry drink, resulting from the high concentrations of polyphenols and flavonoids found in blueberry. Based on these findings, blueberry appears to have potential benefits in terms of prevention of a cognitive decline during stress, and these effects may extend to the cognitive decline associated with other neuropathic diseases.

**CONFLICT OF INTEREST**

The authors declare no potential conflicts of interests.

**REFERENCES**

1. Chen WQ, Zhao XL, Hou Y, Li ST, Hong Y, Wang DL, Cheng YY. Protective effects of green tea polyphenols on cognitive impairments induced by psychological stress in rats. Behav Brain Res 2009;202:71-6.
2. Liu J, Mori A. Stress, aging, and brain oxidative damage. Neurochem Res 1999;24:1479-97.
3. Pike JL, Smith TL, Hauger RL, Nicassio PM, Patterson TL, McClintick J, Costlow C, Irwin MR. Chronic life stress alters sympathetic, neuroendocrine, and immune responsivity to an acute psychologica
4. Kim JJ, Diamond DM. The stressed hippocampus, synaptic plasticity and lost memories. Nat Rev Neurosci 2002;3:453-62.
5. Joëls M, Pu Z, Wiegert O, Otzl MS, Krugers HJ. Learning under stress: how does it work? Trends Cogn Sci 2006;10:152-8.
6. Oh JK, Kim YS, Park HJ, Lim EM, Pyun KH, Shim I. Antidepressant effects of Soyo-san on immobilization stress in ovariectomized female rats. Biol Pharm Bull 2007;30:1422-6.
7. Abidin I, Yarigçioglu P, Agar A, Gümüşlu S, Aydin S, Oztürk O, Sahin E. The effect of chronic restraint stress on spatial learning and memory; relation to oxidant stress. Int J Neurosci 2004;114:683-99.
8. Head E. Oxidative damage and cognitive dysfunction: antioxidant treatments to promote healthy brain aging. Neurochem Res 2009; 34:670-8.
9. Sasaki T, Unno K, Tahara S, Shimada A, Chiba Y, Hoshino M, Kaneko T. Age-related increase of superoxide generation in the brains of mammals and birds. Aging Cell 2008;7:459-69.
10. Shukitt-Hale B. Blueberries and neuronal aging. Gerontology 2012;58:518-23.
11. Shukitt-Hale B, Lau FC, Joseph JA. Berry fruit supplementation and the aging brain. J Agric Food Chem 2008;56:363-41.
12. Stevenson DE, Hurst RD. Polyphenolic phytochemicals–just antioxidants or much more? Cell Mol Life Sci 2007;64:2900-16.
13. Williams CM, El Mohsen MA, Vazdour D, Rendeiro C, Butler LT, Ellis JA, Whiteman M, Spencer JP. Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB.
phosphorylation and brain-derived neurotrophic factor (BDNF) levels. Free Radic Biol Med 2008;45:295-305.
14. Rendeiro C, Vauzour D, Kean RJ, Butler LT, Rattray M, Spencer JP, Williams CM. Blueberry supplementation increases spatial memory improvements and region-specific regulation of hippocampal BDNF mRNA expression in young rats. Psychopharmacology (Berl) 2012; 223:319-30.
15. Krikorian R, Shidler MD, Nash TA, Kalt W, Vinqvist-Tymchuk MR, Shukitt-Hale B, Joseph JA. Blueberry supplementation improves memory in older adults. J Agric Food Chem 2010;58:3996-4000.
16. Bekk K, Veira A. Flavonoid intake and disability-adjusted life years due to Alzheimer’s and related dementias: a population-based study involving twenty-three developed countries. Public Health Nutr 2010;13:1403-9.
17. Prior RL, Cao G, Prior RL, Cao G. Analysis of botanicals and dietary supplements for antioxidant capacity: a review. J AOAC Int 2000; 83:950-6.
18. Malin DH, Lee DR, Goyarzu P, Chang YH, Ennis LJ, Beckett E, Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, JR. Effects of Ginkgo biloba administered after spatial learning on hippocampal-dependent cognitive functions. Arch Biochem Biophys 2015;576:2-7.
19. Muto J, Hosung L, Uwaya A, Isami F, Ohno M, Mikami T. Morinda citrifolia fruit reduces stress-induced impairment of cognitive function accompanied by vasculature improvement in mice. Physiol Behav 2010;101:211-7.
20. van der Loo B, Bachschmid M, Papandreou MA, Dimakopoulou A, Linardaki ZI, Cordopatis P, Kunesová G, Hlavácek J, Petronilho MA, Joseph JA. Modulation of hippocampal plasticity and cognitive behavior by short-term blueberry supplementation in aged rats. Nutr Neurosci 2004;7:309-16.
21. Hajipour S, Sarkaki A, Mohammad S, Mansouri T, Pilevarian A, Rafieirad M. Motor and cognitive deficits due to permanent cerebral hypoperfusion/ischemia improve by pomegranate seed extract in rats. Pak J Biol Sci 2014;17:991-8.
22. Shif O, Gillette K, Damkaoutis CM, Carrano C, Robbins SJ, Hoffman JR. Effects of Ginkgo biloba administered after spatial learning on water maze and radial arm maze performance in young adult rats. Pharmacol Biochem Behav 2006;84:17-25.
23. Dufy KB, Spangler EL, Devan BD, Guo Z, Bowker JL, Janas AM, Hagepanos A, Minor RK, DeCabo R, Mouton PR, Shukitt-Hale B, Joseph JA, Ingram DK. A blueberry-enriched diet provides cellular protection against oxidative stress and reduces a kainate-induced learning impairment in rats. Neurobiol Aging 2008;29:1680-9.
24. Shukitt-Hale B, Carey AN, Jenkins D, Rattray M, Spencer JP, Williams CM. Blueberry supplementation increases spatial memory improvements and region-specific regulation of hippocampal BDNF mRNA expression in young rats. Psychopharmacology (Berl) 2012; 223:319-30.
25. Krikorian R, Shidler MD, Nash TA, Kalt W, Vinqvist-Tymchuk MR, Shukitt-Hale B, Joseph JA. Blueberry supplementation improves memory in older adults. J Agric Food Chem 2010;58:3996-4000.
26. Bekk K, Veira A. Flavonoid intake and disability-adjusted life years due to Alzheimer’s and related dementias: a population-based study involving twenty-three developed countries. Public Health Nutr 2010;13:1403-9.
27. Prior RL, Cao G, Prior RL, Cao G. Analysis of botanicals and dietary supplements for antioxidant capacity: a review. J AOAC Int 2000; 83:950-6.
28. Malin DH, Lee DR, Goyarzu P, Chang YH, Ennis LJ, Beckett E, Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, JR. Effects of Ginkgo biloba administered after spatial learning on hippocampal-dependent cognitive functions. Arch Biochem Biophys 2015;576:2-7.
29. Muto J, Hosung L, Uwaya A, Isami F, Ohno M, Mikami T. Morinda citrifolia fruit reduces stress-induced impairment of cognitive function accompanied by vasculature improvement in mice. Physiol Behav 2010;101:211-7.
30. van der Loo B, Bachschmid M, Papandreou MA, Dimakopoulou A, Linardaki ZI, Cordopatis P, Kunesová G, Hlavácek J, Petronilho MA, Joseph JA. Modulation of hippocampal plasticity and cognitive behavior by short-term blueberry supplementation in aged rats. Nutr Neurosci 2004;7:309-16.
31. Hajipour S, Sarkaki A, Mohammad S, Mansouri T, Pilevarian A, Rafieirad M. Motor and cognitive deficits due to permanent cerebral hypoperfusion/ischemia improve by pomegranate seed extract in rats. Pak J Biol Sci 2014;17:991-8.
32. Shif O, Gillette K, Damkaoutis CM, Carrano C, Robbins SJ, Hoffman JR. Effects of Ginkgo biloba administered after spatial learning on water maze and radial arm maze performance in young adult rats. Pharmacol Biochem Behav 2006;84:17-25.
33. Dufy KB, Spangler EL, Devan BD, Guo Z, Bowker JL, Janas AM, Hagepanos A, Minor RK, DeCabo R, Mouton PR, Shukitt-Hale B, Joseph JA, Ingram DK. A blueberry-enriched diet provides cellular protection against oxidative stress and reduces a kainate-induced learning impairment in rats. Neurobiol Aging 2008;29:1680-9.
34. Shukitt-Hale B, Carey AN, Jenkins D, Rattray M, Spencer JP, Williams CM. Blueberry supplementation increases spatial memory improvements and region-specific regulation of hippocampal BDNF mRNA expression in young rats. Psychopharmacology (Berl) 2012; 223:319-30.
35. Krikorian R, Shidler MD, Nash TA, Kalt W, Vinqvist-Tymchuk MR, Shukitt-Hale B, Joseph JA. Blueberry supplementation improves memory in older adults. J Agric Food Chem 2010;58:3996-4000.
memory in rat. Brain Res Bull 2008;76:109-13.

45. Chen WQ, Zhao XL, Wang DL, Li ST, Hou Y, Hong Y, Cheng YY. Effects of epigallocatechin-3-gallate on behavioral impairments induced by psychological stress in rats. Exp Biol Med (Maywood) 2010;235:577-83.

46. Juárez Olguín H, Calderón Guzmán D, Hernández García E, Barragán Mejía G. The role of dopamine and its dysfunction as a consequence of oxidative stress. Oxid Med Cell Longev 2016;2016:9730467.

47. Bunea A, Rugina DO, Pintea AM, Sconta Z, Bunea CI, Socaciu C. Comparative polyphenolic content and antioxidant activities of some wild and cultivated blueberries from Romania. Not Bot Horti Agrobo 2011;39:70-6.

48. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J 2008;22:659-61.