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Antioxidant and Memory Enhancing Effects of Purple Sweet Potato Anthocyanin and Cordyceps Mushroom Extract

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The effects of purple sweet potato anthocyanin (SPA) and Cordyceps mushroom extract (CME) on lipid peroxidation, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and cognitive deficits were examined. Both SPA and CME exhibited DPPH radical scavenging activities with similar potency. In contrast, only SPA was shown to effectively inhibit lipid peroxidation initiated by Fe^{2+} and ascorbic acid in rat brain homogenates. Furthermore, SPA markedly enhanced cognitive performance, assessed by passive avoidance test in ethanol-treated mice. Combined treatments with SPA and CME did not significantly influence the effects of SPA alone. These results demonstrate that anthocyanin prepared from purple sweet potato exhibits memory enhancing effects, which may be associated with its antioxidant properties.

Key words: Sweet potato, Anthocyanin, Antioxidant, Memory enhancement, Cordyceps mushroom

INTRODUCTION

Memory is the process by which facts and events are kept in mind and made available for later use. Depending on how long a memory lasts; it is classified as either working memory, a limited-capacity, short-term memory that lasts seconds to minutes, or long-term memory, which lasts hours, days, or years. Most demential conditions caused by aging or Alzheimer's disease (AD) are characterized by common psychological phenomena (Brewer, 1998) and oxidative damage was considered to be a likely cause of brain dysfunction (Sohal *et al.*, 1990; Coyle *et al.*, 1993). Recently, many natural compounds such as anthocyanins have been known for their antioxidative effects (Shafiee *et al.*, 2002; Ng *et al.*, 2000; Tanizawa *et al.*, 1992) and are expected to be beneficial for preventing brain dysfunction.

Anthocyanin pigments are not only responsible for the red, purple, and blue colors of many fruits, vegetables, and flowers but also the active components in several herbal folk medicines. Recently, interests in anthocyanin pigments have been intensified because of their potential health benefits. Certain anthocyanins have shown to exhibit antiproliferative, vasoprotective, antiinflammatory, and hepatoprotective activities in cell cultures and animal models (Wang *et al.*, 2000; Lazze *et al.*, 2003). In addition, the anthocyanins of Bilberries (*Vaccinium myrtillus*) have long been used to improve night vision (Jayle *et al.*, 1965) and other diseases (Amouretti, 1972). Furthermore, orally administered anthocyanins are reported to be beneficial for treating diabetes and ulcers and may have antiviral and antimicrobial activities (Sterling, 2001). The chemical basis for these biological properties of anthocyanins is related to their antioxidant capacity to scavenge and trap free radicals that damage biomolecules (Tsuda *et al.*, 1996, 2000; Wang *et al.*, 2000; Ramirez-Tortosa *et al.*, 2001). Recently, new cultivars of sweet potatoes (*Ipomoea batatas* Lam.), also known as "purple sweet potatoes", were introduced (Odake *et al.*, 1992; Goda *et al.*, 1997). These cultivars attracted interests of scientists as well as anthocyanin lovers because of their high anthocyanin contents. Several anthocyanins found in sweet potatoes were chemically elucidated (Odake *et al.*, 1992; Goda *et al.*, 1997; Harborne and Williams, 2001). These include acylated anthocyanins such as 3-*O*-(6-*O*-*trans*-caffeyl-2-*O*-β-glucopyranosyl-β-glucopyranoside)-5-*O*-β-glucosides of cyanidin and peonidin (Goda *et al.*, 1997). The anthocyanin fraction of sweet potato was claimed to enhance blood flow in the rat brain as well as increase ATP level in

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the stratum corneum (Jo *et al.*, 1997). Recently, it has been introduced in combination with Cordyceps mushroom extract into the Japanese market as a supplementary health food enhancing memory.

Cordyceps mushrooms, also known as "insect fungus" or "insect-born fungus", have traditionally been used as medicinal mushrooms in Asian countries such as China, Korea, and Japan (Jianzhe *et al,* 1987). During the last decades, their medicinal effects have been intensively investigated and some of the active components are well characterized. For example, cordycepin, originally isolated from *Cordyceps militaris* and later in *C. sinensis,* is the best-known antitumor compound of Cordyceps mushrooms. In Korea, more than 71 species of insect fungi have been reported (Sung, 1996) and two of these, *C. militaris* (Korean name, bundegi-dongchunghacho) and *Paecilomyces japonica* (Korean name, nunggot-dongchunghacho) are well known to the public. Among these, *C. militaris* is considered to be a representative species of Cordyceps mushrooms.

In an attempt to evaluate antioxidant and memory enhancing effects of purple sweet potato anthocyanin (SPA) and Cordyceps mushroom extract (CME), we investigated effects on lipid peroxidation in rat brain homogenates and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals by *in vitro* assays, and cognitive performance in ethanol-treated mice using passive avoidance test. The ethanol-intoxicated mice model has been used as an animal model for studying AD-associated dementia, since alcoholic dementia is reported to be similar to AD with regard to the influenced mediator systems and damaged brain regions (Raghavendra and Kulkarni, 2001). Furthermore, since SPA is currently introduced into the Japanese market as combined preparations with CME, we also evaluated whether the addition of CME influenced the effects of SPA alone.

MATERIALS AND METHODS

Instruments and chemicals

The Passive/Active avoidance system, PACS-30 (Columbus Co., OH, USA) was used for passive avoidance test. Trizma base, thiobarbituric acid (TBA), trichloroacetic acid (TCA), ferric sulfate, ascorbic acid, and DPPH were purchased from Sigma Chemical Co. (MI, USA). All other chemicals were reagent grade or better.

Preparation of anthocyanin from purple sweet potatoes

Purple sweet potatoes cultivated in Boryung area in Chungnam, Korea, were obtained from Dongyi Co., Ltd. (Seoul, Korea). The anthocyanin was prepared according to Rodriguez-Saona *et al.* (1999), with some modifications. In brief, the tubers (ca 1 kg) were cut in slices (ca 1 cm thick), blended with 70% aqueous ethanol (ca 1.6 L), stood at room temperature for one hour, and filtered through a Buchner funnel. The filter cake was extracted again with the same solvent (ca 1.2 L). The filtrates were combined, evaporated (to ca 800 mL) in a vacuum evaporator, shaken with the same volume of chloroform, and kept at 4°C overnight. The aqueous portion was then collected using a separating funnel, and evaporated in a vacuum evaporator until all residual chloroform was evaporated and the volume was reduced to ca 315 mL. The concentrated aqueous portion was lyophilized to yield a deep purple-blue amorphous powder (ca 51.9 g).

Preparation of hot water extract of cordyceps mushroom

The hot-air dried carpophores of Cordyceps mushroom, *C. militaris*, were obtained from Dongyi Co., Ltd. (Seoul, Korea). The mushrooms (ca 450 g) were pulverized in a blender (Osterizer 16 Speed Blender), mixed with 3 L distilled water, stirred for 30 min, and extracted at 120°C for 60 min. After being cooled down, the extract was separated by centrifugation at 2800 rpm for 20 min, filtered through a Buchner funnel and then lyophilized to yield yellow brown amorphous powder (ca 94.3 g). Some of the mushrooms are kept as a voucher specimen in the Laboratory of Microbiology and Immunology, College of Pharmacy, Chungnam National University.

Animals and dosing regimens

Male Sprague-Dawley (SD) rats (270-300 g) for brain homogenate preparation and male ICR mice (18-20 g) were obtained from Daehan Biolink Co. (Chungbuk, Korea). They were separately housed in automatically controlled animal rooms at 24±1°C, 55±5% relative humidity, ventilation, and controlled lighting (from 6:00 to 18:00 daily) with standard foods and water *ad libitum*. For passive avoidance test, the dried samples were dissolved or suspended in water and administered orally once a day for 7 days in a volume of 1 mL/100 g body weight. Ethanol was given to mice in a single oral administration at a dose of 3 g/kg body weight as 38 v/v% solution in water at 30 min after the last treatment of the extract. The control animals were administered with water for 7 consecutive days without further treatment with ethanol.

Assay of lipid peroxidation in brain homogenates

SD rat brains were homogenized with a Polytron in 10 volumes of ice-cold Tris-HCl buffer (20 mM, pH 7.4) and centrifuged at 14,000 rpm for 15 min at 4°C. Lipid peroxidation was determined in the homogenates by the methods of Liu and Ng (2000) with slight modifications. The incubation mixture containing an aliquot of brain homogenates, 10 μM ferric sulfate, 100 μM ascorbic acid,

and various concentrations of the test samples dissolved in double distilled water was incubated for 1 h at 37°C. The reaction was stopped by addition of TCA (28 w/v %) and TBA (1 w/v %) in succession. The solution was heated at 100°C for 15 min, followed by centrifugation to remove precipitated protein. The color of the malonedialdehyde-TBA complex was detected at 532 nm using $VERSA_{max}$ microplate reader (Molecular Devices, CA, USA). The percent inhibition was calculated from the following equation:

$$\text{Inhibition (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100.$$

IC_{50} values, the concentrations inhibiting 50% of lipid peroxidation, were determined by nonlinear regression using Prism (GraphPad Software Inc., CA, USA).

Assay of DPPH radical scavenging activity

DPPH radical scavenging activities were measured by the method of Blois (1958) with some modifications. In brief, reaction mixtures in a 96-well microtiter plate containing 150 μM DPPH in methanol and various concentrations of the test samples dissolved in double distilled water were incubated at 37°C or 30 min and the absorbance was measured at 520 nm. Percent scavenging activity was determined by comparison with the vehicle-treated control group and calculated from the above equation. IC_{50} values, the concentrations required to scavenge 50% of the free radicals generated by DPPH, were determined by nonlinear regression using Prism.

Passive avoidance test

Memory tests were carried out by the published method (Lee *et al.,* 1999). The apparatus consisted of two equal compartments (15×15×22 cm) separated by a wall with a guillotine door (4×3.5 cm) in the lower middle part. One of the two compartments was illuminated and the other was dark. The animals were placed in the dark room for 1 h before trials began. The test was conducted on two consecutive days at the same time of the day after 18:00. On the first day, each mouse was placed in the illuminated compartment. After 30 s, the guillotine door was raised, allowing access to the dark compartment. Once the mouse entered the dark compartment, the guillotine door was lowered. And the mouse was received an electric shock (0.6 mA for 5 s). On the second day, the testing trial was carried out by the same test procedure of the learning trial. The mouse was again placed in the illuminated compartment, the guillotine door was opened and the step through latency was measured. An upper cut off time was set, allowing mice exposed in the light compartment for 300 s. The latency and the number of mice, which did not enter the dark compartment, were recorded in the testing trial.

Statistics

The data were processed by analyses of variance followed by individual comparisons using Student's *t*-test, and were expressed as the mean±S.E.M.

RESULTS AND DISCUSSION

Effects of SPA, CME, and their mixtures on lipid peroxidation

As shown in Fig. 1, SPA potently inhibited lipid peroxidation initiated by Fe^{2+} and ascorbic acid in rat brain homogenates. The IC_{50} value was 88.2 μg/mL. In contrast, CME exerted only a slight reduction of lipid peroxidation, inhibiting 30% at 1 mg/mL. The mixtures of SPA and CME at ratios of 1:1 and 1:3 also inhibited lipid peroxidation in concentration-dependent manners (Fig. 1). However, their potency was apparently decreased, with the respective IC_{50} values of 193.4 and 504.5 μg/mL. The decreased potency of the mixtures appears to be proportional to the contents of CME, which exerts negligible inhibition of lipid peroxidation. These findings imply that combination of SPA with CME may not be synergistic in the inhibition of lipid peroxidation.

DPPH radical scavenging activities of SPA, CME, and their mixtures

As illustrated in Fig. 2, SPA exhibited potent DPPH radical scavenging activity. Although lipid peroxidation was not effectively inhibited by CME (Fig. 1), DPPH radicals were markedly scavenged by CME with similar potency to that of SPA. Their IC_{50} values were 85.7 and

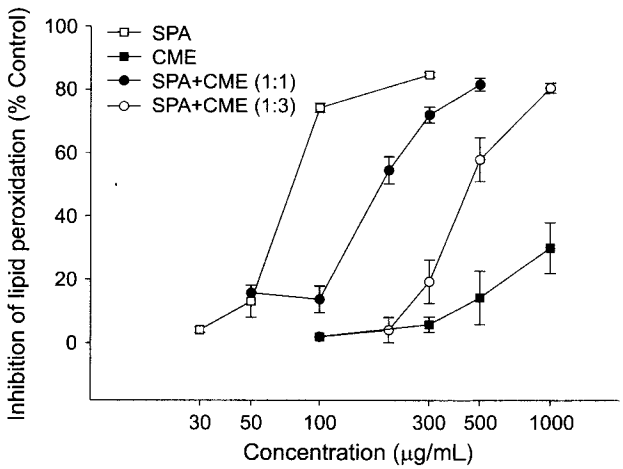

Fig. 1. Effects of SPA, CME, and their mixtures on lipid peroxidation. Lipid peroxidation was initiated by Fe^{2+} and ascorbic acid in the rat brain homogenates in the presence of the indicated concentrations of SPA, CME, or their mixtures, as described in the Materials and methods. Data are expressed as percent inhibition of lipid peroxidation measured in the absence of the test samples. Each point represents the mean±S.E.M. from at least three measurements performed in duplicate.

87.5 μg/mL, respectively. The 1:1 and 1:3 mixtures of SPA and CME also exhibited similar patterns of DPPH radical scavenging activities (Fig. 2), with the respective IC_{50} values of 91.2 and 91.4 μg/mL. Based on these findings, combination of SPA with CME did not exert synergistic actions in scavenging DPPH radicals, either.

Passive avoidance test

The single administration of ethanol reduced the latency of the mice in passive avoidance test. As shown in Fig. 3, the ethanol-treated group showed about 15% of the latency of the control (no ethanol-treated) group. The effect of ethanol on the latency was significantly interrupted by the administration of SPA (25 mg/kg, p.o.) and the latency was increased to 44% of the control. While the same dose of CME showed no interruption against the effect of ethanol. Administration of CME in combination with SPA did not influence the latency of mice treated with SPA only, indicating that CME possessed no synergistic effect on memory enhancement of SPA.

Taken together, we confirmed antioxidant properties of anthocyanin prepared from purple sweet potato, demonstrating inhibition of lipid peroxidation in brain homogenates by SPA and its DPPH radical scavenging activity. Furthermore, SPA was shown to improve cognitive performance in ethanol-treated mice. Cognitive dysfunction is probably due to the vulnerability of the brain cells to increased oxidative stress during aging or after the ethanol treatment (Coyle, 1993). The ethanol-induced amnesia has been reported to be reversed by antioxidants such as melatonin (Raghavendra and Kulkarni, 2001). Therefore,

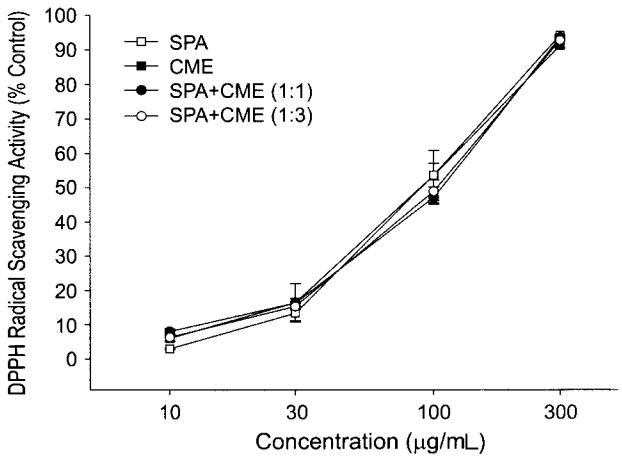

Fig. 2. DPPH radical scavenging activities of SPA, CME, and their mixtures. DPPH radical scavenging activities were determined in the presence of the indicated concentrations of SPA, CME, or their mixtures as described in the Materials and methods. Data are expressed as percent control activity, measured in the absence of the test samples. Each point represents the mean±S.E.M. from three measurements performed in duplicate.

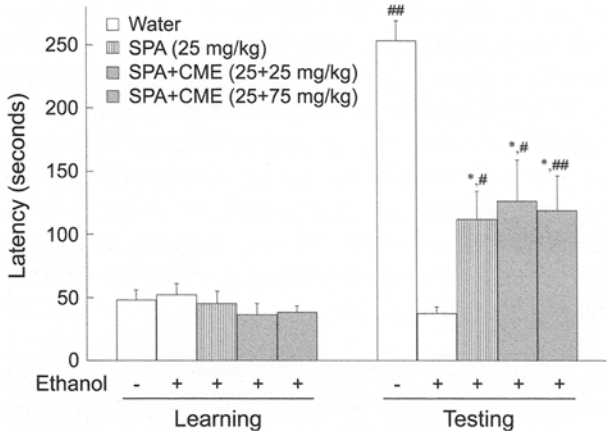

Fig. 3. Effects of SPA, CME, and their mixtures on cognitive performance in ethanol-treated mice. Animals were orally administered with water, SPA (25 mg/kg), or the mixtures of SPA and CME at a ratio of 1:1 (25 + 25 mg/kg) or 1:3 (25 + 75 mg/kg) once a day for 7 days. Cognitive performance was evaluated by passive avoidance test as described in the Materials and methods. Latency indicates the time at which mice entered the dark compartment. Values represent the mean ±S.E.M. of four to eleven mice. *p<0.05 when compared with water group in testing. #p<0.05 and ##p<0.01 when compared with the respective learning latency.

the memory enhancing effects of SPA observed in the present study could be, at least in part, due to its antioxidant action.

In contrast to SPA, CME did not inhibit lipid peroxidation nor improve cognitive performance, but it scavenged DPPH radicals with similar potency of SPA. Based on these observations, DPPH radical scavenging activity may not be sufficient to exhibit memory enhancing effects. The precise mechanisms of action remain to be fully elucidated.

It was found in this study that the combined treatment with SPA and CME did not enhance the effects of SPA alone. These results strongly support that SPA is the active component exhibiting memory enhancement in combined preparations commercially available in Japan. Nevertheless, commercial preparations containing both components seem to be plausible because CME is well known for its immunopotentiating and anticancer activities as well as other medical effects.

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